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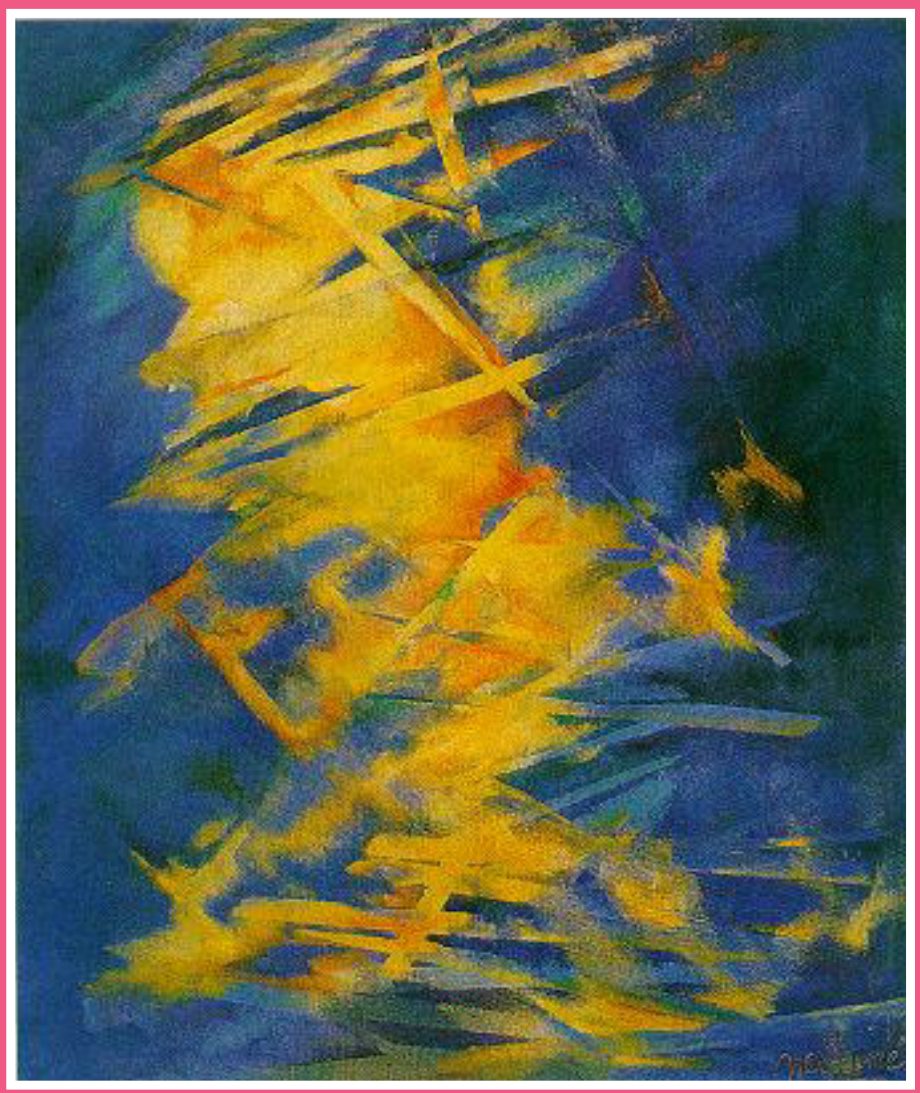

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La Tapa (Ver p. IV)
Imagen ígnea, 1996.
María Esther Gené

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medicina
BUENOS AIRES, VOL 77 Supl. I - 2017

REUNIÓN CONJUNTA DE SOCIEDADES DE BIOCIENCIAS

LXII REUNIÓN ANUAL DE LA SOCIEDAD ARGENTINA DE INVESTIGACIÓN CLÍNICA (SAIC)

LIII REUNIÓN ANUAL DE LA SOCIEDAD ARGENTINA DE INVESTIGACIÓN BIOQUÍMICA Y BIOLOGÍA MOLECULAR (SAIB)

LXV REUNIÓN ANUAL DE LA SOCIEDAD ARGENTINA DE INMUNOLOGÍA (SAI)

REUNIÓN DE LA SOCIEDAD ARGENTINA DE ANDROLOGÍA (SAA)

XLVI REUNIÓN ANUAL DE LA SOCIEDAD ARGENTINA DE BIOFÍSICA (SAB)

XIX REUNIÓN ANUAL DE LA SOCIEDAD ARGENTINA DE BIOLOGÍA (SAB)

XLIX REUNIÓN ANUAL DE LA SOCIEDAD ARGENTINA DE FARMACOLOGÍA EXPERIMENTAL (SAFE)

REUNIÓN ANUAL DE LA SOCIEDAD ARGENTINA DE FISIOLOGÍA (SAFIS)

REUNIÓN DE LA SOCIEDAD ARGENTINA DE HEMATOLOGÍA (SAH)

XXIX REUNIÓN ANUAL DE LA SOCIEDAD ARGENTINA DE PROTOZOOLOGÍA (SAP)

13-17 de noviembre de 2017
Palais Rouge– Buenos Aires

medicina
BUENOS AIRES, VOL 77 Supl. I - 2017

JOINT MEETING OF BIOSCIENCE SOCIETIES

LXII ANNUAL MEETING OF ARGENTINE SOCIETY OF CLINICAL INVESTIGATION (SAIC)

LIII ANNUAL MEETING OF ARGENTINE SOCIETY OF BIOCHEMISTRY AND MOLECULAR BIOLOGY (SAIB)

LXV ANNUAL MEETING OF ARGENTINE SOCIETY OF IMMUNOLOGY (SAI)

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XLIX ANNUAL MEETING OF ARGENTINE SOCIETY OF EXPERIMENTAL PHARMACOLOGY (SAFE)

ANNUAL MEETING OF ARGENTINE SOCIETY OF PHYSIOLOGY (SAFIS)

MEETING OF ARGENTINE SOCIETY OF HEMATOLOGY (SAH)

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November 13 -17, 2017
Palais Rouge– Buenos Aires

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LA TAPA

María Esther Gené, **Imagen ígnea**, 1996.

Acrílico sobre tela, 110 x 95 cm. Cortesía de la Comisión Nacional de Energía Atómica, Predio TANDAR, Centro Atómico Constituyentes. Presidente de la Comisión Organizadora de la Exposición Permanente: Dr. A.J.G.Maroto.

María Esther Gené nació en Buenos Aires. Cursó Historia del Arte y Estética con Blanca Pastor y Nelly Perazo. Se inició en el taller de Centa Bertier y continuó su formación con Miguel Dávila. Participó del grupo de investigación plástica que dirigió Emilio Renart. Integró el Grupo Gen y formó el Grupo Fusión. Realizó numerosas exposiciones colectivas e individuales (Museos Municipal de Bellas Artes de Luján, Fernán Félix de Amador, de Arte Moderno de la Ciudad de Buenos Aires, Fundaciones San Telmo y Banco Mayo, Fundación Andreani, Patio Bullrich, Galería Kristel K., Salón ICCED de Pintura, entre otros). Sus obras se encuentran en colecciones privadas de Argentina, México, Alemania, España, Uruguay y EE.UU.

[1] Comisión Nacional de Energía Atómica. Artistas Plásticos con la CIENCIA, Centro Atómico Constituyentes, Predio TANDAR, Buenos Aires, 1999; En: *http://www2.cnea.gov.ar/xxi/artistas/artistasplasticos.htm*

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OPENING LECTURE

CANCER NEOANTIGENS AS TARGETS FOR NATURAL AND THERAPEUTIC ANTI-TUMOR RESPONSES

ROBERT D. SCHREIBER, PH.D., Andrew M. and Jane M. Bursky
Washington University School of Medicine, St. Louis, USA.

Our previous work led to the elaboration of strong experimental data demonstrating the existence of a cancer immunoediting process that functions in both mouse models of cancer and human cancer patients. In the course of these studies we used a combination of exome sequencing and epitope prediction algorithms to show that tumor specific mutant proteins expressed in highly immunogenic d42m1 tumor cells derived from methylcholanthrene (MCA) treated immunodeficient mice represent immunodominant, tumor specific mutant antigens (TSMA) for $CD8^+$ T cells and that immunoselection is a major mechanism underlying the immunoediting process. More recently, we used a similar approach to identify tumor neoantigens in progressively growing T3 MCA sarcomas that render these mouse tumors susceptible to T cell-dependent checkpoint blockade immunotherapy. This work lead to the identification of two immunodominant neoantigens among the 700 point mutations in T3 tumor cells encoding mutant Laminin α subunit 4 (mLama4) and a mutant glucosyltransferase (mAlg8). Therapeutic vaccination of mice bearing established T3 tumors with synthetic long peptides (SLP) vaccines consisting of mLama4 plus mAlg8 peptides plus poly IC resulted in tumor rejection. The therapeutic protection afforded by the SLP vaccine was equal to that induced by checkpoint blockade therapy. Since we did not see evidence of T cell responses to other T3 TSMA, we explored whether the immunodominant antigens masked responses to weaker TSMA. Using CRISPR/Cas9, we reverted mLama4 and mAlg8 back to their nonimmunogenic wild type counterparts in T3 tumor cells. The tumor cells lacking mutant forms of either Lama4 or Alg8 remained susceptible to checkpoint blockade therapy with evidence of TIL skewing towards the remaining TSMA. We then generated T3 tumor cells lacking both mLama4 and mAlg8. The "fixed" T3 tumor cells also remained susceptible to anti-PD-1/anti-CTLA-4-mediated rejection, with evidence of enhanced T cell responses to at least two additional TSMAs—a point mutant form of Gapvd1 and a second novel mutant protein caused by an indel. We are currently testing whether SLP vaccines comprised of these two subdominant TSMA can effectively treat mice bearing either parental or "fixed" T3 tumors. We have also explored the minimal TSMA requirement for immune mediated tumor rejection. Using oncogene driven sarcomas that were completely devoid of mutant neoantigens, we found that protective immune responses to tumors requires the presence of both MHC-I and MHC-II restricted epitopes. The implications of these findings to development of effective neoantigen vaccines will be discussed.

LECTURE II

INFLAMMATION, METABOLISM AND CANCER LINKED TO AP-1(FOS/JUN) EXPRESSION

ERWIN F. WAGNER et al.
CNIO, Madrid, Spain

Our studies aim to analyze gene function in healthy and pathological conditions, e.g. in tumour development, using the mouse as a model organism, but also employing patient-derived samples. Specifically, the functions of the AP-1 (Fos/Jun) transcription factor complex regulating cell proliferation, differentiation and oncogenesis, as well as the cross-talk between organs are being investigated. The goal is to define molecular pathways leading to disease/cancer development and to identify novel therapeutic targets. We focus on elucidating a causal link between inflammation, cancer and AP-1 (Fos/Jun) expression using cell type-specific, switchable genetically engineered mouse models. We are also developing and characterizing new genetically engineered mouse models (GEMMs) for cancer and human diseases, such as for bone loss, fibrosis and psoriasis, and applying these to preclinical studies.

I will focus in my talk on recent studies towards understanding liver and skin pathologies at the cross-road between inflammation, metabolism and cancer, and will discuss the adverse effects of cancer cachexia, a deadly wasting syndrome and metabolic impairment with systemic manifestations frequently observed in cancer patients.

LECTURE III

CLOSING RIFTS TO OPEN NEW HORIZONS: A ´SWEET TALE´ OF TOLERANCE AND IMMUNITY FROM BASIC DISCOVERY TO DRUG DESIGN

GABRIEL A. RABINOVICH et al

Laboratorio de Inmunopatología, Instituto de Biología y Medicina Experimental (IBYME, CONICET) y Facultad de Ciencias Exactas y Naturales, Universidad de Beunos Aires, Argentina.

In the postgenomic era, the study of the glycome- the whole repertoire of saccharides in cells and tissues- has enabled the association of unique glycan structures with specific physiological and pathological processes. The responsibility for deciphering this biological information is assigned to endogenous glycan-binding proteins or lectins whose expression is regulated at sites of inflammation, infection and tumor growth. Galectins, a family of highly conserved glycan-binding proteins, control immune and vascular signaling programs, by modulating reorganization, clustering and signaling threshold of relevant glycosylated receptors. Our laboratory investigates the molecular interactions between endogenous galectins and glycans leading to the control of immune tolerance and homeostasis. In the past years, we identified essential roles for galectin-1 (Gal-1), a proto-type member of this family, in tolerogenic circuits operating during tumor growth, microbial invasion and resolution of inflammation associated to autoimmune and allergic disorders, neurodegeneration, pregnancy and cardiovascular diseases. Mechanistically, this endogenous lectin acts by selectively dampening Th1 and Th17 responses, instructing the differentiation of tolerogenic dendritic cells, promoting the expansion of regulatory T cells, favoring M2 macrophage polarization and modulating eosinophil trafficking. Moreover, our studies identified a glycosylation-dependent, Gal-1-mediated program that links tumor hypoxia, immunosuppression and vascularization and hinders success of anti-angiogenic and immunotherapeutic modalities. These observations, confirmed in a variety of experimental settings and human patient samples, prompted us to generate and validate a set of anti-Gal-1 mAb aimed at overcoming cancer immunosuppression, preventing aberrant angiogenesis and reinforcing antimicrobial responses. Moreover, using an interdisciplinary approach ranging from computational modeling, protein engineering, and cell biology, we designed and validated stable galectin-tailored variants aimed at potentiating tolerogenic circuits and promoting resolution of autoimmune inflammation. In conclusion, our studies contributed to elucidate novel pathways via which endogenous galectins translate glycan-encoded information into unique signaling programs, findings that bring unifying principles to the diverse fields of immune regulation, oncology and vascular biology. These observations have opened new possibilities for development of therapeutic strategies aimed at potentiating antitumor responses, reinforcing antimicrobial immunity and limiting autoimmune inflammation.

LECTURE VI

THE BIOCHEMISTRY OF G PROTEIN-COUPLED RECEPTOR SIGNALING

JEFFREY BENOVIC

Thomas Jefferson University, Jefferson Health, USA

G protein-coupled receptors (GPCRs) regulate the activity of numerous effector molecules and play an essential role in coordinating the ability of cells to rapidly respond to their environment. Agonist binding to a GPCR activates heterotrimeric G-proteins, which mediate downstream signaling and ultimately a physiological response. GPCR signaling is dynamic and undergoes rapid regulation by GPCR kinases (GRKs), which specifically phosphorylate activated GPCRs, and arrestins, which bind to GRK-phosphorylated GPCRs to promote receptor desensitization and endocytosis as well as arrestin-mediated signaling. Significant structural and dynamic insight on GPCR interaction with G-proteins, arrestins and GRKs has been gained in recent years. In my presentation, I will highlight our recent efforts to gain mechanistic insight on GRK interaction with GPCRs. These studies have focused on GRK5 interaction with the b2-adrenergic receptor and reveal key mechanistic features of how these two proteins interact, and how this interaction leads to conformational changes that are essential for mediating receptor phosphorylation. I will also discuss our recent efforts to utilize small molecules to preferentially bias GPCR signaling to either the G-protein or arrestin pathways. Such biased signaling provides insight on signaling dynamics and has the potential to improve the treatments of diseases involving GPCRs.

LECTURE V

TUMORIGENESIS REGULATION BY STRESS KINASE SIGNALING

ANGEL R. NEBREDA
ICREA and Institute for Research in Biomedicine (IRB Barcelona), Barcelona Institute of Science and Technology, Barcelona, Spain.

The ability to properly interpret stress signals is critical for cell survival. One of the pathways that play a key role in the regulation of stress responses is orchestrated by the MAPK family member p38α. Interestingly, this pathway not only regulates the cellular responses to stress but can also integrate signals that affect many other cellular processes in a cell context- and cell type-specific manner. Genetically modified mice have helped to elucidate in vivo functions for p38α signaling and have also provided insights into how this pathway can suppress tumor initiation. It is now well established that normal epithelial cells rely on p38α signaling to engage various tumor suppressor mechanisms, including cell cycle arrest, apoptosis induction or cell differentiation. However, in contrast with other tumor suppressors, p38α does not seem to be usually mutated in human tumors, and there is evidence that p38α signaling can sometimes facilitate the proliferation, survival and dissemination of cancer cells, therefore enabling tumor progression. Of note, p38α signaling has been also implicated in the resistance of cancer cells to some chemotherapeutic drugs. We have used mouse models to investigate the roles of the p38α pathway in the progression of different types of tumors, and obtained genetic evidence that transformed epithelial cells often rely on p38α signaling for survival and proliferation. Experiments using chemical inhibitors further support a role for p38α signaling in tumor development in vivo. I will present results showing how the p38α pathway can contribute to tumor development, both by regulating the homeostasis of cancer cells and by modifying the tumor microenvironment. I will also illustrate how this information can be used to develop new potential therapies for cancer.

THE STRUCTURE AND FUNCTION OF THE RIBOSOME COMPLEXES WITH VARIOUS PROTEIN FACTORS AND ANTIBIOTICS

Thomas A. Steitz
Department of Molecular Biophysics & Biochemistry and Department of Chemistry at Yale University, and the Howard Hughes Medical Institute, New Haven, CT (USA)

We have obtained many insights into the structural basis of ribosome function in protein synthesis from our structural studies of the large ribosomal subunit as well as the 70S bacterial ribosome, and their complexes with substrates, protein factors or antibiotics. These have elucidated the mechanism by which this ribozyme catalyzes peptide bond formation and the specificity and mode of its inhibition by antibiotics.

We have obtained the structure of the complex of the 70S ribosome with tRNAs and EF-G in a previously unseen compact conformation. This compact conformation of EF-G, unlike the elongated one, allows the simultaneous binding of a tRNA in the A site and EF-G. We propose that the conversion of the compact to the elongated conformations of EF-G is responsible for tRNA translocation. The structures of the 70S ribosome with the factor EF4 (LepA) with tRNA bound in the P site or in the A and P sites provide the first insights into EF4's possible role in protein synthesis. Our structure of the 70S ribosome bound with a ribosome rescue protein (yaeJ) shows how it rescues stalled ribosomes.

The structures of some of our antibiotic complexes have been used by Rib-X Pharmaceuticals, Inc. (now Melinta Therapeutics) of New Haven to develop new potential antibiotic compounds that are effective against MRSA, one of which has successfully completed phase II clinical trials. Recently, we have obtained the structures of the 70S ribosome complexed with various oligopeptides that bind in the peptide tunnel, but in the opposite direction from peptides being synthesized; these structures might enable the creation of other new antibiotics.

CONFERENCE I

BIOINFORMATICS APPLICATIONS: WHERE BIOPHYSICS AND GENOMICS MEET

MARCELO A. MARTI.
Departamento de Química Biológica e IQUIBICEN-CONICET, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires.

Next Generation Sequencing technologies have driven biology in the big-data paradigm, with genomic information growing exponentially on a daily basis. Bioinformatics is the core business that allows conversion of this

information into biological knowledge, and among most important issues are genotype-phenotype inference relationships. Predicting how genetic differences impact a given protein function ate the molecular level, and thus phenotypic outcome, is important for understanding among other issues: antibiotic resistance and development of new drugs, enzyme selectivity and specificity, and human genetic borne diseases.
In the present talk I will show examples of several in-house developed bioinformatic applications that allow structure based physico-chemical characterization of proteins and protein variants at genomic wide scale. Specifically I will show: i)how to select best protein targets from a given pathogen genome to develop new drugs- in the present case Mycobacterium tuberculosis-, and how to seamlessly select possible "commercially available" inhibitors for the desired target; ii) how to predict subtle differences in protein function (mainly oxygen affinity) in the whole hemoglobin protein superfamily; and iii) how to predict whether a newly found human mutation is likely to be pathogenic

CONFERENCE II

LESSONS FROM THE ANALYSIS OF THE IMMUNE RESPONSE TO PLASMODIUM FALCIPARUM

ANTONIO LANZAVECCHIA
Institute for Research in Biomedicine, Università della Svizzera italiana, Switzerland

We use cell culture-based high-throughput methods to interrogate human memory B cell and plasma cell repertoires and to isolate antibodies selected on the basis of their neutralizing potency and breadth. Relevant examples are antibodies that neutralize all influenza A viruses or even four paramyxoviruses (Science 2011, 333:850; Nature 2013, 501:439). By targeting conserved structures, these broadly neutralizing antibodies are less prone to select escape mutants and are promising candidates for prophylaxis and therapy of infections, as well as tools for vaccine design. While searching for antibodies that broadly react with malaria variant antigens, we discovered a new mechanism of antibody diversification, which relies on templated insertions of genomic DNA sequences into immunoglobulin genes, followed by somatic mutations (Nature 2015, 529:105). Indeed, human monoclonal antibodies that recognize erythrocytes infected by different P. falciparum isolates acquired broad reactivity through a novel mechanism of insertion between the V and DJ segments of the entire 98 amino acid collagen-binding domain of LAIR1, an immunoglobulin superfamily inhibitory receptor encoded on chromosome 19. More recently, we studied two large cohorts of individuals living in malaria-endemic regions and found that 5-10% of malaria-exposed individuals, but none of the European blood donors tested, have high levels of LAIR1-containing antibodies that dominate the response to infected erythrocytes (Nature 2017, 548:597). The study also revealed different modalities of LAIR1 insertion that lead to public and dominant antibodies against infected erythrocytes. Sequencing of the switch regions of memory B cells from European blood donors revealed frequent templated inserts originating from transcribed genes that, in rare cases, comprised exons with orientations and frames compatible with expression. Our data suggest that insertion of templated DNA represents an additional mechanism of antibody diversification that can be selected in the immune response against pathogens.

CONFERENCE III

NEDDYLATION AS A NEW ESSENTIAL POST-TRANSLATIONAL MODIFICATION: ITS ROLE ON NEURONAL DEVELOPMENT AND METABOLISM AS A PROOF OF PRINCIPLE

DAMIAN REFOJO
Instituto de Investigación en Biomedicina de Buenos Aires,
CONICET-Instituto Partner de la Sociedad Max Plank, Buenos Aires, Argentina.

Neddylation is anubiquitylation-like pathway that controls proliferation by covalently conjugating Nedd8 a subset of ubiquitin E3 ligases called Cullins, which target different substrates involved in the control of cell cycle and duplication. Even though Nedd8 (NEDD8 neural precursor cell expressed, developmentally down-regulated 8) was originally cloned from brain tissue its role in neurons as well as in others, non-replicating postmitotic cells, remains almost entirely unexplored.
We recently found that Nedd8 is ubiquitously expressed in the brain and that neddylationincreases along postnatal brain development and with neuronal maturation. Nedd8 conjugation is active in mature synapses, where many proteins are neddylated both at pre- and post-synaptic compartments. Interestingly, neddylationis essential for normal development of excitatory (but not inhibitory) synapses during neuronal maturation and as well as spine stability in mature neurons.
Using different biochemical tools, we found that PSD-95 the most abundant scaffolding protein of the post-synaptic density is neddylated and that neddylation on Lys202 in its second PDZ domain is required for the proactive

role of the scaffolding protein in spine maturation and AMPA synaptic transmission. Finally, we developed Nae-1$^{CamKIIα\text{-}CreERT2}$ mice, in which neddylation is conditionally ablated specifically in adult excitatory forebrain neurons and only upon tamoxifen administration. These mice showed synaptic loss, impaired neurotransmission and severe cognitive deficits.

In follow-up studies we found that neddylation also exerts critical roles in early neuronal development and identified new neddylated targets that would help explain the underlying proactive effects of neddylation in neuronal maturation. A new potential mechanism of action of the neddylation pathway in neuronal maturation would be discussed.

These studies prompted us to consider that the Nedd8 conjugation pathway might play, besides its well-known role in cell cycle and proliferation,additional functions along cell differentiation and exert tissue-specific roles in cells with different identities. In order to explore this possibility we studied the potential influence of the neddylation pathway in adipocyte differentiation and biology. By using cell culture and mouse genetics tools we found that neddylation is also a critical controller of adipocyte maturation and that indeed this pathway is essential for developing high-fat diet induced-obesity. Interestingly the administration of a recently developed inhibitor of the neddylationpathway reduces body-weight and fat accumulation in obese mice, opening new opportunities and therapeutic avenues for treating obesityand associated metabolic changes.

Altogether, these results obtained in different cell types and maturation stepsmight arguably represent a proof of principle of a more general possibility: that neddylation operates as a relevant, so far unexplored post-translational regulatory pathway controlling diverse structural and functional aspects of cells undergoing differentiation as well as cell identity-specific functions in fully differentiated cells.

CONFERENCE IV

RECENT CLINICAL STUDIES FOR THE ETIOLOGIC TREATMENT OF CHRONIC CHAGAS DISEASE: PRECLINICAL ANTECEDENTS, RESULTS AND ALTERNATIVES

JULIO A. URBINA, PH.D.

Emeritus Investigator, Venezuelan Institute for Scientific Research, Caracas, Venezuela.

Chagas disease, a chronic systemic parasitosis caused by the Kinetoplastid protozoon Trypanosoma cruzi, is the first cause of cardiac morbidity and mortality in poor rural and suburban areas of Latin America and the largest parasitic disease burden in the continent, now spreading worldwide due to international migrations. Currently available anti-T. cruzi drugs, the nitroheterocyclic compounds benznidazole and nifurtimox, were developed empirically over 40 years ago and have been shown to be highly effective in acute, congenital and early chronic (pediatric) Chagas disease, but observational studies in the prevalent established chronic disease indicated that their efficacy is significantly lower and variable; furthermore, both drugs have frequent adverse effects that can lead to treatment discontinuation in 10-30% of patients. The BENEFIT study (published in 2016), was the first randomized, double-blind, placebo controlled, multi-centric trial to evaluate the efficacy of benznidazole on the clinical evolution of chronic Chagas' disease patients with cardiac compromise. The results indicated that the drug was able to markedly [but transiently] reduce parasite blood levels, while there was no significant effect on clinical deterioration through 5 years of follow-up. Further analysis of these results showed that were marked differences in the parasitological and clinical efficacy of the drug among the endemic regions included in the study, with a clear correlation of both effects, in concordance with the parasite persistence hypothesis of chronic Chagas' disease pathogenesis. The TRAENA trial (to be published in 2017) was a randomized, double blind, placebo controlled trial aimed at evaluate the parasitological and clinical efficacy of benznidazole in chronic patients from Argentina. The results indicated that there was a very marked reduction of the parasite blood levels up to 12-14 months post-treatment but no significant effect of the drug on the clinical evolution of patients, with a mean follow up of 7 years. Such result could be associated to a delayed relapse of the patients' parasite load, as seen in the BENEFIT study. Ergosterol biosynthesis inhibitors, such as posaconazole and ravuconazole, have been shown to have a potent and highly selective anti-T. cruzi activity in vitro and in animal models. Recent studies have evaluated the safety and efficacy of posaconazole in chronic patients in monotherapy (CHAGASAZOL, 2014) and in combination with benznidazole (STOP CHAGAS, 2017); the results indicated that the drug is much better tolerated than benznidazole but is unable to induce a sustained suppression of parasitemia at the end of the 12 months follow up period; this finding is probably explained by the fact that the systemic exposure of the drug at the dose of the liquid suspension formulation used in these studies (400 mg b.i.d.) is an order of magnitude lower than that attained at the curative dose in animal models. Finally, the results of a study on the safety and efficacy of E1224, a prodrug of ravuconazole in chronic patients from Bolivia were recently published (2017) and indicate that 30% of patients treated with the dose of 400 mg/week for 8 weeks had sustained parasitemia suppression at 12 months post-treatment; pharmacodynamics models derived from the data indicate that such percentage can reach to ca. 80% if the treatment period is extended to 12 weeks or if combined with benznidazole for 4-6 weeks.

CONFERENCE V

MANIPULATING THE TUMOR MICROENVIRONMENT FOR ANTI-CANCER THERAPY

LISA M. COUSSENS,

Department of Cell, Developmental & Cancer Biology, Knight Cancer Institute, Oregon Health & Sciences University, 3181 SW Sam Jackson Park Road, Portland OR 97239-3098.

The concept that leukocytes are critical components of solid tumors is now generally accepted, however, their role(s) in regulating aspects of neoplastic progression, and/or response to cytotoxic, targeted and/or immune therapy is only beginning to be understood. Utilizing de novo mouse models of organ-specific solid tumor development (mammary, cutaneous, and pancreas carcinomas and mesothelioma), we now appreciate that adaptive leukocytes differentially regulate myeloid cell recruitment, activation and effector function, and in turn, activated tumor-infiltrating myeloid cells engage tissue-based programs to foster malignancy, and repress anti-tumor immunity by a diversity of mechanisms. Treatment of tumor-bearing mice with therapeutic agents that disrupt lymphocyte-myeloid cell interaction, myeloid cell activation, or myeloid cell functionality generally slow primary tumor growth kinetics when combined with cytotoxic therapy; however, their impact on metastases is variable. Similar to organ-specific regulatory programs co-opted to foster primary tumor growth, regulation of metastatic seeding and outgrowth is also regulated by tissue- and organ-specific mechanisms. Based on this, it stands to reason that therapeutic strategies may not be efficacious in both primary and metastatic locations. To be presented will be our recent insights into organ and tissue-specific regulation of primary and metastatic cancer development by adaptive and innate immune cells, and new studies evaluating how attenuating protumor properties of select lymphoid and myeloid cells can be exploited to enhance therapeutic responses to cytotoxic and immune-based therapy.

LMC acknowledges generous support from the NIH / NCI, the Department of Defense Breast Cancer Research Program Era of Hope Scholar Expansion Award, Susan G. Komen Foundation, the Breast Cancer Research Foundation, the Brenden-Colson Center for Pancreatic Health, and a Stand Up To Cancer – Lustgarten Foundation Pancreatic Cancer Convergence Dream Team Translational Research Grant.

CONFERENCE VI

DEFINING THE CELLULAR AND MOLECULAR PROCESSES CRITICAL FOR PRIMATE OVARIAN FUNCTION AND FERTILITY

JON D. HENNEBOLD

Division of Reproductive & Developmental Sciences, Oregon National Primate Research Center, Beaverton, OR, USA; Department of Obstetrics & Gynecology, Oregon Health & Science University, Portland, OR, USA.

The midcycle surge of pituitary derived luteinizing hormone (LH) initiates cellular activities critical for ovulation, which includes follicle rupture and the release of a fertilizable oocyte, reinitiation of oocyte meiosis, as well as the development of the corpus luteum. While it is well documented that LH is the key endocrine factor responsible for orchestrating the events necessary for ovulation, downstream LH-regulated activities have yet to be fully characterized, especially in the primate ovarian follicle. Our laboratory has recently utilized high-throughput methods to systematically identify LH-dependent processes in the rhesus macaque follicle that play a role in ovulation and the release of an oocyte that is competent to undergo fertilization and embryonic development. Genomic studies were performed on follicles collected from rhesus macaques undergoing controlled ovulation (COv) protocols. The COv protocol is initiated after the selection of a single dominant follicle has occurred, which is determined by the presence of circulating estradiol above a threshold level (~100 pg/ml). At this point, a gonadotropin-releasing hormone antagonist is administered to prevent a spontaneous LH surge. Low levels of gonadotropins (i.e., follicle stimulating hormone, LH) are administered to promote continued survival and development of the follicle. A bolus of human chorionic gonadotropin (hCG) administered 48 hours later initiates events that lead to ovulation. The complete rhesus macaque transcriptome was then characterized in COv derived follicles isolated prior to (0 hour) and 12, 24, and 36 hours after hCG delivery. The 36 hour time point included follicles that were collected just prior to rupture and those that had ovulated. Of the genes whose mRNA levels change significantly through the periovulatory interval, a current area of investigation for the laboratory includes defining the role LH-regulated immune factors and cytokines play in ovulation. A second project in the laboratory includes using a comprehensive metabolomics assessment of follicular fluid obtained 36 hours post-hCG from individual follicles of rhesus macaques undergoing a controlled ovarian stimulation (COS) protocol to identify intrafollicular determinants of oocyte quality. The mature metaphase II oocyte from each follicle was then subjected to in vitro

fertilization, with outcomes being categorized as failed fertilization (did not cleave), arrested post-fertilization development (did not progress past the compact morula stage), or fertilization and development to the blastocyst stage. From these studies, it was noted the follicular fluid concentrations of specific compounds correlated with the potential of the resident oocyte to fertilize and develop to the blastocyst stage. Current efforts in the laboratory include determining the role the molecular and cellular pathways responsible for generating these compounds play in impacting the potential of the resident oocyte to undergo fertilization and embryonic development. Thus, specific cellular and molecular processes that serve to coordinate LH activities in the primate follicle were successfully identified using both genomic and metabolomics approaches, which in turn will likely yield insight into the control of fertility in women.

CONFERENCE VII

HEALTH CARE FOR SEXUAL DIVERSITY AT THE PUBLIC HOSPITAL: 12 YEARS OF EXPERIENCE

DR. ALBERTO NAGELBERG

Since 2005, the Health Care Team for Transgender People (Grupo de Atención de Personas Trans- GAPET) from Carlos Durand Hospital, has been working on a multidisciplinary basis. After the approval of the Gender Identity Law in 2012, medical consultations have increased significantly.

We use the term “trans male” (TM) to refer to a person who was assigned female at birth but with a male gender identity, and “trans female” (TF), to biological males with a female gender identity. However, due to the existence of multiple variants in gender identity beyond this binary, a personalized treatment for each case is required.

Even in current days, there are several myths and stigmas around them, majorly due to misinformation.

In order to know the background of the trans population seen by our team, we have reviewed clinical reports of 510 patients (147 TM and 363 TF) assessed between 2005 and 2017.

Most of them showed an adequate insertion in their family, social, educational and working environments.

High prevalence of tobacco use (mainly among TM), equal prevalence of marijuana consumption in both groups, and higher rates of heavy drugs among TF. No significant alcohol consumption in the majority. Prevalence of AIDS was considerable among TF, but almost non- existent among TM. Both groups had backgrounds on self-medication, much higher among TF. Both TF and TM showed a low desire towards fertility preservation. High levels of ideation and suicidal attempts in both groups, a higher percentage of ideation among TF and a higher prevalence of suicidal attempts among TM.

Most TM would undergo a breast reassignment surgery, in comparison with a low percentage among TF, who showed satisfaction with the cross - sex hormone therapy. Almost 70% seek for genital reassignment surgery, whereas the others prefer to keep their birth assigned genitality.

Are we endocrinologists, skilled and trained enough to deal with consultations of transgender patients efficiently?

In order to find the answer to this question, we conducted two kinds of anonymous surveys on line. One of them aimed at endocrinologists and fellows, the other one aimed at endocrinologists career directors. Most of the endocrinologists surveyed had received consultations from trans patients; this shows a high demand for assistance. We have also observed a positive attitude to see these patients with almost no rejection towards the subject. Training times proved to be insufficient, leading to a middle to low confidence on counselling patients about legal and practice issues. In most cases, the non-medical staff had not received any training at all, making it difficult for the patients to join the health care system. The need to implement programs on transgender medicine was considered relevant among career directors. However, most of them shared the view that the assistance to those patients is not easily available or inclusive in our country due to prejudices on the subject and lack of experience as well, which we understand is contradictory to the “positive attitude” expressed by the surveyed endocrinologists.

CONFERENCE VIII

NOVEL APPROACHES FOR GENOME EDITING IN TRYPANOSOMES

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Genome editing by CRISPR (clustered regularly interspaced short palindromic repeats)/Cas9 (CRISPR-associated gene 9) system has revolutionized biological research because it is highly flexible and allows for targeted DNA cleavage (double-strand breaks) and genome editing (repair usually by non-homologous end joining or by homology-directed repair) in a variety of eukaryotic cells. The endonuclease cleavage specificity

is guided by RNA sequences, allowing editing to be targeted simply by engineering the guide RNA sequence that is transfected along with the Cas9 endonuclease. Initially discovered as an adaptive prokaryotic immune system, CRISPR/Cas9 has been repurposed for genome editing in a broad range of organisms. Protist parasites are unicellular organisms producing important diseases that affect millions of people and animals around the world. Vaccines or effective treatments are not available for many of these diseases, such as malaria, Chagas disease, leishmaniasis and cryptosporidiosis. The adaptation of the CRISPR/Cas9 method to several parasites is becoming extremely useful for functional studies of proteins, characterization of metabolic pathways, and identification of new chemotherapeutic targets. We will present recent studies where the CRISPR/Cas9 system was adapted to protist parasites, emphasizing studies relevant to Trypanosoma cruzi, the agent of Chagas disease, as well as other approaches, such as the use of metabolically activated ribozymes, used for genome modifications in different trypanosomatids.

CONFERENCE IX

CONFERENCIA "ALBERTO TAQUINI"

MECHANISM-BASED TARGET IDENTIFICATION AND DRUG DISCOVERY IN CANCER RESEARCH: RELEVANCE OF CAMP AND COUPLED SIGNALING PATHWAY

CARLOS DAVIO

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The goal of our laboratory is to exploit the emerging information on deregulated signaling circuitries and individual genomic and molecular alterations to develop new precision therapies to prevent and treat cancer. Specifically, we have focused on the study of cAMP and its coupled signal transduction pathway, and recently on the use of different approaches to study Pancreatic Ductal Adenocarcinoma (PDAC) initiation and progression. PDAC is one of the most lethal types of tumors and one of the biggest therapeutic challenges for modern oncology. Despite the recent improvement in survival, the 5-year survival rate remains below 5-8%. Therefore, there is a great clinical need to identify key molecular and cellular regulators of tumor progression and invasion in order to develop new therapeutic strategies. The carcinogenic process of pancreatic cancer accounts for an accumulation of mutations and genetic lesions in signaling pathways linked to cell proliferation, motility and survival. In the last few decades, several mutations leading to the activation of oncogenes (such as KRAS, GNAS, and BRAF) and the inhibition of tumor suppressor genes (such as p16/CDNKN2A, TP53, SMAD4/DPC4, and BRCA2) have been identified. These mutations are present in the earliest-stages and lowest-grade premalignant lesions of the disease, suggesting that they could be essential for its development/progression and therefore, conceivably therapeutic targets. Interestingly, frequent activating mutations in GNAS, gene encoding for the stimulatory Gα subunit (Gαs), are not the only alterations on the canonical cAMP signaling pathway described in PDAC. Recent studies identified that 59% of the tested human PDAC samples exhibit an elevated Gαs expression compared to normal tissues, and 72% show an increment in the expression of phosphorylated substrates of cAMP-dependent kinase (PKA), a downstream effector of the Gαs-protein-coupled receptor-cAMP signaling pathway. Altogether, these observations indicate that cAMP cascade is commonly activated in PDAC, and suggest an oncogenic potential for this signaling pathway. Nonetheless, this assumption points to an intriguing paradox based on the fact that the activation of cAMP pathway has been classically associated with inhibition of cell proliferation and migration in PDAC. Due to the critical role of cAMP in cellular biology, the time-course levels of this second messenger are strictly regulated by several mechanisms, including its degradation by phosphodiesterases, its compartmentalization, the desensitization of GPCR, and its extrusion to the extracellular compartment mainly mediated by the Multidrug Resistance-Associated Protein 4 (MRP4/ABCC4). In recent years, numerous publications describe the anti-proliferative effects of MRP4 inhibition, its potential as a marker of poor prognosis and its up-regulation in several tumors. However, little is known about the mechanisms involved in the anti-proliferative effects of MRP4 inhibition and there are limited studies of its potential as a therapeutic target. We have recently described that enhanced intracellular cAMP levels upregulate MRP4 expression through the activation of cAMP/Epac/Rap1 signaling pathway. Considering the frequent cAMP-signaling deregulation observed in PDAC, we hypothesize that pancreatic cancer cells could be "addicted" to high levels of MRP4, relying on active cAMP extrusion to provide a survival advantage, and therefore highlighting a therapeutically exploitable difference between cancerous and normal cells. In this study we showed that MRP4 expression is upregulated in human pancreatic cancer and may influence patient outcome. MRP4 inhibition abrogated cell migration and proliferation in vitro by both, activating the cAMP/Epac/Rap1 signaling pathway and reducing extracellular cAMP levels. Interestingly, the extruded cAMP acted as an autocrine/paracrine mitogenic signal and silencing MRP4 strongly impaired tumor growth and tumorigenicity in a mouse xenograft model. Our results strongly indicate

that MRP4 could represent an excellent therapeutic target for pancreatic ductal adenocarcinoma.

CONFERENCE X

MOLECULAR BASIS OF T CELL EXHAUSTION: INSIGHTS FOR IMMUNOTHERAPY

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T cell exhaustion is common during chronic infections, cancer and prevents optimal immunity. Exhausted T cells (T_{EX}) are defined by the poor ability to perform effector functions, low proliferative capacity, inefficient survival following antigen stimulation and a unique transcriptional program that is conserved between different chronic infections, species and is also present in cancer. In addition, it has become clear that T_{EX} co-express multiple inhibitory receptors that negatively regulate their function. Indeed, receptors such as PD-1 have become major targets of clinical immunotherapies in cancer and infectious disease aimed at re-invigorating T_{EX}. Our work has defined a transcriptional circuit involving the transcription factors T-bet and Eomesodermin (Eomes) that controls progenitor and terminal subsets of T_{EX}. Moreover, features of these subsets allow tracking of T_{EX} in humans during immunotherapy. One observation from these studies is that a key surrogate of reivingoration of T_{EX} responses is a population of $Eomes^{Hi}$ $PD\text{-}1^{Hi}$ exhausted CD8 T cells. The use of this subset of CD8 T cells as a biomarker is explored. Moreover, we have examined the durability of re-invigoration of T_{EX} in both humans and mice and found that the effectiveness of PD-1-based checkpoint blockade may be limited by the epigenetic inflexibility of T_{EX}. Ultimately, a more precise molecular understanding of T cell exhaustion should lead to novel and more robust clinical interventions to reverse exhaustion in settings of persisting infections and cancer.

CONFERENCE XI

MOLECULAR AND CELLULAR MECHANISMS OF HUMAN VIRAL ONCOGENESIS

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Viral cancers account to 12% of all human cancers and occur in their vast majority in the developing world. Viral oncogenesis is an special case for the exquisite adaptation of persistent viruses that subvert physiological host-regulatory pathways in order to ensure their replication and long term survival on the host (1). Oncoviruses carry viral oncogenes that activate the same host signaling pathways regulating growth and apoptosis driving non-viral cancers. This results in the acquisition of hallmarks of cancers that can initiate the process towards full transformation (1). Although infection and oncogenicity are under the control of the immune system, immunosuppression and HIV/AIDS can facilitate the onset of viral cancers induced by oncoviruses such as High risk HPV, EBV and KSHV (1). KSHV; a gamma2-herpesvirus, is the etiologic agent of Kaposi's sarcoma, an AIDS-associated neoplasm characterized by angiogenesis and the proliferation of spindle-like cells (3,5). KSHV encodes viral oncogenes such as the viral G protein coupled receptor (vGPCR) which can drive oncogenesis and angiogenesis by inducing the expression of VEGF (2,3,4,5). Our latest studies on vGPCR and the mechanisms of KSHV in cell and animal models exemplify how molecular and cellular studies on viral oncogenesis can be exploited for both for therapeutic purposes and gaining novel insights on the pathophysiology of viral transformation. We found that KSHV vGPCRand other viral genes activate the host PDGF signaling axis by upregulating PDGFA and B. This results in viral-mediated activation of PDGF-receptor A (PDGFRA), a well knownsarcomagenic driver and a validated therapeutic target in non viral sarcomas, which we found is the receptor tyrosine kinase most activated in Kaposi's sarcoma. We validated PDGFRA as therapeutic target in KS using receptor tyrosine kinase inhibitory drugs, by the use of specific dominant negative constructs and by showing that the vast majority of KS lesions robustly display PDGFRA in its phosphorylated form. We next reasoned that the potent KSHV activation of PDGFRA suggest that this is a critical host factor for KSHV oncobiology. Therefore, KSHV target cells that are progenitors of KS spindle cells should be PDGFRA expressing cells. This led us to the identification of mesenchymal stem cells (MSCs)as KS oncogenic progenitors and the use of murine mesenchymal stem cells (mMSCs) to develop a reproducible and robust system of tumorigenesis that is dependent on expression of PDGFRA as well as external pro-angiogenenic conditions. Using RNAseq analysis and CHIPSeq we found that under pro-angiogenic conditions mMSCs provide an epigenetic landscape permissive for KSHV oncogene expression and cell transformation.

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CONFERENCE XII

THE LAST FRONTIER OF ANTIBIOTIC RESISTANCE: AT THE HEART OF PROTEIN EVOLUTION

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Protein evolution can be described as a walk on sequence space where a fitness value is assigned to each particular sequence. A major challenge to understand the biological mechanisms of protein evolution is the attempt to correlate genotype with phenotype and fitness at a molecular level. This is a complex challenge, involving the assessment of biochemical, biophysical and structural features to many protein variants.

Antibiotic resistance mediated by β-lactamases is an ideal system to study protein evolution, since the chances of a whole organism to survive depend on the availability of a folded, stable and active protein in the proper cellular compartment (the bacterial periplasm).

Metallo-β-lactamases (MBLs) are Zn(II)-dependent β-lactamases that constitute the latest resistance mechanism of pathogenic and opportunistic bacteria against carbapenems, considered as last resort drugs. Zn(II) binding is critical in the bacterial periplasm, not only to activate these enzymes and provide resistance, but also to stabilize the protein scaffold. This phenomenon is not paralleled by in vitro studies. We developed a strategy aimed to correlate the biochemical and biophysical features in purified enzymes with those in the bacterial periplasm, ultimately leading to the selected phenotype, i.e., resistance to antibiotics. This strategy allows us to dissect the molecular features that are tailored by accumulating mutations during evolution. We have applied this approach to in vitro evolved protein in the laboratory, as well as to natural allelic variants selected in clinical strains. This has allowed us to account for the epistatic interactions between mutations at a structural level.

Finally, we have shown that the natural evolutionary landscape of allelic variants of a clinically relevant lactamase (NDM) has been shaped by Zn(II) deprivation conditions as those induced by the host immune response. As a consequence, natural NDM variants with enhanced Zn(II) binding affinity have been selected, overriding the most common evolutionary pressure acting on catalytic efficiency.

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CONFERENCE XIII

DON'T STRESS! NEW INSIGHTS INTO STRESS INHIBITION OF MAMMALIAN REPRODUCTION

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Stress disturbs many physiological processes, including reproduction. Indeed, multiple types of stressors, including psychological stress, can negatively impact reproductive status in many mammalian species, including humans. Restraint and/or isolation, commonly-used models of psychosocial stress in mammalian models, reduce luteinizing hormone (LH) and follicle stimulating hormone synthesis and secretion, as well as impair reproductive ovarian cyclicity. To date, little is known about underlying mechanisms for stress suppression of GnRH and downstream LH secretion, especially at the level of the brain. While it is known that psychosocial stress inhibits several hypothalamic or pituitary components of the reproductive axis, including LH secretion, it is less clearly understood how stress regulates reproductive circuits in the brain. Emerging evidence suggests that hypothalamic kisspeptin (encoded by Kiss1) neurons are key components for generating and stimulating GnRH pulse secretion as well as the GnRH/LH surge that elicits ovulation. Thus, it is possible that psychosocial stress—through factors and pathways yet to be determined—impacts kisspeptin neurons, thereby altering downstream LH pulsatility or surge secretion. In addition, recent evidence suggests that the peptide, RFamide-related peptide 3 (RFRP-3, encoded by Rfrp), can inhibit GnRH and LH output and may do so under stressful conditions. We recently conducted several projects in mice to ascertain the effects of acute psychosocial stress or stress hormones on pulsatile and

surge LH secretion and the upstream brain circuits regulating the reproductive axis. Our findings indicate that acute stress or short-term exposure to physiological levels of corticosterone can have profound effects on LH pulses and the positive feedback induction LH surge, in part through regulation at levels upstream of GnRH neurons, either directly or indirectly, through hypothalamic Kiss1 and/or Rfrp neurons. This talk will summarize our recent findings of stress impairment of reproductive status in mouse models and discuss remaining gaps in knowledge.

CONFERENCE XIV

ROLE OF THE ENDOCANNABINOID SYSTEM IN FOOD-INDUCED ADDICTIVE-LIKE BEHAVIOR

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An increasing perspective conceptualizes obesity and overeating as disorders related to addictive-like processes that could share common neurobiological mechanisms. We aimed at validating an animal model of eating addictive-like behavior in mice, based on the DSM-5 substance use disorder criteria, using operant conditioning maintained by highly palatable chocolate-flavored pellets. For this purpose, we evaluated persistence of food-seeking during a period of non-availability of food, motivation for food, and perseverance of responding when the reward was associated with a punishment. This model has allowed identifying extreme subpopulations of mice related to addictive-like behavior. We investigated in these subpopulations the Epigenetic and proteomic studies have allowed to identify a significant decrease in DNA methylation of CNR1 gene promoter in the prefrontal cortex of addict-like mice, which was associated with an upregulation of CB1 protein expression in the same brain area. The pharmacological blockade of CB1 receptor during the late training period reduced the percentage of mice that accomplished addiction criteria, which is in agreement with the reduced performance of CB1 knockout mice in this operant training. Proteomic studies have identified proteins differentially expressed in mice vulnerable or not to addictive-like behavior in the hippocampus, striatum, and prefrontal cortex. The use of DREADD techniques in this model has now allowed identifying the crucial role of the prefrontal cortex in the development of eating addictive-like behavior. This model provides an excellent tool to investigate the neurobiological mechanisms underlying eating addictive-like behavior.

CONFERENCE XV

AGING, TDP 43 AGGREGATION AND NEURODEGENERATION

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The aggregation of TDP-43 is the major distinguishing feature of most ALS cases. There is still a significant uncertainty in regards to many aspects concerning the nature of the aggregates and their functional consequences. For example, the biophysical characteristics of TDP-43 aggregates are still unclear, especially with regards to their possible amyloid composition that may be acquired under certain conditions. Furthermore, TDP-43 aggregates have recently been suggested to be responsible for the spreading of disease among neuronal cells in a prion like manner. All these aspects of TDP-43 aggregation need further definition, as new therapies could be directed towards them. At this point, therefore, it is essential to have a comprehensive catalog of the alterations of TDP 43 structure and function and identify those that are critical for the development of the disease. A better understanding of TDP 43 structure and interactions is needed and data on the N-Terminal Domain structure and C-Terminal domain intra and intermolecular interactions will be presented. In addition a focus is needed in the TDP-43self-regulation loop that could have a critical function on the development of the disease. Finally, ALS is a disease that occurs mostly during the fifth to the seventh decade of life. In this respect, we have previously shown that the onset of the locomotion defect in an ALS fly model coincides with an age-related 4-fold drop in TBPH levels (the Drosophila TDP43 orthologous), similar TDP 43 reduction with age was observed in mice brain. Thus, understanding the relationship between aging and TDP-43 production in cell culture, animal models, and human tissues might provide further clues to explain the age of disease onset.

CONFERENCE XVI

FROM FERTILIZATION TO THE IMMUNE SYSTEM

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Fertilization is a key process involving a series of coordinated interactions between the male and female gametes. However, the mechanisms underlying this key process still remain to be elucidated. Our laboratory has been dedicated to underpin the molecular mechanisms involved in the acquisition of sperm fertilizing ability and gamete interaction using CRISP proteins as model molecules. Epididymal protein CRISP1, the first described member of the evolutionarily conserved CRISP (Cystein-Rich Secretory Protein) family, associates with the sperm surface during the maturation process that occurs while passing through the epididymis. Substantial evidence obtained using in vivo and in vitro approaches as well as knockout (KO) models shows that CRISP1 participates in both sperm-egg binding and gamete fusion through its interaction with egg-complementary sites. These observations can be extended to human as judged by the finding that the human CRISP1 also associates with sperm during maturation and participates in fertilization through binding sites in the human egg. More recent results reveal that CRISP1 is a novel regulator of CatSper, the principal sperm Ca(2+) channel essential for male fertility and that the protein is also expressed by the cumulus cells that surround the egg acting as a sperm chemoatractant during cumulus penetration. Thus, CRISP1 escorts both the male and female gamete and plays multiple roles during the fertilization process. In spite of its functional relevance, CRISP1 KO mice are fertile as are mice lacking CRISP4, another epididymal CRISP family member also involved in fertilization. Interestingly, recent evidence from our group shows that the simultaneous lack of both CRISP1 and CRISP4 (double KO) affects not only fertilization but also male fertility, confirming the existence of compensatory mechanisms between homologue proteins to ensure reproductive success. Moreover, the double KO males show severe defects in both the epididymal epithelium and luminal sperm as well as an abnormal presence of immune cells within the organ indicative of a disruption of the characteristic immune tolerance of the epididymis. Together, these observations confirm the relevance of CRISP proteins for fertilization and fertility and reveal novel inmunoregulatory roles for these proteins within the epididymis. We believe these results provide important information for a better mechanistic understanding of the maturation and fertilization processes that will contribute to future research on infertility and contraception.

CONFERENCE XVII

HETEROGENEITY OF THE HUMAN T CELL RESPONSE AGAINST MICROBES

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Center of Medical Immunology, Institute for Research in Biomedicine, Università della Svizzera italiana, Bellinzona, Switzerland and Institute of Microbiology, ETH Zurich, Switzerland.

We study the human system to address fundamental questions in the context of the immune response to different classes of antigens, such as microbial pathogens, allergens or self-antigens, to gain insights into mechanisms that induce host protection or immune-mediated pathology. Our efforts towards human immunology have resulted in the development of high-throughput cell-based screening methods to analyze the human immune response and, in particular, the functional diversity and repertoire of human effector and memory T cells. The T cell library method allows the efficient and high-throughput interrogation of the human T cell repertoire without being limited by the complexity of the antigen, the HLA haplotype and the sample size. The method can be used to predict antigenicity and identify T cell epitopes to guide design of T cell vaccines or optimization of biologicals. When applied to the study of T cells in cancer and autoimmunity, it can provide tools for immunotherapy and insights as to the mechanisms of immune-mediated pathology. The T cell library method, together with high throughput TCR Vβ sequencing, which can analyze millions of T cell clonotypes in blood or tissues, mass spectrometry, and single-cell gene expression analysis, can provide a wealth of information that is expected to contribute to the progress in medical immunology research.

CONFERENCE XVIII

EVOLUTION AND HOST-SPECIFIC FUNCTIONS OF DENGUE VIRUS RNA STRUCTURES

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The Flavivirus genus include a large number of emerging and re-emerging human pathogens that are mainly transmitted by arthropods, including dengue, Zika, yellow fever and West Nile viruses. Dengue is the most prevalent arthropod-borne viral disease around the world. It is endemic in more than 100 countries, with about 390 million infections each year. In 2016, Latin America faced their worst dengue and Zika epidemics, without effective vaccines or antivirals to control infections.

Dengue virus contains a single stranded RNA genome, encoding at least ten viral proteins. We have previously identified the mechanism of viral genome amplification and defined conserved RNA structures that serve as promoters, enhancers and silencers of viral RNA synthesis. These elements are mainly located at the viral 5' and 3' untranslated regions (UTRs) and they communicate by conserved long-range RNA-RNA interactions. Interestingly, in the process of studying viral replication in mosquito and human cells, we identified a novel viral mechanism of host adaptation. This mechanism includes changes of viral RNA structures that play different roles during viral infection in each host. In this regard, replication of dengue virus in mosquito or human cells resulted in the selection of different viral populations with specific mutations in RNA structures in the viral 3'UTR. The selective pressure for this mutations was associated to the accumulation of different patterns of viral non-coding RNAs, directly involved in counteracting the cellular antiviral response. Using epidemic and pre-epidemic Zika isolates, similar patterns of non-coding RNAs were observed in mosquito and human infected cells, but they were different from those observed during dengue virus infections, indicating that distinct selective pressures act on the 3'UTR of these two related human pathogens.

Finally, by extrapolating our observations to other flaviviruses, we found RNA structure duplications at the 3'UTR of viruses that alternate between vertebrate and invertebrate hosts. Two pairs of duplicated structures were found in most mosquito-borne flaviviruses, and each of the duplicated element displayed a different function during viral replication. Interestingly, we found that the RNA duplication allowed to accommodate mutations beneficial in one host but deleterious in the other, providing robustness for viral replication in both cell types. These findings reveal a novel mechanism of viral host adaptation and provide new ideas for flavivirus 3'UTR evolution.

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SIMPOSIA I:
BIOCHEMISTRY AND MOLECULAR CELL BIOLOGY OF PLANTS
Chairs: Paula Casati and Isabel Lacau

BUILDING AND MAINTAINING EPIGENETIC MEMORY: INSIGHTS FROM FLC

JULIA IRENE QÜESTA

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Plants are sessile organisms and need to align their development to specific external cues from the different seasons. To be capable of inferring seasonal information, plants have evolved systems to sense environmental signals and also to 'remember' previous exposure to these signals. The floral repressor *FLORWERING LOCUS C* (*FLC*) has allowed the study of these mechanisms in *Arabidopsis thaliana*. *FLC* expression is repressed by prolonged cold exposure during winter in a process called vernalization. *FLC* repression is then epigenetically maintained until embryo development after flowering. Central to vernalization is the nucleation of a specialized Polycomb Repressive Complex 2 (PRC2) at an intragenic site within *FLC* to deposit the chromatin repressive mark H3K27me3. Our recent work has uncovered the role of the sequence-specific transcriptional repressor VAL1 in targeting Polycomb to *FLC* chromatin to initiate epigenetic silencing. In parallel, the cold-induced, long non-coding RNA *COOLAIR* facilitates *FLC* transcriptional shutdown by coordinating the switch between active and repressive chromatin states. Natural allelic variation at *FLC*, distinguished by polymorphisms in non-coding regions, influences the expression level and the rate of epigenetic silencing of the gene. Thus, modulation of chromatin-based mechanisms and non-coding RNA regulation, both crucial to vernalization, are likely to be generally involved in adaptation to changing environments.

ASCO LONG NONCODING RNA MODULATES ALTERNATIVE SPLICING PATTERNS IN *ARABIDOPSIS*

FEDERICO ARIEL

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Alternative splicing (AS) of pre-mRNAs represents a major mechanism boosting transcriptome and proteome complexity. In Arabidopsis, the long noncoding RNA *ALTERNATIVE SPLICING COMPETITOR* (lncRNA *ASCO*) was previously found to hijack the nuclear AS regulators NSRs to modulate AS patterns during root development. Here, we showed by RNA-seq that the deregulation of *ASCO* impacts the expression levels of stress and defense-related genes, including several members from the AP2/ERF and JAZ families of transcription factors. Furthermore, the knock-down of *ASCO* alters the AS pattern of certain stress-related pre-mRNAs, notably involved in senescence. Noteworthy, several pre-mRNAs alternatively spliced in *ASCO*-deregulated lines were not affected in the *nsra/b* double mutants, suggesting that *ASCO* also modulates alternative splicing in an NSR-independent manner. Therefore, we adapted a chromatin-related technique (called Chromatin Isolation by RNA Purification, ChIRP) to what we called nuclear Protein Isolation by RNA Purification (nPIRP) followed by Mass Spectrometry, in order to identify *ASCO*-associated protein complexes. Our nPIRP results suggest that *ASCO* is recognized *in vivo* by several specific splicing factors, as well as few stress- and phytohormone-related proteins. Altogether, our work indicates that *ASCO* modulates hormone and stress signaling via the regulation of alternative splicing of specific targets.

TMV SYSTEMIC MOVEMENT REQUIRES NEGATIVE MODULATION OF THE IMMUNE SYSTEM

SEBASTIÁN AZURMENDI

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An important aspect of plant-virus interaction, which remains insufficiently understood, is the way viruses dynamically modulate host immunity to achieve a successful infection. The plants have developed different mechanisms of immunity against the attack of pathogens. A well-characterized mechanism involves salicyl-

ic acid (SA), a hormone that has the ability to induce nuclear translocation of NPR1 (NON-EXPRESSOR OF PR GENES 1) and stimulate the transcription of a genes network involved in the immune response. Some pathogens have co-evolved developing strategies that allow them to silence the natural defense systems of plants. In Tobamoviruses, emerging evidences suggest that viral encoded proteins display a great variety of functions beyond the canonical roles required for virus structure and replication. Among them, the modulation of host immunity is arising as a relevant aspect required for the infection progression. We have shown that the Tobacco Mosaic Virus (TMV) capsid protein (CP) negatively modulates the SA-mediated defense pathway. We determined the role of DELLAs proteins in the modulation of signaling events during TMV infection. We demonstrated that during TMV infection, CP stabilizes DELLAs proteins and this stabilization results in the modulation of defense signaling pathway resulting in turn in a de-regulation of the gene network induced by SA-mediated immunity. Lastly and most importantly, we have demonstrated that this function of TMV CP, negative modulation of the plant immune system, is required for the systemic movement of the virus, therefore very important for the success of the infection.

MOLECULAR MECHANISMS OF PLANT FERTILIZATION

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It has been described that most of the pollen proteins are synthetized during pollen development. However, there are reports about some pollen mRNAs that accumulate in dehydrated mature pollen grains but translated only after pollen germinates. Our hypothesis is that they are stored in cytoplasmic granules. We analyzed the cellular localization of the *SKS14* and *AT59* Arabidopsis mRNAs, which are orthologues of the tobacco *NTP303* and tomato *LAT59* pollen mRNAs respectively, by artificially labeling the transcripts with a MS2-GFP chimera. We developed a new MATLAB automated image analysis to identify and quantify the presence of cytoplasmic *SKS14* and *AT59* mRNA granules in mature pollen grains. These mRNA granules partially co-localized with VCS and DCP1, two processing bodies (PBs) proteins. In addition, we found that both VCS and DCP1 are necessary for the formation of *SKS14* and *AT59* mRNA granules. Finally, we found a temporal correlation between SKS14 protein accumulation and the disappearance of *SKS14* mRNA granules during pollen germination. These results would contribute on the understanding of how pollen translational regulation takes place in *Arabidopsis thaliana*. It remains to be elucidated whether these results can be extrapolated to other pollen genes.

SYMPOSIA II
B CELLS AND FOLLICULAR T HELPER CELL
Chairs: Eloisa Arana and Silvia Correa

MOLECULAR AND PHYSICAL MECHANISMS THAT ORCHESTRATE B CELL DEVELOPMENT

CORNELIS MURRE

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It is now well established that the transcriptional regulators EBF1 and Bcl11b specify B and T cell fates, respectively. Here we show that in developing B- and T-lineage cells regulatory regions repositioned from the nuclear lamina to the transcriptionally permissive compartment. In early B cell progenitors the EBF1 and FOXO1 loci switch from the heterochromatic to the euchromatic compartment. Likewise we found that in early T cell progenitors the Bcl11b locus control region repositions from the nuclear lamina to the transcriptionally permissive compartment. In search for candidate factors that repositioned the EBF1 and Bcl11b control regions we identified ThymoD, an enhancer RNA (eRNA). We found that ThymoD-deficient mice are blocked at the DN2 cell stage. We found that ThymoD acts *in cis* to reposition the Bcl11b super-enhancer from the lamina to the nuclear interior. We demonstrate that ThymoD acts to promote CTCF and SMC3 occupancy across the ThymoD transcribed region to form a single loop domain that brings the Bcl11b super-enhancer and promoter within close spatial proximity. We will discuss related studies in the context of EBF1 and B cell development. These data indicate how eRNA-induced structural changes in nuclear architecture dictate with high precision enhancer-promoter communication to specify lymphoid identity

B CELLS AND FOLLICULAR T HELPER CELLS

JOSEPH E. CRAFT

(Yale University School of Medicine, New Haven, Connecticut, USA)

Initiation of a thymus-dependent humoral immune response requires interaction of activated B cells and follicular B helper T (Tfh) cells, a specialized subset of CD4 T helper (Th) cells. Differentiation of Tfh cells leads to their localization to B cell follicles and germinal centers (GC) of secondary lymphoid organs, with their expression of costimulatory proteins and secretion of cytokines promoting GC B cell proliferation, immunoglobulin gene hypermutation, and development of B cell memory and long-lived plasma cells. The transcriptional repressor Bcl6 acts cell autonomously to drive a Tfh cell development program, including upregulation of proteins necessary for function, with environmental signals promoting differentiation of these cells at the expense of other CD4 T subsets. Tfh cells undergo differentiation within the GC, enabling differential help to B cells, with affinity selection of the latter in part promoted by regulatory GC T cells. Therapeutically, blockade to Tfh-GC B interactions interrupts end-organ disease in the systemic autoimmune disease lupus, while resetting the Tfh cell phenotype toward normal homeostasis. Supported by NIH AR40072 and AR068662, and the Alliance for Lupus Research

GALECTIN-3 DEFICIENCY DRIVES LUPUS-LIKE DISEASE

ADRIANA GRUPPI

(Centro de Investigaciones en Bioquímica Clínica e Inmunología, CONICET, Facultad de Ciencias

Germinal centers (GC) are important sites for high-affinity and long-lived antibodies (Abs) induction and their overactivity causes autoimmunity. In our laboratory, we report a novel function by which Galectin-3 (Gal-3) is involved in the development of GC. Gal-3 KO mice exhibit a high frequency of GC B cells and T follicular helper cells that correlated with increased percentage of Ab-secreting cells and high concentrations of IgM, IgG2c, IgG3 and IFN-γ in serum. Likewise, Gal-3 KO mice develop a lupus-like disease, characterized by kidney infiltration and antinuclear Ab secretion. IFN-γ blockade in Gal-3 KO mice reduced GC formation, class-switch recombination, autoantibodies production and renal pathology, demonstrating that IFN-γ overproduction sustain lupus-like disease. Using chimeric mice with B cell-specific deficiency of Gal-3, we demonstrate that intrinsic Gal-3 signaling in B cells control spontaneous GC formation. Together, our data provide the first evidence that Gal-3 acts directly on B cells regulating GCs responses *via* IFN-γ.

AN EXPANDED FAMILY OF B CELL-HELPER T CELLS IN RHEUMATOID ARTHRITIS

DEEPAK A. RAO

(Harvard Medical School, Harvard University, Boston, Massachusetts, USA)

Determining the pathologic functions of T cells that infiltrate target tissues remains a central challenge in autoimmune diseases. Pathogenic CD4+ T cells within affected tissues may be identified by expression of markers of recent activation. Here, I discuss our studies using mass cytometry and multidimensional flow cytometry to interrogate T cell populations in synovial tissue and blood from patients with rheumatoid arthritis, a chronic immune-mediated arthritis that affects up to 1% of the population. Mass cytometric analysis of RA synovial tissue cells revealed a strikingly expanded population of PD-1^{hi} CXCR5^{-} CD4^{+} T cells, which constituted ~25% of synovial CD4^{+} T cells. Surprisingly, these cells are not exhausted, but instead highly express factors that confer the ability to help B cells, including IL-21, CXCL13, ICOS, SAP, and MAF. Like T follicular helper (Tfh) cells, PD-1^{hi} CXCR5^{-} T cells from synovium and blood induce plasma cell differentiation *in vitro* via IL-21. However, RNAseq transcriptomics robustly separate PD-1^{hi} CXCR5^{-} cells from Tfh cells, with altered expression of Bcl6 and Blimp-1 and unique expression of chemokine receptors that direct migration to inflamed sites, such as CCR2, CX3CR1, and CCR5, in PD-1^{hi} CXCR5- T cells. We suggest that PD-1^{hi} CXCR5^{-} CD4^{+} T cells represent a T peripheral helper (Tph) cell population, analogous to T follicular helper (Tfh) cells, that supports B cell responses in pathologically inflamed non-lymphoid tissues. Given their marked expansion in RA joints, these cells may be important in driving pathologic B cell responses and autoantibody production within the inflamed target tissue

SIMPOSIA III
NEW THERAPEUTIC APPROACHES FOR THE CONTROL OF CHAGAS DISEASE
Chairs: Adelina Riarte and Hector Freilij

CHAGAS DISEASE: CLINICAL EVIDENCE FOR ETIOLOGICAL TREATMENT

AND BIOMARKERS FOR ASSESSING RESPONSE

SERGIO SOSA ESTANI
(Drugs for Neglected Diseases Initiative, Buenos Aires, Argentina)

Specific anti-parasitic treatment for Chagas disease using benznidazole and nifurtimox is indicated in the following situations: a) All acute phase patients, including congenital transmission; b) Following reactivation of infection by immune suppression e.g. AIDS and transplantation; c) Patients up to 18 years of age with chronic disease; d) Women of childbearing age with *Trypanosoma cruzi* infection (with contraception during treatment). There is a relative consensus that drug treatment should generally be offered to adults aged 19–50 years without advanced Chagas heart disease, and is optional for those older than 50 years.

To date it is estimated that < 1% of infected patients are currently receiving treatment. This news, is a great challenge, and is opening a new pathway to be addressed to dramatically change access to the drug in endemic countries. Progress will be bolstered by the generation of new clinical evidence on the safety and efficacy of new antiparasitic treatment regimens, especially for adult patients with chronic Chagas disease. Several completed clinical trials have shown a clear trypanocide effect of benznidazole, and a non-sustained response to alternative drugs (TRAENA, BENEFIT, CHAGASAZOL, STOP CHAGAS, E1224). The search for new drugs for Chagas disease is actively underway, ongoing studies will add significantly to our knowledge of how to utilize existing drugs in the safest, most effective manner possible. A proof-of-concept Phase II study (BENDITA study-DNDi) underway in Bolivia is pursuing the evaluation of new regimens of benznidazole, as a monotherapy and in combination with fosravuconazole, for the treatment of adult patients with chronic indeterminate Chagas disease in order to improve tolerability, while maintaining or improving efficacy. Efficacy and safety analyses analysis of the first FEXI trial carried out in 2013 in Bolivia, interrupted due to safety and tolerability issues, suggested high efficacy rates of fexinidazole. These results led DNDi to design a new Phase IIA proof of concept trial to assess short regimens of Fexinidazole to be conducted in Spain.

Meanwhile, the Berenice project, an international initiative coordinated by Spain and with the participation of Argentina, Brazil, and Colombia, has launched the MULTIBENZ trial, which will evaluate lower doses of benznidazole. In Colombia, the CHICAMOCHA trial is assessing the efficacy of benznidazole and nifurtimox in adult patients in the chronic indeterminate stage of the disease. Another clinical study in Argentina is testing an intermittent regimen of standard doses of benznidazole to provide evidence on alternative regimen of this trypanocide. CHICO, a multicenter trial in Argentina, Bolivia and Colombia supported by Bayer, is evaluating the safety and efficacy of a new paediatric formulation of nifurtimox.

Ongoing clinical studies from DNDi and other groups are identifying and validating potential biological markers of therapeutic response in Chagas disease patients to support clinical development. DNDi is fostering and encouraging the testing of four biomarkers to assess response to treatment of Chagas through the Iberoamerican network NHEPACHA.

Continued progress on these various fronts will help ensure that diagnosis and treatment finally reaches the over 99% of people with Chagas disease who have thus far been neglected.

BISPHOSPHONATES A PROPOSAL FOR ANTIPARASITIC TREATMENT

ALICIA FUCHS
(Centro de Altos Estudios en Ciencias Humanas y de la Salud, Universidad Abierta Interamericana, Buenos Aires, Argentina)

Bisphosphonates (BP) are synthetic analogs of pyrophosphate in which two phosphates are connected through carbon instead oxygen. They classify in two groups due by the presence or not of a nitrogen, which favors BP intrinsic activity. The P-C-P are mainly responsible to bone binding, reduce hypercalcemia, and help bone mineralization acting on pH regulation and hydroxyapatite stability. BP are versatile drugs and depending of the target cells present different actions. The alendronate activity on NO· production, calcium deposition and alkaline phosphatase activity is target tissue depending. Moreover, the use of BP have been proposed against parasitological diseases. Studies are focus on its interferences on parasites incomplete metabolic pathways from which survival is depending, but others mechanisms of actions were proposed. The inhibition of farnesyl- geranyl diphosphate synthase, as substrate-like inhibitors, is essential for mevalonate synthesis and protein prenylation and important molecular- dynamics differences found between human or *Plasmodium* origin (Ricci et al, 2016), *T.brucei*, (Yang et al 2015) and *T.cruzi* (Huang et al, 2010). On the other hand, BP action on phosphate accumulation impaired its mobilization in *T.cruzi* acidocalcisome caused by the inhibition of translocating pyrophosphatase (Docampo and Moreno, 2008). In *Leshmania tarentolae* alkyl- alendronate decreased parasite growth *in vitro* (Christensen et al, 2016). In *T.brucei* the solanesyl disphophate synthase is inhibited by an experimental BP, 1-[(*n*-oct-1-yl-amino)ethyl] 1,1-bisphosphonic acid, which impaired ubiquinone 9 synthesis, causing ATP decrease (Lai et al, 2014). The decrease of ATP was also observed on EGPE, cell line, from bovine *E.granulosus*, as consequence of

five BP treatment *in vitro* but, only three of them caused decreased of cell growth. The most effective was etidronate (EHDP). Its deleterious effect was also related with total calcium accumulation, a fact not happening with the use of ibandronate (IB) or olpadronate (Fuchs et al, 2014). Diverse mechanism triggered by BP could regulate available Ca^{2+} suggested by the interaction observed on 1,3 -disphosphonates with L-type calcium channels inhibitors (Rossierq et al 1989). Our results showed that EHDP or amino- etidronate (N-EHDP) and IB (Gador SA, Buenos Aires) differentially modulate $[Ca^{2+}]_{cit}$ and phosphatase activity, depending of cell type, EGPE or HT29 (human colon adenocarcinoma). This could explained N-EHDP deleterious effect on EGPE and not on HT29 cells, at 30μM. Many new studies about anti parasitic effect of BP suggested that the improved of the effect should be achieve by the increasing the molecule lyposolubility (Yang et al, 2015). Other authors suggested that the presence of fluoride or sulfur increases the effect on apicomplexan parasites (Galaka et al, 2017). But, BP action on Helminths would depend of others factors acting on calcium uptake and on its intracellular distribution. Moreover, in spite of the many anti-parasitic possibilities, BP compounds effect *in vivo* would depend of its systemic distribution because their high affinity with the bone surfaces.

MODERN APPROACHES FOR THE DISCOVERY OF DRUGS AGAINST CHAGAS DISEASE

ALAN TALEVI

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According to the latest figure from the Panamerican Health Organization, there are more than 7 million people exposed to the disease in Argentina, and 1.5 million people infected (almost 4% of the total population). Whereas during the last years the "neglected disease" status of Chagas disease has fortunately started reverting, around 90% of the limited investment in research and development linked to Chagas comes from the public sector or non-for-profit organizations. Such scenario imposes the need of using rational approximations in the search of novel diagnostic and therapeutic tools.

Here, we describe different modern approaches towards the discovery of novel therapies against Chagas disease: *in silico* screening, high-throughput screening, drug design and systematic drug repurposing. Focusing on neglected conditions, the advantages and disadvantages of each one of them are discussed in terms of the technological and translational gaps. Some recent examples of successful applications are described.

NEW THERAPEUTIC STRATEGIES FOR THE TREATMENT OF CHAGAS DISEASE

CELINA LAMAS

(Instituto de Química de Rosario, CONICET, Rosario, Argentina)

Chagas disease or American tripanosomiasis is a neglected disease caused by the parasite Trypanosoma cruzi, which enters the body through broken skin and mucous membranes, producing several symptoms including fever, conjunctivitis and skin lesions. *Trypanosoma cruzi* is, found mostly, in blood-sucking triatomine insects (kissing bugs) which lives in the cracks of buildings and substandard houses. Although the vectorial transmission has been reduced Chagas' disease is still a major public health problem and one of the leading causes of morbidity, long-term disability and mortality in Latin America. Additionally to human morbidity and mortality, this parasitic infection place a substantial burden in poorer developing countries, increasing both the poverty and the vulnerability of those people.

Even though Chagas disease is transmitted primarily by insects the dissemination of this parasitic infection may occur by transfusion of blood from persons infected with *T. cruzi* and/or organ transplantation. Infected pregnant women may transmit *T. cruzi* to her newborn, resulting in congenital Chagas disease, also. It is worth mentioning that this neglected infection is becoming a serious concern that can have severe consequences for the public health, principally because of its potential to contaminate the blood transfusion supply as well as, its dissemination through surgical implantation of infected donor organs to patients in non-endemic areas including North America and Europe.

Although some progress focused in the process of drug discovery have been made in recent years, the significant cost of these projects discouraged pharmaceutical companies from investing in research and development programs against Chagas' disease, without assurances that they can make a return on their investment. As a consequence of it, up to these days, there are only two therapeutic agents applied to the treatment of Chagas' disease: Nifurtimox and Beznidazole. Despite of both active compounds are included in the WHO project "Better medicines for children", an effective chemotherapy for paediatric population infected with Chagas' disease is still lacking. Since both Nifurtimox and Benznidazole are formulated only into tablets, the pediatric population is treated by fractioning the solid dosage form in pieces according the body weight of each patient. This quite primitive and non-recommended therapy may cause undesirable side-effects due to the potential erroneous dosage. In addition, since paediatric patients include neonates (0-28 days), infant (1-12 months), and

children (1-12 years), another drawback for the availability of pediatric dosage forms is the particular dosing regime in each age group. In this context, the development of age-adapted dosage forms, in terms of easy administration and dose flexibility would be a remarkable advantage for improving the efficacy of Benznidazole. Thus, this presentation aims at demonstrating how pharmaceutical technology can assist in the development of new therapeutic platforms towards novel treatments using an old drug for Chagas disease.

SYMPOSIA IV
YOUNG INVESTIGATORS IN REPRODUCTION
Chairs: Clara Marín-Briggiler and Leandro Miranda

HORMONAL REGULATION OF INSECT REPRODUCTION

SHEILA ONS
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Insects are the dominant terrestrial life form in Earth. They affect us directly either with harmful or beneficial consequences (vectors of diseases, crop damage/ pollinators, natural predators of pests, etc.). Their evolutionary success is due to particular strategies for development and reproduction, which have facilitated the colonization of a wide range of ecological niches. During post-embryonic development, insect larvae complete each molt by shedding the old cuticle, in order to emerge as the next developmental stage. This process culminates in adults able to produce mature gametes, and is perfectly regulated by endocrine networks composed of lipophilic hormones and neuropeptides. The crucial involvement of neuropeptides in development and reproduction suggests that these molecules could be promising targets for new-generation insecticides. Given their particular characteristics, neuropeptidergic-targeting insecticides will offer species-selectivity and environmental compatibility, with practical advantages compared to current neurotoxic insecticides.

The peptidergic signaling cascade regulating ecdysis at the end of each molt has been well studied for those insect species that undergo a complete metamorphosis (holometabolous) such as flies, moth, beetles, etc. The nodal components of this cascade are *Ecdysis Triggering Hormone* and *Eclosion Hormone*. However, the endocrine factors regulating ecdysis in Hemimetabolous (such as kissing bugs, cockroaches, etc) has not been characterized to date. Strikingly, even though adults do not molt, the hormones regulating ecdysis are expressed in this mature stage, as much in males as in females. This suggests pleiotropic physiological roles for ecdysis hormones. Recent reports indicate that the interrelationships of hormones occurring in juvenile stages to regulate molting and ecdysis is maintained in the adult stage to regulate gametogenesis and fecundity. Hence, the members of this hormonal network would be excellent candidates in the search for next-generation insect pest management strategies. In this context, our research group studies hormonal regulation in insects, focusing in the post-embryonic development and reproduction processes. Our results show for the first time that a particular family of neuropeptides called *Orcokinin* is a critical component of the regulatory network of ecdysis and reproduction in insects. When *Orcokinin* gene expression was silenced, phenotypes of arrested molting in kissing bugs and impaired reproduction in cockroaches were observed. Furthermore, we studied the components and interrelationships within this network in hemimetabolous, and found crucial differences with holometabolous. Our recent work suggests that Orcokinins could be excellent candidates as targets for novel insect pest management strategies.

KISSPEPTIN: IS IT THE GENE OF THE FIRST KISS IN FISH?

ALEJANDRO MECHALY
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In mammals, the kisspeptin system, composed by the kisspeptin neuropeptide ligand and its receptor, *KISSR*, is involved in the regulation of onset of puberty and sexual maturation. In this group, kisspeptin is considered a gatekeeper of reproduction, since mutations in *KISS*/*KISSR* in several species blocked puberty. However its role on fish is not so clear. For example, recent knockout studies in zebrafish have demonstrated that spermatogenesis and folliculogenesis, and subsequently the ability to reproduce are not impaired. In teleost fish, genome duplication made the system more complex, with two kisspeptin genes (*kiss1 and kiss2*) and three receptors (*kissr1, kissr2 and kissr3*). The genome duplications events and the enormous diversity of reproductive strategies make teleost fish interesting models to study the involvement of kisspeptin in reproduction. Also, by using bioinformatics tools, we reported the gene structure organization of two kisspeptin receptors (*kissr2* and *kissr3*) in pejerrey fish. Genomic analysis revealed their gene organization and the presence of alternative

spliced variants of the kisspeptin receptor in several fish species. Modeling and structural analysis of Kissr2 and Kissr3 revealed that truncation of the proteins may lead to non-functional proteins, as the structural elements missing are fundamental for receptor function. These findings may contribute to a better understanding of the many physiological functions suspected to be mediated kiss/kissr signaling pathway. Surprisingly, no evidence of alternative splicing was detected in mammals, suggesting that this mechanism is probably unique for fish. Other differences between fish and mammalian models are in relation with neuroendocrinological regulation of food intake. Although numerous experimental studies in fish suggest a role in reproduction for this system, little is known about its participation in food intake control. In that sense, evidences of a possible link between energy balance and reproduction mediated by kisspeptin signaling in non-mammalian vertebrates were discovered in some fish species such as the Senegalese sole and pejerrey. It is generally believed that fasting inhibits the reproductive axis and kisspeptin gene expression in the mammalian hypothalamus; however, the opposite effect was observed in fish, probably due to the variation of life strategies across different fish species. Thus, the aim of this presentation is to give an up to date review of the kisspeptin system in vertebrates with special emphasis in teleost fish with a particular view of their role in reproduction and others novel functions. Nevertheless, there are many important questions that need to be addressed in future studies of the kisspeptin system in fish and the new genomics technologies, through the next-generation sequencing (NGS), can provide some answers about the roles of alternative isoforms or role of kisspeptin system genes under different nutritional conditions or other external factors such as temperature or photoperiod.

CYCLOOXYGENASE AND PROSTAGLANDINS IN TESTICULAR PHYSIOPATHOLOGY

EUGENIA MATZKIN

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Prostaglandins (PGs) are bioactive lipid substances derived from arachidonic acid. They are produced by almost all nucleated cells and can, therefore, be found in most tissues. PGs are generated in a complex biosynthetic pathway in which cyclo-oxygenase (COX) is the rate-limiting enzyme. Two isoforms of this enzyme have been described: COX-1 and COX-2. They are encoded by two separate genes and exhibit distinct cell-specific expression, subcellular localization and regulation. While COX-1, commonly known as the constitutive isoform, seems to mediate housekeeping functions, COX-2, the inducible isoform, seems to be expressed in response to varying stimuli such as growth factors and cytokines.

Given their ubiquitous nature, PGs are involved in diverse physiological and pathological processes, including regulation of inflammatory and immune responses, cell growth, vascular tone, angiogenesis and tumorigenesis. Regarding reproductive function, interesting findings were described in the early 1990's using mouse models with targeted disruption of COX. While COX/PGs were found to be important regulators of female reproductive function (ovulation, uterine receptivity, implantation and parturition), fertility was not affected in male mice with COX deficiency, thus suggesting that PGs may not be critical for male reproductive function. However, this view has recently been challenged by novel observations stating essential roles for PGs in testicular physio-pathology. We have described a COX-2/PG system in the two key somatic cell types of the testis: Leydig and Sertoli cells. Our studies have provided insights into how several hormones and cytokines (i.e., follicle stimulating hormone, prolactin, testosterone and IL-1β) modulate COX-2 expression and PG production in these somatic cells. Studies performed mainly in rodents indicate that some PGs (i.e., PGD2 and PGF2α) modulate androgen production in Leydig cells, while 15d-PGJ2 regulates glucose transport in Sertoli cells and, consequently, spermatogenic efficiency. Furthermore, using mouse models with delayed (Ames dwarf and growth hormone releasing hormone-knockout mice) or accelerated (growth hormone-transgenic mice) aging we have shown an inverse association between longevity and testicular COX2/PGD2 system. Relevant to male (in)fertility, we have reported that although COX-2 is not detected in human testes with no evident morphological changes or abnormalities, it is expressed in testicular biopsies of men with impaired spermatogenesis and infertility. In addition to its expression in Leydig and Sertoli cells, COX-2 has been detected in the seminiferous tubule wall, and in testicular macrophages and mast cells of infertile patients. These observations stress the possible relevance of PGs in testicular inflammation associated with idiopathic infertility.

Collectively, these data indicate that the COX-2/PG system plays crucial roles not only in testicular physiology (i.e., steroidogenesis, spermatogenesis, reproductive aging), but also in the pathogenesis or maintenance of infertility status in the male gonad. Therefore, the study of COX and PG actions emerges as a promising field of research with potential impact on male fertility. Further advances in the knowledge of the role played by COX, PGs, and their receptors in the human testis, as well as future studies concerning the impact of drugs targeting COX/PGs at the testicular level, could lead to new therapeutic approaches in idiopathic male infertility.

PARTICIPATION OF LIPID MEDIATORS IN HUMAN TROPHOBLAST MIGRATION DURING

THE FIRST TRIMESTER. ROLE OF CAMP

MICAELA SORDELLI
(Centro de Estudios Farmacológicos y Botánicos, CONICET, Buenos Aires, Argentina)

An intricate molecular dialogue between trophoblast and uterus initiates the process of implantation, by which the embryo attaches to the lining of the uterus. After implantation, trophoblast cells begin a proliferative, migrating and invasive process infiltrating the decidua, remodeling uterine vasculature and connecting the mother blood stream with the fetus. These events are accomplished by molecular and cellular interactions controlled by the maternal-fetal interface microenvironment and may fail in certain obstetric complications such as preeclampsia, intrauterine growth restriction and implantation failure. Several years ago, it has been observed that there is a change in the lipid composition at the luminal epithelium adjacent to the area where the blastocyst is attached. This change is interpreted as a mobilization of precursors for the synthesis of lipid molecules that participate in angiogenesis and in the transformation of endometrial fibroblasts into decidual cells. Hence, novel insights with respect to roles played by the participation of lipids in the establishment of pregnancy have sparked a renewed interest in understanding them no longer as simple structural components of the membranes but as reproductive bioactive mediators. In this sense, lysophosphatidic acid (LPA) and prostaglandins play a major role in embryo implantation. LPA has pleiotropic functions by binding to six G-protein coupled receptors. Prostaglandins have a pivotal role in decidualization and vascularization, two main processes during trophoblast migration and invasion. We have previously reported that LPA augments the production of cyclooxygenase-2 derived prostaglandin E2 in the rat uterus during the window of implantation. Trophoblast cells are the main source of LPA in human first trimester, suggesting that LPA actively participates in trophoblast functions. Defects in trophoblast migration and invasion are particularly vulnerable to failure during embryo implantation resulting in pregnancy complications. Therefore, we aimed to investigate the role of LPA on human first trimester trophoblast´s functions and its interaction with prostaglandin E2. To achieve our experiments, we use a well-known immortalized human first trimester trophoblast cell line HTR-8/SVneo. We design a pharmacological strategy using selective and broad range LPA receptors antagonists and cyclooxygenase inhibitors. First, we characterize the mRNA expression of LPA receptors and the enzyme Lysophospholipase-D, the main enzyme involved in LPA synthesis and its principal source during gestation are the placental trophoblast. We observed that LPA stimulates both invasion and migration through LPA1-LPA4 receptors ($p<0.05$, but does not modify proliferation. Then we found that cyclooxygenase-2 derived prostaglandins mediate the increase triggered by LPA on trophoblasts cells since cyclooxygenase inhibitors diminish LPA increased migration ($p<0.05$). Interestingly, LPA stimulates prostaglandin E2 production ($p<0.05$) and immunocytochemistry experiments suggest that trophoblast cells stain positive for cyclooxygenase-1 and 2 in the cytoplasm and plasma membrane. Overall, these results demonstrate that LPA and prostaglandin E2 perform primordial functions at the maternal-fetal interface modulating trophoblast invasion and migration by a prostaglandinmediated mechanism triggered via LPA. Our findings contribute to better understand the significance of LPA signaling in the trophoblast behavior that lead to a successful pregnancy

SIMPOSIA V
INFLAMMATION AND AUTOIMMUNITY
Chairs: Analía Trevani and Gerardo Mirkin

LNCRNAS AND INFLAMMATION

JORGE HENAO-MEJÍA
(Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, USA)

Epigenetic modifications induced by extracellular cues enable cells to rapidly adapt to changes in their environment. Long non-coding RNAs (lncRNAs) are now considered key controllers of epigenetically regulated gene expression. Notably, emerging evidence indicates that lncRNAs play a critical role in integrating extracellular inputs with epigenetic modification pathways to promote changes in gene expression programs. Given the tight regulation that is required to maintain innate immune cells on check and prevent the development immunopathology, we propose that highly inflammatory cells specifically express lncRNAs that are required to finely tune these cells to their environment, to both promote anti-pathogen immunity and prevent immunopathology. In here, we combined a novel bioinformatic workflow with the CRISPR/Cas9 system to provide evidence that rapid and local regulation of gene expression by lncRNAs in response to extracellular cues is a powerful and potentially generalizable mechanism through which optimal immune cell homeostasis is achieved.

NEUTROPHIL DAMAGE AND HYPERCITRULLINATION IN RHEUMATOID ARTHRITIS

FELIPE ANDRADE
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Rheumatoid arthritis (RA) is a chronic autoimmune disease, characterized by inflammatory infiltration of the synovial membrane, destruction of articular cartilage and erosion of bone. While the etiology of RA is still unknown, there has been enormous progress in this area, largely fueled by the discovery that citrullinated proteins are the major targets of autoantibodies in RA. Protein citrullination (or deimination) is the enzymatic conversion of peptidyl-arginine residues to peptidyl-citrulline and is mediated by the family of calcium-dependent peptidylarginine deiminases (PADs), which includes five PAD isoforms (PAD1 to PAD4 and PAD6). The finding that patients with RA have antibodies against citrullinated proteins (known as ACPAs) has suggested the possibility that the disease might be driven by dysregulated citrullination. Yet, the mechanisms that dysregulate and sustain citrullination in RA are not fully understood. In recent studies, we identified that membranolytic damage in neutrophils induced by host and bacterial pore-forming proteins (PFPs) are potent inducers of cellular hypercitrullination and the production of citrullinated autoantigens targeted in RA. PFPs serve as defense mechanisms both in pathogenic bacteria to kill host cells in order to promote colonization and spread, and in vertebrates (e.g., perforin and the complement membrane attack complex, MAC) to kill bacteria and infected cells to limit infections. PFPs create pores on the surface of cells, which initiate membrane destabilization, influx of extracellular calcium, and ultimately cell death. Neutrophils are innate immune cells which are abundant in PAD enzymes. Since the PAD enzymes are activated with calcium, the abrupt exposure to high amounts of calcium during PFP-induced neutrophil death overactivates these enzymes, generating a massive production of citrullinated autoantigens. During this process, citrullinated proteins are released into the extracellular space in a process that is consistent with cell death by leukotoxic hypercitrullination (LTH), a mechanism that distinguishes toxin-induced cytolytic changes from NETosis. We proposed that in RA, bacterial-induced LTH is a potential source of autoantigens that initiate the lack of tolerance to citrullinated proteins. In this regard, we recently identified the periodontal pathogen *Aggregatibacter actinomycetemcomitans* (*Aa*) as a potent induced of LTH and a candidate bacterial trigger for RA. Host PFPs, such as perforin and MAC, appear to be major drivers that sustain LTH in the RA joint. Membranolytic damage and cellular hypercitrullination induced by bacterial and host PFPs may represent a unifying mechanism that initiates and sustains autoantigen production at various disease sites in RA.

OXIDATIVE STRESS DRIVES INNATE-ADAPTIVE IMMUNE CROSSTALK IN HUMAN CHAGAS DISEASE

MARÍA DEL PILAR AOKI
(Centro de Investigaciones en Bioquímica Clínica e Inmunología, CONICET; Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Córdoba, Argentina)

Reactive oxygen and nitrogen species, generated by phagocytic cells, are important microbicidal agents but also, they are involved in lymphocyte unresponsiveness. Within metabolites that mediate many of the biological effects attributed to nitric oxide are peroxynitrites, which induce the nitration of immune cells. Particularly, nitration of tyrosines in the surface of CD8+ T cells results in the inability of these cells to bind MHC and, in consequence, to mount a specific cytotoxic response. Thus, although lymphocytes are in all likelihood the instigators of chronic inflammatory disease, activated monocytes are well equipped to cause its dysfunction. In consequence, the study of the crosstalk between T cell and myeloid cell compartments would be important to clarify the immunopathology of chronic inflammation. The major infectious heart disease in the world is represented by American trypanosomiasis, or Chagas disease, which is the result of human infection by the protozoan parasite *Trypanosoma cruzi (T. cruzi)*. Following infection, both innate and adaptive immune systems induce the clearance of circulating parasites. However, despite infection control, most patients progress to a chronic phase and around 30% of infected people develop chronic cardiomyopathy usually after decades of asymptomatic infection. We found a higher frequency of hypoxia inducible factor (HIF)-1α+ and nitric oxide-producing monocytes in asymptomatic patients with long-term *T. cruzi* infection. The presence of inflammatory monocytes promotes surface-nitration of circulating CD8+ T cell. Underlining the physiological relevance of our findings, increased tyrosine nitration was associated with impaired effector functions of CD8+ T cells in chronic Chagas patients. These findings have important clinical implications, considering that CD8+ T cell-mediated protective immunity has become a center of intense research to find control measures that could be used to produce effective therapeutic vaccines.

UNDERSTANDING THE COMPLEX IMMUNOPATHOGENESIS OF *TRYPANOSOMA CRUZI* INFECTION: THE ROAD TO CLINICAL MANAGEMENT OF CHAGAS DISEASE

MANUEL FRESNO

(Centro de Biología Molecular Severo Ochoa, Universidad Autónoma de Madrid, Madrid, Spain)

Chagas disease (CD) is a complex disease, presenting various clinical outcomes such as spontaneous healing, asymptomatic infection, cardiac, visceral, or cardio-visceral forms. Furthermore, the etiologic agent of CD, the parasite *Trypanosoma cruzi*, exhibits high genetic diversity, proposed to be associated to the diverse clinical outcomes of CD. Despite the discovery of this disease more than 200 years ago, still there are many controversies about its pathogenesis Understanding the pathogenic mechanism may have deep implications in the clinical management of this disease. Parasite replication, inflammation and autoimmunity have been described as responsible mechanisms.

To address this issue, we have employed a murine model system by using 2 different mice strains, Balb/c and C57B6. We also used multiple *T. cruzi* strains, including at least a representative of the 6 different main lineages (DTUs) to infect mice and analyzing the course of infection in the acute and chronic phase. Organ and systemic immune responses, parasite tropism and replication in several tissues, histopathological damages, metabolic changes, etc, were analyzed. Data reveal important differences between the genetic background of the host and of the strains. We found that the parasite genetic background impacts parasite tropism, host responses, and clinical presentations of CD. Thus, systemic and Local Immune Response differs among strain. Adaptative Th17, Th1, Th2 and CD8+ T cytotoxic immune responses, as well as immunosuppressive responses (Treg and Myeloid Derived Suppressor Cells) are induced differentially and may play a different, even opposite, role on the course of the disease depending on the genetic of the host and parasite. Interestingly, in chronic phase, cardiac damage can exist without detectable parasite load whereas chronic intestinal damage was always linked to parasite burden. Moreover, organ damage may decrease or increase in the chronic phase depending of the tissue analyzed and the infecting *T. cruzi* strain.

Our results show a great variability at the level of tropism, organ damage, local and systemic immune responses, illustrating the high diversity of the CD an d more importantly warning against simplification of the problem of this disease. However, combinations of some pathophysiological parameters using a systems biology approach allowed to group parasite strains, while other combinations permitted to gather individuals with respect to the type or level of clinical damage they underwent. This diagnostic significance of some infection factors underlines their importance as markers and/or determinants of the pathophysiology of CD. Finally, some parameters were studied in CD patients, showing that they can be used as biomarkers of clinical manifestations.

SYMPOSIA VI
PABMB CELL BIOLOGY: INTRACELLULAR TRAFFICKING
Chairs: Gustavo Chiabrando and Pablo Schwatzbaum

AUTOPHAGY COOPERATES IN THE DEGRADATION OF KEY MOLECULAR PLAYERS IN ALZHEIMER'S DISEASE

PATRICIA BURGOS

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Brain regions affected by Alzheimer's disease display well-recognized early neuropathological features in the endo-lysosomal and autophagy systems of neurons, including enlargement of endosomal compartments, progressive accumulation of autophagic vacuoles, and lysosomal dysfunction. Although the primary causes of these disturbances are poorly understood, a growing body of evidence suggests that the amyloid precursor protein (APP) intracellular C-terminal fragment-β (C99), generated by cleavage of APP by BACE1, is the earliest initiator of synaptic plasticity and long-term memory impairment. Herein we show that autophagosomes are key organelles for maintaining intracellular C99 and BACE1 levels, strongly indicating a direct relationship between the endo-lysosomal protein degradation pathway and autophagy.

FUSEXINS, A FAMILY OF SEXUAL, SOMATIC AND VIRAL CELL FUSION PROTEINS

PABLO S. AGUILAR

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Cell-cell fusion is essential for fertilization and sculpting of bones, muscles, epidermis and placenta in different or-

ganisms. However, the proteins that mediate membrane fusion between cells have not yet been well-characterized and in some cases are still unknown. HAP2(GCS1) are membrane glycoproteins essential for gamete fusion in plants, *Chlamydomonas*, *Plasmodium*, *Tetrahymena*, and *Dictyostelium*. To investigate whether HAP2 is not only essential but also sufficient for cell-cell fusion, we expressed the *Arabidopsis* sperm HAP2 in cultured baby hamster kidney cells that normally do not fuse. We found that HAP2 expression in these heterologous cells results in the formation of multinucleate cells by cell-to-cell fusion [1]. Genetic and cell biological analyses showed that HAP2 has to be present in only one of the fusion partners (usually the male gamete) [2,3]. However, we found that when expressed in mammalian cells, *Arabidopsis* HAP2 is sufficient to fuse them only when expressed in both fusing cells [1]. Thus, HAP2-mediated plasma membrane merger occurs via a bilateral zippering mechanism reminiscent to intracellular fusions mediated by SNAREs, atlastins and mitofusins. Furthermore, expression of HAP2 on the surface of pseudotyped vesicular stomatitis virus results in virus-cell fusion only to cells that also express HAP2. Thus, we propose that, in addition to sperm HAP2, a HAP2-like protein is needed in the egg for gamete fusion. Structural modeling of the HAP2 protein family predicts that it is homologous to class II viral fusion proteins (e.g. Zika, rubella and dengue) and the somatic cell fusogen EFF-1 from *C. elegans* [1-3]. Moreover, the recently solved crystal structure of *Chlamydomonas'* HAP2 demonstrates structural homology with EFF-1 and class II viral fusion proteins [3]. We name this superfamily FUSEXINS: FUSion proteins essential for sexual reproduction and EXoplasmic merger of plasma membranes. Fusexins mediate enveloped virus entry into cells, sexual reproduction, and somatic cell fusion. These proteins share the same structure and function but use distinct mechanisms to merge membranes. We hypothesize that modern fusexins have existed since the dawn of eukaryotic cells.

REGULATION OF EXTRACELLULAR ATP IN HEALTH AND DISEASE

PABLO J. SCHWARZBAUM

(Instituto de Química y Fisico-Química Biológicas "Prof. Alejandro C. Paladini", CONICET; Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Argentina)

Human red blood cells (rbcs) can release ATP when exposed to adrenergic stimulation, mechanical deformation, cell adhesion, blood flow turbulence, hypoxia and acidosis or, unspecifically, under conditions promoting hemolysis.

A reduction of ATP release has been associated with type II diabetes and cystic fibrosis, while rbcs infection with *Plasmodium falciparum* correlated with activated ATP release. Both, in physiological and pathological conditions, the resulting extracellular ATP (ATPe), and its metabolic products, can activate purinergic receptors which mediate a wide variety of local and systemic responses, such as changes in cell volume or intravascular pressure.

Irrespective of the nature of the stimuli, kinetics of ATPe accumulation depends not only on ATP exit, but also on ATPe hydrolysis by nucleotidases. This is because ATPe concentration can be downregulated by ectonucleotidases of intact rbcs, or by intracellular nucleotidases released to the extracellular space under hemolysis.

Candidate conduits for regulated –non lytic- ATP release include several anionic channels and pore forming proteins. In addition, bacterial toxins such as alpha hemolysin (HlyA) were shown to activate ATP exit of rbcs, presumably by forming pores through which the nucleotide transverses the plasma membrane.

HlyA is an exotoxin secreted by uropathogenic strains of *Escherichia coli*, causing lysis of several mammalian cells, including human rbcs. *In vivo* HlyA has been associated with urinary tract infections and septicemia. HlyA is secreted by bacteria in the intravascular milieu, where it can bind to rbcs and other cells.

During this talk we will analyze ATPe regulation of rbcs exposed to HlyA and analogs.

We made a quantitative estimate of ATPe kinetics, together with a description of the main processes controlling HlyA-dependent ATP regulation of rbcs.

Our results show that, in rbcs:

- Exposure to HlyA produced a nonlinear and biphasic increment of ATPe, caused by simultaneous lytic and non lytic ATP release. The [ATPe] increase showed an acute phase, mainly driven by non lytic ATP release, and a late phase that is mostly dependent on lytic ATP release and ATPe hydrolysis by nucleotidases.
- Moreover, HlyA led to slight swelling and increased sphericity, which was in part responsible for the observed activation of ATP release.
- Both proHlya, the unacylated precursor of HlyA and HlyA-GF(-), an HlyA mutant lacking the segment capable of binding to rbcs glycophorin, showed little or negligible ATP exit.
- Blockage of P2X receptors led to significant reduction of HlyA induced hemolysis, causing downregulation of ATPe kinetics.

Several experiments are under way to test other processes potentially affecting ATPe regulation of HlyA treated rbcs. Among these are aggregability and deformability of rbcs, intravascular pressure, and rbcs adhesion to endothelial cells.

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MOLECULAR CROSS-TALK IN OUTER RETINA; ANGIOCRINE FACTORS SECRETED BY CHOROIDAL ENDOTHELIAL CELLS CONTROL OUTER RETINA-BLOOD BARRIER AND CHOROID INFLAMMATORY RESPONSE

ENRIQUE RODRIGUEZ BOULAN

(Margaret Dyson Vision Research Institute, Weill Cornell Medicine, New York, USA)

The outer retina is composed of a layer of rods and cones, collectively called photoreceptors (PR), closely apposed to a layer of pigmented epithelial cells, the Retinal Pigment Epithelium (RPE). Light is transduced by photoreceptors into electric impulses that travel to specialized neurons in the inner retina and from there to the brain. PR function is intimately dependent on key support functions performed by the RPE, including its key role as a selective Outer Retina-Blood Barrier (ORBB) between the retina and the underlying choroid circulation. The RPE is separated from the underlying choroid capillaries by Bruch's membrane (BM), an elastin- and collagen-rich basement membrane produced by RPE and choroid. Recently, it has become clear that endothelial cells (ECs) are tissue-specific and constitute instructive niches that secrete key angiocrine factors that promote parenchymal cell differentiation and tissue regeneration (Rafii et al, Nature, 2016). To explore this hypothesis in the eye, we purified ECs from developing and adult mouse choroidal vasculature, as well as from neural retina, liver, lung and heart. In a recent study (Benedicto et al, Nature Communications, 2017), we showed that adult choroid ECs display a unique transcriptome enriched in extracellular matrix-related transcripts and regulate RPE barrier function through angiocrine factors. Moreover, we showed that EC-secreted factors control BM assembly, which in turn is sensed by RPE integrin receptors and transduced via small GTPases into changes in the molecular composition and function of RPE tight junctions. Additional studies uncovered a novel Indian Hedgehog (IHH)-dependent pathway in the choroid that may play an important role in immune regulation of the retina. The significance of our findings in the context of Age Related Macular Degeneration, a major retinal blinding disease, will be discussed. Supported by NIH grants, Research to Prevent Blindness, Dyson Foundation and Starr Foundation.

Rafii S, JM Butler and B-S Ding (2016) Angiocrine functions of organ-specific endothelial cells. Nature 529:316-325.

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SIMPOSIA VII
IMMUNOPHARMACOLOGY AND IMMUNOTHERAPY
Chairs: Romina Girotti and Vanina Medin

THE HISTAMINE H_4 RECEPTOR: TRANSLATION OF PRECLINICAL MODELS TO CLINICAL EFFICACY

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Histamine exerts its physiological activities via the activation of four receptors. All of these receptors have been considered targets for the treatment of a variety of diseases, with drugs acting at histamine H_1 and H_2 receptor being available for many years. Ligands for the most recently discovered receptor, the histamine H_4 receptor, have just started to be tested in the clinic. The H_4 receptor mediates chemotaxis and cytokine release of mast cells, eosinophils, monocytes, dendritic cells and T cells. In addition, histamine released from mast cells or from other cell types can influence T cell polarization via activation of the H_4 receptor. The receptor also mediates T cell activity in vivo and has a proinflammatory effect not only in models of the innate immune response, but also in models of asthma, dermatitis and arthritis. Extensive medicinal chemistry and pharmacology efforts have led to the development of modulators with excellent potency and selectivity for the H_4 receptor. Two of these molecules, toreforant and JNJ-39758979, have entered human testing. These compounds have been shown to be anti-inflammatory in mouse models of dermatitis, asthma and arthritis. In a mouse model of atopic dermatitis H_4 receptor antagonists reduced skin edema, inflammation, mast cell and eosinophil infiltration. This was accompanied by a reduction in the levels of several cytokines and chemokines in the skin. In addition to anti-inflammatory effects there was also significantly inhibited the pruritus exhibited in the model. These findings have translated into clinical efficacy. A study assessed the safety and efficacy of JNJ-39758979 in patients with moderate atopic dermatitis. Numerical improvements were observed in the Eczema Area and Severity Index score at week 6 compared to baseline, but these changes did not reach statistical significance. Nominally statistically significant

symptomatic improvements were seen in patient-reported itch severity and duration. In addition to atopic dermatitis, H_4 receptor antagonists also have effects in preclinical eosinophilic asthma models. Treatment with H_4 receptor antagonists improved lung function, reduced lung inflammation and eosinophilia as well as reduced inflammatory cytokines in the lung. In the clinic it has been shown that JNJ-39758979 improved lung function in mild to moderate eosinophilic asthma patients. H_4 receptors are also involved in autoimmune diseases and H_4 receptor antagonists reduce symptoms in mouse arthritis models. Once again the preclinical results appear to predict efficacy in human diseases as an early study with the H_4 receptor antagonist toreforant showed a reduction in signs and symptom in patients with rheumatoid arthritis. Although there is still much work to be done, there has been much progress in using preclinical models to understand the physiological role of the H_4 receptor and the translation of these findings into early success in clinical studies.

CYSTIC FIBROSIS FROM LABORATORY TO CLINIC

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Cystic Fibrosis (CF) is an autosomal recessive disorder affecting 1 in every 2500 live births in the UK. Although most common in Caucasian individuals of Northern European descent it can affect any ethnicity. The underlying pathology of CF is a mutation in the gene coding for the cystic fibrosis transmembrane conductance regulator (CFTR), leading to altered expression and function of this protein. Although many body systems are affected including digestive, reproductive and pancreatic systems; pulmonary disease, characterized by infective exacerbations, bronchiectasis and increasing airway insufficiency is the most serious manifestation of the disease process, currently responsible for over 80% of CF deaths. This first part of the talk will provide a background to the pathophysiology of the disease.

The second part of the talk will concentrate on laboratory methods to investigate airways disease in CF. Data will be presented examining cell lines and isolated primary airway epithelial cells. The prolonged and heightened inflammatory response seen in CF is, in part, mediated by a lack of intrinsic down-regulation of the proinflammatory NF-κB pathway. A20 [TNFα-induced protein 3 (TNFAIP3)] is a central negative regulator of NF-κB activation. We found that naturally occurring plant diterpenes such as gibberellin could induce A20 and had anti-inflammatory effects in vitro. Using gene expression connectivity mapping we were able to identify existing drugs that could induce A20 expression.

The final part of the talk will examine how using isolated primary airway epithelial cells were used to test novel drugs used to correct the defect in CFTR i.e. to increase the delivery and amount of functional CFTR protein at the cell surface or to potentiate CFTR i.e. to increase channel activity of CFTR protein located at the cell surface. Data from clinical trials with the CFTR corrector, Lumacaftor; the potentiating drug, Ivacaftor and the combined treatment will be presented.

This talk will highlight the importance of close collaboration between basic and clinical scientists to help alleviate a chronic condition.

IMMUNOPHARMACOLOGICAL CHALLENGES AND OPPORTUNITIES IN MODERN TARGETED THERAPEUTICS

KATERINA TILIGADA

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Drugs targeting immune pathways have been exploited in the treatment of inflammatory, allergic and autoimmune disorders for more than 50 years. Over the last two decades, the enormous interest of scientists in the interface between pharmacology and immunology and the rapid expansion of biotechnology made available increasingly sophisticated clinically relevant immunopharmacological interventions that are largely tailor-made to specific patient populations. The currently growing field of Immunopharmacology builds on the rapid progress in immunology, and is paralleled by the growing number of new classes of molecular-targeted therapies. The related challenges and opportunities are reflected by the establishment of *ImmuPhar*, the new Immunopharmacology section of the International Union of Pharmacology of Basic and Clinical Pharmacology (IUPHAR) (www.iuphar.org/index.php/sections-subcoms/immunopharmacology), and *GtoImmuPdb*, the Guide to Immunopharmacology database (www.guidetoimmunopharmacology.org).

The novel products include small molecules and biological agents. Compared to conventional pharmacotherapy, they are designed to interact with specific targets and to minimize or even eliminate adverse effects. This

cutting-edge biomedical research promises to offer effective therapeutic approaches for an expanding range of unmet medical needs. Among them, the emergence of therapeutic monoclonal antibodies (mAbs) revolutionized the options for pathologies ranging from cancer, allergies and autoimmune diseases to cardiovascular and neuropsychiatric disorders. More recently, a major breakthrough has occurred by targeting immunological checkpoints that negative regulate the immune system and play critical roles in self-tolerance, tissue protection and autoimmunity prevention. The antibody-based immune checkpoint inhibitors targeting the cytotoxic T-lymphocyte-associated protein 4 (CTLA4) and the programmed cell death protein 1/programmed death-ligand 1 (PD-1/PD-L1) pathway are in clinical development, thus forming the foundation for a new era of immunotherapy. Despite the exponentially growing entry of mAbs and biosimilars into the clinic, several limitations hamper their wide exploitation in conditions characterized by complex aetiology and multifaceted clinical manifestations. Importantly, mAbs lack oral availability and can engender cellular and humoral immune responses. The resulting production of anti-drug antibodies (ADA) may neutralize the therapeutic effects of the drug *in vivo* and/or alter its pharmacokinetic properties. Alternatively, low molecular weight molecules remain an effective approach for immunomodulatory drug development and repurposing. By controlling specific signalling pathways, small molecules such as protein kinase inhibitors (PKIs) often potentiate or replace mAbs in the therapeutic armamentarium. In the case of PKIs, the choice of therapeutic target is certainly a critical issue. The characterization of the broad spectrum of PKI-target interactions and off-target effects is important to define not only the molecular basis of action and of adverse drug reactions, but also the therapeutic indications.

In conclusion, targeted immunotherapeutics are changing the landscape of Immunopharmacology. Despite the success, the long-term clinical benefit of these agents is still limited to a small subset of patients and indications. Interestingly, the idea of pan-modulators exhibiting efficacy in multiple disorders seems incorrect, whereas the generation of new immune-related toxicity profiles requires specific management. Testing a novel concept in this field is extremely complicated and expensive. Yet, given the growing therapeutic impact of targeted immunotherapeutics, it is important to fully characterize their properties and general management.

IMMUNE-ONCOLOGY: YESTERDAY, TODAY AND TOMORROW

DR. CARLOS SILVA.

Servicio de Oncología Clínica, Hospital Británico, CABA, Argentina

The immune system has the potential of destructing tumors causing no damage to normal tissue and displaying a long-term memory. In the last 30 years, immuno-oncology research has provided solid evidence that this fact is true by a process known as immunosurveillance. Immuno-Oncology studies the interface between oncology and immunology and is a growing research area that led to cancer immunotherapy: treatments that harness and enhance the immune system against cancer. Immunotherapy represents the most promising new cancer treatment approach since the development of the first chemotherapies in the earlies 40s. Immunotherapy has been an effective treatment for different types of cancer but especially those that harbor a high rate of mutations as melanoma and lung cancer. One of the advantages of immunotherapy versus chemotherapy and radiation is that side-effects are more tractable and many types do not require treatment interruption. Side-effects are usually related to stimulation of the immune system and can vary from minor symptoms of inflammation to major disruptions as autoimmune disorders. In addition, due to the “immunological memory” cancer immunotherapy overall response is often durable and some patients have achieved cancer remission that can be maintained even after treatment is completed.

A promising avenue of immunotherapy is the use of immune checkpoint inhibitors. These novel drugs in general are monoclonal blocking antibodies that targets specific molecules that negatively regulates the immune response. Thereof, by blocking inhibitory molecules or, alternatively, activating stimulatory molecules, these treatments are designed to unleash or enhance pre-existing anti-tumor immune responses. Several checkpoint inhibitors, targeting multiple different checkpoints, are already been approved for first line treatment and some others are currently being tested in clinical trials.

For example, a very promising immunotherapy to the treatment of metastatic melanoma and lung cancer is the use of PD-1/PD-L1 checkpoint inhibitors. PD-1 is found on activated lymphocytes and upon PD-L1 binding weakens the immune response. In March 2015, the FDA approved the PD-1 checkpoint inhibitor nivolumab (Opdivo®), made by Bristol-Myers Squibb, for the treatment of advanced squamous NSCLC that has stopped responding to chemotherapy. This approval was based on results of a phase III trial in which patients receiving nivolumab lived, on average, 3.2 months longer than patients receiving standard chemotherapy. This translates into a 40% reduced risk of death compared to standard chemotherapy. In October 2015, the FDA expanded its approval of nivolumab to include non-squamous NSCLC. This approval was based on the results of a phase III trial that showed that patients who received nivolumab lived an average of 12.2 months compared to 9.4 months for those receiving standard chemotherapy. In addition, pembrolizumab (Keytruda®), a PD-1 checkpoint

inhibitor made by Merck, was approved in October 2015 for patients with NSCLC. In a phase I clinical trial, about 22% of NSCLC tumors tested had PD-L1 expression at a level of at least 50%. This subset of NSCLC had a response rate of 41%. However, whereas significant clinical responses have been achieved in several patients, others maintain only short-term and new checkpoint inhibitors are intensely being searched.**SIMPOSIA VIII**

MICROBIOLOGY
Chairs: Monica Delgado y Susana Laucella

IMMUNOLOGICAL PROCESSES UNDERLYING THE SLOW ACQUISITION OF IMMUNITY TO BLOOD-STAGE MALARIA

DIANA HANSEN
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Naturally acquired immunity to malaria develops only after many years of repeated exposure to *Plasmodium* parasites. Despite the key role that antibodies play in protection against malaria, the cellular processes underlying the slow acquisition of immunity are unclear. Children in high transmission settings that experience frequent malaria clinical episodes are characterized by a delayed development of parasite-specific memory B cells (MBC), suggesting that the inflammatory factors contributing to disease hinder these responses. We have recently addressed this hypothesis using a pre-clinical model of severe malaria and found that the same inflammatory pathways mediating disease syndromes impair germinal centre (GC) responses and the development of MBC and long-lived plasma cells required for antibody-mediated control of parasitemia. To further define molecular mechanisms by which malaria-driven inflammation impairs the induction of protective immunity, the development of GC responses to *P. berghei* ANKA was examined in mice deficient in the T helper 1-defining transcription factor T-bet. Genetic deletion of T-bet significantly improved T follicular helper (Tfh) cell differentiation rates, which translated in enhanced GC and parasite-specific antibody responses to infection. Interestingly, malaria-driven T-bet expression not only affected the T cell compartment but also directly influenced B cell function, as infection of T-bet$^{fl/fl}$CD23^{Cre} mice revealed that intrinsic expression of T-bet in mature B cells during infection influences the induction of humoral immunity by modulating the cellular dynamics of the GC reaction as well as the quality of the parasite-specific antibody response. Thus, these data suggest that inflammatory pathways mediated by T-bet in response to symptomatic malaria negatively impact the development of long-term humoral immunity by compromising T cell help for antibody formation and by directly influencing B cell activity.

ADAPTIVE IMMUNITY OF BACTERIA

KONSTANTIN SEVERINOV
(Waksman Institute, Rutgers University, New Brunswick, New Jersey, USA)

CRISPR-Cas is a common adaptive RNA-guided prokaryotic immunity mechanism that limits the spread of mobile genetic elements such as phages and plasmids. A CRISPR-Cas system is composed of two seemingly independent modules. Cas proteins from the adaptation module are responsible for recording prior encounters with mobile genetic elements by incorporating fragments of foreign DNA into CRISPR array. Small protective RNAs generated after CRISPR array transcription are used by the interference module Cas proteins to locate complementary nucleic acids and destroy them. I will discuss how the activities and substrate preferences of these two functional modules are tightly coordinated to provide efficient defense against foreign DNA.

FROM SOIL TO CYTOSOL: THE PATHOGENIC TRANSITION OF THE ENVIRONMENTAL BACTERIUM *LISTERIA MONOCYTOGENES*

NANCY FREITAG
(University of Illinois, College of Medicine at Chicago, Chicago, USA)

Listeria monocytogenes is an important agent of foodborne infections and can cause serious invasive disease with a high mortality in susceptible individuals. This bacterium is a ubiquitous environmental organism that can survive in the soil as a saprophyte, but which maintains an ability to invade and replicate within the cells of humans and animals. While environmental microorganisms are numerous, relatively few have the capacity to cause human disease, thus we are interested in how *L. monocytogenes* balances its life as an environmental organism with the lifestyle of a human/animal pathogen. Key to the ability of *L. monocytogenes* to transition to life within mammalian cells is the regulated activity of a transcriptional activator known as PrfA. PrfA is required

for the activation of virulence gene expression within the host and contributes to the physiological adaptation of *L. monocytogenes* to the host cell environment. In addition to regulating bacterial gene products required for host cell invasion, intracellular growth and cell-to-cell spread, PrfA regulates the expression of a bacterial pheromone that enhances bacterial escape from host cell vacuoles by enabling the bacterium to sense the confined space of the vacuole. PrfA also controls the expression of critical secretion chaperones that are required for the folding and stabilization of bacterial virulence factors within the host cell. Overall, our studies highlight the diversity of mechanisms exploited by *L. monocytogenes* to enable bacterial growth within highly diverse habitats ranging from the soil to the cytosol of human cells.

IMPACT OF ANTIMICROBIAL ACTIVITY OF BACTERIAL PEPTIDES ON MULTI-DRUG RESISTANT PATHOGENS: EFFECT IN HUMAN AND VETERINARY MEDICINE

MÓNICA SPARO
(Universidad Nacional de La Plata, La Plata, Argentina)

Resistance to antibiotics has become a threat to global public health and is driving novel research into the development of new antimicrobial drugs. Antimicrobial peptides produced by different gram positive bacterial strains, particularly lactic acid bacteria (lactobacilli, bifidobacteria, lactococci, pediococci, enterococci), has aroused growing interest in the light of the decreasing effectiveness of antimicrobials against severe human and animal infections. Innovation is needed for the development of new drugs and for combination therapies with conventional antibiotics. By targeting different mechanisms of resistance simultaneously, combination therapy might help slow the emergence of resistance. These novel antimicrobial compounds are commonly described as small ribosomally synthesized peptides that are secreted by bacteria and inhibit the growth of closely related species; although some of them exhibit broad-spectrum inhibitory/bactericidal activity (Nisin, AP-CECT7121). Peptides function by inserting themselves into the plasma membrane of target bacteria, forming pores and causing lysis. In addition to their food-safety applications, these peptides can be used effectively in treatment of human and animal bacterial infections. Susceptible pathogens include multi-drug-resistant bacteria as hospital methicillin-resistant *Staphylococcus aureus* (H-MRSA), community methicillin-resistant *Staph. aureus* (Co-MRSA), *Staph. pseudointermedius*, high level vancomycin-resistant *Enterococcus* (VRE) strains and other pathogens resilient to treatment as *Listeria monocytogenes*, *Streptococcus agalactiae* and anaerobic spore-forming bacteria (*Clostridium* spp.). Advantage of these bacterial peptides: diverse repertoire, target specific, food-safe grade substances, synergy with and reduce cytotoxicity of antibiotics. However their main weakness: potential for resistance development and sensitive to proteolysis.

SIMPOSIA IX
MISTERIES OF REPRODUCTION I
Chairs: Monica Muñoz de Toro y Débora Cohen

NOW YOU SEE ME, NOW YOU DON'T! SELECTION STRATEGIES EMPLOYED BY THE OOCYTE TO BE CORRECTLY FERTILIZED

HÉCTOR A. GUIDOBALDI
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Polyspermy is the fusion of two or more spermatozoa with the same oocyte. In some organisms, like birds, it is necessary for a normal fertilization and embryo development, and it is known as physiological polyspermy. However, in many taxa, including mammal's polyspermy results in the death of the embryo, and is called pathological. From an evolutionary point of view, the latter is expensive for females since: i) it reduces the fitness by the loss of viable embryos, ii) the energy investment destined to block the polyspermy could be invested in producing more oocytes, and iii) it can prevent fertilization if the mechanism of blocking is too efficient. Hence females will be under a selection pressure to optimize their response mechanism to reduce the risk of polyspermy. Meanwhile, males compete with others in a race for fertilization. And the selection pressure, promotes the production of many spermatozoa highly efficient to fertilize the oocyte. Being, the pathological polyspermy a collateral damage of that sperm competition. In this context of gender conflicts, communication between gametes, mediated by chemotaxis, arises as a coordinating mechanism that could reconcile the differences of interests between males and females. In the 90's it was discovered that mammalian spermatozoa could be attracted by substances released during ovulation. The characterization of the sperm chemoattraction mechanism showed that only a subpopulation of capacitated spermatozoa (those in optimum physiological conditions for fertilization) could

be chemoattracted by substances released by cumulus cells that surrounds the oocyte (like progesterone or CRISP1). These substances, can diffuse passively along the cumulus and beyond, forming a concentration gradient of attractant that orients the spermatozoa inside the oviduct towards the oocyte. However, once fertilization occurs, this attraction system remains active keeping the oocyte "visible" for those spermatozoa seeking to fertilize. Up to date, the polyspermy blocking mechanisms characterized in mammals (removal of binding proteins or mechanical blockage at the level of the zona pellucida) take several minutes to hours to be effective, and there is no rapid blockage mechanism characterized yet. Recently, we have discovered that capacitated spermatozoa can be chemorepelled, also by a progesterone concentration gradient but in the presence of zinc in the culture medium. This is an interesting discovery, since the oocyte stores zinc in micro-vesicles next to the plasmatic membrane. And, immediately after fertilization, zinc is released outside of the oocyte by exocytosis. Hence, the zinc released could convert the progesterone attraction gradient into a repulsion one to "hide" the oocyte from the sight of the capacitated spermatozoa and reorient them away from the fertilized oocyte. Then, the combination of chemoattraction and chemorepulsion, would allow to regulate the fertilization compensating the interests of both genders. Chemoattraction would allow sperm competition, but once fertilized the oocyte, chemorepulsion could discourage it immediately, preserving the normal development of the embryo. The combination of both mechanisms, optimizes the energy invested by the oocyte to blockade the polyspermy and guarantees the normal fertilization.

SPERMATIC ALTERATIONS AS MARKERS OF HYPERCHOLESTEROLEMIA AND THEIR IMPROVEMENT WITH OLIVE OIL CONSUMPTION

MIGUEL FORNES

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Grease- enriched food is associated to hypercholesterolemia. But, a slight reduction of fat linked to virgin olive oil (VOO) ingestion is enough to decrease diet-induced hypercholesterolemia. Moreover, the seminal and sperm parameters altered under hypercholesterolemic environment were also recovered by VOO. Cholesterol is a key molecule in sperm life because it is involved in several steps of sperm physiology: sperm capacitation, acrosomal reaction among others less recognized. In this sense, an increment in sperm membrane cholesterol would not be strange to interfere with the process, promoting a decrease in: p-Y protein (capacitation marker) and acrosomal index induced by progesterone. Both parameters are also accompanied by teratozoospermia and a reduced sperm cell number in semen. The last detrimental effects of high cholesterol could be attributable to defects at seminiferous tubules, low efficient of the spermatogenetic epithelium and failed spermiogenesis. In morphological detailed analysis it was observed that manchete/acroplaxoma arrangement was disrupted. The acrosomal and nuclear rings normally are pulled down, toward the implantational site of flagellum, by the manchette, a specific tridimensional microtubule complex. Under grease food intake, these structures were asymmetric generating abnormal sperm head and flagella implantation out of the central sperm axis. Furthermore, microtubules and actin filaments, that normally co localize during sperm conformation, does not match under high serum cholesterol. Interestingly, VOO incorporated to diet promotes a re-colocalization and normal sperm development. It is also observed that apoptotic cells appear in seminiferous tubules, as well as uncorrelated spermatogonial and spermatocyte cells frequency; any of them would contribute to explain the tubular decreased efficiency. Fortunately, VOO added to a grease diet recovers the normal spermatogenetic way in adult rabbits promoting normal sperm parameters.

MORPHOLOGICAL AND FUNCTIONAL STUDY OF *LEPTODACTYLUS CHAQUENSIS* TESTIS

LUCRECIA IRUZUBIETA

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Leptodactylus chaquensis is an anuran amphibian belonging to the family *Leptodactylidae* and in our country it is considered as an autochthonous species of the regions of the NOA and NEA. In recent years a marked decline in *Leptodactylus chaquensis* populations has been observed. For this reason, the need arises to implement conservation techniques that consider as main actions the controlled breeding in captivity of the species at risk, with subsequent reinsertion of specimens to their natural habitat. In order to develop these actions, it is important to know the reproductive biology of these species, which in the case of *Leptodactylus chaquensis* is very limited. Taking into account these antecedents in this presentation some morphological and functional aspects of the testis of this species of regional importance it will be comment.

The analysis by optical and ultrastructural microscopy indicates that the histo-architecture of the gonads of *Leptodactylus chaquensis* shows characteristics similar to that of other anuran amphibians and the observations at

the level of transmission electron microscopy, allowed to postulate a diagrammatic sperm scheme.
In the testes of this species, apoptosis was observed as a physiological process, with a higher percentage of occurrence during the reproductive period. An interesting finding was the presence of this type of programmed death in some Sertoli cells with sperm anchored to their cytoplasm, which led us to suggest that this could be an alternative spermiation mechanism.
For the first time in amphibians the presence of Ca^{2+}, calbindin - 28KD and calmodulin could be determined in Sertoli cells, germ cells and gametes indicating the importance of the cation and its homeostasis, at the cellular level, in different physiological processes of the gonad. Thus, in germline cells the cation would be involved in cell growth and differentiation, whereas in the sperm, Ca^{2+} -ATPase activity localized in the acrosomal membranes, associated with Ca^{2+} deposits in the acrosomal vesicle would represent a cation storage system, thus avoiding a premature acrosomal reaction. To corroborate this finding, the biological tests allowed determining that the sperm treatment with 2mM $CaCl_2$ induces the acrosomal reaction effectively.
When studying endocrine regulation of gonadal function, it was determined that FSH, LH and hCG induce spermiation and secretion of steroid hormones in a dose and time dependent manner. FSH is more effective as inducer of spermiation, whereas LH is more effective in stimulating the secretion of steroid hormones.
Overall, both morphological and functional results obtained under our experimental conditions, allow us to suggest that *Leptodactylus chaquensis* would be considered a species with a potentially continuous reproductive cycle.

ENVIRONMENTAL FACTORS AND THEIR EFFECTS ON MALE REPRODUCTION

CECILIA PAPARELLA
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A significant deterioration of male reproductive health has been observed in the last decades. Occupational exposure to toxic substances and environmental contaminants (heavy metals, solvents, agrochemicals, and even heat sources) has been identified as one of the possible causes. These chemical compounds, whether natural or synthetic, affect the male hypothalamic-hypophyseal-gonadal axis or spermatogenesis directly, resulting in an alteration of the seminal canal. The main routes of entry of these toxic substances into the body are inhalation and absorption. Occupational exposure affects the reproductive system, and the resulting damage depends on the length of time of exposure, the concentration of the contaminant in the environment, the protection/safety measures in place, and individual susceptibility. Many of these compounds are known endocrine disrupting chemicals (EDC). They mimic natural hormones, and interfere with endocrine homeostasis by altering the estrogen-androgen-thyroid hormone balance during growth and in future generations. Alterations in different reproductive parameters associated with intrauterine exposure to EDC have been observed in both men and women. The mechanism of transgenerational transmission involves non-genomic modifications in germ cells, changes in DNA methylation or changes in histone acetylation. Alterations in spermatic structures involved in sperm viability and fecundity, such as sperm morphology, DNA integrity, chromatin condensation, and sperm membrane functionality, have been observed in men occupationally exposed to solvents. Agrochemicals (pesticides and fertilizers) interfere with the sperm synthesis and maturation processes. Subjects exposed to these toxic substances show a decrease in sperm concentration and in the percentage of progressive motile spermatozoa, and an increase in germ cell concentration and in morphologically abnormal spermatozoa. In addition, occupational exposure to high temperature sources (as occurs with bakers, cooks, welders, and steel workers) or prolonged exposure of the testes to body temperature (as is the case of bus/truck/taxi drivers and cyclists who remain seated for many hours a day) disturb the synchronous development of the spermatogenic cycle. Seminal analysis shows an increase in germ cell concentration and elevated sperm DNA fragmentation, affecting male reproductive function. Workers exposed to the aforementioned environmental factors are at increased risk of male infertility. The effects of exposure to environmental factors must be evaluated when analyzing male fertility in couples with unexplained infertility. The use of many these toxic substances is currently forbidden in some countries. However, it is necessary to implement strict public health policies aiming to control the use and risk of exposure to these chemicals, in order to reduce their deleterious effect on male reproductive function.

SIMPOSIA X
YOUNG INVESTIGATORS IN BIOPHYSICS
Chairs: Vanesa Herlax and Luciano Galizia

EFFECT OF LIPID-PACKING AND MEMBRANE CURVATURE ON PROTEIN MEMBRANE INTERACTIONS

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The understanding of how membrane physics control biological processes in cells is a key step to better comprehend biology. One aspect is how lipid packing and membrane curvature dictates the rules for lipid/protein interactions and/or protein sorting. Here, and based on previous, I will introduce two different cases of how this physical principles could modulate the association and trafficking of proteins: 1-how lipid packing and membrane curvature, together with membrane order, may impact on the sorting of transmembrane SNARE proteins that reach the golgi and are further transported to the plasma membraen; 2-how in-situ phospholipase activity induces lipid-packing defects that are “sensed” by ALPS, the amphipatic lipid packing sensor motif of ArfGAP1.

HAMPERING THE INACTIVATION OF THE IMMUNOMODULATORY PROTEIN GALECTIN-1 IN INFLAMMATORY MICROENVIRONMENTS

SANTIAGO DI LELLA

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In the past decade, significant efforts have been made to delineate the intracellular and extracellular functions of galectins, multifunctional proteins capable of monitoring glycan changes during fundamental biological processes. Their widely conserved structures, exquisite, although in some cases promiscuous, carbohydrate specificity, and their ability to modulate a variety of biological processes have captivated a broad range of scientists from different disciplines. However, despite considerable efforts in dissecting the functions of these glycan-binding proteins, it is still not clear how structural and biochemical aspects of these proteins can influence their biological activities.

Galectin-1, a proto-type member of the galectin family displays at a glance a structure that appears to be quite simple, although a few peculiarities make it highly versatile. Firstly, even thought its binding domain is a monomer, a subtle homodimerization equilibrium at physiologic concentrations is critical to modulate its biological activity. Moreover, the complex kinetics and thermodynamic equilibria critically govern the function and signaling of this lectin by allowing interactions with a preferential set of glycosylated receptors. Furthermore, the presence of some key amino acid residues, such as an unusual number of cysteine residues or a few environmental sensor residues, appears to govern key properties of galectin-1 functional activity.

We have studied structural and chemical determinants of the galectin-1 protein that in a precise and subtle manner modulate its biological function, by combining experimental and computational approaches. Particularly, inactivation of the protein due to specific physico-chemical conditions present in inflammatory microenvironments will be discussed. We aim to integrate structural, biochemical, and functional aspects of this critical carbohydrate-binding protein and will discuss their implications in physiologic and pathologic settings with the ultimate goal of designing potential galectin-based therapeutic agents.

X-RAY PROTEIN CRYSTALLOGRAPHY AS A TOOL FOR THE STUDY OF BACTERIAL VIRULENCE FACTORS

MARÍA NATALIA LISA

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Cells use signal transduction to sense the environment and regulate central processes in response to environmental stimuli. Reversible protein phosphorylation has evolved as a ubiquitous molecular mechanism of protein regulation. Indeed, the flow of information from the outside to the inside of bacterial cells is largely directed by histidine (His) kinases in two component systems and by eukaryotic-like protein kinases. Work in *Mycobacterium tuberculosis* (*Mtb*) reinforces the idea that phosphorylation on serine (Ser), threonine (Thr) and tyrosine (Tyr) is central to bacterial physiology and pathogenesis, and that the corresponding phospho-systems share similarities to those in eukaryotes (Sherman and Grundner, 2014, *Mol Microbiol*). However, as novel functions and components of bacterial O-phosphorylation are identified, distinct differences between pro- and eukaryotic phospho-signaling systems become apparent, revealing specific protein:protein interfaces that could be exploited for the development of selective drugs with unconventional modes of action. In this talk I am going to present recent advances in the understanding of the signal transduction pathway involving PknG (O’Hare *et al*, 2008, *Mol Microbiol*), a unique multi-domain Ser/Thr protein kinase (Scherr *et al*, 2007, *PNAS*; Lisa *et al*, 2015, *Structure*) that senses amino acid availability to control metabolism and virulence of *Mtb* (Rieck *et al*, 2017, *Plos Pathogens*), and which has a conserved function in amino acid homeostasis amongst the Actinobacteria (Cowley *et al*, 2004 *Mol Microbiol*; Niebisch *et al*, 2006, *J Biol Chem*).

SUSTAINED MECHANICAL STRESS ALTERS THE DYNAMICS AND MOLECULAR RESPONSE OF FOCAL

ADHESIONS IN MAMMARY EPITHELIAL CELLS

LORENA SIGAUT
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Cells are constantly subject to different external mechanical stimuli under normal physiological conditions. Cellular response and adaptation to these mechanical stimuli are crucial in cellular functions and processes[1]. Many of the biological responses to external forces are originated in specialized structures, focal adhesions, which mechanically connect cells with the extracellular matrix via integrin membrane receptors. Focal adhesions are large, transient multiprotein complexes, their formation, development and disassembly are force-dependent[2]. Characterizing how these structures dynamically respond in the presence of a mechanical stimulus is essential for understanding different processes, such as cell adhesion, migration, motility and proliferation.

In this work, we explored the changes generated in the dynamics of focal adhesions, in HC11 non-tumorigenic mouse mammary epithelial cells, in response to a global external mechanical stimulation that resembles the physiological stimuli to which these cells are subjected. To perform controlled and reproducible mechanical stimulus we use a mechanical stretching device[3] that allows sustained equibiaxial stretching of an elastic silicon membrane in which cells are grown, while evaluating the cell-responses by different fluorescence microscopy techniques. We were able to follow focal adhesion dynamics during stretching experiments, by imaging living cells expressing fluorescently tagged proteins, observing an enhanced persistency of focal adhesions as well as an increase in their size. At a molecular level, we explored the effect of external equibiaxial strain on two adhesion proteins postulated as mechanosensors: zyxin and vinculin. We found that increasing normal strain in HC11 living cells, not only induce focal adhesions persistency together with an increase in their size, but also raises molecular tension across the protein vinculin within focal adhesions and significantly decreases dissociation rates of zyxin from the focal adhesion complexes. Although mechanoresponses of zyxin and vinculin have been widely studied, the influence of a direct mechanical change of the substrate on these proteins at a molecular level has not been established before.

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SIMPOSIA XI
PRECISION MEDICINE IN ONCOHEMATOLOGY
Chairs: Leandro Cerchietti and Germán Stemmelin

THE GENETIC BASIS OF DIFFUSE LARGE B CELL LYMPHOMA

LAURA PASQUALUCCI
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Diffuse large B cell lymphoma (DLBCL), the most common subtype of B cell lymphoma in adults, is a molecularly and phenotypically heterogeneous disease that arises from the malignant transformation of B cells arrested at different stages within the germinal center (GC). Over the past decade, considerable progress has been made in our understanding of the molecular pathogenesis of these tumors, owing to the rapid expansion of genomic technologies that allowed us to interrogate the genome/transcriptome of the cancer cell in a comprehensive and unbiased fashion. These studies revealed that DLBCL is characterized by significant genomic complexity, harboring on average 50-100 somatic point mutations and copy number aberrations per case; they also uncovered multiple previously unappreciated genes/pathways that are disrupted by genetic alterations in this disease and may contribute to tumor initiation and expansion. Interestingly, many such lesions hijack transcriptional networks and biological programs that play key roles during the normal GC reaction, where they need to be dynamically and tightly controlled. This wealth of new information led to our current view of the DLBCL genomic landscape, which includes i) alterations that are shared across the two main “cell-of-origin” subtypes of the disease [i.e. the germinal center-like (GCB) DLBCL and the activated B cell-like (ABC) DLBCL]; these involve perturbation of histone/chromatin modifying genes, deregulation of BCL6 activity through multiple mechanisms, and evasion from immune surveillance mechanisms (by loss of B2M/HLA-I and CD58); and ii) lesions that are preferentially or specifically associated with GCB-DLBCL and ABC-DLBCL, indicating that these two subtypes utilize distinct oncogenic pathways.

Among the newly identified “shared” lesions, inactivating mutations in epigenetic modifier genes, including the methyltransferase CREBBP and the acetyltransferase KMT2D, emerged as the most common events in DLBCL, suggesting a central role for epigenetic dysreg-

ulation in the malignant transformation process. Interestingly, mutations of these two genes represent early events during the history of tumor clonal evolution, suggesting an early role in lymphomagenesis. *In vivo*, loss of KMT2D or CREBBP synergizes with BCL2 deregulation to promote tumor development, documenting their role as tumor suppressor genes. Collectively, these findings have provided a molecular framework for the development of therapeutic regimens that may target specific oncogenic addictions in a precision medicine approach.

TARGETING NUCLEAR TRAFFICKING: THE WAY OUT

LEANDRO CERCHIETTI
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The nuclear pore complex is best known for its primary function as the key regulator of molecular traffic between the cytoplasm and the nucleus, however this structure is also an important regulator of gene expression by coordinating the delivery of genetic material to the cytoplasmic protein synthesis machinery. Transport of almost all macromolecules into and out of the nucleus is achieved through a common active mechanism that requires the assistance of soluble nuclear transport factors (NTFs) and transport signals, which together form the "soluble phase" of nuclear transport. Most NTFs belong to the karyopherin family of proteins. Exportin 1 (XPO1) is a conserved and well-characterized karyopherin exporting protein that binds and exports more than 200 proteins from the nucleus to the cytoplasm. XPO1 binds its cargo in the nucleus via the canonical leucine-rich nuclear export signal domain. The complex is then translocated through the nuclear pore complex in the cytoplasm where the cargo is released from XPO1. Since the function of several classes of proteins is highly affected by their intracellular localization, XPO1 is often considered a housekeeping gene whose exporting activity regulates multiple functions in normal cells. However, our group found that XPO1 is frequently amplified and overexpressed in aggressive B-cell lymphomas that are refractory to treatment. Solid tumors and leukemia cells also overexpress XPO1, prompting the development of XPO1 inhibitors as anti-neoplastic agents. The observation that XPO1 inhibitors can be safely used in the clinical setting indicates that cancer cells are more dependent on XPO1 exporting activity for proliferation and survival than normal cells. Although the general consensus is that XPO1 inhibitors exerts its anti-neoplastic function by affecting the nuclear localization of key oncosuppressors proteins, we have reported that a key mechanism by which XPO1 inhibitors kill cancer cells is related to the nuclear entrapment of a sub-set of proteins that binds mRNA, among which eIF4E. As a consequence, XPO1 inhibition reduces the delivery of a sub-group of mRNAs to the cytoplasmic protein synthesis machinery decreasing the expression of a sub-group of oncoproteins that are essential for cancer cell survival. In this presentation, I will discuss the mechanisms employed by XPO1 to selectively regulate the expression of oncogenes and how this has been therapeutically capitalized towards precision oncohematology goals.

DECOUPLING THE PROTEOME FROM THE TRANSCRIPTOME: THE STORY OF THE EUKARYOTIC TRANSLATION INITIATION FACTOR EIF4E

KATHERINE BORDEN
(Department of Pathology and Cell Biology, Faculty of Medicine, Université de Montréal, Montréal, Canada)

Recent advances in cancer research have focussed on genome and transcriptomic understanding of cancer. However. global proteomics studies reveal that the transcriptome does not always reflect the proteome. A major question arises: how is the proteome decoupled from the transcriptome? Indeed, alterations in mRNA metabolism at the mRNA export, mRNA processing or translation levels can all contribute to increased protein expression in the absence of increased transcription. The eukaryotic translation initiation factor eIF4E is a prime example of such regulation. eIF4E is an oncoprotein that plays multiple roles in RNA processing driving the conversion of transcripts to protein without altering transcript levels. eIF4E drives the mRNA export and translation of established oncogenic drivers including c-Myc, MCl1, BCL6, hDM2 etc. Indeed, eIF4E regulates about 3500 transcripts at the mRNA export level many of them involved in functionally related roles. In this way, eIF4E can coordinately drive oncogenic networks. eIF4E is elevated in many human cancers including Acute Myeloid Leukemia (AML). Our studies provide mechanistic insights into eIF4E's biochemical activities and our clinical studies demonstrate that targeting eIF4E in AML patients leads to responses.

THERAPEUTIC TARGETING OF LEUKEMIA STEM CELLS

MONICA GUZMAN
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The majority of patients with acute myelogenous leukemia (AML) die from their disease. Despite that most patients can achieve remission after initial induction therapy, most of them will relapse. Relapse is thought to be the result of ineffective ablation of a population of leukemia cells that are known as leukemia stem cells (LSCs). Elevated levels of LSCs (defined by immunophenotype) at diagnosis are predictive of minimal residual disease (MRD) which, in turn, predicts leukemic relapse, even after myeloablative stem cell transplantation. Thus, in order to improve AML therapy, it is critical to identify therapeutic strategies that effectively eliminate LSCs. We have demonstrated that LSCs possess unique molecular features that distinguish them from their normal hematopoietic stem cells (HSCs) and that such can be exploited to selectively eliminate LSCs without harming HSCs. We have identified novel therapeutic strategies to selectively target LSCs without harming HSCs. Such strategies include compounds derived from natural products, nanoparticles and chimeric antigen receptor T cells.

SIMPOSIA XII
CELL SIGNALING: UBIQUITIN AND AUTOPHAGY IN HEALTH AND DISEASE
Chairs: Pablo wapner and Mario Rossi

STRUCTURAL AND FUNCTIONAL PLASTICITY OF MYOSIN V

SIMONA POLO
(Fondazione Istituto di Oncologia Molecolare, Milano, Italy)

In the last few years, the contribution of alternative splicing in human disease has been widely recognised. Unbalanced expression of splicing variants or failure to properly express the correct isoform is undoubtedly part of cancer cell biology. We recently studied alternative splicing of myosin VI, a motor protein with unique functional properties, to find that myosin VI isoforms have mutually exclusive interactors and adopt different conformations (Wollscheid et al. NSMB 2016). Our structural and functional data pointed to an isoform-specific regulatory helix, named α2-linker that acts like a molecular switch, assigning myosin VI to distinct endocytic (myosin VI_{long}) or migratory (myosin VI_{short}) functional roles. This finding is relevant to ovarian cancer, where alternative myosin VI splicing is aberrantly regulated, and exon skipping dictates cell addiction to myosin VI_{short} for tumour cell migration (Wollscheid et al. NSMB 2016).

Interestingly, an isoform switch occurs during epithelial polarization and determines the selective expression of myosin $VI_{long.}$ Indeed, we observed that epithelial cells expressing both isoforms switch on/off their expression according to the culture conditions. In sparse conditions, lung adenocarcinoma cells (A549) and colorectal adenocarcinoma cells (Caco2) express predominantly myosin VI_{short} (that functions in cell migration) but they switch to an almost exclusive expression of myosin $VI_{long.}$ (with endocytic functions) upon reaching confluency and a fully polarized epithelial architecture. This process is reversible as both cell lines switch back to myosin VI_{short} once disaggregated and grown in sparse conditions. This isoform switch mimics the epithelial to mesenchymal transition (EMT) process that leads to the positive selection of myosin VI I_{short} observed in primary ovarian cancer cells and it represents an informative and simple cellular model to study AS regulation at the endogenous level. We are currently identifying and characterizing common splicing-regulated events that occur during the establishment of a fully polarized epithelial architecture and the responsible alternative spicing regulators, which are possibly deregulated during cancer alternative splicing reprogramming.

We also found that myosin VI depletion in non-tumoral cells (hTERT-RPE1 and BJ-5ta) leads to impairment in cell proliferation, caused by a cell-cycle arrest in G0/G1, followed by senescence. This block in cell cycle progression is due to the activation of the p53-p21 axis which occurs independently from DNA damage and might be triggered by the centrosome surveillance pathway. Indeed, myosin VI depletion leads to several centrosomal defects, namely centriole amplification, separation and random orientation. Recent results will be presented.

CULLIN-RING UBIQUITIN LIGASES: FROM SIGNALING TO DISEASE

DANIELE GUARDAVACCARO
(Utrecht University, Utrecht, The Netherlands)

Research in our laboratory focuses on studying the molecular mechanisms through which the ubiquitin system controls fundamental cellular processes and understanding how deregulation of this network leads to human disease. We are especially interested in the role of Cullin-RING complexes, the largest class of E3 ubiquitin ligases in eukaryotes. Cullin-RING ubiquitin ligases (CRLs) share a common catalytic core consisting of a Cullin scaffold and a RING protein which functions as the docking site for ubiquitin conjugating enzymes. The catalytic core of CRL assembles with numerous substrate receptors, which target specific substrates for ubiquityla-

tion. Due to the great diversity of their substrate receptor subunits, over 350 distinct CRLs are present in eukaryotic cells, establishing these enzymes as major mediators of ubiquitin conjugation and key regulators of a wide array of biological processes. Despite their importance, our knowledge of the biological functions, mechanism of action, regulation and physiological partners for most CRLs remains poor. Moreover, the majority of CRLs remains with no established substrates. Therefore, the major goal of our research program is to study the functions of orphan CRLs and systematically identify their biologically significant substrates as well as investigate how deregulation of CRL-mediated ubiquitylation contributes to pathogenesis.

AUTOPHAGY AND PATHOGEN-CONTAINING COMPARTMENTS: SIGNALING TO CONTROL LIFE AND DEATH

MARÍA ISABEL COLOMBO

(Instituto de Histología y Embriología "Dr. Mario Burgos", CONICET, Universidad Nacional de Cuyo, Mendoza, Argentina)

Intracellular pathogens use sophisticated mechanisms to overcome host cell defenses and replicate successfully. Autophagy, a self-degradative process, is a critical cell defense mechanism against invading microorganisms, but certain bacteria avoid or actively subvert autophagy to promote their own replication. We have previously demonstrated that autophagy plays a very important role against *Mycobacterium tuberculosis.* During infection *M. tuberculosis* secrets proteins to evade host cell defense mechanisms. Two of these proteins CFP-10 and the hemolysin ESAT-6, encoded by the region of differentiation 1(RD1), are key in mycobacteria pathogenesis. We have previously shown that *M. marinum*-containing phagosomes are decorated with the autophagic protein LC3. However, this recruitment was not observed in cells infected with *M. marinum* deleted for RD1, indicating that this region is critical for autophagy targeting. Likewise our studies indicate that ESAT-6 is critical for autophagy recognition of *M. tuberculosis.* As expected, GFP-LC3 was recruited to phagosomes containing *M. tuberculosis* wt but not the ΔESAT-6 deletion mutant. These and other evidences indicate that *M. tuberculosis* damages the membrane of the mycobacterium-containing phagosome.

Coxiella burnetii, the etiologic agent of Q fever, is a Gram-negative obligate intracellular bacterium that develops a large *Coxiella* replicative vacuole (CRV) that has late endosome-lysosome characteristics with autophagic features (i.e. LC3 recruitment). Indeed, autophagy activation favors bacteria replication. Our present results demonstrate for the first time, that in a population of *Coxiella*-containing vacuoles the CRV membrane is damaged and, as a consequence, the CRV loses transiently its acidic pH. We propose that the autophagic pathway favors *Coxiella* infection by contributing to the repair of the damaged replicative compartment.

Staphylococcus aureus is a microorganism that causes serious infectious processes in humans. After internalization, this pathogen escapes from the phagosome to the cytoplasm. We have shown that before escaping *S. aureus* resides in a phagosome that recruits LC3. This recruitment depends on the toxin a-hemolysin (Hla), secreted by the bacteria. Very recent results from our laboratory indicate that *S. aureus* at early times after infection generates dynamic tubular structures that are labeled by LC3. By using specific markers we have determined that those tubular structures correspond to novel membranous compartments. Interestingly, a *S. aureus* strain deficient in Hla was unable to generate the LC3-labeled tubules.

Taken together our results contribute to understand the behavior of pathogens with different intracellular life styles that share some common features on the targeting by the autophagy machinery that somehow allows pathogen survival.

AUTOPHAGY IN PANCREATIC DISEASES

MARÍA INÉS VACCARO

(Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina)

The study of the molecular mechanisms that human cells put in place in response to the disease is a very active area in scientific research in medicine. Our work is focused on the molecular mechanisms involved in the early events that occur in pancreatic cells during non communicable diseases. In the search for new molecules that are differentially expressed during acute pancreatitis we clone a new transmembrane protein that we call Vacuole Membrane Protein 1 (VMP1). We found that VMP1 triggered autophagy in mammalian cells. Autophagy is a highly conserved process in evolution by which the cell degrades cytoplasmic components including organelles. Autophagy has been linked to a variety of pathological processes such as neurodegenerative diseases and tumorigenesis, which highlights its medical importance. We demonstrate that VMP1 mediates a new type of selective autophagy that acts as a defense mechanism in cells producing export proteins. Also, VMP1 participates in the mechanisms of resistance to death of pancreatic tumor cells and is induced by cancer microenvironment and its interaction with specific plasma membrane proteins marks a new initiation site for autophagy in mam-

malian cells. In this lecture, we will discuss the role of autophagy and its molecular steps as a cell response to complex pathologies such as cancer, inflammatory bowel diseases, pancreatitis and diabetes. We think that the study of the molecular mechanisms that the cells activate in response to human disease will allow to develop new and more rational therapeutic strategies.

SIMPOSIA XIII
BIOPHYSICAL APPROACHES
Chairs: Sabina Mate and Axel Hollman

IN-CELL NMR AS A TOOL FOR IN SITU STRUCTURAL BIOLOGY

ANDRÉS BINOLFI
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The structure and function of proteins depend on multiple cellular factors such as localization, post-translational modifications and interactions with biological partners. However, most protein structural studies are typically performed on isolated samples, under conditions that differ substantially from *in vivo* environments of live cells. The question arises whether protein features observed *in vitro* correlate with their intracellular behaviors? In this regard, in-cell NMR spectroscopy represents a powerful method to directly study protein structures in intact cells. Here, we present high-resolution in-cell NMR results on the structural and dynamic properties of the human amyloid protein alpha-synuclein (aSyn) in different mammalian cell types, the aggregation of which correlates with the onset of Parkinson´s disease (PD). Using electroporation to efficiently deliver isotopically enriched aSyn into neuronal and non-neuronal cells, we delineate its intracellular behavior by in-cell NMR spectroscopy.[1] Additionally, we study the cellular repair of oxidation-damaged aSyn by endogenous enzymes and identify C-terminal modifications sites as remaining permanently damaged, which may lead to neuronal toxicity under oxidative stress conditions.[2] These results allow us to better understand the native conformations of aSyn under physiological cell conditions, further enabling future *in situ* investigations of intracellular aSyn aggregation in PD and other related neurodegenerative disease processes.

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DENGUE VIRUS CAPSID DYNAMICS: BRIDGING BIOPHYSICS AND MOLECULAR VIROLOGY BY ADVANCED FLUORESCENCE TECHNIQUES

LAURA ESTRADA
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Intracellular dynamics plays a key role in most molecular processes of biological relevance. Many of the studies in this field have been successfully carried out by methods based on the correlation of the intensity fluctuations. Fluorescence Correlation Spectroscopy (FCS), Scanning FCS, Raster Image Correlation Spectroscopy (RICS) and, more recently, spatial pair Correlation Functions (pCF), are powerful techniques that allow studying the motion of particles inside a cell. The pCF measures the time a particle takes to go from one location to another by cross-correlating the intensity fluctuations at specific distances along a line-scan within the cell. The line-scan acquisition implies, however, that the direction along which motion can be studied is predetermined by the chosen scanning pattern. To overcome this limitation we performed a pCF analysis at every pixel along the entire image creating a visual map of the average path followed by the molecules. In this work, we applied a combination of single particle tracking and correlation-based techniques to monitor the subcellular localization of the dengue virus capsid protein undergoing free diffusion into living BHK cells. To this end, we generated a replicating viral RNA with the coding sequence of mCherry fused to capsid. During dengue virus infection, the capsid protein accumulates in nucleolus. However, because viral morphogenesis takes place in the cytoplasm, it is still unknown why and how capsid associates to nucleolus. We used a time course to follow newly synthesized capsid during initial viral RNA translation and replication. Our results show that the directionality of the motion become greater around the nucleolus and it increases with the time passed after transfection.

WHAT CAN WE LEARN ABOUT DRUG MEMBRANE INTERACTION FROM MODEL LIPID MEMBRANES

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Unlike drug selection to achieve specific interaction with protein receptors, compounds that interact with biological membranes seldom do so through specific interactions, but as a sum of weak interactions and entropic factors. The physicochemical bases that govern this phenomenon are not clearly elucidated today. In this talk, I will expose the advances that our laboratory has had in this subject through the study of the interaction of the alkyl esters of L-ascorbic acid (ASCn) to model lipid membranes. These amphiphilic drugs are potent antioxidants that are self-organized in nanostructures called coageles, which have been proposed as ocular and skin permeation enhancers, among other pharmacological uses. On the other hand, we have also studied the interaction with model lipid membranes of the amphiphilic drug hexadecylphosphocholine (HePC) or miltefosine, currently used clinically for the treatment of cutaneous lymphoma and leishmaniasis.

Our studies have highlighted the importance of the structural and rheological properties of the different model lipid membranes used in the interaction of these drugs with these membranes. When an amphiphilic drug is inserted into a biomembrane it must exert a compression on the rest of the membrane components, in such a way that it opposes the lateral pressure. There, the ability of the lipid film to respond to isometric compression and lateral displacement of the components, as well as electrostatic factors (lateral repulsion or between membrane and charged amphiphilic monomers) would condition the insertion of a new molecule into the membrane. From these studies, the compressibility of the membrane is the main physical parameter that governs the incorporation of these drugs.

ASCs are avidly incorporated into lipid membranes in both monolayer and bilayer systems and such incorporation affects the properties of the acceptor membranes differentially according to the length of the hydrocarbon chain of the compounds. These differences were correlated with different pharmacological uses of this family. On the other hand, we address two main issues with regard to the action of HePC: the bases for membrane selectivity and its mechanism as a perturbator agent, which is related to its pharmacological function of cellular lipid homeostasis modulator. HePC is also sensitive to the compressibility of the host membrane by penetrating more favorably into the more easily compressible membranes and less in the membranes rich in cholesterol or that mimic the stratum corneum of skin. This, in turn, alters the phase equilibrium of lipid membranes, which has the potential to modulate the activity of lipolytic enzymes acting on these membranes.

In conclusion, amphiphilic drugs follow a common mechanism of insertion into lipid membranes but with subtle differences according to their chemical structure and amphipathicity, which results in properties that can be exploited in different aspects of their pharmacological use. From what has been learned from these studies we intend to advance in rationally designed, combined therapeutic strategies based on their effect on biomembranes.

A diferencia de la selección de fármacos para lograr interacción específica con receptores proteicos, los compuestos que interaccionan con membranas biológicas pocas veces lo hacen a través de interacciones específicas, sino como una sumatoria de interacciones débiles y factores entrópicos. Las bases fisicoquímica que rigen este fenómeno no están claramente dilucidado en la actualidad. En esta charla, contaré los avances que nuestro laboratorio ha tenido en este tema a través del estudio de la interacción de los esteres alquílicos del ácido L-ascórbico (ASCn) a membranas lipídicas modelo. Éstos fármacos anfifílicos son potentes antioxidantes que se autorganizan en nanoestructuras llamadas coageles, los cuales han sido propuestos como como promotores de la permeación ocular y de piel, entre otras utilizaciones farmacológicas. Por otro lado también hemos estudiado la interacción con membranas lipídicas modelo del fármaco anfifílico hexadecilfosfocolina (HePC) o miltefosina, actualmente utilizado clínicamente para el tratamiento linfoma cutáneo y leishmaniasis.

Nuestros estudios han resaltado la importancia de las propiedades estructurales y reológicas de las distintas membranas lipídicas utilizadas en la interacción de estos fármacos con dichas membranas. Cuando un fármaco anfifílico se inserta en una biomembrana debe ejercer una compresión sobre el resto de los componentes de la membrana, de forma tal que se oponga a la presión lateral. Allí, la capacidad de la película lipídica de responder ante la compresión isométrica y del desplazamiento lateral de los componentes, además de factores electrostáticas (repulsión lateral o entre la membrana y los monómeros solubles de anfifilos cargados) condicionarían la inserción de una nueva molécula a la membrana. De estos estudios surge que la compresibilidad de la membrana constituye el principal parámetro físico que rige la incorporación de estas drogas.

Los ASCn se incorporan ávidamente a membranas lipídicas tanto en sistemas de monocapas como en bicapas y dicha incorporación afecta las propiedades de la membrana aceptora diferencialmente según el largo de la cadena hidrocarbonada de los compuestos. Estas diferencias se correlacionaron con diferentes usos farmacológicos de dicha familia. Por otro lado, abordamos dos cuestiones principales con respecto a la acción de HePC: las bases para la selectividad de la membrana y su mecanismo como un agente perturbador de misma, lo cual está relacionado a su función farmacológica de modulador de la homeostasis lipídica celular. HePC también es sensible a la compresibilidad de la membrana huésped penetrando más favorablemente en las membranas más fácilmente compresibles y menos en las membranas ricas en colesterol o que simulan el estrato corneo de piel. Esto, a su vez altera los equilibrios de fases de las membranas lipídicas, lo que tiene la potencialidad de modular la actividad de enzimas lipolíticas que actúan sobre estas membranas.

En conclusión, los fármacos anfifílicos siguen un mecanismo común de inserción a membranas lipídicas pero con sutiles diferencias según su estructura química y an-

fipaticidad, lo que resulta en propiedades aprovechables en los distintos aspectos de su uso farmacológico. De lo aprendido a partir de estos estudios se pretende avanzar en estrategias terapéuticas combinadas diseñadas racionalmente con base en su efecto sobre biomembranas.

MAPPING THE DYNAMICS OF TRANSCRIPTION FACTORS IN LIVING CELLS AND WHOLE ORGANISMS

VALERIA LEVI

(Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina)

Gene expression results from the dynamical recruitment of the transcription machinery and regulatory proteins to specific target genes. Many components of this machinery including transcription factors (TFs), coregulators, chromatin remodelers and RNA polymerases do not distribute homogeneously in the cell nucleus. Determining how the highly dynamical and heterogeneous distribution of transcription-related proteins within the nucleus modulates the expression profile remains as a central challenge in the cell biology field.

Here, we show how the combination of fluorescence fluctuations based techniques and numerical simulations can help us to reveal important aspects of this complex process in living cells. Fluorescence correlation spectroscopy (FCS) methods are based on analyzing the intensity fluctuations caused by molecules moving through the observation volume of confocal or two-photon excitation microscopes. The quantitative analysis of these fluctuations provides important clues regarding the mobility of the molecules and their interactions with other species First, we use FCS to quantify the dynamics of TFs that controls pluripotency in single cells of mouse early embryos. Our study demonstrates that TFs undergo temporally distinct binding events with DNA in the living embryo. Furthermore, we observed that developmentally regulated epigenetic changes create differences in the fraction of the TF Sox2 engaged in long-lived DNA–binding between 4-cell blastomeres. Finally, we demonstrate that these differences in Sox2–DNA binding predict the allocation of the first cells to the inner mass of the embryo, which subsequently contributes most cells of the body.

In addition, we investigated the dynamical organization of TFs in living cells focusing on the glucocorticoid receptor (GR), a ligand-activated transcription factor. This transcription factor is involved in the control of gene expression linked to several processes like inflammation, stress responses, glucose homeostasis, lipid metabolism, proliferation and apoptosis development. We present evidences showing that GR and its coregulator NCoA-2 diffuse within the nucleus and interact with PML bodies, DNA-dependent foci or engage in interactions with chromatin targets. Cross-correlation analyses show that GR and NCoA-2 sequentially interact with foci and glucocorticoid response elements. Moreover, we show that the coregulator and the conformation of the receptor tune the distribution of the receptor among the nuclear compartment. Our results suggest that GR partition in different nuclear reservoirs ultimately regulates the concentration of receptor available for the interaction with specific targets.

SIMPOSIA XIV
MALE SEXUALITY: AN INTEGRATED APPROACH
Chairs: Pablo Knoblovits and Omar Layus

SEXUAL EVOLUTION IN THE HUMAN SPECIES

OSVALDO MAZZA

(Servicio de Urología, Hospital de Clínicas "José de San Martín", Buenos Aires, Argentina)

The human species is the result of an evolutionary process that began more than six million years ago and its final product – *Homo sapiens* – reached its developmental peak more than twenty thousand years ago, spreading over the planet from its starting point in Africa and giving rise to a diversity of ethnicities.

All living creatures, including man, sustain life through numerous processes, the most remarkable of which is the ability to reproduce. Reproduction is life's central dogma, allowing its existence and perpetuation. This attribute is known as sexual function. Modern man's sexual function is the result of biological development and cultural influences.

Sexual development in man is rooted in the origins of the species and has changed according to human development. The transformation of animal mating conducts into the diverse and sophisticated ways of man's sexual relations is a consequence of evolution from our primate ancestors, somatic characteristics responsible of our anthropomorphism and sexual characters derived from evolution.

Preservation of the species is a fundamental law of Biology, relying on successful reproductive strategies incorporated along evolution. This biological dogma is so important, that in many species, male, female or both, die after mating or egg release (salmon, squid, bee, mantis). In mammals, mating renders the couple defenseless before predators due to brief loss of control of surroundings and

diminished chances of flight if faced with danger during mating. Thus, mating is remarkably brief. Even in those species closest to man, such as the common chimpanzee *Pan troglodytes*, mating is less than twenty seconds. Man has evolved morphologically and culturally and has transformed his sexuality into an act of pleasure, dissociated from reproductive success, by using techniques already effective in primitive cultures or scientifically controlled in the western world. The search for sexual pleasure has become a conscious and primary aim, above social or moral restraints, leading on occasion to homicide for satisfaction.

Man tries to satisfy his sexuality safely (cave, home, town protect him from external dangers) and his sexual encounters have become longer to increase his pleasure, provided that safety and intimacy conditions are adequate and controllable. Man has experimented extension of pleasure during coitus when compared to simians, an evolutionary trait and unconscious product of what Darwin described as sexual selection.

According to Waldinger, control of preejaculatory vaginal latency (time erect penis remains in the vagina until ejaculation) in the modern human, is an evolutionary trait that betters reproductive strategies. Those who perform adequately, achieve a competitive advantage that makes them desirable in the mechanism of sexual selection. In adverse conditions, sexual performance is shorter, primitive and reflex, lacking cultural contribution. This raises two questions: from which starting point and how has human sexuality developed.

In most simians, except the bonobo chimpanzee, sexual posture involves the male engaging the female from behind. Missionary or face to face position has been probably used since *Australopithecus afarensis*, such as Lucy, 4 million years ago. Lucy's erect posture resulted in a vertical vagina, resembling that of modern woman. A simian's horizontal vagina favors rear copulation with immediate retention of semen, even though the female rapidly becomes active following coitus, even beginning search for another male. If Lucy and her descendants, endowed with a vertical vagina favoring face to face coitus, became active immediately following copulation, loss of semen would probably occur. It was thus biologically necessary for Lucy and the rest of the hominids that followed her up to modern woman, to maintain a resting horizontal position post coitum; an ecstatic period that drove her away from all desire of physical or sexual activity, to allow for semen to coagulate; a period extending pleasure and favoring visual contact with her sexual partner; a post orgasmic period.

This incorporates the idea of generating an identifiable bond with another being to turn to regularly for sexual activity. In contrast to express and uninvolved sex of our ancestors, the great apes, sexual partners will be identified and searched to relive that post orgasmic atmosphere. These bonds gave rise to prolonged and safer infancies, with paternal involvement in the care of the young which, in turn, allowed an evolutionary leap that led to the cerebral and sexual development of *Homo sapiens*.

Which aspects of human nature and sexuality are inscribed in our genes? Which is the contribution of cultural heritage? Which are the biological processes that condition our sex life? Research on the human genome has provided glimpses of the answers to some of these questions, but others remain scientific speculation. Sex, like hunger, is among the main driving forces of human actions and selective drive of cultural evolution.

Like hunger, sex is at the same time, compulsion and appetite. We have dissociated sexual intercourse from its reproductive aim. We exercise our sexuality to satiate a primitive appetite and simultaneously satisfy a social need. We have evolved from communal and carefree sex (chimpanzee?) to possessive sex (gorilla?) to such an extent, that some people are capable of fighting, killing, raping, losing their fortune, their health or even their own life.

Ways and habits in developed societies, intensified in this millennium, allow us to witness the breakup of the human couple. Women practice uninvolved sexuality, choosing an occasional man that will enable them to achieve motherhood without commitment (orangutan sex?)

Culture appeases our sexual needs, but our ancestral impulses often break free from cultural restraint and, upon sexual urge, challenge reason and unwanted pregnancies and sexually transmitted diseases ensue. Medicine needs to look at human sexuality considering both its roots and its present surroundings.

Other questions remain to be addressed. Sexual conducts, other than those socially accepted (anal coitus, cunnilingus, fellatio), sexual diversity (homosexuality and bisexuality) can be observed in monkeys as variations among species and gender distribution in the tribe. Other unacceptable social behaviors, such as incest, rape and paraphilia can also be observed in these species. Much of this, could have been carried down generations during evolution and filtered as sex became more rational and restricted by the culturalization process of the human species.

PREMATURE EJACULATION: DIAGNOSIS AND TREATMENT

ERNESTO GRASSO

(Sección Andrología / Sexología, Sanatorio Trinidad Mitre, Buenos Aires, Argentina)

Premature Ejaculatory Dysfunction is the most prevalent sexual dysfunction in the male, though consultation for erectile dysfunction occurs more often. This dysfunction affects not only the person who ejaculates prematurely, but involves his partner as well, because it does not allow both to enjoy the pleasure of sexual encounter for a longer period of time. In this symposium, I will present various definitions of premature ejaculation dysfunc-

tion which have been in use throughout the years, until the latest one, derived from a consensus organized by International Society of Sexual Medicine. I will classify premature ejaculatory dysfunction from different standpoints and address its physiopathology. I will show how the partner of such a patient is affected. I will illustrate how patients, influenced by myth and urban legend, try to solve this problem on their own. I will present different clinical cases that will help understand the suffering these patients undergo. I will show different therapeutic alternatives and, with the help of all those present, will try to choose the most adequate treatment for each case presented. In this way, I intend to show how these patients can be helped from both a biological and psychological perspective.

FROM ENDOTHELIAL DYSFUNCTION TO ERECTILE DYSFUNCTION. WHERE DO WE STAND IN 2017? THERAPEUTIC POSSIBILITIES

MIGUEL RIVERO
(Equipo de Neurocirugía Endovascular Radiología Intervencionista, Buenos Aires, Argentina)

In a vast majority of patients with Erectile Dysfunction, it is based on a biogenic process together with some psychological aspects. The two way equation 'Erectile Dysfunction vs Endothelial Dysfunction" is the key to be considered. Many illnesses and or risk factors are present around this equation, such as obesity, diabetes, hypertension, hypogonadism, ageing, lower urinary tract symptoms / beningn prostate hyperplasia, etc. One of three patients who consult about ED does not know about them. Therefore, the importance of a global approach. After the diagnosis of ED and the right management of the risk factors, the treatment could be more effective. The modern alternatives for treatment of ED, which must be personalized according to each patient, will be presented.

SEXUALITY IN MENOPAUSE

MARTA RAJTMAN
(Sociedad Argentina de Sexualidad Humana, Buenos Aires ,Argentina)

We can definitely state that for women, sexuality exists until they want it to. Neither medicine nor the psychological sciences have shown that being sexually active all life long, may be harmful or impossible to achieve. Taboos, the ban implicit in the cultural message we receive, are remnants of the belief that sexuality is only useful for reproduction and is no more licit or acceptable when it has lost its reproductive aim. Our first job is, thus, to remove from this topic the idea of forbiddance and shame.

We must be aware of the changes taking place in our body, accept them and learn to enjoy in a different way. Our sexuality will not be the same as before, but it may be excellent. The important thing is to keep active and stand up for our right to life and pleasure.

Every single aspect that has been studied in relation to menopause, has shown that there are significant changes involved, and that they limit quality of life and life expectancy of women, with high morbidity and mortality.

As important as it is for the primary care sexologist to know this process thoroughly, from all its biological, psychological and social standpoints, so it is for the patient. In this contention, it is necessary to individualize each woman and every aspect of her personality.

The menopausal woman belongs to a particular social group with a characteristic epidemiologic profile. Accordingly, she needs special medical care during this very important period of her life where, as has been previously shown and can be easily inferred, there is so much to be done in health prevention and promotion.

SIMPOSIA XV
TUMOR MICROENVIRONMENT
Chairs: Mariana Salatino and Claudia Lanari

MYC IS A GLOBAL REGULATOR OF THE HOST IMMUNE RESPONSE

DEAN W. FELSHER
(Stanford University, Stanford, California, USA)

Many human cancers are as associated with the activation of specific oncogenes. We have used the Tet System to generate transgenic mouse models to model and predict the therapeutic efficacy of targeted therapy of specific oncogenes. We have shown that many oncogenes (MYC, RAS, BCR-ABL) induce tumorigenesis that is completely reversible upon their inactivation. Previously, we have described this phenomenon as oncogene addiction. We have shown that oncogene addiction is associated with proliferative arrest, apoptosis, differen-

tiation, cellular senescence and the shut down of angiogenesis.
Now, we have found that the oncogenes, such as MYC, casually regulates the immune response. MYC transcriptionally regulates immune checkpoint regulators, including PD-L1 and CD47 in murine and human tumor cells. MYC activation can causally suppresses an immune response against tumors. Conversely, MYC suppression, reduces expression of immune checkpoints and elicits and immune response. Finally, blocking the ability of MYC inactivation to down-regulate expression of PD-L1 or CD47 prevents tumor regression. Hence, MYC oncogene addiction is causally associated with ability to regulate immune checkponits.
MYC's regulation of immune checkpoints is causally regulates the recruitment of innate and adaptive immune effector cells that regulate tumor microenvironment. Then, these immune effectors regulate self-renewal programs in tumor cells and host angiogenesis in the tumor microenvironment. Hence, the MYC oncogene globally regulates the host immune response. Therapies that target MYC could be used to restore the immune response against human tumors. MYC overexpression may predict responsiveness to immune checkpoint therapy.

ON THE ROLE OF THE MICROBIOTA AS A KEY MODULATOR OF THE TUMOR MICROENVIRONMENT

ROMINA GOLDSZMID
(Center for Cancer Research, NCI; NIAID, National Institutes of Health, Bethesda, Maryland, USA)

Cancer has historically been viewed as a disease determined by genetic and environmental factors, however, it is now clear that inflammation affects all stages of the disease: initiation, progression and metastasis formation. The inflamed tumor microenvironment is in part sustained by infiltrating mononuclear phagocytes (MPs) [e.g. dendritic cells, monocytes, macrophages] and neutrophils. In cancer, as in infection, MPs can induce adaptive immune responses, but in cancer they mainly promote the tumor's immune evasion, progression, and metastasis. We, and others, have recently uncovered a role for commensal microbes in controlling the response of subcutaneous tumors to cancer immuno- and chemotherapy. In this presentation, we will discuss the role of the microbiota in regulating the composition and function of the myeloid cell compartment in the tumor microenvironment and the role of these cells in the response to cancer chemotherapy. Targeting MPs represents a powerful approach to manipulate the outcome of immune responses; therefore, a clear understanding of their regulation and functional organization may lead to rational novel cancer immunotherapeutic approaches.

BLOCKADE OF FOXP3 IMPROVES THE EFFICACY OF ANTITUMOR VACCINES IN EXPERIMENTAL BREAST CANCER

MARIANELA CANDOLFI
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Breast cancer is the most frequent cancer and the first cause of death by cancer in women worldwide. Immunotherapy has emerged as a powerful therapeutic approach for the treatment of many cancers. Due to the specificity of the antitumor immune response and the possibility of generating antitumor immunological memory, the field of immunotherapy has developed exponentially over the last few decades. Unfortunately, since breast tumors were traditionally thought to be poorly immunogenic, the use of immunotherapy was long considered inappropriate to treat breast cancer patients. However, a growing body of evidence indicates that immunotherapeutic strategies can benefit a larger cohort of breast cancer patients than hitherto anticipated. Mammary tumors are infiltrated with immune cells and the level of tumor infiltrating T lymphocytes positively correlates with good prognosis in breast cancer patients. However, several immunosuppressive mechanisms impair antitumor T cell responses, such as immunological checkpoints and immunosuppressive cytokines that are up-regulated in the tumor microenvironment, skewing the immune response towards Th2 and regulatory T (Treg) phenotypes. Tumor infiltrating Tregs constitute an important therapeutic target, as they suppress effector T cell function in a dose-dependent manner and have been associated with poor survival in breast cancer patients. Tregs have also been involved in the relatively low efficacy of antitumor vaccines. We found that while prophylactic antitumor dendritic cell (DC) vaccines improved the survival of murine models of breast cancer and trigger antitumor immunological memory, DC vaccines administered to mice bearing established breast tumors induce the expansion of Tregs and lack efficacy in this therapeutic setting. In order to improve the antitumor effect of DC vaccines, we used a cell penetrating peptide (P60) that inhibits Foxp3, a transcription factor required for Treg function. Treatment with P60 inhibited tumor progression and improved the therapeutic efficacy of DC vaccines in experimental breast cancer models. Monotherapy with P60 inhibited tumor growth not only in immunocompetent mice, but also in immuno-compromised tumor-bearing mice. We found that Foxp3 is expressed in human and murine breast tumor cells, in which P60 exerted a direct antitumor effect by reducing cell viability and proliferation.

P60 also inhibited IL-10 secretion in breast cancer cells that expressed Foxp3. Our findings suggest that Foxp3 blockade improves the therapeutic efficacy of DC vaccines by inhibition of Tregs and through a direct antitumor effect. This strategy could prove useful to neutralize the immunosuppressive microenvironment and to boost antitumor immunity in breast cancer.

CONTROL OF INFLAMMATION - DRIVEN LIVER TUMORIGENESIS BY ADAPTIVE IMMUNITY: A TUG OF WAR BETWEEN IMMUNOSURVEILLANCE AND IMMUNOSUPPRESSION

MICHAEL KARIN

(University of California at San Diego, San Diego, California, USA)

Chronic hepatitis caused by hypernutrition (NASH) or alcohol consumption (ASH) increases the risk of hepatocellular carcinoma (HCC), the major form of primary liver cancer and the second leading cause of cancer related death. By investigating clinical specimens a highly appropriate mouse model of NASH in which HCC development depends on ongoing oxidative stress we found that chronic liver inflammation and fibrosis results in accumulation of IgA^+ immunosuppressive plasmocytes (ISP) that express the immunoregulatory molecules PD-L1 and IL-10. By engaging PD-1, ISP induce the exhaustion of liver infiltrating $CD8^+$ T cells, some of which recognize tumor specific antigens that are expressed by HCC progenitor cells (HcPC). This allows the growth of HcPC into established HCC, a malignant progression that is even further accelerated by total $CD8^+$ T cell ablation. Conversely, genetic and pharmacological interventions that reactivate exhausted $CD8^+$ T cells and unleash their cytotoxic activity result in the regression of established HCC. These findings establish the importance of immunosurveillance in preventing liver cancer and the contribution of a specific immunosuppressive mechanism in alcohol and obesity induced HCC.

SIMPOSIA XVI
BIOPHYSICAL INSIGHTS INTO PROTEIN STRUCTURE, FUNCTION AND DYNAMICS
Chairs: Monica Montes and Pablo Tomatis

THE NICOTINIC ACETYLCHOLINE RECEPTOR AND ITS SURROUNDING LIPIDS: A LONG-STANDING RELATIONSHIP

SILVIA ANTOLLINI

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The muscle nicotinic acetylcholine receptor (AChR) is one of the key players of the post-synaptic components in neuromuscular junction. It is an integral membrane protein that belongs to the Cys-loop superfamily of ligand-gated ion channels and is composed of four subunits in a pentameric arrangement ($\alpha_2\beta\gamma\delta$ and $\alpha_2\beta\varepsilon\delta$ in embryonic and adult muscle of vertebrates, respectively). Each subunit has a large N-terminal extracellular domain, four transmembrane segments (M1-M4), a small cytoplasmic domain between M3 and M4, and a short C-terminal extracellular domain. Summing up, the AChR has two well defined structural domains: the neurotransmitter-binding site extracellular domain and the transmembrane domain containing the ion pore. Whereas the extracellular domain is the location site of agonists or different activators/inhibitors, the transmembrane region exhibits extensive contact with the surrounding lipids through structural motifs remarkably conserved along phylogenic evolution. It is known that a correct allosteric coupling between both domains is crucial for AChR function, which is strongly dependent on lipid surrounding. We have previously demonstrated that exogenous hydrophobic molecules, such as free fatty acids or steroids, disturb this coupling through the lipid-AChR interfase. It is also known that the AChR is present in high-density clusters at the top of folds in the muscle cell membrane, and that these clusters localize in heterogeneous membrane domains highly enriched in cholesterol (Chol) and sphingolipids. We studied the influence of different lipid host compositions on the distribution of purified AChR reconstituted in membrane containing Lo domains by fluorescence resonance energy transfer efficiency between the AChR intrinsic fluorescence and Laurdan or dehydroergosterol fluorescence, and by analyzing the distribution of AChR in detergent-resistant and detergent-soluble fractions (1% Triton X-100, 4°C). When the AChR was reconstituted in a brain sphingomyelin (bSM), Chol and POPC (1:1:1) model system it lacked preference for Lo domains. However, the change of bSM by 16:0-SM or 18:0-SM resulted in the preferential partitioning of AChR in Lo domains, which was not the case with 24:1 SM. Although all these SM formed Lo domains, differences in size, amount and/or lipid order of each Lo domain were observed, showing a direct correlation with the tendency of the AChR to localize in such domains. We further studied another membrane condition resulting from inducing transbilayer asymmetry. Enrichment in bSM in the external hemilayer resulted in an increase of the amount of external domains with a higher lipid order and a marked increase of AChR in these Lo domains. Other asymmet-

ric conditions were also studied. Thus, a change in the properties/size/location of Lo domains impacts on the AChR preference for this fraction, clearly indicating that membrane lipid surrounding influences both the coupling between agonist-binding and channel-gating domains and the spatial localization of the AChR in the membrane.

A METASTABLE H-BOND ZIPPER TRANSMIT INFORMATION ACROSS THE CELL MEMBRANE

LARISA CYBULSKI

(Instituto de Biología Molecular y Celular, Universidad Nacional de Rosario, Rosario, Argentina)

Transmission of information across cell membranes is an essential activity for every cell. Bacterial thermosensor DesK is a histidine-kinase endowed with a five-span transmembrane domain that detects changes in environmental temperature and controls the phosphorylation state of its cytosolic catalytic domain. The challenge for most systems is to understand the mechanistic basis of signal transmission across the cell membrane, in order to either inhibit or potentiate signaling. To address the challenge, we constructed a sensor with a single chimerical transmembrane segment that retained full sensing capability. Genetic and biophysical dissection of this minimized DesK version enabled identification of three structural determinants of thermo-detection (DOTs). Here we combine and retune these DOTs to understand their interplay, dominance and over-writing effects according to a functional hierarchy. We found that a trans-membrane zipper represents the master DOT as it forms environmentally-controlled intermolecular hydrogen bonds driving a reversible dimerization of the transmembrane domain. Changing the pattern of dimer-promoting bonds allowed us to control signal sensitivity and even promote inverse regulation. These findings enabled the reverse engineering of a synthetic sensor built upon a poly-valine scaffold to which DOTs were incorporated. Our construct retains regulated thermosensing capacity. This modular switch may be incorporated in genetic circuits to control spatio-temporal gene expression.

STRUCTURE, FUNCTION AND FRUSTRATION IN PROTEINS: A CONFLICTING ORIGAMI

DIEGO FERREIRO

(Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina)

Natural protein molecules fold, move and function according to the information encoded in their energy landscapes. For most architectures, this information is still difficult to deconvolute from the linear sequence of amino acids, as the energy contributions are small, numerous and distant. Since natural proteins are a distinct set of apparently random amino acid polymers, evolution provides the basis for the loop closure of informational flow in coded polymers. Different 'teleonomic goals' converge in the evolution of protein sequences, inevitably conflicting with each other. I will present and discuss the application of statistical analysis of structural and genomic data to extract physically meaningful information about natural proteins' physiology.

CONFORMATIONAL DIVERSITY ANALYSIS REVEALS THREE FUNCTIONAL MECHANISMS IN PROTEINS

GUSTAVO PARISI

(Universidad Nacional de Quilmes, Bernal, Buenos Aires, Argentina)

Protein motions are a key feature to understand biological function. Recently, a large-scale analysis of protein conformational diversity showed a positively skewed distribution with a peak at 0.5 Å C-alpha root-mean-square-deviation (RMSD). To understand this distribution in terms of structure-function relationships, we studied a well curated and large dataset of ~5,000 proteins with experimentally determined conformational diversity. We searched for global behaviour patterns studying how structure-based features change among the available conformer population for each protein. This procedure allowed us to describe the RMSD distribution in terms of three main protein classes sharing given properties. The largest of these protein subsets (~60%), which we call rigid (average RMSD = 0.83 Å), has no disordered regions, shows low conformational diversity, the largest tunnels and smaller and buried cavities. The two additional subsets contain disordered regions, but with differential sequence composition and behaviour. Partially disordered proteins have on average 67% of their conformers with disordered regions, average RMSD = 1.1 Å, the highest number of hinges and the longest disordered regions. In contrast, malleable proteins have on average only 25% of disordered conformers and average RMSD = 1.3 Å, flexible cavities affected in size by the presence of disordered regions and show the highest diversity of cognate ligands. Proteins in each set are mostly non-homologous to each other, share no given fold class, nor functional similarity but do share features derived from their conformer population. These shared features could represent conformational mechanisms related with biological functions.

SIMPOSIA XVII
MYSTERIES OF REPRODUCTION II
Chairs: Fernanda Parborell and Maria Susana Theas

SEX UNDER THE WATER: MYSTERIES HIDDEN BY CRUSTACEAN DECAPODS

LAURA LÓPEZ GRECO
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Crustaceans represent the major group of aquatic arthropods with more than 67000 species. Decapods, commonly known as shrimps, prawns, lobsters, crayfishes and crabs (among others) comprise over 15000 species, 90% of them being marine species. The environment where the group has diversified has a profound effect in many biological aspects, including reproduction and development, moreover, the conquest of brackish, freshwater and even terrestrial habitats has modeled brooding, larvae/juveniles development and mating behavior, as much as the diversity of signals involved. In this context it is not easy to trace general statements about decapod´s reproduction, although some ideas can be pointed out. Mostly, decapods have *primary* maternal care implicated in brooding until larvae/juvenile hatching, and certain freshwater species (mainly freshwater crayfish) extend this care up to one or two post-hatching stages, known as *secondary* maternal care. The brooding is associated with modified female appendages (pleopodos) that carry the brood. In caridean shrimps there is a special molt previous to mating, *to prepare* these appendages to carry on the embryos. This is called the *nuptial molt*; whereas another particular molt, after embryos hatched females, is named *postparturial* molt. Decapods have also very unusual spermatozoa: non motile spermatozoa, and neither condensed chromatin with huge sperm cells morphotypes grouped under the terms of reptantia *versus* natantia spermatozoa, actually considered a relevant tool for taxonomy. Spermatozoa are packaged with vasa deferentia secretions forming the spermatophore/s. The form, number, time, and mechanisms of dehiscence are quite different among species, and profoundly affected by the time the spermatophore is attached to the female as well as the position in the female (internal or external to the female body). The number of packaged spermatozoa is likewise related to the number of female mature oocytes, along with planktotrophic *versus* lecithotrophic larvae. The number and size of hatched larvae (indirect development), or juveniles (direct development) is also linked with the habitat. In many decapods species, females have special morpho-functional structures for sperm storage, the seminal receptacles. They temporarily separate mating from conception, so sperm storage can be adaptive in ecologically diverse habitats, and affect life histories, mating systems, cryptic female choice, sperm competition, sexual conflict, not forgetting that it provides increased opportunity for postcopulatory sexual selection. During mating, chemical and physical signals are involved either in the rather “simple” mating systems (as many caridean species), or in the more “complex” mating systems (as many true crabs). In the more complex systems, courtship can be extended and sophisticated, spending much of the time in pre and postmating activities. This leads to a high cost of mating in some species that could be even greater than gamete production. Mating could be similarly related to somatic growth, since in many species females are recently molted at mating, while males are always in intermolt, in order to favor sperm transfer by the specialized appendages, the gonopods. Gonopods are very diverse too depending on the mode of sperm transfer. These are some of the mysteries hidden by crustacean decapods under the water, and scarcely studied.

THE MATING SYSTEM OF THE GREATER RHEA: ROLE CHANGE AND PROMISCUITY

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The Greater Rhea (Rhea americana) is a near threatened ratite whose wild populations are decreasing and exhibit low polymorphism levels. The mating system of this flightless bird is complex, simultaneous polygyny and serial polyandry, and very infrequent among vertebrate species, in which several females copulate with different males and lay their eggs in communal nests. The male constructs the nest, fully incubate the eggs and care for the precocial chicks. Here we evaluated the degree of relatedness among and within broods, with the aim of enlightening the reproductive system of rheas from a genetic point of view. We sampled feathers from 145 nestlings and three males that incubated in 5 nests in the wild. The genotype was determined by means of eight microsatellites developed for rheas, while relatedness was analyzed with software Colony. The study revealed that there were multiple progenitors per nest (14,8 females and 11,2 males). On average, a given female laid in 1,9 nests and produced 3,7 nestlings, while each male had descendants in 1,75 nests and produced 4,5 chicks. Two males were the major two male progenitors in the clutch they incubated (28,6% and 34,5% of the chicks were of

their own), whereas the third analyzed male had no descendants, neither in his nest nor in the other ones. Each male successfully mated 3,4 females (range: 1-8), while each female had 2,8 partners (range: 1-5). Most adults (81%) that share descendants in a nest were unrelated, and females were always less related than males. Although most full-sibs occurred within the same nest, both full and half-siblings corresponded to less than 5,5% of all paired comparisons among all chicks (complementary, 94,5% were unrelated). Our study shows that: (i) there are several progenitors of descendants in each nest; (ii) no male that incubate is the progenitor of the majority of chicks in a nest; (iii) not all incubating males leave progeny; and (iv) females do not constitute cohesive and stable groups that lay eggs in the same successively nests. This promiscuous strategy could be preventing further losses of genetic variability, counterbalancing detrimental effects that an impoverished genetic pool can exert on the viability of populations.

PREGNANCY OR LUTEOLYSIS IN RUMINANTS. EMPHASIS IN NUTRITION

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The relationship between nutrition and reproduction in ruminant females has been the center of intense research. For decades, studies on the effects of undernutrition on reproductive failure have focused on central (hypothalamus-hypophysis), ovary and embryo effects. Less information has been produced about the peripheral response of the reproductive tract when nutrition is below the requirements for maintenance (e.g. situations of negative energy balance, NEB). This condition is frequent in sheep and livestock on extensive management where the pasture offer varies according to climatic fluctuations. In dairy cows, this state of undernutrition is physiologic as genetic selection towards great production volumes produces a more or less deep NEB during the peripartum. There is consensus among the international scientific community in that, even if fertilization could be affected, early embryo mortality is the main cause of low pregnancy rates in cyclic females. This embryo loss is attributed to an asynchrony between the embryo and its signals, and the uterus; a communication needed to prevent luteolysis, thus maintaining the progesterone circulating levels needed to sustain pregnancy. In this work, we present recently acquired knowledge about how nutrition affects uterine function during the early and late luteal phase, in cyclic or pregnant females, from experiments with sheep, and beef and dairy cows. It demonstrates how undernutrition to half the requirements for maintenance in sheep changes the expression level of candidate genes and proteins associated to uterine functionality and embryo growth. For instance, undernutrition reduces uterine sensitivity to progesterone (progesterone receptors), which could explain the higher rates of embryo mortality observed in undernourished animals. In the same experimental model, massive analysis of endometrial transcriptome allowed us to understand that the metabolic adaptations of the uterus to undernutrition depend on the presence of an embryo; data suggest that this tissue is able of partitioning the scarce nutrients in order to sustain an embryo. In beef cows, we have observed that nutrition in very early stages of life affects uterine function in adult females at the moment of service. In dairy cows, using candidate gene selection or RNAseq, we have demonstrated solid evidence about the impact of nutritional management during early lactation on the uterine environment at the end of the voluntary waiting period. For instance, we have found a greater expression of genes related to embryo growth and uterine function (IGF system, progesterone and adiponectin receptors) in cows fed with diets rich in energy compared to those that suffered a deep NEB in the early postpartum. Our results show that animals with worse energy balance have a uterine environment unsuitable for embryo growth, and that these mechanisms are in the base of the lower pregnancy rates.

SUCH A PRETTY TAIL…STUDYING THE SPERM FLAGELLUM BY HIGH RESOLUTION MICROSCOPY.

Dr Mariano G Buffone

Sperm acquire the ability to fertilize in the female genital tract in a process called capacitation.. During capacitation sperm undergo a change in the motility pattern called hyperactivation (HA) and acquire the ability to underog acrosomal exocytosis (AR) which are critical to fertilization.

Ca^{2+} is the primary second messenger that triggers HA motility and it depends on CatSper channels. It has been described that CatSper1 proteins form a unique pattern of four linear ‘‘stripes’’ running down the principal piece of the flagellum. Catsper Ca^{2+} domains orchestrate the timing and extent of complex phosphorylation cascades because it colocalizes with Ca^{2+} signaling molecules.

On the other hand AR is controlled by several players and one of them seems to be the actin cytoskeleton. Most studies to evaluate actin polymerization (formation of filamentous actin or F-actin) in sperm were performed using phalloidins, which are toxic and not capable of crossing the cell plasma membrane.

We have used the powerful capabilities of super-resolution microscopy to reveal the location and regulation of signaling molecules in the sperm tail and for the first time,

how are the dynamic changes in of the actin cytoskeleton at the onset of the AR. Our work also emphasizes the utility of live-cell nanoscopy for the study of sperm ultra-structures, where these imaging capabilities will undoubtedly impact the search for mechanisms that underlie basic sperm functions.

SIMPOSIA XVIII
MYOCARDIUM FROM SELF PROTECTION TO REGENERATION
Chairs. Elena Lascano and Celeste Villa Abrillet

FROM A PLURIPOTENT STEM CELL TO A CARDIOMYOCYTE: MICRORNAS AND THEIR ISOMIRS REGULATION

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Cell reprogramming now easily allows to de-differentiate an adult cell in to a pluripotent stem cell (PSC). These cells are then differentiated to any sort of adult cells, including cardiomyocytes (CM). These cells presents many similar features to adult CM, including spontaneous electrical beating and contractility, although protein analyses demonstrated that these cells are mostly fetal. In vitro, it is necessary to recapitulate the major steps during embryo differentiation to obtains CM from PSC, including the differentiation into mesoderm progenitor cells (MPC) through epithelial to mesenchymal transition. Different regulatory processes are involved in this differentiation, including microRNAs as one of the most important. They are short sequences of RNA of approximately 20-22 bases, including a region of 6 to 8 bases (the “seed”) in the 5’ extreme, which is able to recognize complementary sites in the 3’UTR site of mRNAs. By these recognitions, the coupled microRNA-mRNA is loaded into the RISC complex by Argonaute, which ether cleaves the mRNA or induce its degradation without translation. The key feature in this regulation is that any microRNA is able to recognize different mRNAs, and in the oposite, any mRNA may be bound by different microRNAs, and hence, the expansive network is incredible deep. Our lab works on the identification of the microRNAs during cardiac differentiation from pluripotent stem cells. We performed a small-RNA sequencing analysis of the different stages and identified those microRNAs involved in this process. We can identify two additional complexing factors: first, there are numerous isoforms (called “isomirs”) which potentially increase and expand the ability of gene regulation. Second, microRNAs can be aggregated according to sequence (“families”) or according to genome position (“clusters”). The expression pattern of microRNAs during cardiac differentiation closely follows this aggregation. Our work currently focus on the regulation of large number of microRNAs at the same time, either by using RNA sponges or by modulating cluster expression by CRISPR. Our work then contributes to the knowledge of cardiac differentiation. Fine-tuning this process is a key step in cardiac regenerative medicine

REMOTE ISCHEMIC PRECONDITIONING: PRESENT KNOWLEDGE, UNANSWERED QUESTIONS AND FUTURE PERSPECTIVES

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Ischemic heart disease is the leading cause of death worldwide. For this reason, there has been a continued search for better therapeutic strategies that would reduce myocardial ischemia/reperfusion injury. Remote ischemic preconditioning (rIPC) was first introduced in 1993 by Przyklenk et al who reported that brief regional occlusion-reperfusion episodes in one vascular bed of the heart render protection to remote myocardial tissue. Subsequently, different studies have shown that rIPC applied to the kidney, liver, mesentery artery, and skeletal muscle, all exhibited cardioprotective effects.

However, the pathway and the intracellular mechanism underlying rIPC are unknown. Thus, it has been hypothesized that communication between the signals triggered in the remote organs and protection in the target organ may be mediated through humoral factors released from the preconditioned organ and transported via the circulation (humoral pathways), by neural pathways and/or via systemic anti-inflammatory and antiapoptotic response to short ischemic bouts.

Elucidation of the mechanisms of endogenous cardioprotection triggered in the remote organ could lead to the development of diverse pharmacological rIPC mimetics. In this sense, we have shown that rIPC reduces the infarct size through a vagal pathway and a mechanism involving phosphorylation of Akt and subsequently eNOS, followed by mK^{+}_{ATP} channel opening and an increase in H_2O_2 production by the mitochondria.

In the clinical setting, rIPChas been evaluated in pediatric patients undergoing cardiac surgery and in adult patients undergoing elective abdominal aortic aneurysm repair, coronary angioplasty and coronary artery bypass

surgery. Most (but, importantly, not all) of these studies have reported attenuation of the ischemia/reperfusion injury. Despite the compelling evidence of infarct size reduction with rIPC in experimental models, the obvious appeal of exploiting this cost-effective and seemingly simple strategy to improve outcome in patients, and the promising results obtained in initial clinical studies, our knowledge of the fundamental ‘‘biology’’ of rIPC and its variants is limited. Four basic aspects of rIPC have, to date, not been elucidated. First, the choice of rIPC algorithms has been empiric: no insights have been obtained regarding the optimal remote stimulus in terms of the number of cycles of brief ischemia/reperfusion or the duration of each episode. Second, while the use of limb ischemia as the protective trigger (rather than renal or mesenteric ischemia) is undoubtedly the most practical option in terms of clinical application, there is no information as to whether the outcome is superior with arm versus leg ischemia.

rIPC and its variants hold the promise of providing a potent yet cost-effective clinical strategyt o attenuate myocardial ischemia/reperfusion injury and improve patient outcome. However, in order to achieve this potential, focused and rationally designed experiments conducted in clinically relevant models are required to (a) elucidate the ‘‘biology’’ and molecular mechanisms of rIPC, and, as a consequence (b) develop optimal rIPC algorithms for therapeutic use. A final, technical priority in facilitating the clinical application of rIPC will be the development ofc ommercial devices for the standardized administration of these optimized rIPC algorithms.

EXERCISE-INDUCED CARDIOPROTECTION: ITS PROTAGONISTS

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Exercise training is a consolidated approach to reduce cardiovascular morbidity and mortality. Cardiac adaptation to endurance training includes cardiomyocyte hypertrophy and improved contractility by a non-yet clarified mechanism, as the most important events. This response is mediated by the production and release of several growth factors and other humoral mediators among which, the insulin-like growth factor 1 (IGF-1) seems to be the most relevant. IGF-1 increases in response to exercise training both in experimental animal models and in athletes compared with healthy sedentary controls. Furthermore, IGF-1 has been demonstrated to be produced in cardiovascular tissue, where it likely exerts autocrine/paracrine effects. Interestingly, we have reported that exercise training is effective to convert pathological into physiological hypertrophy in the spontaneously hypertensive rats (SHR). In the pathophysiology of this consolidated experimental model of pathologic cardiac hypertrophy the hyperactivity of the myocardial Na+ /H+ exchanger-1 (NHE-1) is critical by increasing intracellular Na+ and Ca2+ concentration, hence facilitating calcineurin activation. Moreover, the hyperactivity of the NHE-1 has been extensively described as a central component of many cardiac pathologies, playing a key role in hypertrophy development and progression to heart failure. In the conversion of pathologic to physiologic cardiac hypertrophy of the SHR through exercise training/IGF-1stimulation the prevention of NHE-1 hyperactivity through an AKTdependent inhibitory phosphorylation of the cytosolic tail of the exchanger, as well as the positive inotropic and antioxidant effects, emerge as key players. With respect to the IGF- 1/exercise training enhancement in cardiac contractility we have substantial data supporting that NO-dependent CaMKII activation plays a critical role, mainly due to phospholamban phosphorylation that results in increased Ca2+ transient amplitude, rate of decay and SR content. Interestingly, this pathway (namely NO-CaMKII) seems not to contribute to the adaptive hypertrophy

ADULT CARDIOMYOCYTE CELL CYCLE MANIPULATION AS CARDIO-REGENERATIVE STRATEGY

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Ischemic heart disease is the main cause of death worldwide. Cardiac ischemia results from reduced oxygen flow and nutrients to the cardiac cells. If ischemia is prolonged or intense, infarction occurs and cells die. As the rate of endogenous regeneration is slow and poorly significant, dead cells are progressively replaced by non-contractile fibrotic tissue, ultimately reducing cardiac function and leading to heart failure.The most recently released post-infarction pharmacological treatments target the neurohormonal physiology of the remaining cardiomyocytes (CMs) without intention to replenish the lost ones. So is that novel approaches for replenishing cardiomyocyteslost during myocardial infarction are in need.

An established paradigm in cardiovascular regenerative medicine is that adultmammaliancardiomyocytes have limited proliferative capacity. However, despite the low turnover rate, with most myocytes having their cell cycle blocked, they continue to increase in size and DNA content, both processes requiring cell cycle activity but not leading to cytokinesis.

To be possible for the heart to work as a sincitium, it is of vital importance that either gene or cell experimental therapies warrant newly incorporated cells' engraftment. So it is thatthere is a growing interest in identifying factors playing relevant roles in the regulation of the cell cycle,to be later used for the induction of therapeuticventricular-cardiomyocyte mitotic division.
One of the current approaches consists in the removal of the molecular brake that prevents the adult cardiomyocyte to overcome de G2/M checkpoint of the cell cycle. So far, the *meis1* gene has been identified in mice as a transcription factor required for the transcriptional activation of the inhibitor proteinsp15, p16 and p21, which inhibit cyclin-CDK complexes inducing cell cycle arrest. But more studies are needed both to confirm that this gene has a similar role in large mammals including human beings, and to find new cell cycle-regulating molecules that can be used as targets for gene therapies aimed at promoting adult cardiomyocyte cell division.
Despite the scientific progress achieved during the last decades, the goal of regenerating a damaged heart, still seems far away. While new approaches have emerged, such as the use of stem cells of different sources, bioresorbable scaffolds to improve cell retention, decellularized matrices, and gene therapies with different targets and vectors, many questions remain to be answered, including how cardiomyocyte proliferation is regulated during heart development, to reveal unknown cell cycle regulation mechanisms and regulating molecules, that can be reproduced in order to achieve myocardial regeneration. Only by unraveling the complexity of the cell cycle processes we will approach the chance of partially regenerating the human heart.

SIMPOSIA XVIII
ENDOCRINE SOCIETY JOINT SYMPOSIUM: INTEGRATING GENOMIC AND NONGENOMIC ACTION OF NUCLEAR RECEPTOR HORMONES IN PHYSIOLOGY AND PATHOLOGY
Chairs: Patricia Elizalde and Claudia Pellizas

EXTRANUCLEAR EFFECTS OF THYROID HORMONES AND ANALOGS AT THE PLASMA MEMBRANE LEVEL

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Extranuclear or nongenomic effects of thyroid hormones are widely recognized, beside the classical effects mediated by nuclear receptors, suggesting the existence of different mechanisms involving plasma membrane, mitochondria, or cytoskeleton. Thyroid hormones and immune system cross-talk, although the signaling pathways of this interaction have not been defined yet. A complex relationship exists between thyroid hormone and immune system cells, and in cases of hypo- or hyperthyroidism the immune response is usually altered. Extra-nuclear effects of thyroid hormones are mainly mediated by integrin αvβ3, although other receptors also are able to elicit fast responses. THP-1 monocytes show high expression of integrin αvβ3, and are therefore a good model to study the short-term effects of thyroid hormones. Typical responses of THP-1 monocytes modulated by thyroid hormones, such as cell migration, have been studied using two different chemoattractants: Insulin-like growth factor-1 (IGF-1) and monocyte chemoattractant protein (MCP-1) a potent chemokine. Our data show that thyroid hormones, T3 and T4, in the physiological concentration range are able to inhibit the migration induced by IGF-1 and MCP-1, but the mechanisms involved appear to be different. In case of IGF-1 the inhibition by thyroid hormone depends on integrin αvβ3, and the effects are inhibited by RGD peptide, by an antibody against integrin αvβ3, and by the thyroid hormone metabolite tetrac, which also has interesting antitumor properties. In case of MCP-1-activated migration the signaling pathway elicited by thyroid hormone appears to be more complex, involving also the modulation of the redox state of the cell.

CLINICAL SYNDROMES OF GLUCOCORTICOID HYPERSENSITIVITY: GLUCOCORTICOID BETA MUTATIONS AND BEYOND

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Steroid hormone action reflects the integrated effects of production and clearance rates, bioavailable plasma concentrations and receptor-mediated target tissue responsiveness. Alterations of receptor-level concentrations or biologically relevant mutations can result in relative tissue resistance or hypersensitivity. A variety of clinical syndromes are associated with glucocorticoid resistance which are caused by receptor mutations which inactivate their function. Less frequent are activating mutations which enhance sensitivity to glucocorticoids. Several mutations of the glucocorticoid receptor, including N363S, the *BCL*I restriction fragment polymorphism,

and ER22/23EK, are reported to cause hypersensitivity. Predominant clinical findings in these patients have included obesity/metabolic syndrome, hypertension, altered bone mineral density, vasoconstriction, inflammatory bowel disease, and depression.

Complex receptor systems have been described in which one receptor isoform exerts agonistic actions and another antagonistic. Under these circumstances, inactivating mutations of the antagonistic isoform would result in hypersensitivity. Specifically, with respect to the glucocorticoid receptor, Gα mediates agonistic actions and GRβ a dominant negative isoform, partially antagonizes the effects of GRα. An inactivating mutation of GRβ might then be expected to cause hypersensitivity to the action of glucocorticoids.

We have studied a patient with a compound mutation of glucocorticoid receptor β (hGR βG3134T and hGR βA3669G) with clinical findings associated with hypersensitivity. These included low morning cortisol levels over a six week period (<0.001 vs normal), an enhanced suppression of cortisol in response to dexamethasone, and development of Cushingoid features on replacement hydrocortisone. A mass spectrometry -based assay excluded surreptitious use of an exogenous glucocorticoid. Even though the cortisol levels were low, no signs of adrenal insufficiency were present and potent stimulation tests demonstrated the ability to secrete normal amounts of hydrocortisone. A series of molecular studies in transfected osteosarcoma cells (U-2 OS) demonstrated normal levels of α and β glucocorticoid receptor levels and exaggerated responses of glucocorticoid responsive genes such as GILZ. Comparison of gene expression profiles revealed that the mutant cell line had an altered profile after hydrocortisone stimulation. Studies in the patient's cells are ongoing with preliminary data supporting our findings.

In this presentation, we will review the studies of the glucocorticoid α activating mutations and their clinical manifestations and the studies in our prismatic case of glucocorticoid hypersensitivity due to compound GR β mutations. Taken together, these findings illustrate the complexity of glucocorticoid regulation and diverse clinical manifestations.

CELL TYPE SPECIFIC ACTIONS OF GLUCOCORTICOIDS *IN VIVO*: LESSONS FROM GENETICALLY MODIFIED ANIMALS

JOHN CIDLOWSKI

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Heart failure is one of the leading causes of death in the Western world, and stress is increasingly associated with adverse cardiac outcomes. Glucocorticoids are primary stress hormones that regulate homeostasis through two closely related nuclear receptors, the glucocorticoid receptor (GR) and mineralocorticoid receptor (MR). Cardiomyocytes express both receptors but little is known concerning their coordinated actions in heart physiology and pathology. Here, we show that patients with failing hearts display a disparity in the relative levels of GR and MR compared to non-failing donor hearts. To examine the *in vivo* function of glucocorticoid signaling in the heart, we generated mice with cardiomyocyte-specific deletion of GR (cardioGRKO), MR (cardioMRKO), or both GR and MR (cardioGRMRdKO). The cardioMRKO mice exhibited normal heart function whereas the cardioGRKO mice spontaneously developed cardiac hypertrophy and left ventricular systolic dysfunction. Surprisingly, the cardioGRMRdKO mice were protected from cardiac disease, despite having pathological gene changes that were present in the GR deficient hearts. Genome-wide microarray analysis identified a set of cardioprotective gene changes that occurred uniquely in the double knockout hearts. Re-installation of MR into the cardioGRMRdKO hearts by adeno-associated virus gene transfer completely reversed the cardioprotective gene changes and resulted in cardiac dysfunction. These findings reveal not only a deleterious role for cardiac MR signaling when unopposed by GR but also the molecular targets of MR that contribute to cardiac pathology. Therapies that shift the balance of cardiomyocyte glucocorticoid signaling to favor more GR and less MR activity may provide an improved approach for treating heart disease.

PROGESTERONE RECEPTOR AS A TRANSCRIPTIONAL REPRESSOR REGULATES BREAST CANCER GROWTH

CECILIA PROIETTI

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Progesterone receptor (PR) is a critical mediator of mammary gland development and contributes to breast cancer progression. In its classical mechanism of action PR associates with specific progesterone response elements (PREs) on chromatin. DNA-bound PR recruits transcriptional coactivators and associated cofactors, which modify the local chromatin structure and facilitate transcriptional activation, resulting in activation or repression of PR target genes. PR may also alter gene expression nonclassically, where the receptor tethers to other transcription factors bound to DNA. In addition to its direct transcriptional effects, PR activates signal

transduction pathways in breast cancer cells through a rapid or nongenomic mechanism. PR transcriptional outcome is affected by complex interactions between PR and other regulatory factors which results in different PR cistromes. Till present more attention and study has been devoted to gene activation compared to gene repression mediated by hormones, in particular, progestins. In fact, gene repression is poorly understood, with some authors even considering repression as a secondary effect of activation. In the present study, we addressed a poorly explored PR function: its capacity to repress gene expression.

In this study, we demonstrate that progestin-activated progesterone receptor reduces the expression of the master transcription factor GATA3. GATA3 is involved in mammary gland development and is crucial for the maintenance of the differentiated status of luminal epithelial cells. The role of GATA3 in breast cancer as a tumor suppressor has been established, although insights into the mechanism of GATA3 expression loss are still required.

In the present work, we show that ligand-activated PR modulates GATA3 expression through regulation at the transcriptional and post-translational levels in breast cancer cells. In the former mechanism, the histone methyltransferase enhancer of zeste homolog 2 is co-recruited with activated PR to a putative progesterone response element in the GATA3 proximal promoter, increasing H3K27me3 levels and inducing chromatin compaction, resulting in decreased GATA3 mRNA levels. This transcriptional regulation is coupled with increased GATA3 protein turnover through progestin-induced GATA3 phosphorylation at serine 308 followed by 26S proteasome-mediated degradation. Both molecular mechanisms converge to accomplish decreased GATA3 expression levels in breast cancer cells upon PR activation. In addition, we demonstrate that decreased GATA3 levels are required for progestin-induced upregulation of cyclin A2, which mediates the G1 to S phase transition of the cell cycle and was reported to be associated with poor prognosis in breast cancer. Finally, we show that downregulation of GATA3 is required for progestin stimulation of both *in vitro* cell proliferation and *in vivo* tumor growth.

SIMPOSIA XIX
INFECTION AND IMMUNITY
Chairs: Maria Silvia Di Genaro and Marisa Fernandez

RESIDENT MEMORY T CELLS

DAVID MASOPUST
(Center for Immunology, University of Minnesota, Minneapolis, Minnesota, USA)

Memory CD8 T cells protect against viral pathogens by scanning host cell surfaces, thus infection detection rates depend on memory cell number, migration, and distribution. This talk will discuss several issues related to T cell immunosurveillance efficiency including quantitative analysis of memory T cell subsets, prime boost vaccinations, evaluation of T cell migration, and in vivo imaging of virus specific CD8 T cell mediated immunosurveillance and reactivation in mucosal tissues. The talk will emphasize antiviral resident memory T cells, and will present ongoing investigations into the ontogeny and function of this lineage.

THE ART OF CO-EXISTING: IMMUNE ADAPTATIONS DURING CHRONIC INFECTIONS

ELINA ZÚÑIGA
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During chronic infections, long-term cell adaptation via functional exhaustion has been mostly studied in T cells and B cells but much less is known on how (typically short-lived) innate cells adjust to a persistently infectious milieu. To address this question we studied Plasmacytoid Dendritic Cells (pDCs), which specialize in Type I Interferon (IFN-I) production but often become functionally exhausted in persistent infections and tumors. Our work uncovered novel aspects on the basic regulation of IFN-I producing cells and could have implications for therapeutically manipulating innate responses during chronic diseases. Furthermore, the new knowledge gather from these studies provides a framework to understand how the innate immune system adapts to co-exist with a persistently replicating pathogen for the life of the host.

ZIKA VIRUS CONGENITAL SYNDROME IN EXPERIMENTAL MODELS

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Brazil has recently gone through an unprecedented public health crisis due to the Zika virus epidemics. As many other flavivirus it has never been correlated with human morbidity or mortality. Unfortunately it has changed dramatically as the virus is now responsible for more than 2300 babies born with microcephaly. The so-called Zika Congenital Syndrome has, besides microcephaly, many other relevant features, as retinal damage, intra-uterine growth restriction and arthrogryposis. In fact, babies born without microcephaly but with significant neuronal and retinal lesions have also been reported. In this context, the development of an experimental model is of great relevance for the studies on the pathogenesis of microcephaly. We demonstrated that the infection of pregnant SJL mice with ZIKV results in severe damage of the pups. Most prominently was the significant reduction in size and weight associated with high viral titers in the brains. This was accompanied by high level of apoptotic cell death, probably of neuronal precursor cells (NPCs). In order to confirm this we infected NPCs and human brain organoids with ZIKV, and observed a significant reduction on the number of NPCs, corroborating the apoptotic cell death hypothesis. Currently we are focused on several aspects of the brain inflammation during microcephaly. Interestingly, many pro-inflammatory cytokines are down-regulated, as well as viral receptors and signaling transduction molecules. This is consistent with clinical findings, showing no sign of inflammation, at least through cerebrospinal fluid protein and cellular analysis. This may indicate that the virus controls inflammation in situ. The elucidation of such mechanism would greatly contribute for the understanding of the viral biology in the central nervous system and also for the pathogenesis of microcephaly.

GLYCAN- AND PEPTIDE-BASED VACCINES PROTECT AGAINST HEART INFLAMMATION AND PARASITE BURDEN IN THE NONHUMAN PRIMATE MODEL OF CHAGAS DISEASE

IGOR ALMEIDA

(University of Texas, El Paso, Texas, USA)

Chagas disease (ChD) is a devastating neglected tropical illness caused by the protozoan *Trypanosoma cruzi,* currently affecting 6-8 million people. There is no vaccine available to prevent or treat ChD. *T. cruzi* has a plasma membrane coated by highly immunogenic glycosylphosphatidylinositol (GPI)-anchored glycoproteins, such as mucins and mucin-associated surface proteins (MASPs). Mucins contain the immunodominant glycotope, Galα(1,3)Galβ(1,4)GlcNAcα (KM24), which induces high levels of lytic, protective anti-α-Gal antibodies, which control the parasitemia in both acute and chronic phases of the disease. Recently, we showed that Galα3LN covalently coupled to a carrier protein elicited 100% survival upon parasite challenge in the **α**-galactosyltransferase-knockout (αGalT-KO) mouse model, which mimics the human response against αGal-containing epitopes. MASPs encompass overlapping B- and T-cell epitopes in a peptide named MASPpep (DAENPGGEVFNDNKKGLSRV), which was recently revealed by proteomics of trypomastigote extracellular vesicles and immunoinformatics and shown to induce considerable protection against *T. cruzi* challenge. Here, we evaluated the efficacy of KM24 and MASPpep as potential ChD vaccine candidates in a nonhuman primate model. Four groups of *Papio hamadryas* (baboons; 4 animals each), were immunized subcutaneously with KM24 covalently linked to HSA (KM24h), MASPpep covalently linked to KLH (MASPpep□KLH), KM24h+MASPpep-KLH, or PBS (placebo); all containing liposomal-monophosphoryl lipid A (L-MPLA) as an adjuvant. All groups were immunized four times at weeks 0, 6, 12 and 24, and challenged twice with metacyclic trypomastigotes (isolate HC123, TcI, originated from baboons). Blood, serum, and heart samples were collected for analysis of specific antibody titers (by CL-ELISA), parasite burden (by qPCR), serum and heart cytokines (by Multiplex), and heart histopathology, inflammation-related microRNAs, and metabolomics. We observed a very strong antigen-specific antibody response in immunized baboons with either vaccine alone or combined. Additionally, histopathology and parasite load evaluation of vaccinated baboon hearts clearly indicated that both vaccines, alone or combined, elicited significant protection against inflammation and parasite burden in the cardiac tissue, hallmarks of chronic ChD. Analysis of inflammation-related microRNAs, cytokines, and metabolites in cardiac tissues are underway and will be discussed.

SIMPOSIA XX
BIOLOGY OF LIPIDS IN REGULATING CELL PHYSIOLOGY

THE PHASE OF FAT: MECHANISMS AND PHYSIOLOGY OF LIPID STORAGE

TOBIAS WALTHER

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All organisms face fluctuations in the availability and need for metabolic energy. To buffer these fluctuations, cells use neutral lipids, such as triglycerides, as energy stores. We study how lipids are stored as neutral lipids in

cytosolic lipid droplet organelles. Specifically, we investigate the molecular processes that govern the synthesis of energy storage lipids as well as their storage in and mobilization from lipid droplets. In modern societies, individuals often face metabolic energy excess, leading to metabolic diseases. We will present our studies on the causal link between energy excess and metabolic disease.

METABOLIC RHYTHMS PERSIST IN PROLIFERATIVE GLIOBLASTOMA CANCER CELLS WITH AN ALTERED CIRCADIAN TRANSCRIPTIONAL CLOCK

MARIO GUIDO

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Circadian clocks distributed all throughout the body and present in immortalized cell lines temporarily regulate physiological processes, driving molecular rhythms in gene expression through a transcription/translation feedback loop (TTFL); however, metabolic/redox cycles can persist without transcription in a number of different cell types; they are highly conserved through evolution and present in all kingdoms of life. A body of recent evidence suggests now that under physiological situations the cellular clock can be made up of both the TTFL- and the metabolic oscillators working together and interacting to temporally control physiology. The disruption of circadian rhythms as a consequence of modern life (shiftwork, jetlag, etc.) and continuous artificial illumination conditions may lead to metabolic syndrome and higher cancer risk. Since carcinogenesis is a complex process resulting in the deregulation of cell growth and division, here we investigated whether immortalized human glioblastoma cells maintained quiescent or under proliferation keep a functional cellular clock after synchronization and whether proliferating cells display differential time responses to drug treatment. We examined the expression of key components of the canonical molecular clock (*Bmal1, Per1, Rev-erbα),* and of glycerophospholipid (GPL) synthesizing enzyme genes (*choline kinase α: Chokα* and *CTP:phosphoethanolamine cytidylyltransferase 2:Pcyt-2*), as well as redox metabolism, peroxiredoxin oxidation cycles, GPL-endogenous content and metabolic ^{32}P-GPL labeling over time. Cells grown in 10% FBS-DMEM were synchronized with dexamethasone (DEX, 100 nM) for 20 min (time 0), maintained with (proliferative) or without (arrested) FBS-DMEM for 36-48 h and collected at different times for further assays. In arrested cultures *Bmal1*, *Per1, Rev-erbα, Chokα* and *Pcyt-2* mRNAs and the labeling of ^{32}P-GPLs exhibited a circadian rhythmicity after synchronization. Temporal oscillations were also found in the redox state, and peroxiredoxin oxidation cycles. In proliferating T98G cells, the circadian-controlled rhythmicity of gene expression was lost or its periodicity shortened whereas the redox state and GPL metabolism continued to fluctuate with a similar periodicity to that observed under arrest or over a longer period. Moreover, under proliferation the viability of cells significantly changed over time after treatment with the proteasome inhibitor bortezomib (500 nM), a potent chemotherapeutic agent. With this procedure, the highest levels of cell death (70%) are achieved from 12 h up to 24 h after DEX synchronization compared with only ~50% cell death at other times. Results strongly suggest that an intrinsic cellular metabolic oscillator operates in proliferating tumor cells regardless of the transcriptional circadian clock rhythmicity; the persistence of such temporal control of metabolism likely reflects differential tumor cell suitability for a more efficient, time-dependent drug treatment to be applied at those times at which the cellular redox state is high and GPL metabolism is low.

DECIPHERING MYCOBACTERIAL LIPID BIOSYNTHESIS REGULATION

GABRIELA GAGO

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Besides the relevance of lipid derived molecules in *M. tuberculosis* pathogenicity, little is known about the environmental signals and regulatory cascades involved in the global regulation of lipid metabolism in this bacterium and how lipid homeostasis is maintained for survival and during infection. Our work is directed to fulfill some of these gaps by studying in detail the elements and mechanisms that regulate lipid homeostasis in *M. tuberculosis*, how they impact in the biosynthesis and composition of its cell wall and how host-pathogen lipid metabolisms interact. Mycobacteria are unusual in possessing two fatty acid synthase (FAS) systems, the eukaryotic-like FAS I and the prokaryotic-like FAS II. We initiated our research project with the identification and characterization of the components of the transcriptional regulation network that keeps these two systems tightly regulated and found they were essential for survival. We have constructed and analyzed several conditional mutants in the components identified. The results obtained using lipidomic, proteomic and transcriptomic studies helped us to not only comprehend the physiology and physiopathology of this harmful pathogen but also to identify new targets that can be used for the screening of new antimycobacterial compounds.

A SCIENTIFIC JOURNEY FROM THE BRAIN TO THE RETINA LED BY ISOPRENOIDS AND PROTEIN PRENYLATION

ELENA POSSE DE CHAVES

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Autophagy is a process of lysosome-mediated degradation of cytoplasmic cargos. Small GTPases Rabs, the well-known regulators of membrane trafficking, play key roles in autophagy. Rabs anchoring to cell membranes is achieved by prenylation and is essential for activation of their biological function. Prenylation is the post-translational modification of proteins by covalent attachment of the isoprenoids farnesyl-pyrophosphate or geranylgeranyl-pyrophosphate (GGPP).

We found that when Rab prenylation is defective autophagy flux is significantly impaired. I will present data from two experimental settings.

In Alzheimer's disease (AD) autophagy is dysfunctional and reversing autophagy dysfunction in animal models improves the pathophysiology and rescues memory. Yet, the nature and cause(s) of autophagy dysfunction in AD are unclear, which prevents the development of disease-modifying strategies targeting autophagy. We discovered that amyloid beta peptide (oAβ42) inhibits isoprenoids synthesis decreasing protein prenylation in cultured cells and in brains of the AD mouse model TgCRND8. Using the reporter mCherry-GFP-LC3 in neurons treated with Aβ and in vivo, we found that autophagic flux is blocked. We tested the ability of GGPP to normalize autophagic flux and Rab7 function. Rab7 is required for autophagy progression. In Aβ-treated neurons, Rab7 is hypoprenylated and its localization to autophagosomes is reduced. GGPP prevented Aβ-induced autophagy dysfunction and corrected Rab7 prenylation and Rab7 subcellular localization. Thus, our data indicate that autophagy defects in AD are due, at least in part, to inhibition of protein prenylation secondary to decreased isoprenoids synthesis. Restoration of protein prenylation in cultured cells normalizes autophagy and in vivo could improve the pathophysiology of AD.

Several prenylated proteins are required for normal retinal function and if mutated they cause inherited retinal diseases. We tested the effect of protein prenylation inhibition by statins in a cell model of retinal pigmented epithelium (REP). Simvastatin impaired LC3-Associated Phagocytosis a type of selective autophagy that mediates degradation of photoreceptor outer segments. GGPP prevented inhibition of protein prenylation by statins and corrected photoreceptor phagocytosis. We also examined the effect of simvastatin on retina function in vivo in mice subjected to chronic treatments with simvastatin. Retina function was assessed by electroretinogram recording. At the end of the study we performed biochemical (cholesterol mass, RTPCR of hydroxymetylglutarylcoA reductase, protein prenylation) and histological analysis of the neural retina and the RPE. Simvastatin treatment was associated to impaired retinal signal transmission. Moreover, cryosections of simvastatin-treated mice retinas showed bipolar cells sprouting, a previously reported potential compensatory mechanism in response to reduced photoreceptoral pre-synaptic modulation. RPE flat mounts of simvastatin-treated mice showed the presence of larger RPE cells, a feature observed in retinal degenerative diseases upon RPE cell loss. Levels of plasma and retinal cholesterol mass did not differ between treated and untreated mice. Our experiments suggest that simvastatin treatment might have detrimental effects on retinal function. This may constitute a possible side effect common to all statins and could be particularly relevant to patients with pre-existing retinal diseases.

Our studies identify protein prenylation as a pivotal process for normal autophagy function and highlight its importance in several physiological and pathological paradigms.

SIMPOSIA XXI
TRANSLATIONAL NEUROSCIENCE
Chairs: Francisco Barrantes and Flavia Saravia

SYNAPTOPATHIES: PRE-PRODROMIC STAGES OF NEUROPSYCHIATRIC DISEASE?

FRANCISCO BARRANTES

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Classical views on the neuropathology of neurodegenerative diseases such as Alzheimer disease and Parkinson disease have relied on the assumption that the alterations of neuronal cell bodies (intracellular protein accumulation) and the accretion of metabolic extracellular deposits constitute the initial pathological events. Current views, however, suggest that early alterations of the structure and function of brain synapses play a major role in the pre-symptomatic stages of neurological and neuropsychiatric diseases, certainly before major clinical manifestations are made apparent, and probably before the occurrence of neuronal degeneration and death. Dysregulation of synaptic structure and function, encompassed under the term "synaptopathies", may not only be early aspects of Alzheimer and Parkinson diseases, but also include schizophrenia spectrum and autism spec-

trum disorders, addictions, and depression syndromes. Synaptopathies comprise a wide range of phenotypes, from alterations in the number or size or the dendritic spines, dysmorphic or disarranged spine localization along the dendritic arborizations, biochemical alterations, and combinations thereof. I will review the state-of-the-art knowledge on synaptopathies acquired with the help of current research tools, with special emphasis on superresolution imaging techniques.

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STUDY OF NEUROINFLAMMATION TO IDENTIFY NEUROPROTECTIVE TARGETS FOR PARKINSON´S DISEASE

FERNANDO PITOSSI
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Parkinson ´s Disease (PD) is the second most common neurodegenerative disease in the population. One patho-physiological feature consistently found in animal models and PD patients is robust microglial activation. Identifying parameters that could determine a univocal role of microglial activation on neuronal cell death in the substantia nigra (SN), the main region affected in PD, is crucial to define new therapeutic targets against PD and select PD patients to be enrolled in anti-PD trials based on immunomodulation.

We have found that microglial activation in the degenerating SN is “primed”. Microglial cells can be shifted to a pro-inflammatory state by central and also sub toxic levels of systemic inflammation. This shift can dramatically exacerbate on-going neurodegeneration in the SN leading to increased and earlier motor symptoms, via Interleukin-1beta (IL-1) overproduction. In addition, we have observed that sustained but not acute expression of IL-1 or Tumor necrosis factor-alpha (TNF) in the SN leads to dopaminergic neuronal demise, motor symptoms and microglial activation. TNF effects are dose-dependent since, using a combination of knock-in mice, adenoviral vectors and the CRE/lox system we could demonstrate that low levels of TNF can be neuroprotective for nigral neurons, while higher levels could be detrimental.

In conclusion, we have identified parameters that determine a given effect of pro-inflammatory cytokines on neuronal viability and generated several inflammation-mediated models of nigral degeneration and motor symptoms that could be exacerbated systemically. In addition, these models could serve for the identification of inflammation-derived specific molecules with univocal toxic or protective effects could serve as future protective treatments against PD.

DRUG DISCOVERY IN PSYCHIATRY: FROM GENES TO TARGETS, CIRCUITS AND PATIENTS

PATRICIO O´DONNELL
(Harvard Medical School, Harvard University, Boston, Massachusetts, USA)

Despite strong recent advances in our understanding of brain circuits underlying psychiatric disorders, there has been a paucity of novel drugs entering the market. How can we move forward and take advantage of the wealth of human and animal data that can help the field? A reasonable starting point to identify novel targets is human genetics. However, as the genetic contribution to neuropsychiatric disorders is non-Mendelian, this will not be a straightforward effort. Patricio O’Donnell will provide an overview of the strategy adopted at the Pfizer and other companies to identify and validate novel targets for Psychiatry. Specifically, we started with the recent genomewide association studies (GWAS) for schizophrenia, in which 108 loci were identified as conferring risk for the disorder (Rikpe et al, Nature 2014). We established a workflow that allowed identifying the most likely gene driving the association in most of the 108 loci using linkage disequilibrium analysis. Then, we set an iterative process of selecting a short list of genes in which we considered factors such as whether the GWAS loci were in coding regions of the genome, the potential directionality of the effects, the relevance of these genes to known pathophysiological processes, and the link to testable and targetable biological processes (Schubert et al, Neuron 2015). We identified some transcription factors (ZNF804A), Calcium channels (L and T type), cellular signaling (AKT/GSK3beta) and cell transporters (SLC39A8, a zinc transporter). We followed this up with setting up cell lines and mouse models in which these genes were knocked down and tested the impact on circuitry and function using electrophysiology. One example of how this line of research could contribute to novel pharmacological tools is the AKT signaling. As a consequence of the GWAS data, we explored novel dopamine pharmacology that takes advantage of beta-arrestin signaling (which signals through AKT and GSK3beta). A handful of beta arrestin biased compounds were developed and tested in animal models relevant to psychiatric conditions. These agents showed an antipsychotic like profile, along with cognitive benefits. Electrophysiologi-

cal data revealed that biased dopamine pharmacology was beneficial for restoring activity of cortical inhibitory interneurons (Urs, PNAS 2016). Thus, the use of genetics as a starting point for psychiatry drug discovery may open the door to novel targets and a more biologically informed drug discovery process.

TAUOPATHIES: PERSPECTIVES ON AN EMERGING PATHOBIOLOGY

KENNETH KOSIK
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In several neurodegenerative diseases including Alzheimer disease, tau self-assembles into intracellular aggregates of fibrous polymers. The mechanism by which this critical transition from a soluble protein to an insoluble fibrous state occurs is unknown. We have discovered that tau molecules can become compacted into a protein-rich droplet while maintaining their solubility and native-like protein conformations. This dense liquid droplet state is termed a complex coacervate, and it is held together by the opposite charges of their constituents, ions, and water. In the case of the tau protein, the opposite charge comes from RNA. In human neuronal cell culture, tau selectively binds to a category of RNA known as tRNA. Tau and RNA favorably condense under physiologic conditions to a complex coacervate phase. When the tau-RNA–dense droplets are incubated together over time, tau transitions to a conformation similar to that found in pathological fibers. Current directions include the detection of liquid-liquid phase separated states that incorporate tau in living cells. Additional studies have uncovered an upstream tau pathway that can detect mutant tau before aggregates occur. A repurposed drug to prevent tau inclusions in a mouse model can favorably regulate this pathway.

SIMPOSIA XXII
HOST-PARASITE INTERACTION
Chairs: Maria Teresa Tellez-Iñón and Oscar Campetella

CROSSING BORDERS WITHOUT A VISA: HOW DO AFRICAN TRYPANOSOMES COLONISE THE TSE-TSE FLY VECTOR?

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Trypanosoma brucei undergoes a complex life cycle within the tsetse vector, which involves migration and colonization of different fly tissues. Establishment of a trypanosome infection occurs after the parasites reach the ectoperitrophic space (ES), which is in direct contact with the gut epithelium and is separated from the lumen by the 3-layered peritrophic matrix (PM). Although unproven, the most widely accepted hypothesis on how *T. brucei* reaches the ES is by direct crossing of the tsetse PM. However, PM degradation by trypanosomes is unlikely considering that this is a chitin-rich tissue and *T. brucei* lacks chitinase activity. Furthermore, while previous microscopy experiments show trypanosomes confined between PM layers, evidence of degradation from the midgut lumen or towards the ES have so far been elusive. We re-studied this event by a combination of 3D-fluorescent and -electron microscopy. We found no evidence supporting PM degradation by trypanosomes. Instead, we discovered that trypanosomes invade the ES via the proventriculus (PV - point of PM synthesis), a process that also accounts for the formation of “parasite pockets”, which are then carried along the entire length of the gut as the PM gets synthetized. We propose that after the fly takes an infected bloodmeal, trypanosomes first enter the ES via the PV, which is crucial to establish a midgut infection.

FROM METACYCLICS TO RETROMONADS: A NEW STAGE IN *LEISHMANIA* DEVELOPMENT WITHIN THE SAND FLY MIDGUT IMPACTS VECTORIAL CAPACITY

JESUS VALENZUELA
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Leishmania parasites undergo several developmental stages that give rise to infectious metacyclics, forms adapted for transmission to mammalian hosts. To date, metacyclics have been considered as a final terminally differentiated stage in the sand fly midgut. Here, we reveal a hidden developmental cycle that drives continued augmentation of metacyclics within an infected fly. Blood causes *Leishmania* metacyclics to **dedifferentiate** into a replicative leptomonad-like stage within the thoracic anterior midgut in a process we refer to as **reverse metacyclogenesis.** This newly observed phenomenon resets the infection inside the vector significantly increasing the

number and homogeneity of the parasites through additional rounds of multiplication prior to redifferentiating back to metacyclics. Moreover, haptomonad promastigotes significantly increase in number forming a larger and more distinct spheroid structure, the **haptomonad plug**, that physically blocks the stomodeal valve further promoting transmission. The effect of taking a subsequent blood meal after acquiring *Leishmania* parasites is equally critical for early infections. Our data shows that only a median of 52 (*L. longipalpis*) and 80 (*P. papatasi*) parasites are taken up by a sand fly naturally feeding on an infected host. In the absence of a second blood meal, the large majority of infections are poor or lost. In contrast, the majority of flies that obtain another blood meal 6 days after infection, as they would naturally, develop transmissible infections. Together, our data reveal the critical role of multiple bloodmeals in *Leishmania* development within sand flies that extends beyond metacyclics to expose novel cycles of parasite amplification that enhances vector infectivity with every blood meal.

MULTIPLE ROLES OF MITOCHONDRIA IN INFLAMMATORY CHAGASIC CARDIOMYOPATHY

NISHA GARG

(Department of Microbiology and Immunology, University of Texas Medical Branch, Galveston, Texas, USA)

Chagas disease represents the third greatest tropical disease burden globally, and accounts for ~$8 billion US dollars costs annually in patient care. No vaccines or safe drugs are available for treatment of an estimated >300,000 patients in the US and >20 million patients in endemic countries. Our lab focuses on studying the utility of FDA approved drugs in providing cardioprotection from the severity of progressive cardiomyopathy and ischemic cardiac injury, and set a new direction for clinical treatment of Chagas disease.

Our published data suggest the mitochondrial dysfunction in the heart is linked to suppressed antioxidant response and inflammation-induced immunopathology, particularly due to reactive oxygen intermediates. So far no mechanisms are known as to how these pathways converge to cause clinically symptomatic Chagas disease. In recent studies, we have tested the hypothesis that *activation of cGMP/PKG and SIRT1/PGC1 signal transduction networks will preserve the antioxidant response and mitochondrial function and prevent the oxidative/inflammatory adducts that precipitate cardiomyocytes death and cardiac remodeling in Chagasic disease*. For this, C57BL/6 mice were infected with *T. cruzi* and at the end of acute parasitemia (i.e. 45 days post-infection), treated with sildenafil that inhibits PDE5 (negative regulator of cGMP/PKG) or SRT1720 (SIRT1 agonist) twice per week for 3 weeks. Mice were monitored at 150 days post-infection corresponding to chronic disease phase. The cGMP/PKG and SIRT1 activities were decreased by 2-fold in chagasic myocardium. Transthoracic echocardiography showed the left ventricular (LV) systolic function, i.e., stroke volume, cardiac output, and ejection fraction, were significantly decreased in chagasic mice. Inhibition of PDE5 or activation of SIRT1 preserved the LV function in chagasic mice. The cardioprotective effects of PDE5 antagonist were provided through improvement of mitochondrial health and function, antioxidant/oxidant balance, and inhibition of cardiac collagenosis and chronic inflammation that otherwise were pronounced in chagasic myocardium. The cardioprotective effects of SIRT1 agonist were primarily provided through control of chronic inflammation.

We will discuss the mechanistic links between cGMP/PKG and SIRT1/PGC1 signal transduction networks in providing protection from mitochondrial and inflammatory stress in Chagas disease.

TOXOPLASMA GONDII MOLECULAR MIMICRY: FROM IMMUNE EVASION TO PARASITE PERSISTENCE

HAKIMI MOHAMED-ALI

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The obligate intracellular parasite *Toxoplasma gondii* strikes a subtle balance with the host immune system that not only prevents host death but also promotes parasite persistence. Although being enclosed within a parasitophorous vacuole (PV), the parasite actively interfaces with host cell signaling pathways, thereby directing host cell responses. The PV membrane has been regarded as a sieve limiting the delivery of proteins secreted by the parasite beyond the vacuolar space. However, the discovery of a large variety of effector proteins originating from dense granule organelles (GRA proteins) and their remarkable ability to cross the PV membrane and to accumulate in the host cell nucleus has changed this paradigm. With this new repertoire of molecular weapons, it can target gene expression at both the transcriptional and posttranscriptional levels by regulating the amount of mRNAs encoding proteins and affecting noncoding RNAs, e.g., microRNAs, respectively. These effectors highlight novel mechanisms by which *T. gondii* has learned to harness host signaling to favor intracellular survival and will guide future studies designed to uncover the additional complexity of this intricate host-pathogen interaction.

See review : Hakimi MA, Olias P, Sibley LD. Toxoplasma Effectors Targeting Host Signaling and Transcription. *Clin Microbiol Rev.* 2017 Jul;30(3):615-645.

SIMPOSIA XXIII
HEPATOBILIARY PHYSIOLOGY. BREAKTHROUGHS AND NEW THERAPEUTICAL TARGETS
Chairs: Cecilia Larroca and Mariana Garcia

THE SPARC PROTEIN IN LIVER DISEASE. A NOVEL THERAPEUTIC TARGET?

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Secreted protein acidic and rich in cysteine (SPARC), also called osteonectin, is a 43-kDa glycoprotein highly conserved among different species. SPARC is secreted into the extracellular matrix (ECM), and although it has no structural function SPARC facilitates cell-cell and cell-ECM interactions . SPARC is expressed during tissue development, in tissues with high ECM turnover and in injured tissues. A receptor for SPARC was not identified yet, however, the binding of leukocyte-derived SPARC to VCAM-1 was demonstrated to be necessary for leukocyte transmigration through endothelial monolayers in vitro. It has also been observed that SPARC binds to integrin beta 1 and that this interaction enhances integrin-linked kinase activation and pro-survival activity. In normal livers, SPARC is slightly expressed in some spindle-shaped cells located in periportal areas and in scarce sinusoidal cells within the lobule. Expression of SPARC in normal hepatocytes was not clearly observed. Our group has been involved in the study of the role of SPARC in liver diseases for many years. We have observed that SPARC expression levels were increased in liver samples from patients and animals with acute liver failure (ALF). In SPARC knockout (SPARC-/-) mice a decreased hepatic damage was observed in different ALF animals models (e.g. Concanavalin A, LPS, APAP). Mechanisms of protection involved resistance of liver sinusoidal endothelial cells damage and reduced ROS induction. Inhibition of SPARC by siRNA administration reduced the degree of liver damage in mice.

On the other hand, the role of SPARC in hepatic fibrosis has been particularly investigated in our laboratory. We observed that SPARC knockdown protects mice against the damage induced by chronic intoxication with thioacetamide (TAA). By using an adenovirus encoding for an antisense against SPARC mRNA (AdasSPARC), in a TAA-induced liver fibrosis model in rat, we were able to reduce the amount of liver damage and the number of hepatic stellate cells (HSC), a key cell population involved in hepatic fibrosis. Rat that received AdasSPARC showed thinner and immature collagen fibers. They also showed less TGF-β1 levels. We also observed in an in vitro assay that SPARC knockdown in HSC attenuated profibrogenic response induced by fibrogenic cytokines. Consistent with these results, the use of SPARC-/- mice confirmed a reduction in the level of inflammation and fibrosis after treatment with TAA. In this context, microarrays results demonstrated that SPARC-/- mice showed over-expression of genes related to DNA repair and detoxification.

All in all, these results suggest that SPARC is a key player in acute and chronic liver diseases. Therefore, we propose SPARC as a potential therapeutic target for patients with liver injury.

THERAPEUTIC AQUAPORIN GENE TRANSFER IN HEPATOCELLULAR CHOLESTASIS

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Aquaporins (AQPs) are a family of membrane channels that facilitate the osmotically-driven transport of water. The first AQP (i.e., AQP1) was identified in 1992 by Dr. Peter Agre, 2003 Nobel Prize in Chemistry. The mammalian AQPs, which number about a dozen, are expressed in many cell types, including the hepatocyte. Bile formation by hepatocytes is an osmotic secretory process. The excretion of bile salts via the canalicular bile salt transporter BSEP/ABCB11 is thought to be a major driving force for the osmotic, AQP-mediated, water movement into the bile canaliculus. Moreover, a defective canalicular AQP expression, as observed in experimental models of hepatocellular cholestasis (e.g., that induced by estrogens or lipopolysaccharides), is considered an important contributing factor in the development of bile secretory dysfunction. Based on this, we designed and performed studies to assess whether the adenovector AdhAQP1, which encodes for human AQP1 (hAQP1), can promote AQP1-mediated canalicular water secretion and thus improving bile secretory failure in hepatocellular cholestasis. AdhAQP1, administered by retrograde bile ductal infusion, induced hepatocyte canalicular hAQP1 expression and a concomitant increased in canalicular osmotic water permeability [1]. Bile flow as well as choleretic efficiency of endogenous bile salts (i.e., volume of bile per micromol of excreted bile salts) were significantly augmented in cholestasis. This indicates that bile flow improves to some extent by hAQP1-facilitated canalicular water transport [1]. An unexpected finding in hAQP1-transduced cholestatic rats was a significant improvement in the biliary bile salt output, which in turn normalized the elevated serum bile salt levels in cholestasis [2]. Canalicularly expressed hAQP1 unaltered BSEP/ABCB11 protein expression but markedly increased its

transport activity inducing its redistribution to cholesterol-rich canalicular microdomains [2]. Our observations suggest that the hepatic adenoviral transfer of hAQP1 gene to cholestatic rats improves bile secretory failure by increasing both biliary output and choleretic efficiency of bile salts. This finding may have potential as novel treatment for certain liver cholestatic diseases [3].

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BILIRUBINOMICS 2017

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Since the first documented observation of a jaundiced patient more than 3000 years ago, bilirubin was considered as a waste product of heme with limited biological activity, a part of being a sign of liver disorders. Gaining scientific attention in the 18th century, the interest on the biological effects of bilirubin longer rotated around its toxic and harmful effects to the brain. At concentrations exceeding the binding capacity of plasma albumin, such as in severe neonatal hyperbilirubinemia and in Crigler-Najjar type I syndrome, (free) bilirubin accumulates in brain inducing mild-temporary (acute bilirubin encephalopathy) or severe-permanent damage (kernicterus). Abnormal motor control, movements and muscle tone, auditory disturbance with or without hearing loss, and oculomotor impairments, are the classical sign of bilirubin neurotoxicity. If untreated, kernicterus is still a cause of death in low-income countries. At the molecular level, bilirubin interplays with multiple targets, modulating a plethora of genes, proteins, and signaling pathway, leading to a pro-inflammatory and pro-oxidant status, excitotoxicity, autophagy, ER stress, cell death, interfering with proliferation and maturation, and showing a remarkable topography for specific brain regions and CNS developmental stages. Only more recently, the advantages of having a moderately higher blood level of the pigment (such as in Gilbert subjects) have been proved. Epidemiological evidence demonstrates benefits (in term of lower incidence and improved conditions) in cardiovascular diseases, certain cancers, Crohn disease, diabetes, and autoimmune and neuropsychiatric diseases. All these conditions presenting inflammation and increased oxidative stress. Paradoxically, at low concentration bilirubin protection occurs via an anti-inflammatory and antioxidant action. Bilirubin is the most potent endogenous antioxidant in the cell (more than tocopherol), due to its continuous recovery by the bilirubin-biliverdin redox cycle. Powered thought the simultaneous activity of heme-oxygenase and biliverdin-reductase, the cycle supplies the cell with fresh bilirubin, consumed (oxidized back to biliverdin) by the presence of intracellular ROS. Notably, the most recent studies indicate a low serum bilirubin level as a potential marker of ongoing chronic pro-oxidant diseases, such as atherosclerosis (risk cut-off 10micromol/liter), Alzheimer disease, dementia, multiple sclerosis, and cerebral infection. Of great interest, and supporting a key role of bilirubin as part of the intracellular anti-oxidant mechanisms, is the recent discovery of UnaG, a protein binding and protecting bilirubin toward oxidation, as an attempt to preserve and store this antioxidant molecule. Notably biliverdin reductase, heme oxygenase, their products (bilirubin, biliverdin, Fe and Co), as well the precursors alanine, behave as signaling molecules not only on the enzymes involved in bilirubin metabolism but also on important cellular controller such as kinases (e.g.. PI3K: phosphatidylinositol-4,5-bisphosphate 3- kinase, IRK: insulin receptor tyrosine kinase, MAPK: mitogen-activated protein kinases) and transcription factors (e.g.: AhR: aryl hydrocarbon receptor, PPAR: peroxisome proliferator-activated receptors, Nrf2: Nuclear factor erythroid-derived 2-like 2). In conclusion, a century of research has multiplied the mechanisms of actions and the biological functions of bilirubin, multiplying the pieces of an intriguing puzzle, resumed by the name “bilirubinomics

LIVER VASCULAR PHYSIOLOGY AND ITS CLINICAL APPLICATION

GABRIEL GONDOLESI

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The hepatic physiology and circulation have unique and differential characterististics compared to any other organ in the human body.

Some of the unique features include: the dual vascular supply; a mechanism of intrinsic regulation of the hepatic artery; a portal blood flow supplying 2/3 of liver blood flow, which is not controlled directly by the liver; and the fact of receiving 20% of the cardiac output. At histological level, it has a unidirectional blood flow with an extremely distensible capacitance and venous resistance that regulates parenchymal cell metabolic specialization.

The development of chronic liver disease causes a total body circulatory adaptation, opening anatomical and non-anatomical porto-cava shunts, in order to adequate

systemic flow to the increase intrahepatic vascular resistance.
The need to reduce the consequences of this pathophysiological changes, translated into the evolution of therapies from surgical to percutaneous endovascular porto-systemic shunts. The understanding, and the development of the current knowledge on the close relationship between liver flow and hepatic regeneration, favored recent developments in liver surgery; allowing to plan liver resections, expanding not only the indications but also increasing the possibility of applying surgery with curative intent. The applications of those advances in the transplant field allowed to evolve from using whole cadaveric liver transplant to segmental living or cadaveric liver donation, increasing transplant applicability in all ages.
The current available surgical developments that apply to the liver had become one of the best examples of the use of translational physiology and medicine over the last century and become the aim of this presentation.

SIMPOSIA XXIII
CURRENT TOPICS IN GPCR SIGNALING
Chairs: Juan Carlos Calvo

ALLOSTERIC RECEPTOR-RECEPTOR INTERACTIONS IN HOMO-AND HETERORECEPTOR COMPLEXES GIVE A NEW DIMENSION TO MOLECULAR NEUROSCIENCE AND UNDERSTANDING LEARNING AND MEMORY AND BRAIN DISEASE

KJELL FUXE
(Department of Neuroscience, Karolinska Institutet, Solna, Sweden)

concept of allosteric receptor–receptor interactions in G protein-coupled receptor (GPCR) homo- and heteroreceptor complexes of the central nervous system (CNS) gave a new biological principle to understanding brain integration and neuropsychopharmacology. Allosteric receptor–receptor interactions made possible through receptor oligomerization lead to novel receptor dynamics during which the receptor protomers change their recognition, pharmacology, signaling and trafficking and novel allosteric binding sites can develop. GPCR heteroreceptor complexes can also involve ion channel receptors, receptor tyrosine kinases (RTKs), sets of G protein interacting proteins, ion channels and/or transmitter transporters. There is a need to improve our understanding of the molecular organization of the receptor oligomers, their allosteric communication and the features of the receptor interface. It appears clear that synaptic transmission and volume transmission (extracellular fluid and CSF) in the brain can become integrated in heteroreceptor complexes in the synaptic and perisynaptic membranes built up of ion channel receptors , GPCRs and receptor tyrosine kinases. It enables modulation of synaptic strength and synaptic plasticity in a dynamic way and receptor plasticity is accomplished. Integration of the molecular receptor circuits in the postsynaptic and perisynaptic membranes, respectively can take place through integration of their intracellular molecular signaling pathways in the dendritic spines.
Based on this concept, we proposed that the molecular basis of learning and memory was based on the reorganization of the homo- and heteroreceptor complexes in the postsynaptic membrane of synapses associated with changes also in the presynaptic receptor complexes to facilitate the pattern of transmitter release to be learned. Long-term memory may be created by the transformation of parts of the heteroreceptor complexes into unique transcription factors which can lead to the formation of specific adapter proteins which can consolidate the homo-and heteroreceptor complexes into long-lived complexes with conserved allosteric receptor-receptor interactions. Thus, the homo-heteroreceptor complexes are regarded as highly dynamic assemblies formed or disrupted by integrated synaptic and volume transmission signals. These events are regarded as necessary for learning, and can become transformed into a consolidated rigid state with conserved allosteric communication representing molecular engrams resulting in a major long term modulation of the neuronal networks. This molecular plasticity change, whether transient or long term, can then alter the patterns of outflow in the brain circuits and induce transient and long-term changes in behaviors and cognitive functions. It is now clear through the demonstration of the GPCR heterodimer network (GPCR-HetNet) that the allosteric receptor-receptor interactions dramatically increases GPCR protomer diversity and biased recognition and signaling leading to enhanced specificity in signaling.
Dysfunction or disruption of the heteroreceptor complexes can lead to brain disease. Understanding the D2R heteroreceptor complexes and their dysfunction in schizophrenia can lead to new strategies for its treatment and for avoiding side-effects of antipsychotics known to mainly act as D2R antagonists. This includes a way to optimize combined treatment or single use of heterobivalent drugs targeting distinct D2R heteroreceptor complexes in schizophrenia. This is inspired by current findings on the existence of various types of D2R heteroreceptor complexes in nuc accumbens core and shell.
It was also proposed that novel antiparkinson drugs for the future may be heterobivalent drugs e.g. built up of an A2AR antagonist pharmacophor linked to a D2R agonist pharmacophor specifically targeting the A2AR-D2R het-

erodimer. Other exciting novel targets for antiparkinson drugs are A2AR-D2R-mGluR5 Hets , where A2AR and mGluR5 synergize to put a brake on D2R Gi/o mediated signaling. Emerging new concepts in Parkinson's disease are that dysfunction of the allosteric receptor-receptor interactions and dysbalance of multiple Hets codistributed in the same neuron and synapses of the direct and indirect pathways of the basal ganglia contributes to disease progression and levodopa- induced dyskinesias. Furthermore,the D1 and D2 heteroreceptor complexes in the reward and anti-reward GABA pathways from the nuc accumbens have become exciting new targets for treatment of substance use disorder.The changes found in these receptor complexes in cocaine self-administration opens up a new understanding what goes wrong in cocaine addiction.

HARNESSING THE PLASTICITY OF G PROTEIN-COUPLED RECEPTORS THROUGH ALLOSTERIC REGULATION BY G PROTEINS, SMALL MOLECULES AND CATIONS

ROGER K. SUNAHARA

(Department of Pharmacology, University of California at San Diego, San Diego, California, USA)

G protein-coupled receptors (GPCR) are conduits that sense and communicate extracellular stimuli and transduce their signals across the plasma membrane into intracellular compartments. GPCRs can detect environmental stimuli such as light, odors and tastes, and serve as receptors for hormones, chemokines and even proteases, and modulate downstream effectors that synthesize or regulate second messengers such as cAMP, inositol phosphates and cations. Communication between the receptors and effectors is mediated through the heterotrimeric family of G proteins. Advances in the structural biology of GPCRs have helped unravel the complexities of ligand binding and G protein activation. Analysis of the structural data, along with support from pharmacological and biochemical studies, have aided in developing models of how hormone binding leads to G protein activation. Here we describe the allosteric relationship between G protein binding and the hormone binding site, where G proteins potently enhance the affinity of hormone agonists. Through understanding the structural basis of this cooperativity we further provide a rational foundation for the efficacy of allosteric modulators, as well as a novel allosteric modulator binding site on the beta$_2$ adrenergic receptor. An understanding of the mechanisms underlying this allostery was also useful to describe a major role of additional co-factors, such as cations, as allosteric modulators in the stabilization of inactive and active states of GPCRs. These data highlight the complex nature of the cooperativity existing between small molecules and G proteins in stabilizing specific GPCR conformations and illustrate their impact on drug discovery and development.

MOLECULAR AND STRUCTURAL DETERMINANTS OF GPCR FUNCTIONAL SELECTIVITY AND SIGNAL PROPAGATION

MICHEL BOUVIER

(Department of Biochemistry and Molecular Medicine, Faculty of Medicine and Institute for Research in Immunology and Cancer, Université de Montréal, Montréal, Canada)

It is now clear that G protein-coupled receptors (GPCRs) are not uni-dimensional switches that turn 'on' or 'off' a single signaling pathway. Instead, each receptor can engage multiple signaling partners to form dynamic complexes that can engage various downstream effector systems that may or may not involve G protein activation. Individual ligands can have differential efficacies toward specific subsets of the signaling effector repertoire engaged by a given receptor. This phenomenon, known as ligand-biased signaling or functional selectivity, opens new opportunities for the development of new drugs with increased selectivity profiles and less undesirable effects. Yet, this pluridimensional nature of signaling efficacy presents a challenge to establish the complete signaling profile of drugs. To better assess multiple signaling outcomes and unravel the structural basis of GPCR functional selectivity, we developed a diversity of biosensors based on bioluminescence resonance energy transfer (BRET) that allow real-time monitoring of multiple GPCR effectors and downstream signaling events. We generated more than 40 BRET-based sensors that monitor a large diversity of signaling pathways with unprecedented spatio-temporal resolution in living cells. In addition we developed label-free approaches that permit assessing the integrated cellular responses and dissecting the relative contribution of the contributing signaling pathways. Their combined use with BRET sensors revealed unexpected signaling texture and provide a new tool-set to monitor signal transduction from the membrane to intracellular organelles including endosomes, opening new avenues for the screening and profiling of drug candidates with favorable functional selectivity. At the molecular level, molecular modeling and site-directed mutagenesis revealed that specific domains of receptors play crucial roles for the activation of selective pathways, providing insights into the rational design of biased ligands with desired signaling properties.

DIFFERENT CHEMICAL BIOLOGY APPROACHES TOWARDS MODULATION OF GPCR SIGNALING

ROB LEURS
(Vrije Universiteit, Amsterdam, The Netherlands)

GPCRs are one of the most effective signaling devices, that regulate a plethora of physiological responses via the effective coupling to a diverse set of signaling pathways. The histamine H_3 receptor (H_3R) is a GPCR that regulates neurotransmitter release and is consequently involved in various CNS functions via the coupling of a set of GPCR isoforms to various signal transduction pathways. To elucidate the role of these isoforms and their various signaling pathways, our lab has been developing new tools to distinguish H_3R isoforms and signaling pathways in order to selectively affect G-protein and/or non-G-protein signaling pathways.

Photoswitchable ligands are starting to emerge in the field of GPCRs as another set of pharmacological tools to control receptor activityat will. It has been shown that photoisomerization can change ligand affinity for the receptor as well as its efficacy. Photoswitchable ligands often contain an azobenzene moiety that is able to reversibly photoisomerize from the *trans* to *cis* isomer upon illumination with specific wavelengths. neurological disorders. In this presentations, a novel series of photoswitchable antagonists for the histamine H_3 receptor will be presented. Within this series binding affinity shifts of over 10-fold were found upon treatment with light. In this presentation, a highly complementary bidirectional photo-antagonist and agonist toolbox is presented, which allows dynamic regulation of histamine induced H_3R activation with high temporal resolution. These photopharmacological tools can be of aid in spatio-temporal studies to study H_3R signalling.

SIMPOSIA XXIV
METABOLISM AND THE IMMUNE RESPONSE
Chairs: Carolina Montes and Daniel Gonzalez-Maglio

MACROPHAGE IMMUNOMETABOLISM IN METABOLIC AND INFLAMMATORY DISEASES

PEDRO M. MENDES DE MORAES VIEIRA
(Istituto de Biologia, Universidade Estadual de Campinas, Campinas, Brazil)

The interface between immunology and metabolism leaded to the understanding of many metabolic disorders in which the immune system function is essential, such as in diabetes, atherosclerosis and autoimmune diseases. A key finding that emerged from this context is that the metabolism integrates all other biological systems. The adipokines are one example of molecules that integrates the metabolism with the immune systems. The adipokines include leptin, adiponectin, retinol binding protein 4 (RBP4), among others. RBP4, a retinol transporter, is elevated in insulin resistance and contributes to increased diabetes risk. RBP4 has an essential role in inflammation-induced insulin resistance. Insulin resistance is a major cause of diabetes and is highly associated with adipose tissue (AT) inflammation in obesity. RBP4 elevation causes AT inflammation by activating innate immunity that elicits an adaptive immune response. RBP4 overexpression leads to insulin resistance and increased AT macrophage and CD4 T cell infiltration. RBP4 overexpression changes AT $CD206^+$ macrophage phenotype, enhancing the expression proinflammatory markers and activation of CD4 T cells while maintaining alternatively activated macrophage markers. These effects result from direct activation of AT antigen-presenting cells (APCs) by RBP4 through a JNK-dependent pathway. Blockade of antigen presentation or reduction on RBP4 levels improves insulin resistance and adipose tissue inflammation. RBP4 promotes a metabolic switch favoring glycolysis in adipose tissue M2 macrophages. Glycolysis inhibition block RBP4-induced macrophage activation. RBP4 effects on the metabolic switch of macrophages rely on the Hypoxia inducible factor 1 alpha (HIF-1α). HIF-1α expression is elevated in adipose tissue M1 and M2 macrophages from RBP4 overexpressing mice, which display a differentiated metabolomic phenotype. Thus, RBP4 has a central role in macrophage metabolic fitness during inflammation-induced insulin resistance.

MTOR SIGNALLING IN MACROPHAGES

THOMAS WEICHHART
(Center of Pathobiochemistry and Genetics, Institute of Medical Genetics, Medical University of Vienna, Vienna, Austria)

The interface between immunology and metabolism leaded to the understanding of many metabolic disorders in which the immune system function is essential, such as in diabetes, atherosclerosis and autoimmune diseases. A key finding that emerged from this context is that the metabolism integrates all other biological systems. The adipokines are one example of molecules that integrates the metabolism with the immune systems. The

adipokines include leptin, adiponectin, retinol binding protein 4 (RBP4), among others. RBP4, a retinol transporter, is elevated in insulin resistance and contributes to increased diabetes risk. RBP4 has an essential role in inflammation-induced insulin resistance. Insulin resistance is a major cause of diabetes and is highly associated with adipose tissue (AT) inflammation in obesity. RBP4 elevation causes AT inflammation by activating innate immunity that elicits an adaptive immune response. RBP4 overexpression leads to insulin resistance and increased AT macrophage and CD4 T cell infiltration. RBP4 overexpression changes AT $CD206^+$ macrophage phenotype, enhancing the expression proinflammatory markers and activation of CD4 T cells while maintaining alternatively activated macrophage markers. These effects result from direct activation of AT antigen-presenting cells (APCs) by RBP4 through a JNK-dependent pathway. Blockade of antigen presentation or reduction on RBP4 levels improves insulin resistance and adipose tissue inflammation. RBP4 promotes a metabolic switch favoring glycolysis in adipose tissue M2 macrophages. Glycolysis inhibition block RBP4-induced macrophage activation. RBP4 effects on the metabolic switch of macrophages rely on the Hypoxia inducible factor 1 alpha (HIF-1α). HIF-1α expression is elevated in adipose tissue M1 and M2 macrophages from RBP4 overexpressing mice, which display a differentiated metabolomic phenotype. Thus, RBP4 has a central role in macrophage metabolic fitness during inflammation-induced insulin resistance.

REGULATION OF IMMUNE FUNCTION BY LACTATE AND SHORT CHAIN FATTY ACIDS

MARTÍN RUMBO

(Instituto de Estudios Inmunológicos y Fisiopatológicos, CONICET, La Plata, Argentina)

The interface between immunology and metabolism leaded to the understanding of many metabolic disorders in which the immune system function is essential, such as in diabetes, atherosclerosis and autoimmune diseases. A key finding that emerged from this context is that the metabolism integrates all other biological systems. The adipokines are one example of molecules that integrates the metabolism with the immune systems. The adipokines include leptin, adiponectin, retinol binding protein 4 (RBP4), among others. RBP4, a retinol transporter, is elevated in insulin resistance and contributes to increased diabetes risk. RBP4 has an essential role in inflammation-induced insulin resistance. Insulin resistance is a major cause of diabetes and is highly associated with adipose tissue (AT) inflammation in obesity. RBP4 elevation causes AT inflammation by activating innate immunity that elicits an adaptive immune response. RBP4 overexpression leads to insulin resistance and increased AT macrophage and CD4 T cell infiltration. RBP4 overexpression changes AT $CD206^+$ macrophage phenotype, enhancing the expression proinflammatory markers and activation of CD4 T cells while maintaining alternatively activated macrophage markers. These effects result from direct activation of AT antigen-presenting cells (APCs) by RBP4 through a JNK-dependent pathway. Blockade of antigen presentation or reduction on RBP4 levels improves insulin resistance and adipose tissue inflammation. RBP4 promotes a metabolic switch favoring glycolysis in adipose tissue M2 macrophages. Glycolysis inhibition block RBP4-induced macrophage activation. RBP4 effects on the metabolic switch of macrophages rely on the Hypoxia inducible factor 1 alpha (HIF-1α). HIF-1α expression is elevated in adipose tissue M1 and M2 macrophages from RBP4 overexpressing mice, which display a differentiated metabolomic phenotype. Thus, RBP4 has a central role in macrophage metabolic fitness during inflammation-induced insulin resistance.

IMMUNE REGULATION AT BARRIER SURFACES

DAVID ARTIS

(Weill Cornell Medical College, Cornell University, New York, USA)

Employing models of microbial colonization, pathogen infection, chronic inflammation and tissue repair, research in the Artis lab is examining how mammalian host genetics and signals derived from the environment and commensal microbial communities influence innate and adaptive immune cell responses at the body's barrier surfaces. We are employing gnotobiotic mice to examine the influence of defined commensal microbial communities on intestinal and peripheral immune cell development, function and influence on tissue homeostasis. Our recent findings indicate that commensal microbes have a significant regulatory influence on lymphocyte, innate lymphoid cell and granulocyte function associated with susceptibility to multiple infectious, inflammatory and metabolic disease processes. We have also developed a number of translational immunology projects, including analysis of tissue samples from patients with atopic dermatitis, food allergy, obesity and IBD. It is hoped that the results of these basic and translational studies will advance understanding of the pathophysiology of multiple chronic inflammatory diseases, including asthma, allergy, inflammatory bowel disease and obesity, and provide a framework to test new therapeutic pathways to prevent and treat these diseases.

SIMPOSIA XXV
GENOMIC INSTABILITY AND DNA REPEAIR FROM CANCER, NEURODEGENERATION AND AGING
Chairs: Maria Jimena Ferraris and Gastón Soria

GENOMIC INSTABILITY & DNA REPAIR: LESSONS FROM CANCER, NEURODEGENERATION AND AGING
THE NASCENT DNA: A SIGNALING TOOL BELT THAT INTEGRATES MULTIPLE PATHWAYS IN CELLS

VANESA GOTTIFREDI
(Fundación Instituto Leloir, CONICET, Buenos Aires, Argentina)

The duplication of DNA is an essential process required to produce viable daughter cells. To achieve such an endeavor, the replication machinery needs to readily copy almost two meters of template DNA in approximately 5 hours (the length of an S phase). Such difficult task is further challenged by the frequent chemical modifications of the template DNA. Hence, a signaling network known as DNA damage response (DDR) is activated to guarantee replication adaptation to damaged DNA. While the DDR preserves healthy cells from unnecessary death, it also aids tumor cells when escaping from the harmful effects of DNA damaging agents. Hence, understanding the DDR may serve to identify important targets in cancer therapy.

Our current understanding of the DDR indicates that it gets activated when the replicative DNA polymerases are blocked by DNA lesions. A first DDR option is to tolerate the DNA lesion, meaning that the nascent DNA is synthesized by alternative methods, including the synthesis of DNA by specialized DNA polymerases that can use the damaged DNA as replication templates. A second DDR option is activated when the DNA lesion is not tolerated and the DNA replication is halted. In this case, the stalling of the replisome generates checkpoint signals that spread throughout the nucleoplasm, preventing further initiation of DNA replication events elsewhere. Such reduction in the DNA replication speed creates a temporal window to repair DNA lesions, diminishing the global frequency of encounter of DNA lesions by active replication forks. If option 1 and 2 fail, the replication fork breaks, requiring homologous recombination repair for its reconstitution. Such a linear pathway represents an oversimplification of the sophisticated way in which DDR works. In fact, I will present data demonstrating unanticipated levels of complexity in the interaction between DDR pathways. Such findings may facilitate the identification of key proteins of this network that should be targeted to dismantle the DDR of tumor cells.

REGULATION OF BASE EXCISION REPAIR IN THE AGING BRAIN

RICARDO GREDILLA
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Aging is characterized by reduction in physiological functions. The detrimental effects of aging are best observed in postmitotic tissues, where cells that are irreversibly damaged or lost cannot be replaced. Among such tissues the brain is most important one due to its main role in homeostasis of the organism. Brain aging is characterized by increased susceptibility to neuronal loss and functional decline leading to cognitive impairment. Mitochondrial DNA (mtDNA) mutations are thought to play an important role in these processes. Increased levels of oxidative DNA damage and accumulation of mtDNA mutations have been linked to mitochondrial dysfunction, thus contributing to the aging process. Moreover, increased mtDNA instability has been also associated with the pathogenesis of neurodegenerative diseases such as Alzheimer's disease.

In order to maintain genomic stability, different DNA repair pathways have evolved. Base excision repair (BER) is the primary DNA repair pathway for small DNA modifications caused by oxidation, alkylation or deamination, both in nuclei and mitochondria. During the last years we have investigated the potential role of DNA repair mechanisms in the aging process and age-related neurodegenerative disorders. Brain regions involved in cognition processes, such as cortex and hippocampus, as well as specific subcellular fractions like synaptosomes have been analyzed during normal aging and different animal models of neurodegeneration. Our investigations suggest that a decline in the BER capacity is an important factor in the brain aging process and in the onset and course of age- related neurodegenerative diseases. A better understanding of the underlying mechanisms of the aging process would lead to significant advances in the search of strategies to improve brain aging as well as therapeutic treatments of neurodegenerative diseases.

OVARIAN HORMONE DEPRIVATION AND HORMONE REPLACEMENT THERAPY EFFECTS ON MITOCHONDRIAL DNA REPAIR PROCESSES IN THE BRAIN

SANDRA ZÁRATE
(Instituto de Investigaciones Biomédicas, CONICET; Facultad de Medicina,

Universidad de Buenos Aires, Buenos Aires, Argentina)

Sex steroids exert pleiotropic effects in the brain, preserving neural function and promoting neuronal survival. Not surprisingly, loss of ovarian hormones during natural or induced reproductive senescence in the female is characterized by synaptic and cognitive impairments and increased risk of neurodegeneration, processes that are now recognized to be highly associated with mitochondrial dysfunction. Multiple lines of evidence point for brain mitochondria as targets of steroid hormone action. In fact, a link between sex-dependent susceptibility and decline in brain mitochondrial function has been identified in normal aging as well as in neurodegenerative diseases both in preclinical animal studies as well as in women after natural or surgical menopause. Mitochondria are key players in cellular bioenergetics and survival, they are the main source of ATP production and also the primary sites for cellular reactive oxygen species (ROS) generation. These organelles are unique in terms of having their own genome, which is more prone to oxidative damage than the nuclear genome in part due to its close proximity to the source of ROS production. Accumulative damage in mitochondrial DNA (mtDNA) over time, if not properly repaired, leads to mitochondrial dysfunction and disease. As an organ with a high demand of energy and low antioxidant capacity, the brain is particularly vulnerable to mitochondria dysfunction and oxidative stress. Base excision repair (BER) is the major DNA repair pathway in mitochondria, thereby constituting an important mechanism to avoid accumulation of mtDNA mutations. Still, little is known about how hormonal status affects mtDNA repair mechanisms in the brain. Moreover, the heterogeneity of organization, function, and biochemical mechanisms in the brain implies that some regions may be particularly vulnerable to ovarian hormone loss and accumulation of mtDNA damage. In this context, we are studying the effects of long-term ovarian hormone deprivation and early hormone-replacement treatments on BER pathway in mitochondria from rat cortex and hippocampus, areas primarily affected in aging and neurodegenerative diseases and highly responsive to ovarian hormones. Our data suggest that hormonal status regulate BER pathway in a region-specific manner and poises the hippocampus as a highly vulnerable area to ovarian hormone loss regarding impaired BER capacity. Considering the importance of preserving mtDNA integrity for normal cell function, this study provides insights into the role of early hormone replacement therapy in mtDNA repair capacity and could help find new therapeutic targets to promote a healthier lifespan for women after natural or induced menopause.

MAJOR ROLES OF PYRIMIDINE DIMERS, NUCLEOTIDE EXCISION REPAIR AND ATR IN THE ALTERNATIVE SPLICING RESPONSE TO UV IRRADIATION

MANUEL MUÑOZ

(Instituto de Fisiología, Biología Molecular y Neurociencias, CONICET; Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina)

We have previously found that UV irradiation promotes RNA polymerase II (RNAPII) hyperphosphorylation and subsequent changes in alternative splicing (AS). We show now that UV-induced DNA damage is not only necessary but sufficient to trigger the AS response and that photolyase-mediated removal of the most abundant class of pyrimidine dimers (PDs) abrogates the global response to UV. We demonstrate that, in keratinocytes, RNAPII is the target, but not a sensor, of the signaling cascade initiated by PDs. The UV effect is enhanced by inhibition of gap-filling DNA synthesis, the last step in the nucleotide excision repair pathway (NER), and reduced by the absence of XPE, the main NER sensor of PDs. The mechanism involves activation of the protein kinase ATR that mediates the UV-induced RNAPII hyperphosphorylation. Our results define the sequence UV-PDs-NER-ATR-RNAPII-AS as a pathway linking DNA damage repair to the control of both RNAPII phosphorylation and AS regulation.

SIMPOSIA XXVI
METABOLIC SYNDROME: NOVEL KNOWLEDGE AND NEW CHALLENGES
Chairs: Silvia Villanueva and Luis Cuniberti

ADIPOSE TISSUE INFLAMMATION AND METABOLIC SYNDROME: PROTECTIVE ROLE OF BIOACTIVE GRAPE-DERIVED COMPOUNDS

MARCELA VÁZQUEZ PRIETO

(Instituto de Medicina y Biología Experimental de Cuyo, CONICET, Universidad Nacional de Cuyo, Mendoza, Argentina)

Metabolic syndrome (MetS) is a cluster of symptoms that include increased waist circumference (abdominal adiposity), plasma triglycerides, and fasting glycemia; reduced high-density lipoprotein (HDL) cholesterol; and

hypertension. The increasing incidence of MetS, which occur in parallel with the increase of obesity, currently affects 34% of the world population and is associated with the development of insulin resistance, type 2 diabetes (T2D), and cardiovascular disease, constituting a major public health concern worldwide. Hypertrophy of adipocytes, particularly from visceral adipose tissue, causes an increase production and secretion of pro-inflammatory adipocytokines such as tumor necrosis factor alpha (TNFα), monocyte chemoattractant protein 1 (MCP-1), resistin, interleukin 6, among others. Although the mechanisms are not completely understood, these pro-inflammatory cytokines drive the recruitment and activation of macrophages and other immune cells that completes a cycle of inflammation and impaired metabolic function, contributing to adipose insulin resistance. Diet can play a major role in the prevention of MetS and its associated pathologies. Epidemiological studies show that consumption of fruits and vegetables in humans decreases the risk of MetS. Grape pomace (GP) is a waste product, containing predominantly a left-over of skins and seeds, generated in the winemaking process which contains relatively high amounts of bioactive compounds like polyphenols and dietary fiber. Polyphenols are naturally occurring plant compounds that have a multiplicity of biological effects. The most abundant polyphenols identified in malbec GP are flavanols such as (-)-epicatechin, flavonols, anthocyanins, hydroxybenzoic and hydroxycinnamic acids, and stilbenes. The protective effect of certain polyphenols on adipose tissue dysfunction are attributed to the capacity to modulate cell signaling cascades involved in inflammation and insulin resistance, interaction with enzymes, transcription factors, and receptors among others. Overall, bioactive grapederived compounds constitute an interesting alternative to modulate/mitigate adipose tissue inflammation associated with MetS and obesity.

EFFECTS OF ANTIOXIDANTS ADMINISTRATION IN EXPERIMENTAL METABOLIC SYNDROME

CARLOS REYES TOSO

(Facultad de Medicina, Universidad de Buenos Aires, Buenos Aires, Argentina)

Metabolic syndrome (MS) is a cluster of abnormalities, including among others, abdominal obesity, hypertension, glucose intolerance or diabetes and dyslipidemia (hypertriglyceridemia and/or low HDL cholesterol). The US National Cholesterol Education Program–Adult Treatment Panel III (ATPIII-2005) and the Joint Interim Statement -2009- (International Diabetes Federation; American Heart Association; National Heart, Lung, and Blood Institute; World Heart Federation; International Atherosclerosis Society; and the International Association for the Study of Obesity) defines MS as the combined occurrence of at least three of the above mentioned five risk factors. This syndrome increase the risk for cardiovascular disease and type 2 diabetes, and recently it has been associated with other pathological entities such as Nonalcoholic Fatty Liver Disease (NAFLD), renal dysfunction, cancer (liver, pancreas, bladder and breast) and dementia. It is estimated that approximately 20 % of world's adult population has MS.

Visceral obesity and insulin resistance (IR) are currently considered the primary underlying factors in the development of this syndrome. It has been reported that proinflammatory state of visceral obesity induces IR, leading to clinical and biochemical manifestations of MS. Furthermore, oxidative stress is associated with IR, visceral fat accumulation, and MS. In the last decade, excessive oxidative stress has been proposed as a common pathological mechanism for the development of systemic lesions.

The MS induced in rats by the administration of high-fructose and high-fat diets mimics the syndrome described in humans, but in contrast to patients rats' body weight is not significantly increased (although visceral adipose tissue mass is expanded).

A major contributor to the development of IR is an overabundance of circulating free fatty acids (FFA). These FFA induce IR in muscle by inhibiting insulin-mediated glucose uptake. Increased levels of circulating glucose augment glycolysis and production of diacylglycerol (DAG). DAG stimulates the activity of protein-kinase C (PKC) and so interferes with intracellular insulin signaling. The metabolic alterations above mentioned increase oxidative stress through an increase in reactive oxygen species (ROS), specially mitochondrial and extra-mitochondrial ($^{\cdot}0_2^-$). PKC also induce NADP(H)-oxidase with the production of more ($^{\cdot}0_2^-$). Furthermore the activation of the polyol pathway results in a decrease of intracellular levels of NADPH which leads to reduced synthesis of antioxidant substances. ROS produced, as mentioned earlier, can cause DNA and mitochondrial damage, lipid peroxidation and therefore disrupt the membrane lipid bilayer arrangement that may inactivate membrane-bound receptors and enzymes and lead to cell death.

Several studies of chronic administration of antioxidants substances to fructose-fat fed rats have shown a reduction in blood pressure, triglycerides, total cholesterol and FFA and increased plasma HDL-cholesterol concentration. Even though the exact mechanism that triggers these effects has not been fully elucidated, the results mentioned above suggest that upregulated oxidative stress promotes the development of MS. Even though a great number of clinical trials show that most antioxidants exhibit a wide range of effects in protecting against ROS, results are still controversial. At this point no strong recommendation can be made for the use of these compounds in clinical practice.

PRO-ATHEROGENIC ALTERATIONS IN METABOLIC SYNDROME

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The metabolic syndrome is defined as the concurrence of mutually associated cardiovascular risk factors including abdominal obesity, impaired glucose tolerance, hypertriglyceridemia, decreased high density lipoprotein (HDL)-cholesterol, and/or hypertension. In association with these factors, many investigators have described the activation of the sympathetic nervous system, renin-angiotensin system, and increased levels of proinflammatory adipokines and cytokines. The original conceptualization of this syndrome focused on a central role of insulin resistance, and this is clearly a concurrent and associated feature. More recently, the focus has been on the metabolic syndrome as an epidemiologic tool related to cardiovascular disease risk, and therefore traditional cardiovascular disease risk factors have been adopted as the defining features. Although the precise definition of what clinically constitutes the metabolic syndrome has generated considerable debate, it is well accepted that these comorbidities represent a pathological state that substantially augments risk for the development of type 2 diabetes mellitus and atherosclerotic cardiovascular disease. In previous studies, we have described diverse proatherogenic alterations associated with metabolic syndrome which include: a) hypoadiponectinemia, b) high soluble vascular cell adhesion molecule (VCAM)-1 and leukocyte adhesion molecule expression (monocyte CD54 and lymphocyte CD49d), c) an atherogenic lipid and lipoprotein profile, and d) altered HDL chemical composition and cardioprotective functions, accompanied by higher cholesteryl ester-triglyceride interchange carried out by cholesteryl ester transfer protein (CETP). In this pathological condition, CETP seems to be highly determinant of the metabolic syndrome-associated dyslipidemia. Of clinical relevance, CETP activity may be acceptably predicted by non-HDL-cholesterol and HOMA index. Moreover, CETP would also have a remodelling action on very low density lipoprotein (VLDL) in circulation, favouring, together with hepatic lipase, the formation of highly atherogenic small and dense low density lipoprotein (LDL) particles in patients with metabolic syndrome-associated hepatic steatosis, who, despite the presence of intrahepatic fat, also secreted higher number of VLDL particles. Hepatic steatosis was further associated with larger and triglyceride over-enriched circulating VLDLs, of greater atherogenicity. However, when hepatic steatosis progressed to severe fibrosis, circulating VLDL features apparently improved, probably due to early alterations in hepatic synthetic function. Furthermore, simple hepatic steatosis would play an important role increasing cardiovascular risk, independently of insulin resistance. Thus, metabolic syndrome was shown to be closely related to alterations of the whole lipoprotein spectrum as well as to many other proatherogenic metabolic disturbances.

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METABOLIC SYNDROME AND MALE HYPOANDROGENISM

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The prevalence of metabolic syndrome is significantly increasing over the years in direct relation to negative changes in eating habits and lifestyle. Several studies have shown that men with metabolic syndrome have significantly lower testosterone levels and a higher prevalence of hypogonadism. In recent years, great advances have been made in understanding the pathophysiological mechanisms underlying this association.

Patients with metabolic syndrome are considered to have lower testosterone levels due to multiple mechanisms secondary to obesity and insulin resistance; however, there is increasing evidence of an effect of hyperglycemia per se on the gonadal axis.

In obesity and in hyperinsulinemia secondary to insulin resistance, often present in patients with metabolic syndrome, there is a decrease in total testosterone related to lower sex hormone binding globulin levels resulting from decreased hepatic synthesis of this protein. However, in obesity, as well as in hyperinsulinemia, there is also a decrease in free and bioavailable testosterone levels, which implies a real decline in testosterone production.

In hypogonadal patients with metabolic syndrome or type 2 diabetes, gonadotropin levels are usually normal or low, which supports the diagnosis of hypo- or nor-

mogonadotrophic hypogonadism in most of these men. This reinforces the possibility of a failure at central level, which may be due to a hypothalamic defect and/or to an absence of pituitary response to GnRH.

Other proposed mechanisms is the negative-feedback inhibition of the gonadal axis at the hypothalamic-pituitary level due to higher estradiol concentrations secondary to a greater conversion of androgens into estrogens by the increased aromatase enzyme activity in adipose tissue.

Cell culture studies show that insulin stimulates the gonadal axis activity at all three levels: hypothalamic, pituitary and testicular. Patient with metabolic syndrome have hyperinsulinemia with hypoandrogenism. Anyway, this apparent paradox may be explained by the decreased insulin sensitivity observed in insulin-resistant states.

Leptin is also involved in the regulation of the activity of the gonadal axis. It has been proposed that kisspeptin neurons would play an important role in the nutritional/metabolic/reproductive integration. Kisspeptins are peptide ligands of the receptor GPR54, with stimulating effects on GnRH neurons. It has been demonstrated that kisspeptin neurons have leptin receptors and experimental data would allow us to conceive kisspeptins as mediators of leptin action on GnRH and as being part of the pathophysiology of hypogonadism in obesity. Hypothalamic NPY (a potent inhibitos of gonadal axis) neurons also express leptin functional receptor and might exert at this level an effect similar to that of insulin inhibiting NPY expression. Thus, resistance to leptin in obese subjects lower central effects of leptin with lower GnRH secretion and, in addition, hyperleptinemia secondary to leptin resistance inhibits testosterone secretion at the testicular level.

Inflammatory cytokines produced in adipose tissue may also contribute to the lower synthesis of testosterone at testicular level. By last, recent studies demonstrate the direct effect of hyperglycemia on the pituitary hypothalamus axis, with inhibition of hypothalamic activity.

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SAIC - YOUNG INVESTIGATOR AWARD - FUNDACIÓN HONORIO BIGAND

USE OF A PERSONALIZED MEDICINE STRATEGY TO IMPROVE THE SAFETY AND EFFECTIVENESS OF ISONIAZIDE TREATMENT

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Isoniazid is metabolized by genetically polymorphic NAT2. Different alleles are associated altered acetylation capacity and risk for drug decreased efficacy or toxicity. NAT2 polymorphism is also involved in the metabolism of many chemical compounds with clinical consequences. Methods: An oral isoniazid dose was prescribed to subjects, and three hours later blood was extracted for NAT2 genotyping (PCR-RFLP) and phenotyping (quantification of isoniazid and metabolite concentrations). Results: A chromatographic technique was validated for the simultaneous determination of isoniazid and acetyl-isoniazid. A total of 188 subjects (1 month to-44 years old), with no history of allergy or adverse reactions to isoniazid were analyzed. They received a weight-adjusted dose of 4.56 ± 0.89 (3.05-5.99) mg / kg. The present alleles were determined as: *4 (35.00%), *5 (41.00%), *6 (21.00%) and *7 (2.50%). Concentrations of isoniazid and its main metabolite were 3.16 ± 1.21 (1.51-5.83) µg / mL and 6.57 ± 4.82 (0.10 - 18.24) µg / mL, with an acetyl-isoniazid / isoniazid (MR) molar metabolic coefficient of 2.56 ± 2.23 (0.02-9.04). Antimodes of the trimodal distribution of MR (Rapid> 5.4, Intermediate 1.2 to 5.4 and Slow <1.2) were calculated for genotype/phenotype concordance analysis. A maturation process in pediatric subject was identified, reaching full metabolic capacity at 4 years. Discussion: The allelic distribution of the local population shows its own frequency and slightly different from that reported in other populations and studies. Both genotyping and phenotyping allow the differentiation of metabolic profiles that could be linked to the development of problems of lack of efficacy and/or toxicity and can be used in the provision of therapeutic individualization services. Genotype accurately predicts metabolic activity. Conclusions: pharmacogenetic NAT2 study is a useful tool for the differentiation of metabolic behaviors and eventual therapeutic individualization.

AN INCREASE IN NMDAR-GLUN2A EXPRESSION AS A LONG TERM MEMORY CONSOLIDATION MARKER

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NMDA receptors (NMDAR) play a critical role in synaptic plasticity, memory encoding and storage. These receptors are composed by two obligatory GluN1 subunits and two regulatory subunits: GluN2 (A-D) or GluN3 (A-B), being GluN2A and GluN2B the major regulatory subunits in central areas related to cognitive functions. It was already shown that there is an increase of GluN1 and GluN2A 70' after 5' exploration leading to habituation to a new environment (an open field: OF), in the hippocampus of 1, 2 and 3 month old Wistar rats. As this NMDAR subunits increase could be related to memory tracing; hence, we investigated if those changes would take place following other learning paradigms like object recognition (OR) or inhibitory avoidance (IA) tasks. In this work we show that 70' after OR training there is a significant increase in hippocampal GluN1 and GluN2A NMDAR subunits. As OR task depends on prefrontal cortex (CPF) we investigated if there is a change in CPF NMDAR subunits, however we found that GluN1, GluN2A and GluN2B subunits remains similar to controls, at least at the analyzed times.

Then we decided to investigate if similar changes occurs after IA training. As IA memory is related to amygdala, we decided to analyze also this structure. Western blot analysis shown that there is an increase in GluN1 and GluN2A subunits 70' after IA training in hippocampal protein extracts and also in amygdala extracts. However, these results does not demonstrate that NMDAR subunits changes is associated with memory consolidation. For this reason, we work with McGill APP rats that are an Alzheimer disease's model. In those rats, where IA LTM is not consolidated, NMDAR subunits did not change after IA training. These results strongly suggest that changes in hippocampal NMDAR subunits could be part of the trace of new spatial memories.

ROLE OF RAC3 COACTIVATOR IN CELLULAR DIFFERENTIATION

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Abstract: RAC3 is a member of the p160 family of Steroid Receptor Coactivators and is highly expressed in several human cancers, contributing to cell proliferation and cellular transformation. In this work, we have studied the role of RAC3 during adipogenesis. This is a highly regulated process which involves the cell cycle arrest and changes in the gene expression pattern, which is required for morphological remodeling. We found that RAC3 expression levels are downregulated during specific stimulus induced-adipocyte differentiation. Moreover, a constitutive decrease of RAC3 expression through a shRNA potentiates adipocyte differentiation shortening the time at which the adipocyte markers Perilipin, PPAR- and Oil Red O staining are detected. The RAC3 down regulation favors cell arrest and autophagy. Early and late autophagy inhibitors block adipocyte differentiation of control cells, but inhibit the shRAC3 differentiation partially. Although autophagy is required for adipogenesis, these results suggest that additional signals could be triggered by RAC3 down regulation. We conclude that RAC3 is a key regulator of adipogenesis whose down regulation generates cellular arrest and a permissive environment for autophagy allowing the early adipocyte differentiation.

STUDY OF ISOMERASE PROTEINS AS NEW REGULATORS IN THE ACTIVATION OF TRANSCRIPTION FACTORS RELATED TO STRESS AND INFLAMMATION

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Abstract: The study of proteins involved in biological response is important in order to promote the discovery of new regulators in signalling pathways. In certain malignancies, it is known that some pathways are altered, such as Nuclear Factor Kappa B (NF-kB) and Activator Protein 1 (AP-1) in chronic inflammation. In particular, we study new regulators for the activation of transcription factors related to stress and inflammation such as NF-kB and AP-1, with the final goal of provide new molecular target as a promising approach in therapeutic field. FK506-binding proteins (FKBPs) are Hsp90 Co-chaperones with peptidylprolyl-isomerase enzymatic activity, suppressed by the FK506 drug. Previously, FKBP51 and FKBP52 were characterized as steroid receptors modulators. We demonstrated that FKBP51 impairs NF-kB (p65/p50) activity, while FKBP52 enhances it. Furthermore, NF-kB target genes were highly induced by FKBP52, and its isomerase activity was decisive in this regulation. Importantly, both basal phospho-p65 and total p65 protein level were increased by FKBP52. In this context NF-kB activity was favored by a higher FKBP52/FKBP51 ratio. Moreover, in trophoblast cells AP-1 has an important regulatory role. Preeclamptic placentas show alterations on c-fos and FKBP52 expression. Our findings revealed that FKBP52 stimulated AP-1 transcriptional activity and promoted greater c-fos protein level. Interestingly, the overexpressed FKBP52 produced higher level of pERK/ERK for longer time. We propose FKBP52 as new key regulator on AP-1 and NF-B signalling, emphasizing its isomerase activity as essential. Thus, we suggest FKBP52 isomerase activity as an interesting target to prevent AP-1 and NF-kB biological activity.This findings represent a novel contribution in the development of new therapeutic agents for inflammatory diseases. Financial support: UBA, CONICET and PICT 2015-1603

CÉSAR MILSTEIN AWARD

CRYSTAL VIOLET CHEMICAL ANALOGUES INHIBIT THE Trypanosoma cruzi PROLINE TRANSPORTER TcAAAP069 AND EXERT TRYPANOCIDAL EFFECT.

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Trypanosoma cruzi, the etiological agent of Chagas disease, has a metabolism largely based on the consumption of glucose and proline. Proline is also involved in differentiation processes, cellular invasion and resistance to oxidative, metabolic and osmotic stress. We have previously identified the proline permease TcAAAP069 in *T. cruzi* and we showed the importance of this transporter on parasite survival. Crystal violet (CV) was used for several years as a blood additive for prevention of transfusion transmitted Chagas disease. Among the trypanocidal mechanisms proposed for CV, it was reported the protein synthesis inhibition related to inhibition of methionine and proline transport. The aim of this work was to validate CV as a proline transport inhibitor in order to use it as reference in a virtual screening protocol. CV entered at least in part through the proline permease TcAAAP069 and it also inhibited proline transport. Transgenic parasites overexpressing proline transporter (Tc069 parasites) were more sensitive to CV trypanocidal action (12.7μM for control parasites and 0.27μM for Tc069 parasites). The protective effect of proline in oxidative stress situations led to a 3-fold increase resistance for Tc069 parasites when proline was added along with CV. In order to obtain other drugs that might have similar effects to CV, a similarity-based virtual screening was performed, using the CV as query and different drug databases of compounds approved for use in humans. So far, three of the obtained compounds proved to be effective as proline transport inhibitors that also had trypanocidal effect. Taken together, these results show that it is possible to identify potential trypanocidal compounds by virtual screening using CV as a reference. Drug repositioning is a recommended strategy by the World Health Organization to fight neglected diseases, like Chagas disease, since costs and development time are significantly reduced for its application in therapy.

DAMAGE ASSOCIATED MOLECULAR PATTERN HMGB1 EFFECTS IN NEURONAL SYNAPTOGENESIS AND PROPAGATION OF REACTIVE GLIOSIS

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High-mobility group box-1 (HMGB1) is a DAMP released after focal brain injury that activates local innate immunity cells but also behaves as cytokine acting on the professional immune organs of the periphery. Local brain innate immunity cells are microglia and astrocytes that express pattern recognition receptors such as TLR2-4 and RAGE. We here dissected the HMGB1 effects on astrocytes, microglia and neurons and studied the downstream signaling. Primary astroglial cultures containing different amounts of microglial cells were obtained from postnatal 3-4 days rats or wt/TLR2KO/TLR4KO mice and primary neurons were from E18 rat embryos as described in detail in Rosciszewski et al., Mol.Neurobiol. (2017). In vivo, focal hemorrhagic brain ischemia was induced by cortical devascularization and animals received sulphazalazine (200mg/kg every 12hours) for 3 days. Our results showed that purified primary cortical neurons exposed to 50 or 500 ng/ml HMGB-1, presented a rapid increase in the number of synaptic puncta per neuron without showing significative neuronal loss. However, primary neurons exposed to conditioned medium secreted by 500 ng/ml HMGB1-treated astrocytes showed a reduced survival. In primary astrocytes, HMGB-1 exposure induced a dose-dependent NFkB activation and reactive gliosis; however astroglial response to HMGB1 required the presence of microglia. Loss of function studies showed that the HMGB1 effects are TLR2-TLR4 dependent and partially RAGE-dependent. In addition, HMGB-1 application on astrocytes induced TREM-2 expression. Finally, in vivo NFkB blockage with sulphazalazine significantly reduced reactive gliosis and neuronal degeneration induced by focal brain ischemia. We conclude that DAMP HMGB-1 has a major role in the propagation of proinflammatory response but also acts directly on the surviving neurons after focal brain injury. Supported by grants: PICT2012-1424; PICT2015-1451; UBACYT; PIP CONICET.

MODULATION OF EXPRESSION AND ACTIVITY OF P-GLYCOPROTEIN BY THE PROGESTIN NOMEGESTROL ACETATE IN HEPG2 CELLS. INVOLVEMENT OF MEMBRANE PROGESTERONE RECEPTOR.

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Introduction: Nomegestrol acetate (NMGA) is a synthetic progestin increasingly used for oral contraception. Due to long-term NMGA therapy, concurrent use with other drugs is highly feasible. P-glycoprotein (P-gp; ABCB1) is a key player in drug excretion. Alterations in its activity represent a common cause of drug-drug interactions (DDI). Up to date, there is scarce data about modulation of ABC transporters by NMGA.

Aims: (1) to evaluate the modulation of P-gp expression and activity by NMGA (0.5 – 500 nM) in the human hepatic cell line HepG2 and (2) to identify the underlying mechanisms.

Materials & methods: Protein expression was assessed by western blot and
corresponding mRNA by qRT-PCR. mRNA stability was evaluated using actinomycin D. Pgp activity was evaluated by quantifying the intracellular accumulation of the model substrate calcein by flow cytometry. Progesterone receptor (PR) and Gi protein were inhibited using RU486 and pertussis toxin, respectively. Membrane progesterone receptor a (mPRa) was knocked down using siRNA.

Results: NMGA (5, 50 and 500 nM; 48 h) increased P-gp protein expression (+83%, +77% and +93%, $p < 0.05$) with concomitant increase in P-gp activity. NMGA increased *ABCB1* mRNA expression (+34%, +35% and +36%, $p < 0.05$) without affecting its stability, suggesting a transcriptional mechanism. Regarding hepatic progestin receptors, NMGA mediated P-gp induction was not prevented by RU486, ruling out PR participation. By contrast, P-gp induction by NMGA was prevented by pertussis toxin and by mPRa knock down, confirming participation of both Gi protein and mPRa.

Conclusion: NMGA up-regulates P-gp expression and activity through a mPRa-Gi protein dependent transcriptional mechanism. This is the first reported genomic effect of a progestin on ABC transporters that is independent of the classical PR. The data suggest the possibility of DDI, potentially affecting the therapeutic efficacy of drugs coadministered with NMGA.

A PHYSIOPATHOLOGICAL STUDY OF THE MATURATIONAL CHANGES OCCURRED IN HUMAN AND RHESUS MONKEY TESTIS FROM BIRTH TO PUBERTY - TRANSCRIPTIONAL AND EPIGENETIC MODIFICATIONS

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To date, data regarding the maturational changes occurring in primate testes is poor, mainly due to the scarce studies in non-human primates and the limited normal human testes available. In contrast to primates, where a long period exists between birth and puberty, in rodents, childhood is practically non-existent making it inappropriate to extrapolate testicular development studies between species. For all this, our objective is to carry out a thorough analysis of the histological, transcriptional and epigenetic modifications of the human and Rhesus monkey testis from late fetal to pubertal subjects and contrast it with Disorders of Sex Development.

Leydig cell differentiation stages will be analyzed in depth, focusing on their morphological and gene expression changes (LHCGR, STAR, CYP11A1, HSD3B2, CYP17A1, HSD17B3, SRD5A1, SDR5A2, AKR1C1-4, HSD17B6, DLK1, AR) and insulin-like factors (IGFs) will be studied as possible regulators at prepuberty. The primary testicular androgen synthesis pathway (either front or back-door) will be investigated throughout postnatal life.

Patients with Androgen Insensibility Syndrome are excellent models to study the androgenic actions in the human testis. A comparison of the expressed genes and histological characteristics, focusing on germ cells differentiation and malignization (TSPY, MAGE-A4, OCT4, KIT, GFRA1), would help elucidate the role of androgens at prepuberty.

The Rhesus monkey is an excellent experimental model to determine the testicular transcriptome of the juvenile primate. The first gonadotropin associated changes in testicular gene expression produced by an in vivo induction of puberty (FSH+LH for 2 and 4 days) will be determined, as well as their regulation by miRNAs.

This plan uses: RT-qPCR, Western Blot, RNA-Seq, miRNA-Seq, bioinformatic pipelines, pathway analysis software, human primary cell and organotypic culture, immunohistochemical staining, steroid extraction and

concentration determination by HPLC-MS/MS.

YOUNG RESEARCHERS IN PHYSIOLOGY, SAFIS 2017 AWARD

ADENOVIRUS-MEDIATED HUMAN AQUAPORIN-1 EXPRESSION IN HEPATOCYTES IMPROVES LIPOPOLYSACCHARIDE-INDUCED CHOLESTASIS.

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Lipopolysaccharides (LPS) are known to cause cholestasis in sepsis. There is evidence that a defective expression of canalicular aquaporin water channels contributes to bile secretory failure in LPS-induced cholestasis. Thus, we studied whether the hepatic adenovirus-mediated transfer of human aquaporin-1 gene (*haqp1*) can improve the cholestasis induced by LPS. Adenoviral vector encoding hAQP1 (AdhAQP1) or control vector was administered to rats by retrograde intrabiliary infusion. Hepatocyte canalicular hAQP1 expression was assessed by liver immunostaining and immunoblotting in purified plasma membranes.LPS significantly reduced bile flow and biliary bile salt (BS) excretion by 30% and 45%, respectively. AdhAQP1-treatment normalized both bile flow and biliary BS excretion in LPS-induced cholestasis. Moreover, markedly elevated serum BS levels in cholestatic rats, were almost restored with the AdhAQP1 hepatic transduction (control(C):34±4;C+AdhAQP1:29±6;LPS:114±11*;LPS+AdhAQP1:58±12 μM; *P<0.05).Bile flow and serum or biliary BS in normal rats were not significantly altered by AdhAQP1. AdhAQP1-treatment unaffected the downregulated protein expression of canalicular bile salt export pump (BSEP/ABCB11) in cholestasis, However, AdhAQP1 delivery recovered the biliary output of an intravenous tracer dose of [^{3}H]taurocholate (C:20.1±1.4;C+AdhAQP1:18.8±1.6;LPS:7.3±2.1*;LPS+AdhAQP1:17.1±2.7 cpm.10^{-3}; *P<0.05), thus suggesting an improvement of BSEP transport activity. These may be associated to the finding that hAQP1 expression restores reduced canalicular cholesterol content(C:0.25±0.01;C+AdhAQP1:0.26±0.02;LPS:0.18±0.01*;LPS+AdhAQP1:0.26±0.02 μmol cholesterol/mg protein; *P<0.05).Our data suggest that the adenovirus-mediated hepatocyte hAQP1 expression improves LPS-induced cholestasis in rats by stimulating the BSEP/ABCB11-mediated biliary BS excretion; a finding that might contribute to the understanding and treatment of sepsis-associated cholestatic diseases.

A NEW ROLE FOR LYSOPHOSPHATIDIC ACID IN VASCULAR PROCESSES AT THE MATERNAL-FETAL INTERFACE DURING EARLY GESTATION.

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Successful implantation requires that the invading trophoblast acquires an endovascular phenotype and remodels uterine spiral arteries. Defects in this mechanism correlate with obstetric complications as implantation failure and preeclampsia. Lysophosphatidic acid (LPA) participates in embryo implantation and contributes to vascular physiology in different biological systems. However, the role of LPA on vascular processes at the implantation site has not been investigated. We adopted an *in vitro* and an *in vivo* pharmacological approach to study LPA action on trophoblast endovascular response and uterine transformation. The HTR-8/SVneo cell line (H8) was used to model the acquisition of the trophoblast endovascular phenotype. LPA (10 μM) increased H8 tube formation (6h), migration (wound healing, 18h) and proliferation (MTT assay, 48h) ($p<0.05$). By using selective antagonists, we showed that enhanced tubulogenesis was mediated by LPA3 receptor. In addition, COX-2 and iNOS pathways participated in LPA-stimulated tubulogenesis, being nitric oxide the last effector. When we investigated if the master hormones that orchestrate crucial events at the implantation sites, modulate trophoblast angiogenesis via LPA/LPA3, we observed that estradiol (10^{-5} M) + progesterone (10^{-7} M) increased H8 tube formation via LPA3 ($p<0.05$). The interaction between the trophoblast and the endothelium is relevant during the remodeling of the spiral arteries. We observed that trophoblast LPA-triggered secreted factors increased the migration of the EA.hy926 endothelial cell line ($p<0.05$). Finally, the administration of a selective LPA3 antagonist to Wistar rats in day 5 of gestation increased the rate of embryo resorption (60%) associated to macro and micro-

vascular defects. Our results demonstrate a new role for LPA during spiral artery remodeling at the maternal-fetal interface, and could help to understand obstetric complications as implantation failure and preeclampsia.

THE ROLE OF THE DENTATE GYRUS AND PERIRHINAL CORTEX IN PATTERN SEPARATION OF NON-SPATIAL MEMORIES

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Successful memory involves not only remembering information over time but also keeping memories distinct and less confusable. The ability to separate the components of memories into distinct memory representations relies on pattern separation, a computational process by which differences are amplified to disambiguate similar events. Despite the importance of this mnemonic function, the molecular mechanisms and signals necessary for the behavioral manifestations of this process remain unknown. Although pattern separation has been localized to the dentate gyrus (DG) of the hippocampus and shown to occur in a spatial domain, this cognitive function is thought to take place also during processing of other types of information. The perirhinal cortex (PRH) is involved in the acquisition and storage of object memories, and it was shown to be crucial for the resolution of tasks with ambiguous features. However, there is some debate on whether the DG participates in pattern separation of non spatial representations. Here we use a modified version of the object recognition task and manipulated the load of pattern separation during encoding of the task. We show that two plasticity-related proteins, BDNF and Arc, are required for pattern separation of object memories in the PRH and that Arc is also required in the DG for this process. In addition, while exogenous BDNF was able to enhance pattern separation when injected into PRH, it was not effective when infused within the DG. Finally, BDNF injected into PRH was able to rescue the memory deficit produce by blockade of Arc in the DG. These results reveal a complex interaction between plasticity mechanisms in the PRH and the DG for non spatial pattern separation and posit the Prh as the key structure where unique object representations are stored.

MATERNAL DIETARY INTAKE OF LIPOIC ACID DURING PREGNANCY AND LACTATION INFLUENCES METABOLISM AND WEIGHT GAIN IN THE WISTAR RAT OFFSPRING.

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Lipoic acid (LA) is an antioxidant that easily diffuses trough biological membranes after oral intake. The anti-obesogenic properties of LA are given by a modification of genes related with metabolism and energy expenditure. On the other hand, the fetal period is characterized by strong changes in gene expression that if altered, can influence the phenotype of the organism in the postnatal life. The objective was to determine the effect of maternal intake of LA -during the period that the appetite regulatory network is established in the fetal rat- on lipid deposition and metabolism in the adult offspring. Pregnant Wistar rats were fed ad-libitum with ground food + LA (200 mg/kg) from day 14 of gestation to day 20 of lactation (n=3) or ground food (n=3). After litter reduction, 12 males and 12 females born from the LA fed rats (LA, n=24) or not (Control, n=24) were used to evaluate weekly body weight, food intake, plasmatic levels of cholesterol, glucose and triglycerides and IL6 levels in liver homogenates trough ELISA at 3 months of age. The effect of group was analyzed by t-test for each gender. Compared to Control rats, overall body weight for AL rats was 11.4% heavier for males ($p<0.001$) and 7.5% heavier for females ($p=0.01$), and daily food intake tended to be higher for AL rats ($0.05>p<0.1$). However, LA rats presented lower liver weight ($p=0.024$), lower rate of liver weight/body weight ($p=0.02$) and lower levels of IL6 in liver (males: 2.1± 0.03 pg/mlvs 2.5± 0.07 pg/ml, $p=4.1x10^{5}$; females: 1.2± 0.03 pg/mlvs 1.7± 0.02 pg/ml, $p=3.5x10^{-8}$). Also, AL rats had lower triglycerides levels (males: 74.3± 23.6mg/dlvs 136.6± 25.7mg/dl, $p=0.036$; females: 94.6± 13.0mg/dlvs 125.3± 9.7mg/dl, $p=0.031$) and no differences were detected in cholesterol and glucose levels. In conclusion, these results suggest that maternal intake of LA improves energy expenditure in the offspring, probably stimulating protein synthesis in the muscle and reducing fat deposition.

SAIC- FUNDACION GADOR AWARD

THE SUBPOPULATION OF GABAERGIC PROOPIOMELANOCORTIN NEURONS OF THE HYPOTHALAMUS REGULATE ENERGY BALANCE

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Obesity is an epidemic disease that affects 600 million adult people worldwide, which is a consequence of alterations in energy balance. The arcuate nucleus of the hypothalamus is a key area that integrates peripheral signals of energy status and regulates energy homeostasis by projecting to second order neurons. Arcuate proopiomelanocortin (POMC) neurons inhibit food intake and increase energy expenditure. Pomc mutant humans and mice develop early onset severe obesity, what demonstrates that POMC neuropeptide is one of the main regulators of energy balance. Some studies reveal the existence of subpopulations of POMC neurons secreting the antagonistic neurotransmitters glutamate or GABA, but their physiological roles and targets remain to be dissected. In order to elucidate the role of GABAergic POMC neurons, we intercrossed a reversible Pomc knockout mouse line (in which Pomc expression can be conditionally reactivated), with another line bearing a GABA-specific Cre driver. These mice developed obesity but, after the rescue of Pomc in GABAergic neurons, they normalized food intake and metabolic efficiency. Notably, females significantly decreased their body weight and reached an energy balance that matches that of lean control mice. Surprisingly, these physiological improvements were achieved with the recovery of Pomc expression in only 25% of total hypothalamic POMC neurons. Immunohistochemical analysis showed that GABAergic POMC neurons preferentially project to the dorsomedial hypothalamus (DMH), a nucleus that induces food intake by releasing NPY. In order to emphasize the role of GABAergic subpopulation, by using another mouse line expressing a ubiquitous Cre, we found that the partial rescue of Pomc, unrestricted to any subpopulation, failed to improve body weight, food intake or metabolic efficiency. Altogether, these results show that GABAergic POMC neurons have a major role in the establishment of energy balance, probably, through an arcuate-DMH circuit.

CAFETERIA DIET INDUCES PROGRESSIVE AND DIFFERENTIAL CHANGES IN BRAIN MECHANISMS INVOLVED IN FOOD INTAKE CONTROL OVER TIME

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We aim to determine the effects of Cafeteria diet (CAF), rich in palatable and energy dense foods, on the expression of key genes of the brain reward system (RW) in the short and long term. Female Wistar rats were fed chow or CAF for 4 or 11 weeks. Animals were sacrificed and 2 regions of the Accumbens Nucleus (NA – Core, NAC; and shell, NAS), Ventral Pallidum (VP) and Ventral Tegmental Area (VTA) were dissected. Serum leptin was assessed by RIA. mRNA expression of genes of the dopaminergic and GABAergic pathway, and the leptin receptor (ObRb) was evaluated by qPCR in the nuclei. Data was statistically analyzed by two-way ANOVA followed by Tukey post-test. Four weeks of CAF increased energy intake and adiposity, not affecting circulating leptin or body weight. In VTA, 4 weeks of CAF increased the expression of the dopamine active transporter (DAT) and decreased both isoforms of the enzyme involved in the synthesis of GABA (glutamate decarboxylase, GAD 1 and 2), without altering tyrosine hydroxylase (TH) expression. CAF decreased dopamine receptor (DR) 2 expression in NAS and increased DR1 levels in NAC. Also, CAF increased GAD2 levels in VP. After 11 weeks of CAF, animals sustained the hyperenergetic intake and further increased adiposity, leading to hyperleptinemia and higher body weight, only concomitant to an increased expression of ObRb in VTA. Our results indicate that the higher energy intake of CAF animals in the short-term would respond to hedonic mechanisms, given by molecular deregulations in the RW. The palatability of the diet could lead to a hypodopaminergic state, as DAT expression increase in VTA and DR2 decrease in NAS. Besides, the increment in GAD2 expression in VP indicates an inhibitory GABAergic input to dopaminergic and GABAergic VTA neurons that may, inhibit dopamine and GABA release, in line with the low expression levels of GAD1 and GAD2. Conversely, in the long-term the hypercaloric intake could respond to an altered homeostatic control.

METFORMIN PROMOTES ß-CATENIN AND E-CADHERIN PLASMA MEMBRANE TRANSLOCATION

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Colorectal cancer (CRC) is the third most common diagnosed cancer globally and the third most frequent cause of cancer-related deaths in Argentina. In colorectal tumors the first oncogenic mutation provides selective advantage to the epithelial cell that multiplies and generates a microadenoma. In the majority of sporadic colorectal tumors these "initiatory" mutations frequently occur in genes associated to the Wnt pathway. Subsequent mutations in other genes, such as TP53, PTEN, SMAD4, KRAS and PIK3CA are followed by clonal expansion of transformed cells. Despite advances in surgical techniques, improved chemotherapy and early detection, CRC is still associated with a relatively poor prognosis. At least 40% of patients who undergo resection of the primary tumor die within 5 years. CRC is a major health problem demanding new approaches for its prevention and treatment.

Several lines of evidence indicate that metformin, a first-line oral anti-diabetic agent for type 2 diabetes, has an antitumor effect. For example, a recent meta-analysis that included 23.255 participants from six cohort studies revealed that diabetic patients with CRC taking metformin achieved an estimated overall survival benefit of 44% compared with non-metformin users. The mechanism mediating this effect has not been elucidated.

Results from our laboratory show that metformin inhibits the proliferation of human cells derived from primary adenocarcinoma of the colon by a mechanism that involves ß -catenin and E-cadherin. Specifically, we found that metformin: 1- inhibits the phosphorylation of ß-catenin Ser552, 2- promotes the plasma membrane redistribution of ß-catenin and E-cadherin, 3- inhibits ß-catenin-mediated transcription, and 4- inhibits cell's motility. Overall, our studies reveal two novel metformin targets and suggest a mechanism by which this anti-diabetic drug interferes with CRC development.

VMP1-RELATED AUTOPHAGY CONFERS RESISTANCE TO TREATMENT AND HYPOXIA IN TUMOR CELLS FROM HUMAN GASTROINTESTINAL ADENOCARCINOMAS

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Autophagy is an evolutionarily conserved degradation process of cytoplasmic cellular constituents. It has been suggested that autophagy plays a role in both tumor suppression and tumor progression. Tumor cells develop autophagy as an adaptation to stressful conditions such as hypoxia and treatment. VMP1 is a transmembrane protein necessary for autophagy induction. Previously, we demonstrated that the PI3K-AKT1 signaling pathway activates the GLI3-p300 complex that binds to the VMP1 promoter to regulate its activity in pancreatic tumor cells. Here we characterize new molecular pathways mediated by VMP1, by which gemcitabine, in human pancreatic tumor cells and hypoxia, in colon cancer cells, can trigger autophagy and resistance to treatment. We demonstrate that gemcitabine requires VMP1 expression to induce autophagy in highly resistant pancreatic cancer cells PANC-1 but not in less resistant BxPC-3 cells. Analysis of the mechanisms identified E2F1 as an effector of gemcitabine. We show that E2F1 regulates the expression and promoter activity of VMP1. Moreover, our study demonstrates that the pharmacological stabilization of HIF-1α significantly increases VMP1-related autophagy through binding to hypoxia responsive elements in VMP1 promoter. Moreover, HIF-1α-induced autophagy increases cell survival after photodynamic therapy of CaCo2 and Sw480 cells. Chromatin immunoprecipitation assays showed that E2F1 and HIF-1 bind to the VMP1 promoter in PANC-1 and Caco2 cells, respectively. Finally, we demonstrate that downregulation of VMP1 expression and pharmacological modulation of autophagy sensitize cells to antitumor treatments. We described two novel transcriptional regulation mechanisms of autophagy mediated by HIF-1α/VMP1 and E2F1/VMP1 pathways that may explain resistance in colon and pancreas adenocarcinomas. These findings are of high clinical relevance since they integrate VMP1-related autophagy to the complex network of events involved in tumor cell resistance.

CYTOKINE-INDUCED ENDOPLASMIC RETICULUM STRESS IN β-CELLS IS AMELIORATED BY COMPOUND A

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Type 1 diabetes (T1D) is a T cell-mediated autoimmune disease that selectively destroys insulin producing β-cells. ER stress and subsequent insulin secretory deficiency in β-cells precede the onset of autoimmune diabetes. Pro-inflammatory cytokines (IL-1+IFN-; CYT) signaling leads to activation of ER stress in β-cells. We reported that Compound A (2-(4-acetoxyphenyl)-2-chloro-N-methyl-ethylammonium chloride; CpdA), a dissociative glucocorticoid receptor (GR)-ligand, is an effective modulator of T and dendritic cells. The aim of this study was to explore the beneficial effects of CpdA on CYT-induced β-cell ER stress; the classic GR-ligand dexamethasone was used for comparison. CpdA significantly reduced CYT-induced NO secretion by the insulinoma INS-1E cells ($p<0.01$). CpdA treatment inhibited CYT-triggered IB phosphorylation protecting its degradation and hampering, in consequence, NF-B nuclear translocation in INS-1E cells ($p<0.05$). CpdA treatment impaired eIF2α phosphorylation enhancement and the increase of ER stress protein markers expression, such as ATF4 and CHOP, in CYT-challenged INS-1E cells ($p<0.05$). The expression of chaperones involved in protein folding and processing (PDI, ORP150) was enhanced in the presence of CpdA ($p<0.05$). CpdA administration (i.p.) to NODscid mice adoptively transferred with diabetogenic splenocytes (from diabetic NOD mice) led to a delay of disease onset ($p<0.001$ vs control). CpdA-treated mice showed a reduction in islet leukocytes infiltration and preserved insulin expression in comparison with veh-treated group, assessed by immunohistochemistry. In summary, we demonstrate that CpdA directly improves the UPR attenuating ER stress in -cells challenged by CYT. The latter together with our previous reports on immune cells modulation, might warrant the administration of CpdA as a novel therapeutic strategy with dual activity on autoimmune diabetes.

SAIC - RUBÉN CHERNY AWARD

PROTEIN KINASE D1 REGULATES MITOSIS PROGRESSION.

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Protein kinase D1 (PKD1) is a serine/threonine kinase that is activated by pharmacological agents such as phorbol esters and physiological stimuli including GPCRs stimulation, growth factors and oxidative stress among others. During the interphase PKD1 play an important role in the regulation of a remarkable variety of biological processes including signal transduction, cell migration, differentiation, proliferation and the development of different types of cancer. Nevertheless very little is known about the role of this kinase during other phases of the cell cycle including cell division.

Cell division is a complex process regulated by many macromolecules that are controlled by kinases and phosphatases which in turn are regulated by mitotic signaling pathways that remain poorly defined.

Hence, the principal aim of this study was to determine whether PKD1-mediated signaling contributed to mitosis regulation. We employed cells lines expressing eGFP or eGFP-tagged PKD1, under the control of an inducible promoter, to examine by real-time imaging of single-cells maintained under physiological conditions the role of PKD1 during mitosis. This methodology was complemented by indirect immunofluorescence of fixed cells, Western blot and flow cytometry (FACS). Using these methodologies we obtained the following results: 1- Catalytic active PKD1 colocalizes with mitotic spindle, centrosomes and midbodies 2- Over-expression of the kinase retards mitosis progression, specifically lengthening metaphase duration by 50% ($p<0.001$) 3- Inhibition of PKD1 activity prior to entry into mitosis stops cell cycle progression (Coefficient of variation (CV)<8, 0) 4- Cells that were incubated in the presence of PKD1 specific inhibitors, after entrance into the mitotic phase, do not progress through the cycle preventing the completion of mitosis (CV<8.0). These data, therefore, indicates that PKD1 regulates the progression of cellular mitosis.

RSPO3, A POSITIVE WNT PATHWAY MODULATOR, INDUCES MESENCHYMAL FEATURES IN MAMMARY CELLS AND IS DIFFERENTIALLY EXPRESSED BY THE BASAL-PROGENITOR SUBPOPULATION AND TRIPLE-NEGATIVE BREAST CANCERS.

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Abstract: Breast cancer is a leading cause of death among women worldwide. R-spondin3 (RSPO3) is a member of a family of secreted proteins that enhance canonical and non-canonical Wnt signaling pathways. Due to their Wnt-potentiating activity, RSPOs have been postulated as stem cell growth factors and are implicated in diverse processes like embryonic development, tissue differentiation and human diseases including cancer. However, the role of RSPO3 in mammary gland and breast cancer development remains unclear. We identified MMTV insertion sites close to Rspo3 gene, promoting its overexpression and the consequent tumor formation. Therefore, our goal was to investigate the biological function of RSPO3 in normal and tumor mammary cells. Recently, we found that RSPO3 is expressed in the basal stem cell-enriched compartment of normal mouse mammary glands whereas it is absent from committed mature luminal cells, in which exogenous RSPO3 impairs lactogenic differentiation. In addition, RSPO3 knockdown in basal-like mouse mammary tumor cells reduces canonical Wnt signaling, epithelial-to-mesenchymal transition-like features, migration capacity, and tumor formation in vivo. Importantly, RSPO3 overexpression, which is associated with leucine-rich repeat-containing G-protein coupled receptors 4, 5 and 6 (LGR4-6) levels, highly correlates with the basal-like subtype among breast cancer patients, particularly with triple-negative claudin-low tumors. Thus, we identified RSPO3 as a novel key modulator of breast cancer development that may become a potential target for treatment of the basal subtype which lacks efficient therapeutic options to date.

ACQUISITION OF METASTATIC PROPERTIES IN CARCINOMA CELLS: IDENTIFICATION OF A NOVEL PHOSPHORYLATION SITE IN CIP4 REGULATED BY THE PROTEIN COMPLEX AKAP350/PKA WITH A CENTRAL ROLE IN TUMOR INVASIVENESS.

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Cancer malignancy is associated to cancer cells capacity to invade neighboring and distant tissues. Carcinomas are defined by their epithelial origin and constitute the most common type of cancer. Carcinoma metastasis requires epithelial derived tumor cells to acquire a mesenchymal phenotype (EMT). During EMT, cells lose contacts due to ablation of E-cadherin and acquire increased motility. CIP4 is a cdc42 effector essential for EMT, whose expression levels correlate with cell invasiveness in breast cancer. CIP4 structure and role in membrane plasticity has been characterized; however, how CIP4 function is regulated remains elusive. We have previously shown that CIP4 is a PKA substrate that interacts with AKAP350, and that this interaction is essential for CIP4 promotion of hepatocellular carcinoma (HCC) cell migration. The aim of this study was to evaluate if CIP4 pro-invasive ability is regulated via phosphorylation by PKA. Using in silico analysis and in vitro phosphorylation assays, we characterized that CIP4 has a unique PKA phosphorylation site (CIP4T225). Expression of its phosphomimetic mutant CIPT225E decreased E-cadherin levels (-51%*) and increased migratory efficiency (+44%*) in HCC cells. Conversely, expression of CIP4 not phosphorylatable mutant CIP4T225A inhibited HCC cell migration (-43%*). Pharmacological inhibition of PKA decreased HCC migratory efficiency in control (-40%*), but not in CIP4T225E or CIP4T225A cells. Transwell invasion assays showed that HCC invasiveness was increased in CIP4T225E (+800%*) and inhibited in CIP4T225A (-90%*) cells. In vivo studies confirmed

the in vitro results, showing increased formation of metastatic nodules in lungs of athymic mice injected with CIP4T225E cells. These findings unveil a novel-signaling pathway involving CIP4T225 phosphorylation by PKA as a crucial event in the acquisition of metastatic properties in HCC cells. We are currently evaluating this regulatory pathway in breast cancer cells. *p<0,05

HIGH RAC3 EXPRESSION LEVELS ARE REQUIRED FOR INDUCTION AND MAINTAINING OF CANCER CELL STEMNESS.

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RAC3 is a transcription coactivator, usually overexpressed in several tumors and required to maintain the pluripotency in normal stem cells. Although its involvement in tumor development has been amply investigated its potential role inducing or maintaining cancer cell stemness has not been determined.

In this work we studied the association between RAC3 overexpression and cancer cell stemness and the capacity of this protein to induce cancer stem properties in non tumoral cells.

We performed in vitro and in vivo experiments using two strategies: by overexpressing RAC3 in the non tumoral cell line HEK293 by transfection with a RAC3 expression vector and by silencing RAC3 in the human colorectal epithelial cell line HCT116 with a specific shRAC3. Furthermore, we analysed public repository microarrays data from 19 human colorectal tumors in different developmental stages.

In the microarrays analysis, we found that RAC3 overexpression was mainly associated to early and advanced stages of colon cancer ($p \leq 0,05$), involving increased expression of RAC3, Vimentin, cMYC, OCT4 and Nanog mRNA ($p \leq 0,05$). In turn, RAC3 silencing in HCT116 induced diminished tumoral properties and cancer stem cells (CSC) as determined by Hoechst efflux, tumorspheres and clonogenic growth ($p \leq 0,01$), which correlated with decreased Nanog and OCT4 expression ($p \leq 0,05$). Moreover, RAC3 overexpression was mainly associated to CD133+ side population ($p \leq 0,05$). In non tumoral cells, RAC3 overexpression induced tumoral transformation; mesenchymal phenotype, migration, invasion, metalloproteinases production ($p \leq 0,05$), proliferation under low serum ($p \leq 0,05$), clonogenic tumorspheres growth ($p \leq 0,05$), OCT4 and Nanog expression ($p \leq 0,05$). Moreover, these transformed cells generated tumors in vivo.

Our results demonstrate that RAC3 is associated to cancer stem phenotype not only maintaining the stemness in cancer cells, but also inducing cancer stem like cells by overexpression in non tumoral cells.

ENGINEERING AN ENDOGENOUS LECTIN TO TREAT AUTOIMMUNE DISEASES.

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Glycosylation is a highly regulated process capable of modulating the fate and function of T cells. In the present work, we studied how differential glycosylation can modulate regulatory (Treg) and effector T (Teff) cell function and capitalized on this information for the design of a novel therapeutic strategy for autoimmune diseases. We found that Galectin-1 (Gal1) treatment ameliorates the clinical symptoms of mice bearing experimental autoimmune encephalomyelitis (EAE) ($p<0.05$), animal model of multiple sclerosis (MS), by dampening Th1 and Th17 responses while enhancing the Treg cell compartment ($p<0.01$). Mechanistically, Tregs but not Teff cells, are protected from Gal1-induced apoptosis ($p<0.001$) through differential N-glycan branching and α2,6 sialylation (α2,6SA) of cell surface glycoproteins. In MS patients, we found that during the relapse phase of the disease they show low levels of circulating Gal1 ($p<0.001$), and identified a subpopulation of CD4 T cells –absent during the remitting phase– characterized by low levels of α2,6SA ($p<0.001$) and susceptibility to Gal1-induced apoptosis ($p<0.001$). To capitalize on this information for therapeutic purposes, we studied physicochemical properties that hinder translation of this immunoregulatory lectin to clinical settings. We found that Gal1-induced apoptosis of T cells is impaired by both acidic ($p<0.05$) and oxidative conditions ($p<0.01$), typical hallmarks of inflammatory settings. Thus, we genetically engineered Gal1 protein to generate stable mutants capable of circumventing these limitations. These mutants showed enhanced capacity to induced apoptosis of activated T cells ($p<0.05$), IL-10 secretion by T cells ($p<0.001$) and IL-27 secretion by DCs ($p<0.001$). Finally, these mutants showed enhanced therapeutic potential in mouse models of MS (EAE) ($p<0.001$), arthritis (CIA) ($p<0.01$) and colitis (TNBS-IBD) ($p<0.05$). Our findings provide novel therapeutic strategies for treating a broad range of autoimmune diseases.

SAI – SATZ AWARD

BIOINFORMATICALLY GUIDED DISCOVERY OF NOVEL Trypanosoma cruzi EPITOPES RECOGNISED BY CD4⁺ AND CD8⁺ T CELLS FROM CHRONIC CHAGAS DISEASE PATIENTS

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T cell-mediated response has been proven to play a major role in controlling *T. cruzi* infection and parasite burden. It also seems to be involved in the progression from asymptomatic Chagas disease (ACD) to chronic Chagas cardiopathy (CCC) in chronically infected patients. However, the complexity of the parasite-host interactions hampers the identification and characterization of T cell activating epitopes. We approached this issue by combining *in silico* and *in vitro* methods to interrogate patients' T cells specificity.

The sequences of the 53 *T. cruzi* proteins annotated on the Immune Epitope Database (IEDB) were split in all possible 15-aminoacid long peptides, and 50 candidate peptides were selected using a bioinformatic pipeline (MHCpan, MHCIIpan, PopCover), based on conservation between annotated parasite strains, non-identity with *H. sapiens* sequences, class I and II MHC molecules binding affinity and HLA polymorphic variants coverage. Candidate peptides were randomised in 5 pools of 10 peptides each, which were used to challenge peripheral blood mononuclear cells (PBMC) from chronic Chagas disease patients, in IFN-γ ELISPOT assays. Positive pool-patient pairs were re-assayed in a single-peptide fashion to identify individual active peptides. A total of 7 peptides induced IFN-γ secretion in at least one patient's PBMC, 4 of which do not contain any previously described epitope. In combination, response to these peptides covered 33% of the patients cohort in this study (n=51), 40% of the ACD group (n=25) and 27% of the CCC group (n=26). IFN-γ ELISPOT with CD8- or CD4-depleted PBMC showed that 6 of the peptides contain MHC class II epitopes, while 1 contains an MHC class I epitope. The fact that most of these subjects responded to 1 or 0 peptides is a strong sign of HLA restricted epitopes. In summary, we predicted, validated and characterized 7 T cell-activating peptides from *T. cruzi* antigens, 4 of which contain epitopes first described in this work.

THE BTK INHIBITOR IBRUTINIB IMPAIRS THE INNATE IMMUNE RESPONSE AGAINST Mycobacterium tuberculosis MEDIATED BY MACROPHAGES AND γδ T CELLS.

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The Bruton's Tyrosine Kinase (Btk) inhibitor, ibrutinib (ibru), was recently approved for the treatment of Chronic Lymphocytic Leukemia (CLL) patients. Previously we found that ibru affected macrophage polarization and TNF-α secretion in response to *M. tuberculosis* (Mtb). Considering that ibru is being introduced in countries with high incidence rates of tuberculosis (TB), such as Argentina, we aimed to extend the study of the effect of ibru on the immune response to Mtb mediated by macrophages and γδ T cells, which are central players in TB innate immunity.

Macrophages were differentiated by culturing human monocytes with M-CSF. TNF-α, IL-8 and IL-10 secretion were measured by ELISA after macrophage stimulation with irradiated Mtb, Pam3CSK4 or LPS for 24h. Phospho-p65 of NFkB was evaluated by Western blot. Phagocytosis of Mtb-FITC by macrophages was evaluated by flow cytometry. Bacillary loads were determined by colony-forming units assay. Human γδ T cells were purified by using MicroBead isolation kit and after 24h of Mtb stimulation, IFN-γ was evaluated by ELISA and CD69 expression by flow cytometry. Statistical significance was determined using the Friedman test and the Dunn's post-test.

We found that ibru significantly reduced TNF-α, IL-10 and IL-8 secretion (n=10, $p<0.05$) and diminished phospho-p65 (n=5, $p<0.05$) in response to Mtb. Moreover, ibru impaired TNF-α secretion by macrophages in response to LPS and Pam3CSK4 (n=10, $p<0.05$). Then, we found that ibru significantly enhanced Mtb phagocytosis (n=14, $p<0.05$) and preliminary results showed a slight increase in the bacillary loads obtained from ibru-treated macrophages. CD69 expression and IFN-γ secretion was impaired in ibru-treated γδ T cells in response to Mtb (n=8, $p<0.05$).

Our results suggest that the innate-immune response to Mtb might be compromised in ibru-treated patients. Thus, increased awareness should be taken during ibru treatment especially in countries with high incidence rates of TB.

SAFIS - CAMILIÓN DE HURTADO AWARD

SILENCING OF CARDIAC EPIDERMAL GROWTH FACTOR RECEPTOR REDUCES CARDIAC HYPERTROPHY OF ADULT SPONTANEOUSLY HYPERTENSIVE RAT.

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Epidermal growth factor receptor (EGFR or ErbB1) is an integral membrane protein, that together with other members of the family, ErbB2 and ErbB4 are necessary for a normal adult heart function. EGFR transactivation was linked to pathological cardiac hypertrophy (PCH). Experimentally, its pharmacological inhibition prevented PCH. The objective of this study was to limit PCH by a specific and localized silencing of cardiac EGFR of spontaneously hypertensive rats (SHR) with small interference RNA (siRNA). Animals were treated with a sequence that inhibits EGFR expression (l-shEGFR, n=9) or with the non-silencing disordered sequence (l-shSCR, n=10) as control. To promote siRNA entry into the myocardium, it was integrated into the lentivirus genome and injected ($1x10^7$ TU / 200 µl) attwo sites of the anterolateral heart wall. After 30 days, animals were sacrificed and hearts removed. Heart left ventricle was weighted (LVW) and normalized to body weight (BW) and tibia length (TL). Morphometric analysis showed that LVW/BW and LVW/TL respectively were reduced in l-shEGFR (2.92 ± 0.05 mg/g and 20.25 ± 0.25 mg/mm) compared to l-shSCR group (3.15 ± 0.08 mg/g and 22.42 ± 0.57 mg/mm; $p<0.05$). This reduction was accompanied by a decrease of the myocytes cross sectional area:246.3 ± 20.6 μm^2l-shEGFR vs.298.33 ± 9.8 μm^2l-shSCR, ($p<0.05$), evaluated by hematoxylosin-eosin staining of LV histological sections. Since EGFR inhibition could reduce redox-sensitive pathways, myocardial lipid peroxidation (by thiobarbituric reactive substances, TBARS) and basal reactive oxygen species (ROS) were evaluated in both experimental groups. Both TBARS and ROS were reduced in LV myocardium of SHR injected with l-shEGFR

vs. l-shSCR ($p<0.05$). These results demonstrate that 1) normal EGFR expression is necessary to mediate cardiac hypertrophy, 2) suggest specific silencing of EGFR in the myocardium as an alternative therapeutic strategy for PCH treatment.

CARDIOVASCULAR MODIFICATIONS IN OVARIECTOMIZED RATS: ROLE OF THE SODIUM/PROTON EXCHANGER (NHE) AND THE SODIUM/BICARBONATE (NBC) COTRANSPORTER.

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During post menopause, women are exposed to an increased cardiovascular (CV) risk. Although the main cause seems to be the loss of estrogen, the precise intracellular modifications are still unknown. The sodium/proton exchanger (NHE) and the sodium/bicarbonate cotransporter (NBC) regulate intracellular pH (pH_i). NBC has two isoforms: electrogenic ($1Na^+/2HCO_3^-$; NBCe1) and electroneutral ($1Na^+/1HCO_3^-$; NBCn1). Hyperactivity of NHE and NBCn1 and down-regulation of NBCe1 are associated with several CV diseases. The aim of this work is to investigate the alterations of these transporters during the postmenopausal period, which could explain the increase in CV risk. Bilateral ovariectomy (OVX) was performed in 3 months old Wistar rats. Sham animals were generated by surgical incisions without ovariectomy. The increase in body weight and left ventricle mass was greater in OVX than in Sham. Systolic blood pressure was higher in OVX (122,29±3.06mmHg*, n=16; vs 117.45±1.85mmHg n=23). Cardiac function after an ischemia/reperfusion protocol showed a clear alteration in OVX (i.e. developed left ventricle pressure: 5.66±2.30% vs 21.01±5.36%; n=6, $p<0.05$). The OVX infarct size was 57.21±2.50%* vs 29.40±2.50% in Sham; n=6. NHE and total NBC activity were assessed during recovery from acidosis induced by an NH_4^+ pulse. NHE was hyperactive in OVX (dpHi/dt at pH_i 6.8, pH units/min: 0,083±0,008*, n=19 vs 0,061±0,006, n=16), whereas total NBC activity was not different between groups. NBCe1 activity measured in isolation (depolarization with 45 mM KCl) was decreased in OVX (ΔpH=0,076±0,013*; n=8 vs 0,149±0,018; n=9). Consistently, protein expression of NHE was greater and NBCe1 was lower in OVX compared to Sham. Interestingly, NHE activity measured in platelets was also greater in OVX (0,016±0,002*, n=13 vs 0,010±0,002, n=7), representing a valuable biological marker. We propose that the hyperactivity of NHE and NBCn1, accompanied with the decrease in NBCe1 activity could explain the cardiac dysfunctions found in OVX.

THE CALCIUM INFLUX THROUGH P2X RECEPTORS MODULATE THE BKCa CHANNEL ACTIVITY IN VASCULAR SMOOTH MUSCLE CELLS.

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Human vascular smooth muscle cells (SMC) exposed to extracellular ATP (ATPe) evoked a transient inward ionic current, carried by Na+ and Ca2+, characterized by a fast activation followed by a peak current decrease. Among the different roles this Ca2+ influx could have on the SMCs, we focus on its possible functional coupling with the large conductance voltage and Ca2+ activated K+ channels (BKCa). Since this K+ channel has a high Ca2+ sensitivity, it could be activated by the ATP-induced-calcium-influx, producing hyperpolarization of SMC and subsequently modulate the contractile state of these cells.

Whole-cell patch clamp experiments were performed applying consecutives voltage protocols designed by a 0.5 s voltage step from -50 to +80 mV (which evokes a BKCa current), followed by a 2 s voltage ramp from +80 to -80 mV. ATPe was added at voltage values below 0 mV, where the impulse force is favorable to produce an inward cationic current through P2X channels. Immediately after 100 μM ATPe addition we observed a significant increase in BKCa current and a significant left shift of the ramp evoked current(n=6, $p<0.05$, paired Student's t test). To exclude a direct activation of BKCa channel by ATP, we worked in cell attached patch clamp configuration, where the membrane being under recording it is not in contact with ATPe and cell integrity is maintained. We observed BKCa channel activation, translated as a significant higher open probability value (NPo), after application of 100uM ATPe (control NPo: 0.0197 ± 0.0128; after ATPeNPo: 0.0552 ± 0.0239 at 0 mV, n=6, $p<0.05$, paired Student's t test).

We also tested100 μM ATPe in a Ca2+ free extracellular solution observing no effect on BKCa channel activity, which suggests that Ca2+ influx through P2X receptors is essential to activate the K+ channel.

These results show that extracellular ATP could play an important role as a regulatory mechanism of the vascular resistance involving BKCa channel as a new functional partner.

PERICARDIAL RECONSTRUCTION USING EXTRACELLULAR MATRIX BIOLOGICAL SCAFFOLDS IN A PRECLINICAL PORCINE MODEL.

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Closing the pericardium after cardiac surgery is recommended to prevent postoperative adhesions to the sternum. However, it is frequently not possible. Synthetic materials have been used as substitutes, with limited results due to impaired remodeling and fibrotic tissue formation. Extracellular matrix (ECM) scaffolds, recruit and facilitate the incorporation of native cells to promote constructive remodeling that resemble more the native tissue.

Aims: Evaluate the host response to ECM scaffolds in pericardium defects closure after cardiac surgery, with focus in magnitude, strength and quality of adhesions and epicardial retraction.

10 Landrace pigs were subjected to median sternotomy and a 5x7cm pericardial defect was created. The test group (n=6) was subjected to ECM repair with an 8layer device of 5x7cm. Animals in control group (n=4) were left with an open defect. Animals were euthanized at 8 weeks. Endpoints included cardiac function (Doppler ultrasound), gross morphology with adhesion assessment, mechanical testing and histology.

Softer adhesions were found in ECM group, but strong adhesions and injury of the coronary bed were found in control group. The load at failure showed no differences between the ECM and native pericardium (199.9±59,2g vs. 405.3± 99.89g P=0.0536), but tissue was weaker than native pericardium (44.23±15.01g/mm vs. 146.5±24.38g/mm P<0.01).In ECM pigs, histology resembled that of native pericardium. Control pigs showed fibrotic tissue with mononuclear infiltrate and no organized collagen fibers. Both groups had normal results without cardiac motility disorders.

In conclusion, ECM may contribute to enhance pericardial repair with tissue characteristics that may protect against the formation of postoperative retrosternal adhesions, whereas cardiac function is not affected by the implant.

SAFE – ORAL PRESENTATION AWARD

MODULATORY EFFECT OF NATIVE AND NATURALIZED PLANT EXTRACTS ON P-GLYCOPROTEIN MULTIDRUG TRANSPORTER IN CHRONIC MYELOGENOUS LEUKEMIA CELLS.

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P-Glycoprotein (P-gp) is a unique ATP-dependent transmembrane efflux pump that plays a significant role in the intracellular decreasing of anticancer drugs. Over-expression of this protein leads to the development of multidrug resistance (MDR) and constitutes nowadays one of the main obstacles to successful chemotherapy. Natural product constituents are diverse and they have potential to offer novel chemical scaffolds and leads. With the aim of finding new novel P-gp modulators, one hundred and twenty native and naturalized plants collected from central region of Argentina were screened over K562 human myelogenous leukemia cell line and its MDR counterpart Lucena 1.

Ethanolic extracts dissolved in DMSO at 100 and 50 mg/ml were assayed for their cytotoxicity activity by MTT. Intracellular doxorubicin accumulation was determined, at non-cytotoxic concentration of each extract, using high throughput flow cytometry. Fluorescence intensity radio (FIR) was calculated as the ratio between the fluorescence intensity of each cell line treated and the fluorescence intensity of its respective DMSO control.

Nineteen extracts exhibited significant P-gp modulating effects (**p*-values ≤ 0.05) at 100 mg/ml, being the most relevant: *Ligaria cuneifólia* (FIR: 1,75±0,10*); *Jarava ichu* (FIR: 1,70±0,24*); *Senecio viravira* (FIR: 1,70±0,30*); *Melinis repens* (FIR: 1,62±0,22*); *Senecio madagascariensis* (FIR: 1,62±0,17*). On the other hand, *Aloysia gratissima* (FIR: 1,30±0,10*) and *Eupatorium viscidum* (FIR:

1,23±0,06*) were effective at 50 mg/ml.
The minimum inhibitory concentration of the extracts tested will be presented.
According to the results obtained, these plants could be source of compounds capable to reverse the MDR phenotype and sensitize the leukemia cells to conventional chemotherapy.

NMDA RECEPTOR ANTAGONIST KETAMINE INDUCES A REGULATORY MACROPHAGE PROGRAM ON HUMAN MONOCYTES

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Background: Ketamine, a NMDA antagonist, is still used as an anesthetic but recently is emerging as a fast and potent antidepressive drug. The biology of this effect is poorly understood but new research implicates that one of the main target of its action is the immune response. This associates with the concept that inflammation contributes to the physiopathology of depression.
Macrophages are key players in the balance of pro and antiinflammatory response; therefore, the aim of this study was to elucidate the effect of ketamine, type of receptor involved and gene program elicited on human macrophages during differentiation and polarization. **Methods:** Blood samples were drawn from healthy volunteers and mononuclear cells were isolated from the cellular fraction by Ficoll gradient. After CD14 positive selection, monocytes were cultured for 7 days and treated with ketamine at different time points (day 1 or 5). LPS+INFγ, IL-4 or dexamethasone were used as positive controls of macrophage polarization. To measure the inflammatory response, LPS was added ON in some experiments at day 6. Phenotype characterization was performed by FACS analysis and gene expression by qPCR. **Results**: We found that ketamine (0.1, 1 and 10 μM) induced an antiinflammatory profile on macrophages with high expression of CD163 and MERTK, intermediate levels of CD64 and no expression of CD206, compatible with a regulatory M2c-like phenotype. mRNA levels of TGM2 and CCL22 genes, related to a M2 profile, were also up-regulated in ketamine treated macrophages. Moreover, ketamine dampens macrophage activation, with lower expression of CD80 and HLADR and diminishes TNF-α production induced by LPS. Addition of MK801, a glutamate receptor antagonist, showed that the effect is mediated by NMDA receptors. **Conclusions:** These results show that ketamine not only dampens acute inflammation but also induces an antiinflammatory program on human monocytes skewing macrophages to a regulatory M2c-like profile.

TOXOPLASMOSIS DRUGS REPURPOSED FOR CHAGAS DISEASE: IN VITRO AND IN VIVO EVALUATION OF REFERENCE DRUGS.

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Chagas disease is endemic in Latin America and a global burden due to migration. Benznidazole (BZ) and nifurtimox (NFX) are the only drugs available for treatment but are not exempt of severe adverse events. Thus, better drugs for Chagas disease are needed. Drug repositioning is a cost-effective strategy to identify active compounds that may move forward to clinical use. Taking advantage of phylogenetic similarities and common metabolic pathways between *Toxoplasma gondii* and *Trypanosoma cruzi*, drugs currently used for Toxoplasmosis were sequentially assessed for anti-*T. cruzi* activity in *in vitro* and *in vivo* models. Azithromycin, sulfadiazine, sulfamethoxazole, pyrimethamine (PYR), atovaquone (ATV) and paromomycin were initially evaluated *in vitro* by establishing the percentage of relative activity (%RA) compared to BZ and NFX on amastigote and trypomastigote stages at a fixed concentration (10 µM for each drug). PYR and ATV showed more than 98 %RA compared to NFX on amastigotes. Hence, these drugs were further evaluated to obtain the inhibitory concentration 50% (IC_{50}) on amastigotes, and citotoxicity on Vero cells. IC_{50} values were 0.47 µM for PYR and 6.92 µM for ATV; citotoxicity was not significant. Due PYR well-known favorable human pharmacokinetics and safety properties, there was rationale enough to continue its evaluation in a murine model of acute infection. Female 5 week old BALB/c mice were infected and, at onset of patent parasitaemia, treated with PYR 50 mg/kg for 20 consecutive days. Non-treated (NT) and BZ- or NFX-treated groups (100 mg/kg/day) were included as controls. PYR did not show a significant effect on parasitaemia levels or mortality (100%) compared to NT group at the end of the study. In this screening model, PYR was the most potent drug *in vitro* but this could not be demonstrated *in vivo*.

Further studies will be conducted studying ATV as monotherapy and combined with BZ or NFX.

THERAPEUTIC EFFICACY AND BIOCOMPATIBILITY OF TRIAMCINOLONE ACETONIDE-LOADED LIPID NANOCAPSULES

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Triamcinolone acetonide (TA) is a synthetic corticosteroid used to treat a broad spectrum of ocular diseases. The development of TA dosage forms is limited due to its poor solubility in physiologically acceptable solvents. The aim of this work was to evaluate therapeutic efficacy *in-vitro* of novel "solvent free" TA-loaded lipid nanocapsules (LNC).

An optimized phase inversion temperature method was used to obtain TA-loaded LNC with oleic acid or glyceryl triacetate into lipid matrix. The average particle size (APS), polydispersity index (PI) and loading capacity (LC) of LNC were measured. Cell viability of human corneal epithelial (HCE) cells after TA-loaded LNC exposure was evaluated using MTT assay. In addition, the secretion of the inflammatory cytokine IL-6 by HCE cells in response to tumor necrosis factor (TNF)-α exposure (inflamed cells) was measured after treatment with TA-loaded LNC. Inflamed cells were exposed to TA-loaded LNC for a 1h and IL-6 secreted into the culture medium was measured by ELISA.

Obtained TA-loaded LNC showed acceptable APS, PI and LC. Results of biocompatibility assay did not show any significant cytotoxic effect (cell viability>70%) of TA-loaded LNC in HCE cells over studied period.

Experimental findings exhibited efficacy of TA-loaded LNC to reduce cell inflammation. IL-6 levels secreted by TNF-α inflamed cells were upper compared to those basally secreted by non-inflamed cells($p \leq 0,05$). Exposition of inflamed cells to TA-loaded LNC (1μg/ml) for the 1h, significantly reduced IL-6 production compared to control inflamed untreated cells($p \leq 0,05$). Empty LNC increase the cell inflammation significantly($p \leq 0,005$) while that TA trademark suspension showed a significant IL-6 secretion decrease($p \leq 0,05$) in comparison to both controls: non-inflamed and inflamed untreated cells.

This strategy allowed the decrease of IL-6 level of HCE cells and it could be a potential alternative to treat several ocular diseases with less adverse effects.

INDIVIDUALIZED ANTIRETROVIRAL THERAPY. IMPACT OF PHARMACOGENETIC AND THERAPEUTIC DRUG MONITORING IN THE SAFETY AND EFFICACY OF FIRST LINE ANTIRRETROVIRAL THERAPY IN PATIENTS WITH HIV INFECTION. PRELIMINARY REPORT

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Up to 20% of patients discontinue first-line antiretroviral therapy at currently standard doses. We explored the utility of prior pharmacogenetic (PG) analysis and ongoing drug monitoring (TDM) to individualize the dose of efavirenz or atazanavir in naïve HIV patients.

A multiplex approach was developed including main polymorphisms of *CYP2B6, CYP2A6, CYP3A4* y *ABCB1* for efavirenz; and *UGT1A1, ABCB1 and CYP3A4* for atazanavir. Drug plasma levels were analyzed with UPLC. Median turnaround time was 22 days.

First 40 patients included in the protocol -coming from 4 clinical sites in Buenos Aires- were randomized to standard of care –SC-(25 patients) or pharmacological adaptation –PA: PG + TDM- (15 patients).

One patient in the pharmacological adaptation arm received decreased individualized doses atazanavir with proper tolerance and maintaining treatment efficacy, in 69 patient/months of follow up.

Two patients from SC arm had adverse events, and both of them had to discontinue therapy.

Pharmacological adaptation of initial and ongoing doses of first line antiretrovirals appears feasible and useful for the individualized therapeutic approach of patients with HIV infection starting treatment.

PRECLINICAL PHARMACOKINETIC OF BENZNIDAZOLE-LOADED INTERPOLYELECTROLYTE COMPLEXES FOR IMPROVED TREATMENT OF CHAGAS DISEASE

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Chagas disease, caused by *Trypanosoma cruzi*, is an important public health problem in Latin America and worldwide. Benznidazole (BZ) is the selected drug for its treatment. However, it shows very low solubility, several side effects and toxicity, which compromise the efficacy and safety. Moreover, the available pharmaceutical dosage forms are immediate release tablets and the treatment is administered 2 or 3 times daily for 60 days. The high frequency of administrations and long-term treatment are issues that contribute to the abandonment of therapy, affecting therapeutic success. In a previous work, we have developed BZ-loaded interpolyelectrolyte complexes (IPEC) based on polymethacrylates (EE-EL-BZ) and polysaccharides (Ch-AA-BZ) that showed controlled release of BZ. These systems would reduce the adverse effects of BZ and/or allow reducing its frequency of administration. Therefore, this work aimed to evaluate the preclinical pharmacokinetics of BZ-loaded IPECs compared to the currently available tablets (Abarax). The studies were performed six healthy adult mixed breed dogs, with a 3×2 cross-over design. Each dog received all orally administrated treatments after three experiments, with a washout period of 15 days. They received 100 mg of BZ by single oral dose of EE-EL-BZ, Ch-AA-BZ or Abarax, with a randomization schedule. BZ quantification was performed in plasma by high performance liquid chromatography validated method. The AUC of BZ-loaded IPECs was higher than reference treatment ($p<0.01$). The C_{max} of Ch-AA-BZ and t_{max} of both IPECs were higher than Abarax ($p<0.05$). The k_a, $t_{1/2}$ and MRT were similar for all the treatments evaluated. Our results indicate that bioavailability of BZ was adequate in the administration of both IPECs. The pharmacokinetic parameters demonstrated that the BZ-loaded IPECs prolonged drug release and provided an increase in the maintenance of drug concentration *in vivo*, which would allow reducing

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RESÚMENES DE LAS COMUNICACIONES

ONCOLOGY-ONCOIMMUNOLOGY 1

(1049) **β-LAP HALOGENATED-DERIVATIVE PFB AS A PROMISING NQO1-DEPENDENT ANTICANCER CHEMOTHERAPEUTIC AGAINST MELANOMA**

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The elevated expression of the NQO1 in many human solid tumors along with its ability to activate quinone-based anticancer agents, such as the natural product β-Lapachone (β-Lap), makes it an excellent target for enzyme-directed drug development. NQO1 plays an important role in the initiation stage of melanoma and its correlation with poor patient outcome make it an intriguing target. The most dangerous aspect of melanoma relay on the metastatic ability in later stages. Therefore, new effective and safe therapeutic agents for metastatic melanoma are needed. In this study, metastasis-derived human melanoma cell lines exhibited higher resistance to β-Lap (LD50 SkMel-2 and 1205Lu: not reached) compared to primary-site derived melanoma cells (LD50 SkMel-28: 1.48 μM) ($p<0.05$). The cytotoxic effect of β-Lap was reversed with coadministration of dicumarol, a specific inhibitor of NQO1. NQO1 inhibition preferentially prevented cell death of non-metastasis-derived human melanoma (94.5% of reversion; $p<0.001$). Interestingly, dicumarol had no effect as an antagonist of β-Lap in the murine melanoma cell line B16, but stable overexpression of NQO1 completely restored chemosensitivity ($p<0.001$). These results are in agreement with earlier studies suggesting that β-Lap-induced cytotoxicity might involve differential NQO1 status required for its bioactivation. In a previous report, we proposed halogenated β-Lap derivative PFB as an excellent NQO1 substrate and anticancer agent. Here, PFB had considerable higher potential chemotherapeutic activity than β-Lap on SkMel-2 (LD50 β-Lap: 3.50 μM; LD50 PFB: 2.89 μM; $p<0.05$). These data suggest that PFB possess properties that make it more attractive than the parent β-Lap for treating metastatic-derived melanoma cells. Our preliminary results support the necessity of design new strategies to induce NQO1 activity in cancer cells as a promising approach for bioreductive anticancer drugs.

Keywords: Melanoma; NQO1; β-Lapachone; PFB

(724) **A *N*^{4}-ARYL SUBSTITUTED THIOSEMICARBAZONE DOWN-MODULATES METASTASIS-RELATED PROPERTIES OF TRIPLE NEGATIVE 4T1 MOUSE MAMMARY CANCER CELLS**

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Triple negative breast cancer (TNBC) is defined by a lack of expression of estrogen (ER) and progesterone (PgR) receptors as well as HER2/neu. This pathology represents about 15% of all types of breast cancer and among all subtypes TNBC is associated with a worse prognosis and a higher incidence of visceral metastasis. The lack of targeted therapies and the poor prognosis of TNBC patients have fostered a major effort to develop new alternative treatments. Thiosemicarbazones are synthetic compounds that exhibit several pharmacological activities. Previously, we found that T_2, a *N*^{4}-aryl substituted thiosemicarbazone, had cytotoxic activity on 4T1 mouse mammary tumor cells. Now, we have investigated the action of T_2 on metastasis-related properties of 4T1 cells.

First, we found that T_2 significantly diminished 4T1 clonogenic capacity in a dose-dependent manner (T_2 2.5 mM= 58.8%; T_2 5 mM= 33.3% and T_2 10 mM=0.014% vs control cells). We also observed that 4T1 migratory capacity was down-regulated by T_2 (T_2 2.5 mM= 61.8 ± 2%; T_2 5 mM= 25.4 ± 4% and T_2 10 mM=19.6±2% respect to control cells, pÂ0.05). In order to evaluate T_2 action on cancer stem cells sub-population, we quantified Lin-/CD24+/CD29^h cells by cytometry and found that T_2 reduced this population (T_2 2.5 mM= 54.4 ± 2%; T_2 5 mM= 29.8 ± 14.6% and T_2 10 mM=27.9±14.6% vs control cells, pÂ0.05). This effect was in accordance with a significantly lower mammosphere-forming capacity shown by 4T1 cells after T_2 treatment ($p<0.05$). Finally, we evaluated pluripotential genes expression by real time qPCR and found that T_2 induced a significant down-regulation of Nanog, Oct-4 and Sox-2 expression ($p<0.05$).

In conclusion, our results show that T_2 inhibits some of the metastasis-related properties of 4T1 cells, placing this compound as a candidate for in vivo experiments to investigate its anti-metastasic potential against TNBC.

(1112) **ANTIMETASTATIC EFFECT OF THE PENICILLIN DERIVATIVE TAP6 IN MELANOMA CELLS**

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Abstract: Metastatic dissemination of cancer cells is the main cause of death in melanoma patients. In the search of new antimetastatic compounds, in a previous work, we found that the penicillin derivative named TAP6, formed by a penicillin linked to the dipeptide Phe-Leu through a triazole group, inhibited adhesion and migration of B16F10 metastatic murine melanoma cells. In order to continue studying the antimetastatic properties of TAP6, we investigated the effect of the penicillin derivative on B16F10 cells invasion. Transwell invasion assay results demonstrated that a 10 μM concentration of TAP6 inhibited cell invasion after 18 h treatment (96.1±0.4%, $p<0.001$). Gelatin zymography of supernatants collected after incubating B16F10 cells for 24 h in the presence of 10 μM TAP6 showed a reduction in matrix metalloproteinase (MMP)-2 and -9 proteinase activity of 33±4% and 43±5%, respectively. Additionally, under the same incubation conditions, we observed a significant decrease in the expression levels of MMP-2 (48.2±9.8%, $p<0.001$) and MMP-9 (81.7±6.8% $p<0.001$) by Western blot assay. Wnt/β-catenin signalling pathway is activated in various cancers and promotes metastatic spread by increasing invasion, however, it has controversial roles in the metastatic spread of melanoma. By Western blot analysis, we found that a 10 μM concentration of TAP6 inhibited 92.5±2.8% the expression of β-catenin after 18 h of treatment. Under these conditions, we have also observed a reduction in the cytosolic levels of β-catenin by immunofluorescence microscopy. In conclusion, we demonstrated that TAP6 exhibits antimetastatic properties through the inhibition of cell invasion and MMP-2 and 9 expression and activity. Furthermore, we found that TAP6 significantly inhibited β-catenin expression. These findings encourage us to continue studying this penicillin derivative as a potential candidate for the treatment of melanoma.

Keywords: Melanoma, metastasis, penicillin derivative, invasion,

metalloproteinases

(681) **ANTITUMORAL IN VIVO EFFECT OF MICROSPHERES FOR CHEMOEMBOLIZATION**

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Introduction: The Bariloche Atomic Centre (CAB-CNEA) develops Porous silica Microspheres (PME) as a drug carrier matrix. Our purpose is to evaluate the in vivo effect of Doxorubicin loaded PME (D-PME) on: (I) development and (II) growth of DHD/K12 rat colonic adenocarcinoma.

Materials and Methods: 600mg of 25-45um diameter PMEs were loaded by shaking for 24 hours in Doxorubicin Hydrochloride solution (Doxo, 2mg/ml) or saline (empty PME, Controls).

Two groups of BDIX rats were inoculated subcutaneously with 10^6 DHD/K12 tumor cells (TC) for two different experiments (Exp): Exp (I) TCs were inoculated mixed with D-PME (n=13) or mixed with empty PME (n=14). Exp (II) established tumors (60-80mm^3) were injected intra-tumor: with empty PME (n=14) or with D-PME (n=16).

In both Exp, animals were sacrificed thirteen days after treatment. Tumors were measured and processed for histopathology.

Results: Tumors volume at sacrifice were: Exp (I) Control (with empty PME): 60.99±34.66mm^3 vs Experimental (D-PME): 1.72±1.57mm^3 ($p < 0,0001$). Exp (II): Control (empty PME): 139,81±100,16mm^3 vs. Experimental (D-PME): 35,41± 19,13 mm^3 ($p < 0,0001$).

Histologically, in both experiments, tumors treated with D-PME showed more necrotic areas than those treated with empty PME (Controls). Only Exp II with D-PME showed damaged epidermis.

Conclusion: We demonstrate that the PMEs developed by CAB-CNEA, load and release Doxo enough to produce an antitumor effect on both development and growth of experimental DHD/K12 rat colonic adenocarcinoma.

Key words: Tumor, Microspheres, Chemotherapy, Embolization

(818) **CLOZAPINE AS A POTENTIAL ANTI-CANCER DRUG**

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We have previously demonstrated that clozapine, a well-known antipsychotic inhibited proliferation of human breast cancer and melanoma cells. The aim of this work was to investigate the effect of clozapine alone or in combination with a widely used chemotherapeutic agent (doxorubicin, Dox), on an immunocompetent triple negative breast cancer (TNBC) model. For that purpose, proliferation and apoptotic assays and DNA damage markers were studied in murine 4T1 TNBC cells. *In vivo* studies were performed in syngeneic mice, inoculated orthotopically with 4T1 murine TNBC cells. Results indicate that clozapine reduced proliferation in a concentration dependent manner. At 10 μM concentration it produced a 2.5-fold decrease in clonogenic proliferation and in BrdU incorporation of 4T1 cells ($P<0.01$). This effect was associated with an induction of apoptosis, evidenced by an increased Annexin-V staining, an increase in the differentiation marker Nile red, and enhanced ROS levels, all assays evaluated by flow cytometry. Accordingly, *in vivo* treatment of 4T1 tumors with clozapine (1 mg/kg.day, *sc*) reduced tumor weight (1.3±0.1 vs. 2.1±0.3 g, $P<0.05$). Histopathological analysis indicate that clozapine-treated tumors show extended areas of differentiation and reduced mitotic index. Combined treatment of clozapine and doxorubicin was more effective in reducing growth *in vitro* and *in vivo* than single treatments. Clozapine potentiated doxorubicin-induced apoptosis and proliferation reduction ($P<0.01$). We conclude that clozapine could be an effective drug for the treatment of TNBC. Prospective clinical trials are warranted to confirm the clinical efficacy of this currently used drug for breast cancer therapy.

Keywords: clozapine, breast cancer, apoptosis, combined therapy

(1230) **OCT-4 EXPRESSION WAS INCREASED IN RESISTANT CANCER STEM CELL-LIKE SUBPOPULATION IN COLORECTAL CANCER**

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Radiochemoresistance of tumors has been associated with the presence of a small subpopulation of tumor cells called cancer stem cells (CSCs). These cells had been shown not only linked with high resistance to chemotherapeutic drugs but also are associated with metastatic recurrences. Embryonic markers such as Oct-4, Nanog, Sox-2 are associated with resistance to chemotherapeutic drugs in some cancer. However, little has been reported about the expression of these embryonic markers in colorectal (CRC) CSCs subpopulation. The aim of this work is the study of the mentioned embryonic marker in sensible and oxaliplatin-resistant (OXA) human CRC cancer lines, and in cultures derived of them enriched with CSC-like subpopulation, in order to detect novel markers associated to resistance in these cells. We characterized the expression of Oct-4, Nanog and Sox-2 by immunofluorescence, cell cytometer and real time-PCR in sensible or resistant human CRC cell to oxaliplatin (T-84 and HCT-116) and in cultures enriched by CSC-like subpopulation. The last cultures were obtained by growing tumor cells as colonospheres (CSPHE) in minimum medium in low adherence surfaces. On the other hand, we also measured the reactive oxygen species (ROS) levels as a characteristic associated to CSC-like subpopulation. The expression of Nanog and Sox-2 was not changed in sensible and resistant cell lines grown in monolayer or as CSPH cultures. However, the expression of Oct-4 was positive in 6-12% and 40-50% of sensible cells that were grown in monolayer or as CSPHE respectively. About chemoresistant cells lines, 40-60% and 75-85% of cells were positively for Oct-4 depending if cells were grown as monolayer or as CSPHE respectively. About ROS level, cells that were grown as CSPHE had shown significant lower levels in respect to cells that were grown as monolayer. These results showed that Oct-4 could be a promising marker to identify chemoresistant cells with CSC-like characteristics in CRC.

Keywords: Oct-4, Cancer Stem Cell, Chemoresistance

(1139) **HUMAN RENAL ADIPOSE TISSUE FROM NORMAL AND TUMORAL KIDNEY REGULATES THE BEHAVIOR OF RENAL EPITHELIAL CELLS**

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Abstract: Tumor stroma is one of the emerging topics of interest in cancer research. In the present work, we evaluated the effects of Conditioned Media (CMs) of human adipose tissue explants from normal (hRAN) and tumor (hRAT) kidney on proliferation of tumor (786-O, ACHN) and non tumor (NK-2) renal epithelial cells. We also evaluated: 1) leptin, adiponectin and versican mRNA expression in hRAN and hRAT; 2) adiponectin, ADAMTS1 and perilipin expression in hRAN- and hRAT-CMs; and 3) adiponectin and perilipin expression in hRAN- and hRAT.

Human renal adipose tissues were obtained from patients with re-

nal cell carcinoma (hRAT, n=6) and kidney donors (hRAN, n=8). The CMs from hRAN and hRAT were collected after 24h of incubation. 786-O, ACHN and NK-2 cells were grown and incubated with CMs for 24 and 48h. Proliferation was quantified by MTT; mRNA expression was evaluated by real-time PCR, protein expression by WB and immunohistochemistry. Statistical differences between experimental conditions were evaluated by one-way ANOVA or t test. Tukey´s post hoc tests were performed.

We found a higher RNA expression of leptin (a major adipokine with tumorigenic effects) and versican (a proteoglycan that is essential in epithelial-stromal interaction and intracellular signaling) in hRAT *vs.* hRAN explants (p<0.01). Also, we observed that adiponectin (a major adipokine with anti-tumorigenic effects), and perilipin expression were significantly increased in hRAN *vs.* hRAT explants (p<0.05) and in addition we observed a lower protein expression of perilipin (marker for mature differentiated adipocyte) in hRAT-CMs *vs.* hRAN-CMs (p<0.05). Added to this, hRAN-CMs significantly increased the proliferation of 786-O and HK-2 compared to control- and hRAT-CMs after 24h (p<0.05).

In conclusion, the changes observed in the adipose microenvironment could be favoring tumor progression and therefore, the tumor stroma should be taken into consideration when dealing with a malignancy.

Keywords: human adipose tissue, renal epithelial cells, cancer, epithelial-stromal interactions.

(830) **TISSUE CULTURES FROM PATIENT DERIVED XENOGRAFTS (PDX): A NEW FAST METHOD TO TEST THE EFFECT OF THERAPEUTIC AGENTS.**

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Precision medicine needs to be accompanied by diagnostic tools to determine which patient may benefit from therapeutic agents. For this purpose, to test drug efficacy, patient derived xenografts (PDX) or *ex vivo* assays are currently being developed. We have recently shown a 32% of success evaluating the effects of antiprogestins in tissue cultures (BCTC). However, with this technique few treatments can be simultaneously tested due to the small size of breast cancer samples. The main aim of this study is to develop PDX from luminal breast cancers and evaluate if BCTC from these PDX reproduce the observed drug effects. In this study, we evaluated the effect of doxorubicin (Dox; 1 μM) and/or paclitaxel (Pax; 50 nM) on 16 BCTC. In addition, 20 PDX were attempted inoculating breast cancer cells into NSG female mice treated or not with estrogen pellets (0.5 mg). The study was approved by institutional review boards. In the BCTC exposed to chemotherapeutic agents we observed histological and cytological changes related to Dox or Pax exposure such as macronuclei, necrotic foci and apoptosis. Most of the PDX developed small tumors and remained quiescent. PDX-485 started to grow similarly in E2-treated or untreated mice. Tumor 485 was a tumor relapse (luminal B; ER: 40%, PR: 30%, Ki-67 90%, HER2: negative). Dox inhibited tumor growth (p<0.05), and histological features of Pax effects were registered although no significant difference was observed in tumor size as compared with control group. Mifepristone (MFP), an antiprogestin, did not exert any effect. Sensitivity to Dox, Pax and MFP was evaluated in PDX-485 tissue cultures. Dox proved to be the most effective drug (p<0.05) inducing a decrease of Ki-67 expression and an increase in Casp-3, both evaluated by immunohistochemistry (p<0.05). We conclude that BCTC derived from PDX represent a promising tool that may be used for drug testing or to increase the number of drug combinations to be tested in short periods of time.

Keywords: breast cancer, patient derived xenografts, therapeutic agents

(1594) **CHLORPYRIFOS PROMOTES CELL INVASIVENESS AND ACTIVATES P-38/GSK3B PATHWAY MEDIATED BY ROS INCREMENT IN BREAST CANCER CELL LINES.**

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Chlorpyrifos (CPF) is one of the most common pesticide used for control pest in agriculture in our country. Previously, we reported that 0.05 uM CPF induced cell proliferation in an estrogen receptor dependent way in MCF-7 cells and 50 uM CPF inhibited cell proliferation via p-ERK induced by an increment of reactive oxygen species (ROS) in MCF-7 and MDA-MB231 cells. Objective: Our aim was to analyze if CPF may promote cell invasion and the associated signaling in breast cancer cell lines. Methods: MCF-7 and MDA-MB231 cells were exposed or not to CPF (0.05 or 50 uM). Cell invasiveness was examined by the Matrigel® invasion assay and c-SRC, p38 and GSK3β phosphorylations by Western Blot. Results: 50 uM CPF induced the invasion in MDA-MB231 after 24 h, (60% over control, p < 0.01) and MCF-7 cells after 48 h (400% over de control, p< 0.001). CPF at 0.05 and 50 uM increased c-SRC phosphorylation (p-cSRC) after 60 min in MCF-7 cells (19% and 14% over control respectively, p< 0.05) and this effect was reverted by catalase (30 IU/mL). In MCF-7 cells, 50 uM CPF enhanced phospho-p38 (p-p38) after 30 minutes of exposure (27% over control, p<0.05) and both, CPF 0.5 (145% over control, p <0.01) and 50 uM (70% over control, p< 0.01), incremented p-p38 after 1 h. In MDA-MB231, 50 uM CPF induced p-p38 after 1h of exposure (34% over control, p<0.05). In both, MCF-7 and MDA-MB231 cells, 50 uM CPF action on p38 was reversed by adding catalase (30 IU/mL). In MCF-7 cells, CPF 0.05 uM induced phospho-GSK3β (p-GSK3β) after 5, 30 and 60 min (67%, p<0.01; 75%, p<0.05%; 55%, p<0.05 over control respectively); CPF 50 uM induced p-GSK3β after 5 min (26% over control, p<0.05) and could be reverted by catalase (30 IU/mL). In MDA-MB231, p-GSK3β was also induced by CPF 50 uM (p< 0.05). Conclusions: All together, our results indicate that CPF 50 uM induces cell invasion in MCF-7 and MDA-MB231 attended with c-SRC phosphorylation and/or the activation of p-38/GSK3β pathway mediated by ROS.

ONCOLOGY-ONCOIMMUNOLOGY 2

(031) **CIRCADIAN CONTROL OF LIPID AND REDOX METABOLISMS IN PROLIFERATIVE GLIOBLASTOMA CANCER CELLS**

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Immortalized cell lines contain circadian clocks conducting transcriptional/translational rhythms in gene expression whereas metabolic rhythms can persist without transcription. Circadian rhythm disruption by modern life may cause higher cancer risk; however, little is known about clock functioning in tumor cells. Here we evaluated glycerophospholipid (GPL) and redox metabolisms in cultures of glioblastoma T98G cells under proliferation (P) or partial arrest (A), synchronized with dexamethasone (100 nM) (time 0) and collected at different times. In arrested cultures, mRNAs for clock- (*Bmal1, Per1, Rev-erbα)* and GPL enzyme genes, and ^{32}P-GPL labeling exhibited circadian rhythmicity; oscillations were also found in the redox state/peroxiredoxin oxidation cycles. In proliferating cells, circadian rhythms of gene expression were lost or their periodicity shortened whereas the metabolic rhythms persist with a similar or longer period to that observed under A Also, cell viability significantly changed over time after bortezomib (500 nM) treatment. Nevertheless, cell viability and redox state rhythms were altered when *Bmal1* expression was knocked down by CRISPR/Cas 9 genomic editing

technology. Results support that a metabolic clock operates in proliferative tumor cells regardless the molecular clock; property that may confer tumor susceptibility for a time-dependent chemotherapy.

Keywords: circadian rhythm, tumor cell, glioblastome, clock gene, glycerophospholipid metabolism, redox state.

(1363) **IN VITRO EFFECTS OF T_4 ON APOPTOSIS AND PROLIFERATION OF MAMMARY TUMORS**

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Our aim was to study the genomic effects produced by thyroid hormones (TH) on mammary tumors from hypo-, eu- and hyperthyroid rats. Female Sprague Dawley rats were treated with a single dose of dimethylbenzathracene (15 mg/rat) at 55 days of age, and were divided into three groups: hypothyroidism (HypoT; 0.01% 6-N-propyl-2-thiouracil in drinking water, n=10), hyperthyroidism (Hyper, 0.25mg / kg / day Tyroxine -T_4- via sc; n=10) and EUT (untreated control, n=10). At sacrifice, tumor explants were obtained and treated with 10^{-10} M T_4 and/or 10^{-9} M 17β-estradiol (E_2) in DMEM/F12 without phenol red with 1% Charcoalized Fetal Bovine Serum (SFBc) for 24 h to evaluate changes in the expression of hormone receptors and proteins related to apoptosis and proliferation by immunohistochemistry and Western Blot. Statistical analysis was performed using ANOVA I and Bonferroni's test as post hoc. Administration of T_4, E_2 and T_4+E_2 induced a significant increase in KI67 expression in all tumors. Only T_4 stimulation decreased total and cleaved caspase 3 and PARP expression in HypoT tumors while a slight increase of both apoptotic markers was observed in the Hyper tumors. No significant apoptotic changes were observed in the tumors of EUT. Regarding hormone receptors, administration of T_4 increased cytoplasmic progesterone receptor (PgR), nuclear expression of thyroid hormone receptor β (TRβ) and favored the translocation to the nucleus of estrogen receptor (RE) β in HypoT tumors. T_4 enhanced the nuclear and cytoplasmic expression of REα and REβ in Hyper tumors. T_4 and T_4+E_2 augmented the expression of REα and PgR in EUT. E_2 did not significantly modify the expression of hormone receptors. In conclusion, the administration of T_4 induced a differential expression of hormone receptors according to the thyroid state of the tumor. It has a proliferative effect on all mammary tumors independent of the thyroid state but it only showed an antiapoptotic role in Hypot tumors.

Keywords: caspase 3, PARP, tyroxine, KI67, dimethylbenzathracene.

(1168) **PHOSPHORILATED S6 (pS6) EXPRESSION AND TUMOR LOCALIZATION IN RESPONSE TO NEOADJUVANT CHEMOTHERAPY IN BREAST CANCER**

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Neoadjuvant treatment benefits include *in vivo* assessment of tumor response to treatment as well as tumor reduction before surgery. In a recent study, we observed that neoadjuvant endocrine therapy in luminal breast cancer induced a reparative stromal reaction, including neovascularization, immune cells infiltration and carcinoma-associated fibroblasts that stain for phosphorylated S6 (pS6), a protein downstream PI3K/AKT/mTOR pathway. We also demonstrated that stromal levels of pS6 correlated with tumor shrinkage percentage after treatment ($p<0.05$). Here, we aim to analyze if pS6 expression and localization are also associated with the therapeutic response to chemotherapy in breast cancer. To accomplish this, we assembled tissue microarrays with pre and post neoadjuvant chemotherapy samples of 129 invasive luminal breast carcinomas obtained from Hospital Provincial de Neuquen, and performed immunohistochemistry for aSMA (smooth muscle actin) and pS6. We confirmed that stromal fibroblastic-like pS6+ cells are mainly aSMA+ whereas epithelial pS6 was reduced after therapy. To study the mechanisms involved in tumor response to chemotherapy, in a mouse model of luminal breast cancer we used two chemotherapic regimens: FAC (fluorouracil, adriamycin and cytoxan) and CMF (cyclophosphamide, methotrexate and fluorouracil). After 7 days of treatment, we observed high levels of apoptosis, cytostasis and necrosis that cover nearly 50% of the tumor area, together with some stromal reaction. Immunohistochemistry assessment showed a switch of pS6 stain from epithelial to stromal cells, along with higher levels of aSMA. We conclude that, as reported for endocrine therapy, chemotherapy induces S6 activation in the stroma. Our efforts are now set on trying to identify the pS6+ stromal cell population and to understand its regulation in order to evaluate its potential use as a predictive biomarker.

Key Words: breast cancer; chemotherapy; PI3K/AKT/mTOR pathway.

(514) **THYROID HORMONE MEMBRANE RECEPTOR INHIBITION IN THE TREATMENT WITH REXINOIDS OF CUTANEOUS T CELL LYMPHOMA**

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CTCL are exposed to a complex paracrine and endocrine environment that influence their progression from skin to visceral disease. One of the most common treatments for CTCL, the rexinoid bexarotene (Bex), is associated with hypothyroidism being patients candidate for thyroid hormone (TH) replacement therapy. We recently found that TH, mostly through the action on its membrane receptor (mTR, integrin $\alpha V\beta 3$) is required for the proliferation of T cell lymphomas (TCL), including CTCL. The consequences of TH administration on the activity of Bex in CTCL cells are unknown. Our aim was to study the effect of TH on the anti-lymphoma activity of rexinoids.

We first evaluate cell viability and apoptosis induction of human CTCL cells HuT78 and MJ and murine TCL EL4 cells treated with Bex in the presence and absence of physiological levels of TH. As expected, Bex decreased the viability and induce apoptosis on all the cell lines, but in presence of TH both effects decreased by 15-40% ($p<0.01$). These results support the notion that Bex should not be administered with TH replacement. However, beside the metabolic dysfunction, hypothyroidism could also favor tumor progression.

We thus determine if the inhibition of the mTR would be sufficient to decrease the pro-survival effect of TH on cells treated with Bex. We found that the inhibitor of the integrin $\alpha V\beta 3$, cilengitide, not only avoided the pro-survival effect of TH but increased the activity of Bex *in vitro* on HuT78, MJ and EL4 cells. We also found that the combination of both drugs results in a significant increase in the inhibition of the transcriptional expression of anti apoptotic (*BCL2, BCL2L1)* and cell cycle genes (Cyclins) and in the induction of pro apoptotic genes (*BID, BAX)* (in all the cases at least $p<0.05$ vs Bex alone).

These results will be the rationale to perform *in vivo* experiments using a murine model of EL4 LCT tumor to evaluate if this mechanism can be therapeutically capitalized to improve Bex treatment.

REXINOIDS, T CELL LYMPHOMA, CILENGITIDE, THYROID HORMONE

(1201) **TREATMENTS WITH THE SELECTIVE LIGAND SAFIT1 SUGGESTS THAT THE HSP90-BINDING IMMUNOPHILIN FKBP51 IS A NOVEL TUMOR FACTOR**

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Immunophilins (IMMs) are a family of proteins that bind to immunosuppressive drugs and show peptidylprolyl isomerase (PPIase) activity. FKBP51 is an Hsp90-binding cochaperone able to bind the macrolide FK506, which in turn, abolishes PPIase activity. We have previously demonstrated that FKBP51 forms complexes with the reverse transcriptase subunit of telomerase, hTERT, via de molecular chaperone Hsp90. hTERT is highly expressed in cancer cells, where it is required to compensate the loss of telomeric DNA after each successive cell division, and also shows the antiapoptotic action in tumor cells. The IMM FKBP51 also shows antiapoptotic action and is overexpressed in cancer cells. Therefore, we investigated the potential anticancer activity of the IMM ligand FK506. In vitro treatment of HeLa tumor cells with FK506 decreases cell viability, possibly due to inhibition of the PPIase activity of the IMM. Importantly, in vivo treatments with FK506 of xenographic tumors generated in Squid/Nod mice significantly decreases tumor growth. Inasmuch as FK506 shows similar affinity for both IMMs FKBP51 and its close-related partner FKBP52, we tested a new synthetic drug named SAFit1, which is a potent and selective inhibitor for FKBP51. We evaluated the possible effect of this synthetic drug compared to FK506 in tumor processes, and demonstrate that SAFit1 exhibits a significant antitumor activity. Interestingly, the molecular chaperones Hsp90 and Hsp70 relocalize in the cytoplasm of drug-treated cells generating aggregation bodies that are not classified as stress granules since they do not contain the stress granule RNA-binding protein TIAR-1. Taking together, these studies confirm the hypothesis that FKBP51 is a novel antiapoptotic factor related to tumor development.

Keywords: hTERT, FKBP51, SAFit1, FK506, tumor

(1698) **MCF7 CELLS OVEREXPRESSING A NOVEL HUMAN EPITHELIAL CADHERIN SPLICE VARIANT ACQUIRE A TRIPLE NEGATIVE PHENOTYPE. STUDIES *IN VITRO* AND *IN VIVO***

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Breast cancer (BC) is the fifth most common cancer and the leading cause of cancer death in women worldwide. Alterations in Epithelial cadherin (Ecad) expression and functions have been associated with BC progression. However, the molecular mechanisms underlying Ecad deregulation have not been fully elucidated. We have recently reported the identification of a novel human Ecad alternative splice variant (Ecadvar) that differs from the wild-type mRNA (Ecadwt) by lacking the first 34 nucleotides of exon 14. MCF7 cells stably transfected with pcDNA3-Ecadvar (MCF7-Ecadvar) showed a fibroblast-like morphology, downregulation of Ecadwt (transcript/protein) and changes in other epithelial-to-mesenchymal transition markers, reduced cell-cell adhesion and increased cell migration and invasion, when compared to MCF7-pcDNA3 (control) cells.

To further characterize the relevance of Ecadvar expression in BC progression and aggressiveness, mRNA levels of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2), three key markers of BC prognosis and treatment, were evaluated. As result, a decrease ($P<0.05$) in ER alfa, PR and HER2 mRNA levels was found in MCF7-Ecadvar compared to control cells, while no changes were observed in ER beta mRNA levels. Moreover, no detectable levels of ER alfa protein were registered in MCF7-Ecadvar cells.

Injection of MCF7-Ecadvar and MCF7-pcDNA3 cells into immunodeficient female mice receiving exogenous estradiol supplementation led to tumor generation with sustained growth over time in both cell lines. However, only MCF7-Ecadvar cells produced tumors without exogenous hormone supplementation, which were similar in size to those obtained for this cell line in mice injected with estradiol.

These findings revealed the acquisition of a triple negative phenotype in MCF7-Ecadvar cells. Alterations in Ecadvar expression levels may promote tumor progression and resistance to hormone therapy in women with BC.

Key words: cancer, breast, Epithelial Cadherin, alternative splicing, triple negative

(051) **MULTIDIMENSIONAL ANALYSIS OF IN VITRO TRIDIMENSIONAL (3D) CULTURES: COMPUTATIONAL TOOLS DEVELOPMENT FOR MICROSCOPY IMAGES**

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Understanding cellular processes such as tumor progression and asymmetric division requires biological models that replicate as close as possible the conditions found in living tissue. Tridimensional (3D) cell culture technologies such as spheroids and organoids provide this model systems but create new methodological challenges to efficiently characterize their growth and internal structure. In this work, we present a modular, extendable and easy to use open source Python toolbox to obtain parameters of interest in this kind of culture. Using glioblastoma multicellular spheroids in suspension and embedded in agarose, we demonstrate a pipeline of automatic segmentation of transmission or fluorescence images, recognition of cells clusters, and morphological characterization with the possibility to apply it to tridimensional reconstructions. By making this available to the scientific community, we expect that improving the characterization of 3D cell systems provides a way to better optimize and standardize culture and assess the effect of drug in screening assays.

Supported by ANPCyT, CONICET, INC and FOCEM (COF 03/11)
Keywords: stem cells, tridimensional cell culture, software, image analysis

(1300) **OPTIMAL LACTATION EARLY IN LIFE ACTIVATES THE EXTRINSIC APOPTOTIC PATHWAY IN MAMMARY TUMORS IN ADULTHOOD**

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Studies in rodent models show that exposures to diet during the posnatal life, in periods when the mammary gland undergoes extensive modeling and re-modeling, alter susceptibility to carcinogen-induced mammary tumors. The objective of the present study was to analyze if adequate breastfeeding generates changes in the biology of mammary tumors developed in adult life. To do this we used Sprague Dawley female litters divided into 3 (L3), 8 (L8) or 12 (L12) pups per mother, and, at the age of 55 days, they were treated with a single dose of dimethylbenzanthracene (15 mg / rat) to study the latency, incidence, progression and tumor bilology. *We performed a microscopic analysis of mitosis and apoptosis to asses the effect of differential lactation on the development of the tumors. We additionally evaluated mitosis by IHC of PCNA and determined the expression of caspase 8, Bid, Bax, Bcl2 and caspase 9 by western blot from the tumors.* Incidence was expressed as percentages and compared by Fisher test. Other comparisons were performed by ANOVA I and Bonferroni's test as post hoc. *There was a lower incidence and a higher latency in L3 compared to the other groups. The mitotic index was significantly augmented in tumors of L12 rats compared with L3 and L8 ($p<0.001$), while the apoptotic index was augmented in tumors of L3 versus L12 ($p<0.05$). We observed an increased expression of PCNA in tumors of L12 compared to L8 ($p<0.05$).* The expression of Bax, Bcl2, Bid and caspase 9 were similar in the three groups. However, cleaved caspase 8, was higher in tumors from L3 than L12 ($p<0.01$). All this results indicate that optimal lactation early in life is associated with the activation of the extrinsic apoptotic pathway in mammary tumors. Moreover, we showed a greater cell proliferation of tumors in rats with a deficient lactation (L12). These results are in accordance with the augmented tumor incidence and the tendency of tumors to grow faster in this group of animals.

Keywords: mammary cancer, apoptosis, proliferation, lactation

(123) **THYROID HORMONES INDUCES CHEMOSENSITIVITY TO DOXORUBICIN IN JURKAT CELLS THROUGTH THE MODULATION OF ENZYMES INVOLVED IN CHEMOTHERAPY DRUG METABOLISM**

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Thyroid hormones (TH) – 3,3´,5-triiodo-L-thyronine (T3) and L-thyroxine (T4) – are important regulators of the metabolism and physiology of most normal tissues. We recently showed that TH stimulate the proliferation and metabolism of T cells.

To assess the effect of TH on the response to conventional chemotherapy, we determined the Doxorubicin (DOX) dose that inhibits proliferation by 50% (IC_{50}) in presence or absence of TH. As expected, DOX induces cytotoxicity in a dose-dependent manner in cells treated or not with TH. It is important to note that under proliferative conditions, pretreatment with TH sensitizes Jurkat cells to DOX treatment. Noteworthy pretreatment with TH significantly decreases chemosensitivity to DOX by 50% ($p<0,01$). It is well known that CYP3A4 is the major enzyme involved in the metabolism of chemotherapeutic drugs. We have previously demonstrated that TH induce a significant increase of CYP3A4 mRNA synthesis, protein expression and metabolic activity through both the canonnical (TR) and membrane (integrin $\alpha v\beta 3$) receptors. We reasoned that TH-induced CYP3A4 modulation may act as an important regulator in the metabolisms of DOX. To further explored the role of CYP3A4 in TH-chemosensitivity to DOX we used siRNA knock down of CYP3A4 in Jurkat cells. As expected, in CYP3A4 knocked down cells, no TH-mediated chemosensitivity was observed. We also found that TH modulate these functions by activating the membrane receptor integrin $\alpha v\beta 3$. We found that inactivation of integrin $\alpha v\beta 3$ by either a chemical inhibitor or siRNA inhibit TH-induced DOX chemosensitivity.

These results present a new mechanism by which TH could modulate chemotherapy response. These findings highlight the importance of evaluating thyroid status in patients during application of T cell lymphoma therapeutic regimens.

Thyroid Hormones; CYP3A4; Doxorubicin

GENETICS AND MOLECULAR BIOLOGY 1

(213) **DEVELOPMENT OF BIOTECHNOLOGICAL STRATEGIES INVOLVING CDPKS AND PGPB TO GENERATE POTATO PLANTS TOLERANT TO SALINITY AND *Phytophthora infestans***

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INGEBI-CONICET

Abstract: Salinity of soils affects plant development and is responsible for great losses in crop yields. Potato plants can grow well in soils with an electrical conductivity (EC) of 1,7 dS/m, however tuber yield decreases more than 50% at 5.9 dS/m. Calcium is a universal second messenger that responds to biotic and abiotic stimuli. Calcium dependent protein kinases (CDPKs) sense the fluctuation in cytosolic Ca2+ levels and participate in stress responses. Particularly, StCDPK2 promoter contains elements responsive to ABA, dehydration and desiccation. Transgenic plants 35S::StCDPK2 lines B and E presented higher biomass and greater root development under 50 mM NaCl. In order to compare the expression profile of CDPK2 in both normal and saline conditions we analyzed two transgenic lines (St2B and St2K) harboring reporter gene beta-glucuronidase (GUS) under the control of CDPK2 promoter. These plants were grown in solid MS media (2% agar) and were then treated with 150 mM NaCl for 1, 4 and 12 hours. Histochemical and fluorometrical GUS activity was conducted in leaves and roots. GUS activity was higher on the cells of the root cap under saline stress. Fluorometrical analysis correlated with this observation in line St2B. On the other hand, line 35S:CDPK2 A that exhibited a stronger tolerance to saline stress presented bacterial colonies associated to the root. The microorganism was isolated and 16S rDNA partial sequencing identified it as belonging to *Methylobacterium sp*. Treatment with 50 mM NaCl produced a reduction in chlorophyll content and root development which was mitigated with *Methylobacterium* inoculation. Moreover, a preliminary study showed its capacity to antagonize the phytopathogenic oomycete *Phytophthora infestans* the causal agent of late blight. Taken together, our results suggest that potato StCDPK2 could mediate the response to salt stress and that this plant growth promoting bacteria (PGPB) could play a significant role in saline tolerance and biocontrol.

Keywords: CDPKS, PGPB, salinity, *Phytophthora infestans*

(1851) **ADVANCES IN THE FUNCTIONAL STUDY OF THE *ARABIDOPSIS* DNA GLYCOSYLASE MBD4L**

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DNA glycosylases play important roles in the life of all organisms, acting at initial stages of the base excision DNA repair system that excises and replaces damaged bases from DNA. Interestingly, some particular DNA glycosylases remove 5-methylcytosine (5-mC), whose replacement by cytosine (C) can produce DNA demethylation. In Arabidopsis, DNA glycosylases from the DEMETER family have such capacity, and act over some well characterized targets. Here, we studied a novel DNA glycosylase recently described named MBD4L (methyl-binding domain protein 4 like), that is homologous to the human DNA glycosylase MBD4. Curiously, MBD4L does not recognize 5-mC *in vitro*. Even so, we evaluated if MBD4L can affect DNA methylation of some particular genomic regions. For that, we selected genomic targets with different chromatin states (euchromatic- heterochromatic) and used CHOP-PCR assays to determine their methylation level. Studies were conducted in wild-type, *mbd4l* mutant and MBD4L over-expressing plants, under both basal and stress conditions. We found that MBD4L controls DNA methylation at loci having different chromatin states. Moreover, the enzyme affects DNA methylation both at basal and stress condition. These results suggest that the action of MBD4L is not restricted to a particular chromatin state and probably contributes to stress-induced responses. The putative effects of MBD4L on the different genomic sites and on transcriptional regulation will be discussed.

Keywords: epigenetics, DNA demethylation, chromatin state, gene expression, DNA glycosylases, stress.

(1610) **CHANGE IN LIPID COMPOSITION OF *Bradyrhizobium* CELL ENVELOPED REVEAL A RAPID RESPONSE TO WATER DEFICIT INVOLVING LYSOPHOSPHATIDYLETHANOLAMINE SYNTHESIS IN OUTER MEMBRANE**

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Universidad Nacional de Río Cuarto

Cell enveloped is the primary targets affected by variations in the medium. Membrane behavior of *Bradyrhizobium* sp. SEMIA6144 during adaptation to water deficit induced by polyethylene glycol (PEG) was evaluated. *B.* sp. SEMIA6144 was exposed to PEG shock (1 h, 5 h and 24 h).

To investigate if PEG caused changes in cell morphology we used AFM. Fractions of internal and external cell membrane were obtained and lipids were extracted. The fatty acids (FA) were analyzed by GC and phospholipid (PL) were identified by TLC. For its quantification, [1-14C] sodium acetate was added to culture. Fluidity membrane cell and multilamellar vesicles (MVLs) from lipids cell was determined by measuring fluorescence polarization of DPH. To determine the PLA activity during PEG shock, outer membrane was used as enzyme source.

A dehydrating effect on the morphology of the cell surface as well as a fluidizing effect on the membrane was observed 10 min after PEG shock ($p<0.05$). The bacteria were able to restore optimal membrane fluidity. MVLs exposed to 1 h shock presented higher

rigidity compared to the control ($p<0.05$). A rapid response (1 h) was mediated by an increase in lysophosphatidylethanolamine (LPE) ($p<0.05$) at the expense of phosphatidylethanolamine(PE) ($p<0.05$) and an increase in saturated fatty acids ($p<0.05$). After 5 h and 24 h of PEG shock, the amount of LPE decreased ($p<0.05$) while PE increased ($p<0.05$). Shock of 1 h caused an increase of LPE ($p<0.05$) in the outer membrane through an increase in PLA activity ($p<0.05$) on PE. LPE amount did not remain constant during the shock. The inner membrane composition showed an increase of PC at the expense of PE ($p<0.05$) after 1 h of shock. However, the proportions of these PL reach values similar to the control after 24 h of shock.

Our results suggest that during PEG shock, the membranes composition of *B.* sp. SEMIA6144 is rapidly remodeled to counter fluidizing effect; then, this response is dynamically modified via PL turnover.

Keywords: Water deficit, Morphology cell, Membrane response, Phospholipase activity.

(538) **GENERALIZED MUTAGENESIS AND TAIL-PCR FOR THE IDENTIFICATION OF COMPONENTS INVOLVED IN THE INVERSE REGULATION BETWEEN T3SS AND MOTILITY IN *Mesorhizobium loti***

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IIB-UNSAM

Mesorhizobium loti presents a type 3 secretion system (T3SS) that is involved in the determination of bacterial competitiveness for the nodulation of *Lotus* spp. It was found that TtsI, the transcriptional factor that induces T3SS expression, inhibits bacterial motility in soft agar plates. This effect is accompanied by a down regulation of flagellar genes and flagella production. Besides, it was found that T3SS must be localized in membrane for that inhibition to occur. The objective proposed was to determine which mechanisms are involved in the motility inhibition. A generalized mutagenesis was performed by using mini-Tn5 transposon in order to isolate mutants that do not show this inhibition under T3SS induction conditions. A bacterial suspension was inoculated in soft agar plates containing naringenin (for T3SS induction) and those mutants that presented a higher motility were transferred to a new motility plate. After 5-6 passages, isolated colonies were obtained. In order to identify the gene in which the transposon was inserted, a TAIL-PCR method was performed for each mutant. We used a combination of random primers and transposon specific primers for the amplification of the region flanking the Tn5 sequence. By the evaluation of the presence or absence of the pili protein in culture supernatants, as indicative of T3SS functionality, mutants are being classified in two groups, those which the mutation affected a component of the T3SS and those in which a different gene was affected.

(1593) **GENERATION OF HYBRID BACTERIOCINS VARIANTS WITH ENHANCED ANTIMICROBIAL ACTIVITY**

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Bacteriocins are bacterial antimicrobial peptides of ribosomal synthesis active on phylogenetically related microorganisms. The chimerical BACTERIOCIN-HINGE-MICROCIN named PedA1-GGG-ColV and Ent35-GGG-MccV are new hybrid peptides with broader antimicrobial spectrum than the parental molecules. Based on the hypothesis that changes in the hinge connecting the bacteriocins could improve the activity of the hybrids, the inter-peptide region was subjected to gene-based bioengineering to generate novel derivatives with enhanced bioactivity. In this work, we generate a bank of randomly mutated hybrid bacteriocin genes by saturation mutagenesis. The employed site-directed saturation mutagenesis method uses two primers containing a degenerate mixture of the four bases at the central codon of the three-amino-acids hinge-encoding region. These primers were added to starting plasmid template and thermal cycled to produce mutant DNA plasmids, which were subsequently transformed into competent *Escherichia coli* DH5α. The plasmid DNA was purified from all the obtained colonies and the preparation containing a plasmid mix was analyzed by DNA sequencing. Approximately equal quantities of the four bases were seen at each position of the mutated codon. The plasmid mixture was used to obtain an expression library in *E. coli* BL21. One hundred colonies were randomly selected and the expression of hybrid peptides was further induced with 0.01 mM IPTG. The antimicrobial activity of cellular extracts was determined against the Gram (+) and Gram (-) indicator strains (*L. monocytogenes* FBUNT and *E. coli* MC4100). Some of the extracts showed higher antibiotic activity compared to the Ent35-GGG-MccV extract (parental hybrid). In particular, the extract obtained from the strain SN35 was four times more active on both *E. coli* and *L. monocytogenes*. The approach was highly successful to obtain mutants with improved bioactivity with respect to the original hybrids.

(211) **STNDPK3 IS A MITOCHONDRIAL NUCLEOSIDE DIPHOSPHATE KINASE FROM *Solanum tuberosum***

Elisa Fantino, Cecilia Eugenia María Grossi, Franco Santin, Marcelo Sciorra, Rita María Ulloa
INGEBI-CONICET

Abstract: Nucleoside diphosphate kinases (NDPKs) are capable of altering the set of nucleoside triphosphates, participating in the intracellular energy communication network and have been described in several signaling cascades associated to stress responses in plants. Plant NDPKs are organized in four distinct types (I to IV) according to their subcellular localization and their phylogenetic relationships. The cytosolic isoform Type I NDPK (NDPK1) has been shown to be the major NDPK isoform in plants (Dorion et al., 2006). An *in silico* search of the*Solanum phureja* genome (PGSC) and ClustalW alignment with NDPKs from other plants, animals and microorganisms allowed us to identify two chloroplastic (type II) and two mitochondrial (type III) isoforms that belong to group I and one secreted isoform (type IV) that belongs to group II. In this work we characterize one of the potato mitochondrial isoforms. The complete coding sequence of StNDPK3 was cloned from potato sprouts and the recombinant protein was expressed. The analysis of its expression profile reveals that it is an ubiquitous isoform. RT-qPCR assays of plants infected with *Phytopthora infestans* suggest that it could be involved in biotic responses.

Keywords: Solanum tuberosum, nucleoside diphosphate kinases, mitochondria, biotic stress

(1589) **PCR-BASED DETECTION OF RESISTANCE TO ACETYL-COA CARBOXYLASE-INHIBITING HERBICIDES IN JOHNSONGRASS (*Sorghum halepense* L. Pers*).***

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Herbicide-resistant weeds is one of the main problems of modern agriculture. Timely diagnosis is crucial for resistance management and mitigation, so confirming resistance in weed populations requires rapid and accurate methods to identify resistant biotypes. Bioassays are commonly used for detecting resistances. However, these methods are laborious, time consuming and do not discriminate between different mechanisms of resistance. When target site resistant mechanism is involved, it is possible to develop DNA-based assays, and thus, the polymerase chain reaction (PCR) technique can be used to detect resistant genotypes.

In this work, we cloned the CT domain of the plastidic acetyl-coenzyme A carboxylase (ACCase) encoding gene, target enzyme of herbicides group A, from a subpopulation of *Sorghum halepense* found in production fields of Gobernador Crespo (Santa Fe province). This subpopulation showed a resistance factor higher to 14.41 to haloxyfop-p-methyl (FOP) compared to a susceptible subpopulation from Zavalla (Santa Fe province).

The study of the sequences from bulks of genomic DNAs allowed to identify two alleles in the resistant subpopulation. The R1 allele carries the previously reported substitution (I2041N) as responsible for the resistance to FOP, and the R2 allele has a novel mutation that

could contribute to the resistance phenotype. To evaluate this last hypothesis, genetically identical Johnsongrass plants were obtained by clonal dispersion of rhizome after the application of the herbicide at the field dose. Then, these vegetative clones were subjected to specific allele PCR assays to screen the presence of each allele and to characterize the individual phenotype.

Accordingly, a simple method based on allele-specific PCR was developed to detect point mutations in the *accase* gene, allowing the rapid identification of different models of studies to elucidate resistance mechanisms.

Keywords: PCR, resistance, herbicide, weeds, ACCase

(1861) **CHARACTERIZATION OF A GENETICALLY ENCODED BIOSENSOR TO MEASURE NADP(H) REDOX POTENTIAL IN LIVING CELLS**

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Detection of $NADP^+$/NADPH in living organisms is a major analytical resource in biology. However, available methods are not efficient enough and are not able to distinguish existing levels in different cellular compartments. In this work, a non-invasive sensor was designed to determine the $NADP^+$/NADPH fraction based on the ratiometric properties of roGFP2: a GFP variant sensitive to reduction/oxidation which has two fluorescence excitation maxima at 390 and 485 nm and shows reversible ratiometric changes at 510 nm emission both *in vitro* and *in vivo*, responding to thiol-containing redox metabolites. We designed a biosensor to determine the cellular redox state of NADP(H), by fusing roGFP2 to the coding region of rice NADP(H)-thioredoxin reductase C (NTRC) by spacer arms of variable lengths. The resulting construct, NTRC-roGFP2, was expressed in *Escherichia coli* strains which over-express molecular chaperones, and purified by affinity chromatography. Fluorescence ratios reflecting NTRC-roGFP2 redox state varied with NADP(H) concentrations, indicating that NTRC-roGFP2 is capable to poise with NADP(H) and works as a sensor, therefore translating pyridine-nucleotides redox variations to the universe of thiols. The redox potential of this couple was determined by fluorescence measurements of the sensor redox status, applying the Nernst equation adapted to the $NADP^+$/NADPH pair.

The biosensor was also characterized by studies *in vivo* in tobacco plants and bacteria using confocal microscopy. Measurements on *E. coli* were assayed in cultures that were grown in defined minimal media as well as in anaerobic conditions. Concurrently, we are also working to improve electronic exchange between NTRC and roGFP2, to achieve a more efficient detection which can be then used for the study of fundamental redox mechanisms in living beings.

Keywords: biosensor, fluorescence, redox potential, NADP(H)

(843) **STRUCTURAL AND FUNCTION ANALYSES OF D199A MUTATION PROVIDE NEW INSIGHT INTO *XCC*BPHP BACTERIOPHYTOCHROME SIGNALING IN *XANTHOMONAS CAMPESTRIS PV. CAMPESTRIS***

Valeria Paola Conforte (1), Hernán Ruy Bonomi (2), Lisandro Otero (2), Jimena Rinaldi (2), Sebastián Klinke (2), Fernando Goldbaum (2), Adrián Vojnov (1), Florencia Malamud (3)

(1) ICT Milstein - Fundación Cassará, (2) Fundación Instituto Leloir, (3) IIB INTECH –UNSAM.

Many organisms possess photoreceptors that are capable of detecting light wavelengths and transduce this information within cells. Phytochromes constitute a major superfamily of light-sensing proteins that are reversibly photoconverted between a red-absorbing (Pr) and a far-red-absorbing (Pfr) state. *Xanthomonas campestris* pv. *campestris* (*Xcc*), the causal agent of black rot disease, codes for a functional bacteriophytochrome (*Xcc*BphP). Previous work of our group have demonstrated that *Xcc*BphP is a bathyphytochrome that acts as a negative regulator of virulence. Here, we go deeply into the study of the *Xcc*BphP structure and function. In this work, we analyze the role of the conserved residue Asp_{199} located in the photosensory domain, which is crucial for photochemical Pr-to-Pfr conversion. For this aim, we purified the recombinant *Xcc*BphP-D199A full-length mutant, crystalized it and solved the crystal structure, revealing a Pr state identical to the wild-type Pr structure. We also evaluated its UV-Vis absorption spectroscopic properties showing that this mutation locks *Xcc*BphP in Pr state. Once the effect of the mutation was established, we tested the effect of D199A during infection. We first complemented an *Xcc*bphP null mutant strain with *Xcc*BphP-D199A (D199A). We inoculated 10-day-old *Arabidopsis* plant seedlings with bacterial strains cultured under red, far-red or dark conditions. After 2 days p.i., bacterial CFU per plant mg were determined. We found that D199A was less virulent than the wild-type and the *XccbphP* strains in all cases regardless of the light treatment. D199A ability to infect plants remained comparable to null mutant strain carrying the plasmid with an intact *XccbphP* copy. Exopolysaccharide production, a known virulence factor, correlated with the infection results. Taken together, these results indicate that D199A mutation locks *Xcc*BphP in an activated Pr-like state, irresponsive to light, inhibiting virulence factors and plant infectivity.

Key words: *Xanthomonas*, bathy-type phytochrome; virulence factors

(468) **DEVELOPMENT OF METHODS TO VALIDATE SYNTHETIC LETHALITY INDUCTION *IN VITRO* AND *IN VIVO***

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One of the current challenges in the development of anticancer therapies lies in the identification of drugs that selectively kill cancer cells with minor impact on normal cells. A strategy of selective cytotoxicity that has yielded promising results is the induction of synthetic lethality (SL). SL induction involves tumor-acquired mutations that sensitize cells to a given treatment when they are in combination. We are focused in the study of drugs that cause SL in BRCA1/2-deficient cancer cells (Bd-cells). Herein we show the validation of potential drugs which have been previously identified as SL-inducers using a cell-based high-throughput screening platform based on flow cytometry. Three validation methods were developed to assess the performance of these drugs on a Bd-cell model in comparison to the wild type cell line (Bw-cells). The first method evaluates the clonogenic potential of Bd-cells and Bw-cells after the treatment with the drugs, using an adaptation of the colony formation assay. The second method involves the use of 3D chimeric spheroids constituted by an equal proportion of Bd-cells and Bw-cells. Both cell populations are tagged with different fluorescent proteins and can therefore be quantified by flow cytometry after the treatments with the drugs. The third model is a double tumor xenograft approach in Nude mice. This model consists in the generation of flanking tumors in a single mouse, one from Bd-cells and the other from Bw-cells. Mice are then treated with potential SL-inducers or vehicle and the progression of each tumor-type is assessed in order to expose the SL phenomena *in vivo*.

Keywords: Synthetic lethality, BRCA1/2-deficient cells, DNA damage

GENETICS AND MOLECULAR BIOLOGY 2

(521) **NUCLEAR TRANSLOCATION OF PROTEINS *VIA* IMPORTIN PATHWAY IN PROTOZOAN PATHOGEN *TRYPANOSOMA CRUZI***

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In higher eukaryotes, active transport of proteins to the nucleus

occurs through the nuclear pore complexes (NPCs), directed by nuclear localization signal (NLS) sequences. The NLS is recognized by importin α in the cytoplasm that then binds to importin β which transports this trimeric complex across the NPC. The importin α consists of two functional domains, a short basic N-terminal domain (the IBB domain) sufficient for importin β binding, which also acts as an autoinhibitory domain, and a NLS-binding domain comprised of armadillo (arm) repeats. There is limited amount of information about nuclear protein targeting in lower eukaryotes such as trypanosomatids. Therefore, we aimed to study the importin pathway in *Trypanosoma cruzi* (*T. cruzi*), causative agent of Chagas Disease, using the N-terminal domain of the poly(ADP-ribose)polymerase from *T. cruzi* (TcPARP-NT) as a tool, which is known to be sufficient for nuclear translocation. Through a bioinformatic approach we found a sequence encoding for a putative importin α (TcIMPα) in this parasite. This gene was cloned and expressed as a full length protein (TcIMPα FL) or lacking the IBB domain (TcIMPα ΔIBB). A pull down assay using His-tag capture confirmed binding of TcPARP-NT with TcIMPα ΔIBB. In a Size Exclusion Chromatography this protein complex did not elute together, but by using centrifugal filters, with a cut off higher than the TcPARP-NT molecular weight, binding was observed. In addition, interaction of TcIMPα with TcPARP was confirmed by Bio-Layer Interferometry that enables real-time, label-free analysis for determination of affinity, which resulted in μM dissociation constant. It was established that TcIMPα FL and TcPARP-NT do not bind reinforcing the idea of importin α autoinhibition, also present in the parasite. Taken together, these results give us a first insight into the presence of an operative importin system in *T. cruzi* which could play an active role in protein translocation.

Keywords: Importin α, *Trypanosoma cruzi*, nuclear translocation of proteins

(516) **ROLE OF THE ESCRT COMPLEX IN TRYPANOSOMATIDS: FUNCTIONAL CHARACTERIZATION OF Vps32**

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INGEBI; CONICET; UBA

The ESCRT (Endosomal Sorting Complex Required for Transport) is a machinery that drives a diverse collection of membrane remodeling events such as endocytosis, multivesicular body biogenesis, autophagy, release of enveloped viruses, reorganization of the nuclear envelope and cytokinetic abscission during mitosis exit. This complex is composed of four subcomplexes (0-III) been ESCRTIII the effector of the complex. Vps32 is the most abundant protein in ESCRTIII and the abscission capability is given for its molecular structure alternating between a monomeric-closed state to polymeric-open state. Here we investigate the conservation of Vps32 in *Trypanosoma cruzi* and *Trypanosoma brucei*. The *Saccharomyces cerevisiae* gene corresponding to the Vps32 (Snf7) was used to screen TriTrypDB databases. We found the *T. cruzi* orthologue (TcVps32) which have two alleles in CL Brener strain: TcCLB.511589.250 (Esmeraldo) and TcCLB.511229.100 (Non Esmeraldo). These sequences were used to screen *T. brucei* database resulting in a high-scored target: Tb427tmp.01.1390. Protein domains, secondary structure and isoelectric point were determinated showing the presence of Snf7 motif, alpha helices with the MIM motif in the last helix and basic N-terminal and acid C-terminal which implies a structure conservation among eukaryotic cells. To perform a functional characterization, TcVps32 coding sequence was amplified by PCR fused to a hemagglutinin tag at N-terminal (HA-TcVps32) or without tag (TcVps32). The PCR products were subcloned into pRIBOTEX and the constructs (pRibo-HA-TcVps32 and pRibo-TcVps32) were transfected in *T. cruzi* epimastigote cells. Simultaneously, in *T. brucei* procyclic form we designed an RNAi strategy where a 405bp of TbVps32 was cloned into the p2T7 vector (TbVps32-RNAi) which allow a tetracycline inducible silencing of this protein. The TcVps32 overexpressing *T. cruzi* epimastigotes and the procyclic form of *T. brucei* transfected with TbVps32-RNAi are under evaluation.

Keywords: membrane remodeling, multivesicular body biogenesis, autophagy, cytokinetic.

(1854) **THE ROLE OF SUMO CHAINS IN NUCLEAR FOCI FORMATION AND CHROMATIN ORGANIZATION IN *Trypanosoma brucei* PROCYCLIC FORMS**

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SUMOylation is a post-translational modification conserved in eukaryotic organisms that involves the covalent attachment of the small ubiquitin-like protein SUMO to internal lysine residues in the target proteins. This tag usually alters the interaction surface of the modified protein and can be translated into changes in its biological activity, stability or subcellular localization, among other possible outputs. SUMO can be attached as a single moiety or as SUMO polymers in case there are internal acceptor sites in SUMO itself. These chains have been shown to be important for proteasomal degradation as well as for the formation of subnuclear structures such as the synaptonemal complex in *Saccharomyces cerevisiae* or promyelocytic leukemia (PML) bodies in human cells.

In this work, we have examined SUMO chain formation in the ancient protozoan parasite *Trypanosoma brucei*. Using a recently developed bacterial strain engineered to produce SUMOylated proteins we confirmed the ability of *Tb*SUMO to form polymers and determined the type of linkage using site-directed mutational analysis. By generating transgenic procyclic parasites unable to form chains we demonstrated that although not essential for normal growth, SUMO polymerization determines the localization of the modified proteins at the nuclear periphery. In addition, FISH analysis of the telomeres showed a differential localization depending on the polySUMOylation abilities of the cells, and MNase accesibility assays revealed structural changes in the chromatin. A bioinformatic analysis showed that the *T. brucei* proteome contains about a hundred of good SUMO interactor candidates. Specifically, these proteins are sequences containing SUMO interactive motifs enriched in nuclear processes. Thus, our observations indicate that SUMO chains play an important role in establishing interaction platforms at the nuclear periphery likely contributing to chromatin organization.

Keywords: polySUMOylation, Chromatin, Telomeres, *Trypanosma brucei*

(1095) **UNRAVELLING THE FUNCTION OF THE UNIQUE N-TERMINAL DOMAIN OF *TRYPANOSOMA CRUZI* HIGH MOBILITY GROUP B PROTEIN.**

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High Mobility Group B (HMGBs) are a family of chromatin proteins capable of binding non-canonical DNA structures through one or more "HMG box" domains. These proteins are highly conserved among eukaryotic organisms and are involved in key nuclear processes like transcription, replication, recombination and DNA repair. The HMGB from *Trypanosoma cruzi* (*Tc*HMGB) contains two "HMG box" domains and a unique N-terminal sequence present only in trypanosomatids' HMGBs. *In silico* studies showed that within this region there is a nuclear localization signal (NLS) and a DEK-C terminal domain, a putative third DNA-binding domain in trypanosomes´ HMGBs.

We obtained parasites capable of overexpressing truncated forms of TcHMGB (N-terminal domain and *Tc*HMGBΔN) and the full length *Tc*HMGB as well. Immunofluorescence assays showed that the wild type version is located in the nucleus but when we overexpressed TcHMGB lacking the N terminal domain (*Tc*HMGBΔN) the protein is distributed along the whole parasite but excluded from the nucleus. On the other hand, when we overexpressed only the N-terminal region, this fragment accumulated in the nucleus but also it was detected in the cytoplasm. These results suggest that the NLS is necessary but not sufficient to drive the protein to the nucleus.

We previously determined that overexpression of TcHMGB results in changes in chromatin structure that is detrimental for the parasite fitness. We now evaluated the effect of overexpressing the N-termi-

nal region alone, which caused a dramatic decrease in epimastigotes growth. In order to clarify this phenotype we analyzed the cell cycle by flow cytometry with synchronized cultures in the G1 phase, showing a clear delay in the cell progression compared to the negative control. *In vitro* infection assays showed that amastigotes replication can also be affected. We propose that the DEK-C terminal domain may be another contact point of TcHMGB with nuclear DNA contributing to chromatin structure remodeling.

Key words: TRYPANOSOMA CRUZI, DEK-C, NLS, CHROMATIN.

(561) **PROTEOMIC ANALYSIS OF EXTRACELLULAR VESICLES FROM *Trypanosoma cruzi* TRYPOMASTIGOTES REVEALED THAT CONTAIN A LARGE AMOUNT OF PROTEINS INVOLVED IN PATHOGENESIS**

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The trypomastigote is the circulating bloodstream and non-replicative stage of *Trypanosoma cruzi*, the causative agent of Chagas disease. Many of the surface proteins of the trypomastigote, involved in cell invasion and/or in the infection process, are also secreted. We have previously found that trypomastigotes secrete into extracellular vesicles (EVs) virulence factors that should be hidden from the immune system. Data currently available from exoproteomes were derived from total secreted material (free and vesicle-contained, Y strain) or from a mixture of EVs from parasites and host cells (Tulahuen strain). Here we present a proteomic analysis of trypomastigote EVs (reference CL Brener strain), to search for putative virulence factors secreted into trypomastigotes EVs. To isolate EVs, conditioned media from cell-derived trypomastigotes were sequentially ultracentrifugated to obtain, after 2 and 16 hs, EVs fractions V2 and V16, respectively. Both fractions were analyzed in a Q Exactive HESI-Orbitrap coupled to a nano HPLC Easy-nLC 1000. MS/MS data were used to search *T. cruzi* database (Tritrypdb.org, v.30). Each EV subpopulation presented a differential set of major proteins (V2: n=270; V16: n=228), and a minor core of 142 common proteins. GO Analysis (UniprotKB) revealed that V2 and V16 had mostly proteins involved in pathogenesis (37,8%; 56,7%) and cellular process (22,1%; 12%). We also found known virulence factors like KMP-11, several members of multigene families (TS, TcTASV) and immune system modulators (i.e. TcHMGB), some of them were among the common core like TcTASV-C, TS group I and TolT. This data demonstrate that EVs contain a large number of key proteins involved in multiple regulatory and pathogenic processes. Characterization of trypomastigote EVs cargo may be helpful to identify novel vaccine or drug targets for the control of Chagas disease.

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Keywords: *Trypanosoma cruzi*, trypomastigote, extracellular vesicles, proteome, pathogenesis

(293) **ANALYSIS OF THE NADPH CYTOCHROME P450 REDUCTASE GENE: ITS RELATIONSHIP WITH PYRETHROID RESISTANCE IN THE CHAGAS DISEASE VECTOR *Triatoma infestans***

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Abstract: Pyrethroid resistance has been detected in the Chagas disease vector *Triatoma infestans* (Hemiptera: Reduviidae), which has been attributed to target site insensitivity and increased oxidative metabolism of the insecticide by cytochrome P450s. NADPH cytochrome P450 reductase (CPR) plays an essential role in transferring electrons from NADPH to the P450-substrate complex. In this study, the full length CPR cDNA of *T. infestans* was isolated and gene expression was determined by quantitative PCR. The open reading frame is 2046 bp long, encoding a protein of 682 amino acids. Amino acid sequence analysis indicates that the *T. infestans* CPR and the putative *Rhodnius prolixus* and *Triatoma dimidiata* CPRs, present conserved ligand-binding domains. The expression of the CPR gene at transcriptional level was determined in muscle, heads, gonads and fat body of fifth instar nymphs and adults. In both stages, gonads and fat body presented significant levels of expression. It was detected a higher level of expression in gonads of nymphs than in adults, while the fat body presented a higher level of expression in adults. On the other hand, congruently with a previous study of our laboratory, in which the expression of three cytochrome P450 genes (CYP4EM7, CYP3085B1, and CYP3092A6 genes) was induced by deltamethrin, the levels of *T. infestans* CPR mRNA were up-regulated in the fat body of fifth instar nymphs after topical application of deltamethrin. Besides, similarly to the observed in the CYP4EM*7* gene, it was detected overexpression of the CPR gene in the most resistant strain of *T. infestans* included in the study. These results suggest that CPR plays an essential role in P450-mediated resistance of *T. infestans* to insecticides.

Keywords: chagas disease, *Triatoma infestans,* pyrethroid resistance, CPR gene

(305) **ANALYSIS OF THE CLOCK GENES PERIOD AND TIMELESS IN CHAGAS DISEASE VECTORS**

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In *Rhodnius prolixus* and *Triatoma infestans* circadian rhythms seem to control basic biological processes. In order to better understand the molecular bases of the circadian rhythms, we explored the biological central clock at the molecular level in triatomine insects. With this purpose, we identified coding regions of the clock genes *period* (*per*) and *timeless* (*tim*) from the genome of *R. prolixus* and analyzed their expression at mRNA level in *T. infestan*s. The *per* and *tim* genes expression in nervous tissue of adults *T. infestans* varies with a daily rhythm in groups of individuals maintained under photoperiod (LD), showing a significant peak of expression at sunset. These rhythms of expression agrees with those described in *Drosophila melanogaster* and would be compatible with the daily oscillation in the levels of PER and TIM proteins detected by other authors in *R. prolixus*. Contrary, in the group maintained under constant light (LL), no daily increase were detected in *per* and *tim* transcript level. This result suggests a disruptive action of light in the transcription of the clock genes *per* and *tim*. Besides, the presence of *per* transcript in different tissues of adult individuals and in nervous tissue of nymphs evidenced activity of peripheral and central clocks in adults and activity of the central clock in nymphs of *T. infestans*.

Keywords: Chagas disease, vectors, clock genes, *period* and *timeless*.

IMMUNOLOGY (ADAPTATIVE IMMUNITY) 1

(466) **REGULATION OF DENDRITIC CELLS INTRACELLULAR TRAFFICKING BY SNX17 DURING ANTIGEN CROSS-PRESENTATION**

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Cross-presentation by MHC class I molecules allows the detection of exogenous antigens by CD8+ T lymphocytes. This process is crucial to initiate cytotoxic immune responses against many pathogens (i.e. *Toxoplasma gondii*) and tumors. To achieve efficient cross-presentation, dendritic cells (DCs) have developed highly specialized adaptations of their endocytic network. Consequently, DCs are the most potent antigen presenting cell type to accomplish this immunological process. However, a complete view of the many molecular effectors involved in antigen cross-presentation is still missing. Sorting nexin (SNX) proteins are characterized by the presence of a phox-homology (PX) domain that interacts with elements of the endocytic pathway enriched with phosphatidylinositol-3-monophosphate. In this way, SNXs control key features of endocytosis, as well as endosomal signaling, sorting and tubulation. In this study, we fo-

cus on the role of SNX17, which associates with compartments of the early endocytic network and participates in several processes of intracellular recycling. Here, we identify SNX17 as a crucial regulator of antigen cross-presentation by DCs. By silencing the expression of SNX17 with shRNAs, we evaluated in DCs the role of this molecule in the fast and slow recycling pathways by flow cytometry. Furthermore, the knock-down of SNX17 hampered the cross-presentation of soluble, particulate and *T. gondii*-associated antigens. Our results also demonstrate that SNX17 is actively recruited to the vacuole containing *T. gondii* parasites in DCs, as observed by immunofluorescence labelling and confocal microscopy analysis. Our findings provide compelling evidence that SNX17 plays a central role in the endocytic trafficking of DCs and is crucial to guarantee an efficient antigen cross-presentation.

Keywords: Dendritic cells, cross-presentation, SNX17, recycling, *Toxoplasma gondii*.

(192) **CHARACTERIZATION OF TONSILLAR IL10 SECRETING B CELLS AND THEIR ROLE IN THE PATHOPHYSIOLOGY OF TONSILLAR HYPERTROPHY**

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The immune actions of tonsillar B cells (Bc) must be tightly regulated to balance the protection against virulent germs and the tolerance to harmless flora and innocuous Ags in food and air. Despite the logic of such presumption, the potential role of tonsils in oral tolerance induction has not been completely elucidated so far. The aim of our work was to test the ability of tonsillar Bc to secrete IL10 (regulatory cytokine) and to assess the physiological relevance of this population. All measurements were done by FACS. We determined the phenotype of tonsillar IL10 secreting Bc (tBregs). Also, we found that the frequency of tBregs as determined by FACS upon 72 hs stimulation *ex vivo,* via TLR9 and CD40L, depended on the cause of surgery. The hypertrophied (HT) tonsils´ samples showed a significantly ($p<0.05$) lower percentage of tBregs (4.4±0.4) compared to those from recurrent tonsillitis (RT, 9.9±1.5) as well as a significantly ($p<0.05$) higher proportion of the germinal center (GC) population (23.8±4.3) than those from children with RT (7.9±1.8). A comparative increment of the GC percentage was not accompanied by a proportional growth of the memory B cell population in those HT samples. In contrast, these samples displayed a significantly ($p<0.05$) lower percentage of the eBm5 subset (7.8±0.5) than RT samples (15.5±2.1), indicative of a putative blockade between the GC and memory B cell stages on HT patients. Collectively, our results demonstrate that a defective tBregs compartment indicates an increase in the proportion of GC *in vivo* and therefore unrestrained Tfh function, suggesting that Tfh are a target population of tBreg function. Finally, tBreg modulated TNFα intracellular expression by CD4+ cells in co-cultures of autologous tonsillar lymphocytes. Our findings provide greater insight into the role of tBregs in GC reactions and characterized for the first time their involvement in the pathogenesis of tonsillar dysfunction.

Keywords: Regulatory B cells, Mucosal immunity, Tonsillar disorders

(202) **CHARACTERIZATION OF CYTOKINE SECRETING B CELL SUBPOPULATIONSIN HUMAN TONSILS**

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Tonsils are secondary lymphoid organs which must grant immunologic protection against pathogens and tolerance against inoffensive Ags in air and food. They are mostly B-cell (Bc) maturation and differentiation sites. Bc are able to secrete multiple cytokines under different stimuli, independently of their role as Ag presenting and Ab producing cells. It is likely that alteration of the immune equilibrium activation/regulation may trigger recurrent tonsillitis (RT) and/or hypertrophy (HT) leading in turn to tonsillectomy. Hence, the aim of our work was to characterize such unconventional immune functions of tonsillar Bc, *i.e* different Bc subsets with regulatory and pro-inflammatory profiles, within the human oropharyngeal cavity. All measurements were done by FACS. We determined tonsillar Bc expression of IL6, IL8, IFNγ, IL5, TNFα, IL21, IL10 and TGFβ at different time-points post stimulation. Also, we established the kinetics of appearance of IL10 (regulatory cytokine) expressing Bc (B10) and compared it with that of pro-inflammatory cytokine-expressing Bc (IL6 and IL8) for 5 patients upon 0; 16; 32 and 72 hs stimulation, via TLR9 and CD40L. The proportion of B10 increased between 16 and 72 hs in all cases. Notably, the percentage of B10 detected at the different time points depended on the cause of surgery, an issue that is addressed in a different poster in the same Congress. The results varied across individuals; hence, we showed the contour plots of a single patient and graph lines plotting the results of 5 different patients for the cytokine kinetics. Importantly, we also found a relevant proportion of IL17-expressing B cells in tonsils which is, to our knowledge, the first time that is reported in literature. Collectively, these results demonstrate that despite disease-associated changes, Bc from those damaged tonsils preserve their immune competence to secrete a number of cytokines. This study advances our understanding of the immune functions of the tonsils.

*Keywords:*Regulatory Cytokines, Pro-inflammatory cytokines, Mucosal Immunity.

(1282) **LOW TLR9 EXPRESSION IN ADAPTIVE CELLS PROTECTS AGAINST PROGRESSION OF NONALCOHOLIC FATTY LIVER DISEASES**

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T cells play a key role for the progression of Nonalcoholic Fatty Liver Diseases (NAFLD). Signaling through toll-like receptors (TLR) co-stimulates activation/ differentiation and generation of memory T cells. No study correlates clinical facts of NAFLD patients with TLR9 expression and functionality of adaptive cells. We aimed to study TLR9 expression in peripheral and liver adaptive cells during NAFLD progression, its link with metabolic changes and the differentiation of effector and memory cells. Biochemical/anthropometric parameters were measured. PBMC were obtained by Ficoll-hypaque gradient and liver cell suspensions by mechanical disruption. Cells were stained with anti-CD4, -CD8, -TLR9 or -IFNg mAbs and studied by flow cytometry (FC). Analysis of $CCR7^{+/-}$ subpopulations of $CD4^+$ and $CD8^+$ memory cells was performed after negative selection of CD45RO cells. Mann-Whitney, Kruskal-Wallis and Spearman´s correlation tests were used. Blood and liver samples came from 13 patients with simple steatosis (SS), 15 steatohepatitis (SH) and 21 healthy controls (CO). We found a correlation between plasmatic AST and ALT levels with TLR9 expression in peripheral $CD8^+$ cells ($r=0.645$, $p=0.037$; $r=0.645$, $p=0.034$), and between plasma triglycerides levels with TLR9 expression in hepatic $CD4^+$ cells ($p=0.034$, $r=0.821$) from NAFLD patients. Liver $CD4^+$ cells (p=0,020) and peripheral $CD4^+$ and $CD8^+$ cells ($p=0.022$; $p=0.002$) from SS patients

showed a low TLR9 expression vs. CO. $CD8^+$ cells from SS patients exhibited a low frequency of $CD8^+IFNg^+$ cells (p=0,002 vs SH). A higher frequency of $CD4^+CCR7^-$ cells (p=0.013, vs. CO) was found but unrelated to a higher expression of TLR9 in the $CCR7^+$ and $CCR7^-$ cells. The overall lesser expression of TLR9 and particularly its association with cytotoxic cells differentiation in SS, but not with memory cell generation, might confer protection against NAFLD progression.

Keywords: TLR9, NAFLD, adaptive cells

(1116) **ALPHA-(2,6) SIALYLATION INFLUENCES B CELL FUNCTIONALITY IN THE MUCOSAL COMPARTMENT**

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Glycosylation is a common post-translational modification that has the potential to regulate cellular processes relevant to immune tolerance and disease. Here, we aim to examine the impact of α(2,6) sialylation (α2,6sia) on B-cell functionality, specifically in the context of intestinal immunity. In a α(2,6) sialyltransferase (ST6Gal1) KO mouse model, we found that the animals exhibited a decreased percentage of IgA+ plasma cells (PCs) in the intestinal lamina propria and alterations in fecal bacterial coating with IgA. Moreover, 16S rRNA gene sequencing analysis of fecal microbiota also showed significant differences in bacterial composition and a reduction in diversity compared to wild type (WT) mice. In order to study α(2,6)sia influence on B-cells during intestinal inflammation, we established a chronic T-cell transfer model of colitis in $RAG2^{-/-}$ mice. Co-transfer of CD4+CD45RBhi T-cells and WT B-cells reduced colonic inflammation and IFN-γ expression in lamina propria (LP). However, co-transference with $ST6Gal1^{-/-}$ B-cells resulted in decreased percentage of B-cells in mesenteric lymph nodes, lower amounts of IgA+ plasma cells and increased number of CD4+ T-cells in LP. Additionally, we observed a decreased percentage of IgA-coated bacteria when compared to co-transference of WT B-cells. These results show an unrecognized role of sialic acid in the mucosal compartment, B-cell functionality and reveal potentially novel mechanistic roles for B-cell glycobiology in intestinal inflammation.

Keywords: B cell, sialic acid, mucosal compartment, microbiota

(1650) **CHARACTERIZATION OF THE TSLP AND IL33 AXIS IN THE INFLAMMATORY COLONIC TISSUE OF FOOD ALLERGIC PATIENTS WITH JUVENILE POLYPS**

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Juvenile polyps are common in paediatric patients, leading to rectal bleeding and diarrhea. The aetiology is largely unknown and we have previously characterized the allergic inflammatory infiltrate of the polyp tissue in children with clinical suspicion of milk allergy. We found high frequency of eosinophils, IgE-producing plasma cells, Th2 cells, high level of type 2 cytokines and augmented expression of ST2 and IL33. These results lead us to investigate the production of thymic stromal lymphopoietin (TSLP) and IL-33 as contributors to the development of the Th2 local immune response.

TSLP and IL-33 expression were assessed in the epithelial compartment and lamina propria of colonic polyps (P) and surrounding colonic tissues (SCT). Biological samples were obtained in 7 patients by colonoscopy and analysis were performed by confocal microscopy and qPCR.

We found a significant increased expression of IL-33 in epithelial cells from P compared with SCT (p<.04), while no expression was observed for TSLP in P and SCT. In contrast, TSLP (p<.07) and IL-33 (p<.05) were increased in the lamina propria of P compared with SCT. Confocal microscopy revealed the presence of $IL33^+$ epithelial cells (11.50±3.96 vs 1.00±0.41 cells in 600x field, P vs SCT respectively) (p<.04). TSLP was not detected in epithelium of P or SCT. In addition, the lamina propria revealed the presence of $TSLP^+$ cells (11.75±2.81 vs 1.50±0.30 cells in 600x field, P vs SCT respectively) (p<.01) and $IL33^+$ cells (27.40±4.02 vs 2.25±0.95 cells in 600x field, P vs SCT respectively) (p<.001).

In conclusion, we found different cell sources of TSLP and IL-33 with a strong production of IL-33 in the epithelial compartment of the inflammatory tissue. These findings may shed light to understand the etiology of the allergic infiltrate found in the inflammatory colonic tissue of patients with food allergy.

Keywords: juvenile polyps, food allergy, IL33, TSLP, colonocytes

(1890) **BLIMP-1: A NEGATIVE REGULATOR OF IFN-g EXPRESSION IN THE IMMUNE RESPONSE TO *Mycobacterium tuberculosis*.**

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Tuberculosis now ranks alongside with HIV as the leading cause of death from an infectious disease. Protective immunity against *Mycobacterium tuberculosis* (*Mtb*) is mediated by Th1 immune response and IFN-g secretion.

Blimp-1 plays key roles in many cell lineages and in early development, promotes Th2 lineage commitment by opposing the differentiation of IFN-g-secreting Th1 cells. Overexpression of Blimp-1 in T cells attenuates Th1 cell expansion through downregulation of IFN-g.

Therefore, we evaluated Blimp-1 protein expression in PBMCs from healthy donors stimulated with *Mtb* at different time points. We found the highest Blimp-1 expression after 3 days of *Mtb*-stimulation. Blimp-1 expression decreased at 4 days and showed the lowest expression after 5 days of *Mtb*-stimulation. Blimp1 kinetic was opposite to IFN-g; with the highest levels at 5 days of *Mtb* stimulation (as determined by ELISA).

We further studied Blimp-1 expression levels in $CD3^+$ $CD4^+$ T cells by flow cytometry. We observed high levels of Blimp-1 on CD4+ T cells. Moreover, *Mtb*- stimulation decreased Blimp-1 expression after 5 days. Interestingly, Blimp-1 expression among IFN-g producing cells was reduced after 5 days of *Mtb*-stimulation.

Finally, to evaluate if Blimp-1 directly regulates IFN-g expression, we determined Blimp-1 binding to the IFNG gene regulatory sites CNS-22 and -242 by ChIP-qPCR. At CNS-22 site of IFNG gene we found higher binding of Blimp-1 at 3 days of *Mtb*-stimulation compared to 5 days. We did not find binding differences at -242 binding site.

Taken together, our results demonstrate that *Mtb* stimulation regulates Blimp-1 expression on T cells and further suggest that Blimp-1 could act as an IFN-g repressor, trough the binding to CNS-22 site during *Mtb* stimulation.

During the immune response against *Mtb*, IFN-g is regulated by epigenetic modifications. Blimp1 could be responsible to recruit histone modification complex to the IFN-g regulatory sites.

Keywords: *Mycobacterium tuberculosis*, IFN-g, Blimp1, Epigenetics

(241) **B CELLS SUSTEIN *Trypanosoma cruzi* SPECIFIC CD8+ T CELL RESPONSE VIA IL-17**

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CD8+T cells are key in the defense against *T.cruzi* infection. Then, factors that promote the generation and maintenance of CD8+T cell response need to be studied in deep. The aim of our work was to analyze the role of B cells on CD8+T cell response during *T.cruzi* infection.

For this, 8 days before intraperitoneal (ip) infection with 5000 trypomastigotes of *T.cruzi* Tulahuén strain, C57BL/6 mice were ip injected with anti-CD20 (BcD mice), to deplete B cells, or with control isotype. At 20 days post infection (dpi), tissue parasitic DNA quantification was assessed by real time PCR and *T.cruzi*-specific CD8+T cell response was measured by FACS using tetramers loaded with the parasite immunodominant peptide Tskb20. Phenotype and function of CD8+ T cells were also analyzed by FACS.

Infected BcD mice exhibited higher parasitism than controls. Further, infected BcD mice had a significant lower frequency and number of Tskb20+CD8+T cells in blood, spleen and liver (p=0.01), lower frequencies of short-lived (p=0,03) and memory (p=0,02) effector cells, and a significant higher frequency of naïve CD8+T cells, than infected controls. Total and *T.cruzi*-specific CD8+T cells from infected BcD mice exhibited a lesser extent of activation and proliferation but higher levels of inhibitory receptors. In agreement, CD8+T cells from infected BcD mice presented reduced cytotoxicity, degranulation, IFN and TNF production (p=0,02). When infected mice with a settled down specific-CD8+T cell response (12dpi) were depleted from B cells, interestingly, they exhibited the same characteristics than those depleted before infection. Injection of recombinant IL-17 rescued the frequency, phenotype and function of CD8+T cells generated in B cell absence.

Results indicate that B cells are key for *T. cruzi* specific CD8+T cell maintenance and function, but are not necessary for their induction. Considering B cells produce IL-17 in *T. cruzi* infection probably its function on CD8+ T cells depends on IL-17.

Keywords: B cell, CD8 T cell, *T. cruzi*, IL-17

IMMUNOLOGY (INNATE IMMUNITY) 1

(368) THE ROLE OF IL-10 IN FOAMY MACROPHAGE DIFFERENTATION IN TUBERCULOSIS

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The ability of Mycobacterium tuberculosis (Mtb) to persist relies on its numerous immune evasion strategies such as the dysregulation of the lipid metabolism leading to foamy macrophages (FM). Although FM provide an environment which is essential for bacilli to escape from host immune system, the specific host factors leading to FM induction are unknown. We aimed to characterize which host factors may contribute to FM differentiation in Mtb infection. For that we use the acellular fraction of tuberculous pleural effusions (PE) as a source of local factors released during *Mtb* infection. We treated human macrophages with PE and evaluated the accumulation of lipid bodies by oil O red staining, cytokines by ELISA, ACAT and pSTAT-3/STAT-3 by western blot, cholesterol by enzymatic assays, CD36 and CD210 by FACS, and bacillary loads by colony-forming units assays.

PE induced FM differentiation as observed by the increased in lipid bodies ($p<0.01$), intracellular cholesterol ($p<0.05$), CD36 expression –receptor which mediates lipids uptake- ($p<0.05$), and ACAT expression –enzyme that esterifies cholesterol- ($p<0.05$). All these parameters could be prevented after IL-10-depletion unlike the depletion of IL-1**β**, IL-6, IFN**γ**, IL-4, or TNFα ($p<0.05$). In line with it, while PE induced the activation of STAT-3 –transcription factor activated by IL-10- ($p<0.05$) and the expression of IL-10 receptor (CD210; $p<0.05$), the inhibition of STAT-3 prevented FM differentiation ($p<0.05$). Interestingly, PE treatment enhanced the bacillary loads in an IL-10 dependent manner ($p<0.01$). To confirm the role of IL-10, we evaluated whether BMDM from IL-10 KO mice could indeed become FM. IL-10 deficiency prevented FM induction after Mtb lipids exposure in comparison with wt BMDM ($p<0.05$). In conclusion our results provide evidence for a role of IL-10 in promoting FM differentiation in the context of Mtb infection contributing to our understanding of the host metabolic alterations driven by Mtb.

Keywords: IL-10, foamy macrophages, lipids, tuberculosis.

(488) PROKARYOTIC RNA MODULATES ENDOTHELIAL-NEUTROPHIL INTERACTIONS

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Human neutrophils (PMN) are able to sense bacterial viability, activating and generating a response only against live bacterial stimuli. In this regard, we have demonstrated that prokaryotic RNA (pRNA), present only in live bacteria, is capable of triggering PMN activation and the generation of bactericidal responses. Because endothelial cells can actively regulate the migration of PMN through the endothelium by the expression of adhesion molecules and chemokines, our objective was to determine if pRNA can directly activate the endothelium and modulate the interaction with PMN. In all experiments, a microvascular endothelial cell line (HMEC-1) was stimulated with RNA (1 μg/ml) extracted from *E. coli* (pRNA) or from human eukaryotic (mononuclear) cells (eRNA), or left untreated, for 24 hs. After stimulation, the expression of ICAM-1 (CD54) in HMEC-1, measured as the MFI by FACS was increased in pRNA-treated cells compared to the other groups ($p<0.001$). Moreover, the secretion of IL-8 after pRNA exposure was higher ($p <0.05$) measured by ELISA. To determine the effect on PMN, stimulated-HMEC-1 free supernatants were incubated for 30 min with PMN isolated from healthy donors. Both, cell size (% of high FSC) and CD11b expression (MFI) measured by FACS were increased in PMN incubated with cell free supernatants obtained from pRNA-treated HMEC-1 ($p<0.05$). Through the use of a chemotaxis chamber, a significant migration of PMN was observed only when supernatants from HMEC-1 that had been pre-treated with pRNA were used as the chemotactic stimuli ($p<0.05$). We also evaluated the adhesion of PMN to HMEC-1 treated cells, by measuring the activity of PMN alkaline phosphatase with the substrate p-nitrophenylphosphate in the adhered PMN after washing. Higher adhesion was observed only when HMEC-1 were pre-treated with pRNA ($p<0.05$). Our results indicate that the presence of pRNA directly activates the endothelium, which releases signals for the recruitment and adhesion of PMN.

Keywords: Prokaryotic RNA, Neutrophil, Endothelium, HMEC-1

(625) PROSTAGLANDIN E2 INTERFERES WITH TGF-β SIGNALLING IN MONOCYTE-DERIVED DENDRITIC CELLS

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Abstract: Monocytes infiltrate infection sites and tumors, where they sense microenvironmental cues and become dendritic cells (DCs) or macrophages that will be key players of the ensuing immune response. TGF-β and prostaglandin E2 (PGE2) are two ubiquitous immunomodulators, known to influence differentiation of DCs. Presence of TGF-β during differentiation of DCs with IL-4 and GM-CSF leads to DCs with enhanced inflammatory properties. Conversely, PGE2 drives differentiation towards generation of myeloid-derived suppressor cells.

In this study we aim to investigate the impact of PGE2 on the profile induced by TGF-β during differentiation of DCs. To this end, we incubated human monocytes for 5 days with IL-4 and GM-CSF alone (control DC) or with TGF-beta (T-DCs), with or without the addition of PGE2 (10-7M) at the beginning of the culture.

Compared to control DCs, T-DCs showed higher CD1a and decreased CD14 expression, as well as enhanced LPS-induced IL-12 production. Notably, LPS-induced expression of IL-10 was completely abolished. However, simultaneous addition of PGE2 led to DCs showing a CD1a-CD14+ phenotype and unable to fully mature or produce IL-12 after LPS stimulation (12 vs 1347 pg/ml in T-DCs). These DCs, which produced higher levels of IL-10 (1410 vs 35 pg/ml in T-DCs) and IDO (22 times more than T-DCs, by qPCR), elicited the expansion of CD25+FoxP3+ T cells when cultured with allogeneic CD4+ lymphocytes (17.9% vs 8.0% in T-DCs). These results indicate that the presence of PGE2 can override TGF-β signaling and drive DCs towards a tolerogenic phenotype. Furthermore, we demonstrated that inhibition of TGF-beta by PGE2 requires prostanoid receptors EP2 and EP4, elevation of cyclinc AMP and PKA activity.

Taken together, these results suggest that potential TGF-β pro-inflammatory actions on myeloid differentiation in the context of tumor or inflammatory microenvironment could be masked by the simultaneous presence of PGE2.

Keywords: inflammation, monocytes, dendritic cells, PGE2, TGF-β

(626) SEMINAL PLASMA INHIBITS SPERM-INDUCED ACTIVATION OF NEUTROPHILS

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Semen deposition on the genital mucosa induces an infiltration of neutrophils to the vaginal lumen, where they rapidly outnumber sperm cells. Previous reports have shown that neutrophils interact with sperm cells, but details are lacking regarding the impact of this interaction on their function. This study analyzes the influence of spermatozoa and seminal plasma (SP) on the immune properties of neutrophils, in paticular their ability to modulate dendritic cell (DC) phenotype and thus influence adaptive immunity.

Semen samples from healthy donors were used to obtain SP and spermatozoa (purified by density gradient centrifugation). Neutrophils were purified from blood by Ficoll-Paque centrifugation followed by dextran sedimentation. DC were obtained from monocytes cultured with GM-CSF and IL-4 for 5 days. Neutrophil and DC phenotype were analyzed by flow cytometry and cytokine production was assessed by ELISA.

Incubation of neutrophils with sperm cells led to increased expression of CD11b (MFI 548 +/- 34 vs 1048 +/- 51, n=5), CD66 (MFI 987 +/- 144 vs 1348 +/- 151, n=5), and increased production of IL-6 (pg/ml: 701 +/- 105 vs 387 +/- 51, n=4) and TNF (pg/ml: 1038 +/- 234 vs 617 +/- 42, n=4). Addition of SP in dilution 1:20 inhibited neutrophil phenotypic activation and secretion of cytokines. Interestingly, this result was mimicked by addition of prostaglandin E2 (10^{-6} M), a known inhibitor of neutrophil function that is abundant in SP. In a second set of experiments, neutrophils pre-incubated with SP were co-cultured with DCs. When compared to DCs co-cultured with control neutrophils, DCs co-cultured with SP-treated neutrophils had diminished production of LPS-induced IL-6 (pg/ml: 2456 +/- 344 vs 677 +/- 98, n=3) and IL-1β (pg/ml: 1415 +/- 168 vs 419 +/- 84, n=3).

Taken together, these results indicate that SP can block the sperm-induced activation of neutrophil and also educate them to modulate the function of DCs, suggesting a novel immunoregulatory function of SP.

Keywords: seminal plasma, PGE2, neutrophils, semen, dendritic cell

(675) THE SPLEEN TYROSINE KINASE SYK PARTICIPATES IN THE ACTIVATION OF GAMA DELTA T CELLS BY MONOSODIUM URATE CRYSTALS

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γδ T cells act as sensors of cellular stress and infection. They recognize danger and pathogen-associated molecular patters, such as phosphoantigens. Uric acid is a cellular stress signal, which is released from dying cells and promotes the generation of innate and adaptive immune responses. The inflammatory effect of uric acid depends on its precipitation into monosodium urate (MSU) crystals. MSU crystals activate different intracellular pathways, i.e. p38 MAPK, Syk kinase and PI3K. We have previously reported that MSU crystals activate γδ T cells and that this activation was reduced in presence of a Syk tyrosine kinase inhibitor. Now, we aim to investigate more in detail the participation of Syk tyrosine kinase during the intracellular signaling triggered by MSU crystals in γδ T cells. To this purpose, γδ T cells were purified from human peripheral blood mononuclear cells by using an anti-TCR γδ MicroBead isolation kit. After purification, γδ T cells were incubated or not with MSU crystals (200 μg/ml) at different time points, and then we analyzed the expression of phospho(p)-Syk (the active form of the tyrosine kinase) by immunofluorescence and confocal microscopy analysis. We observed an increase in p-Syk expression at 5 and 15 min post-stimulation by MSU crystals ($p<0.05$, n= 2), followed by a decrease in the level of expression at 45 min. The maximum values obtained after MSU crystals stimulation were similar to that observed in cells treated with peroxide oxygen (positive control). Our results suggest that, in γδ T cells, MSU crystals induce the activation of the tyrosine kinase Syk pathway.

Key words: γδ T cells, MSU crystals, Syk kinase

(976) RELEVANCE OF PLATELETS IN NETS-INDUCED ENDOTHELIAL DAMAGE IN HEMOLYTIC UREMIC SYNDROME.

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Hemolytic Uremic Syndrome (HUS) is the most common cause of pediatric renal failure. Linked to Gram (-) Shiga toxin-producing (Stx) infections, lipopolysaccharide (LPS) and neutrophils (PMN) can potentiate the disease. Since endothelial damage is characteristic of HUS, PMN-platelet (Plts) interaction in the context of Stx could promote netosis causing endothelial damage. We have shown that Stx2 increases netosis induced by LPS-treated Plts and endothelial cytotoxicity in vitro. The aim of this work is to study in detail this phenomenon and determine the relevance of netosis in a murine model of HUS. We determined in vitro by FACS that Stx2 stimulation of LPS-treated Plts increased the formation of PMN-Plts mixed aggregates (% CD11b+CD61+ p≤0,05) and increased the activation of both PMN (CD11b p≤0,05) and Plts (P-selectin p≤0,05). Additionally, the increased netosis promoted by Stx2 stimulation of LPS-treated Plts was dependent on the PMN-Plts-mediated P-selectin junction. In order to corroborate in vitro findings, mice administrated with LPS and Stx2 showed an increased % of PMN-Plts blood aggregates (%Gr1+CD61+ p≤0.05) in LPS+Stx2 treated mice. Also the NETs in this group were increased measured using an ELISA kit (PMN Elastase-DNA levels p≤0.05).Correlating with this, endothelial damage was higher in LPS+Stx2 treated mice (von Willebrand Factor, vWF levels in plasma p≤0.05). NETs were digested and levels of Elastase-DNA decreased correlating with vWF diminution. Finally, in order to evaluate the role of circulating Plts mice were depleted of Plts. We observed in LPS+Stx2 mice without Plts that, both netosis (Elastase-DNA levels p≤0.05) and endothelial damage levels (vWF

p≤0.05) decreased compared to LPS+Stx2. These results demonstrate the relevance of LPS+Stx2 induced netosis on vascular damage and the fundamental role of Plts in this phenomenon.

Key-words: HUS, platelets, NETs, endothelial damage.

(1002) **ROLE OF ALPHA-GLUCANS FROM *Mycobacterium tuberculosis* IN DENDRITIC CELL MATURATION AND PHAGOCYTOSIS**

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Tuberculosis remains the single largest infectious disease with two million deaths estimated to occur yearly. The spread of *Mycobacterium tuberculosis (Mtb)* from the lungs to other sites occur before the development of adaptive immune responses. The major carbohydrate constituents from *Mtb* surface are α-glucans, a sugar which has been implicated in fungal virulence, and represent up to 80% of the extracellular polysaccharides in Mycobacteria. Previous results demonstrated that reactive oxygen species (ROS) in neutrophils are generated by non-opsonized mycobacteria through α-glucans and that the lack of a-glucans in *Mtb* leads to the loss of their ability to induce ROS and also dendritic cell (DC) maturation and antigen presentation. Here we evaluated the role of α-glucans in the entrance of bacteria in DC and the endogenous production of ROS as effectors of the maturation of DC. Results Monocyte-derived DC were incubated 24 hours with LPS and thereafter were treated with *Mtb* (H37Rv) subjected or not to a-amyloglucosidase treatment (*Mtb-e*) for 15 min with or without H2O2. Thereafter maturation markers were evaluated by cytometry. Expression of CD86 ($p<0.001$) and MHC-II ($p<0.01$) were induced by *Mtb* but significantly less induced by *Mtb-e* ($p<0.001$) whereas treatment with H2O2 restored parameters of maturation ($p<0.05$). In addition, LPS primed DC were incubated with 123Dihidrorhodamine (123DHR) treated *Mtb* and thereafter emission of oxidized DHR was evaluated by flow cytometry as a measure of phagocytosis. Whereas *Mtb* induced significant ROS production ($p<0.002$), *Mtb-e* induced less but significant signal ($p<0.002$). Conclusion Here we show that lack of the ability to induce DC maturation by a-glucans in *Mtb* is due to the loss of their ability to induce ROS. In addition, a-glucans in *Mtb* participate in entrance to DC being essential for the onset of the protective immune response because maturation and function of DC are critical against tuberculosis disease.

Key Words: tuberculosis, dendritic cell, alpha-glucans

(1372) ***Tc*13Tul ANTIGEN FROM *Trypanosoma cruzi* INDUCES B-CELL PROLIFERATION IN SPLENOCYTES FROM *NAIVE* BALB/C MICE**

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Several soluble factors of *Trypanosoma cruzi*, the ethiological agent of Chagas´ disease, modulate host immune responses, among them the enzyme trans-sialidase (*Tc*TS), a member of group I of the trans-sialidase superfamily (TS). Here, we evaluated the effect of *Tc*13Tul antigen on splenocytes from *naive* BALB/c mice. *Tc*13Tul belongs to group IV of TSs, characterized by C-terminal EPKSA repeats.

Splenocyte proliferation were assessed by CFSE staining after 72 h of incubation with recombinant *Tc*13Tul, EPKSA repeats or the carrier protein MBP as a control. Flow cytometry analysis showed an increase in the % of divided cells when incubated with *Tc*13Tul (11.65 ± 0.07 % vs. 0.93 ± 0.12 % for EPKSA or 1.29 ± 0.05 % for MBP; $p<0.001$). *Tc*13Tul induced B-cell (CD19+) proliferation (12.9 ± 0.28 % vs. 1.50 ± 0.34 % for MBP, $p<0.0001$). Conversely, no differences were observed in the % of divided T-cells (CD3+) respect to MBP. Stimulation with *Tc*13Tul also resulted in an increased IgG secretion (16.26 ± 5.21 µg/ml vs. 3.57 ± 1.84 µg/ml for MBP; $p<0.05$). However, no IL-17 was produced as it has been reported for *Tc*TS.

*Tc*13Tul also induced B-cell proliferation and IgG secretion in *naive* splenocytes from the mouse strain C3H/HeJ, deficient in the LPS receptor, confirming that these effects are due to *Tc*13Tul and not to LPS in the antigen preparation.

Splenocyte-antigen interaction assays using FITC conjugated proteins showed 2.6-fold higher labeling in lymphocyte population with *Tc*13Tul-FITC than with MBP-FITC. *Tc*13Tul-FITC bound to 96.06 % of CD19+ and 78.73 % of CD3+ populations, while the control protein MBP-FITC bound to 34.6 % and 11.1 % of CD19+ and CD3+ cells, respectively. Therefore, these results suggest that *Tc*13Tul may interact with surface antigens on both B and T cells.

Our results indicate that *Tc*13Tul antigen may participate in the innate immunity against *T. cruzi* by favoring immune system evasion through B-cell activation and non-specific IgG secretion.

Keywords: Chagas´disease, innate immune response, lymphocyte.

(1802) **AUTOPHAGY IS REQUIRED FOR THE *TRYPANOSOMA CRUZI* ELIMINATION IN MACROPHAGES BY XENOPHAGY**

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Trypanosoma cruzi is the etiologic agent of Chagas, an endemic disease of Latin-American countries. Autophagy is a cellular process required for the removal of aged organelles and cytosolic components through lysosomal degradation. In previous studies, we observed that mice deficient in autophagy displayed a more aggressive infection indicating that this process could be participating as a component of the immune response against the parasite. The main objective of this work was the study the participation of xenophagy in the elimination of *T. cruzi* in macrophages. Xenophagy is a specialized type of autophagy responsible for the capture and elimination of intracellular microorganisms in macrophages. To study this process we detect specific xenophagic-related proteins in peritoneal cells obtained from C57wt mice and C57 Beclin-1 +/- mutant mice (deficient in autophagy) that was previously infected with trypomastigotes of *T. cruzi* Y strain. We also infected Raw cells under different conditions of autophagy inhibition. Data showed that infected macrophages displayed higher levels of LC3 expression compared with non-infected cells. This LC3 was recruited surround the parasitic bodies in the cell cytoplasm. We also observed that when autophagy is inhibited, the number of amastigotes per cell increased. Other proteins involved in xenophagy were found decorating the parasites. We conclude that the autophagic pathway is participating in the elimination of intracellular amastigotes in macrophages by xenophagy.

Key words: Autophagy, Xenophagy, *Trypanosoma cruzi*, macrophages.

(1873) **THE BACULOVIRUS ACMNPV INDUCES THE PRODUCTION OF INTERFERONS IN THE PORCINE MODEL**

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Baculoviruses (BVs) are dsDNA viruses that infect insects and belong to the *Baculoviridae* family. The model species *Autographa californica nucleopolyhedrovirus* (AcMNPV) is able to transduce mammalian cells, but not to replicate, and to induce a strong innate immune response. Because of the high frequency of CpG motifs present in its genome, they stimulate the production of inflammatory cytokines and type I and II interferons (IFNs) via the Toll-like receptor 9 (TLR9)/MyD88-dependent signaling pathway in mice. However, there are cytosolic DNA sensing pathways involved in the IFNs induction in some cells. Mice inoculated with BVs were protected at short term against challenges with influenza H1N1, encephalomyocaditis virus and foot and mouth disease virus. There are not studies about the effects of the BVs in the porcine innate immune response.

The aim of this work is to evaluate if the BV AcMNPV is able to induce the production of IFNs in swine peripheral blood mononuclear cells (PBMCs), macrophages and the epithelial cell line of kidney PK15. Each cell type was infected with different multiplicities of infection of BVs. After 16 hours, the supernatants were collected and the presence of IFNs was determined by a bioassay in MDBK

cells based on their antiviral effect against vesicular stomatitis virus (VSV) infections. The specific presence of IFN-alpha in supernatants was detected by ELISA. The BV was able to promote the production of IFNs in the supernatants of all the cell types studied ($p \leq 0.01$), and quantities of IFN-alpha were detected in supernatants of PBMCs ($p \leq 0.01$).

In conclusion, AcMNPV induces the production of high levels of IFNs in porcine cells and the specific presence of IFN-alpha could be identified. Therefore, this work showed that BVs are a potential tool that deserves to be studied for the development of antiviral strategies in pigs.

Biología Animal 1

(080) **BRAIN DISTRIBUTION OF IMMUNOREACTIVE NEURONS AND FIBERS EXPRESSING GnIH IN PEJERREY, Odontesthes bonariensis.**

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Gonadotrophin-inhibitory hormone (GnIH) is a RFamide peptide, originally characterized in the japanese quail as an inhibitor of gonadotropins, changing the classical view of the regulation of vertebrate reproduction. Later, it was also found in others vertebrate species, with similar function. However, the effects in fish remain controversial. In this context, as a first step, the objective of this work was characterized the neuroanatomic distribution of GnIH neurons and fibers in the brain of adult pejerrey by immunohistochemistry. Our results showed that neuronal somata with immunoreactivity to GnIH (ir-GnIH) were localized at the olfactory bulbs, the junction of the olfactory bulbs and telencephalon (Terminal Nerve Ganglion or *Nucleus Olfacto Retinalis*) and the *Nucleus Posterioris Periventricularis* (NPPv). This distribution was similar to that reported in other teleost fish species. Moreover, some isolated neuronal somata were detected in the *Nucleus Paraventricularis Posterioris* (NPP) in the preoptic area (POA). While ir-GnIH fibres were present in all brain areas examined, and also at the pituitary gland. In addition, double labelling with GnIH and GnRH I (also known as pjGnRH) antisera was performed, in order to analyze a possible relationship between GnIH and the hypophysiotropic GnRH variant of this species. In double labelling immunocytochemistry, ir-GnIH fibers were localized very close to ir-GnRH neurons in the preoptic area, suggesting that both neurons could interact. This is the report on the GnIH distribution in the brain of pejerrey and its relation with GnRH.

(1732) **COMPARATIVE MORPHOLOGICAL STUDY OF BRISTLES ON CATERPILLARS WITH MEDICAL RELEVANCE IN MISIONES.**

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Caterpillars (larval stage of butterflies and moths) have urticating bristles which protect them from predators and are also responsible for the cutaneous reactions observed when human beings inadvertently lean against them. In recent years, accidents caused by different species of caterpillars have become more common among people living in Misiones. Taking into account that the biology of these animals remains to be poorly understood, we conducted a morphological study of the urticating bristles in representatives of the two families that are most frequently involved in accidents in this province: Saturniidae (*Automeris naranja, Hylesia nigricans, Leucanella memusae, Lonomia obliqua*) and Megalopygidae (*Megalopyge lanata, Podalia orsilochus and Podalia* aff. *fuscescens*). The external morphology of the caterpillars (scoli and spines) was observed and photographed under a stereomicroscope. The median dorsal region of caterpillar bodies were dissected and isolated, cleaned, critical-point dried, coated with a thin layer of gold, and finally examined by scanning electron microscopy. In this study, all caterpillars exhibited scoli with spines that bear a hollow canal. In *L. obliqua*, we could observe some spines that possessed a circular groove similar to a ring-like structure, and other spines that lacked this groove. The latter were the only type of spines that we could observe in *A. naranja, H. nigrincans and L. memusae*. The scoli of *P. orsilochus*, *P.* aff. *fuscescens* and *M. lanata* exhibited similar structures, showing long hairs with transverse striations and spicules, and short and hollow spines. It is noticeable that the saturnid *H. nigricans* also exhibited long hairs with spicules. Altogether, this is the first comparative morphological study showing the way in which different species of Argentinian caterpillars can induce an irritant or toxic dermatitis in humans.

Key words: caterpillars, urticating bristles, Saturniidae, Megalopygidae.

(1864) **COMPARATIVE ONTOGENY OF THE TEGUMENTARY SYSTEM OF RHINELLA SCHNEIDERI (ANURA: BUFONIDAE), *LEPTODACTYLUS CHAQUENSIS* (ANURA: LEPTODACTYLIDAE) AND *SCINAX ACUMINATUS* (ANURA: SCINAXIDAE)**

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Profound changes and complexation of anurans tegument accompany morphological, physiological and behavioral modifications that take place during metamorphosis. Also, its structure allows interpreting specific biological characteristics of the habitat of each species. The objective of this work was to analyze comparatively the external morphology and histology of the tegument during the ontogeny of *Rhinella schneideri* (Rs), *Leptodactylus chaquensis* (Lc) and *Scinax acuminatus* (Sc). Larval, juvenile and adult specimens of the species were analyzed in stereoscopic magnifying glass and scanning electron microscopy (SEM) to establish macro-structural comparisons. Histological sections were obtained by a conventional technique and were stained with H&E, Mallory Trichrome, and PAS for contrast tissue aspects. The three species coincided in general tegument configuration (epidermis and dermis), and its changes that go through ontogeny. Significant differences were found in presence and time of appearance of specialized structures. Giant cells associated with gregarious behavior were recorded at different larval stages for Rs and Ls. Multicellular glandular outlines appeared in pro-metamorphosis for Rs and Sa, but just in the metamorphic climax for Lc. Tubercles and spines were described for Rs, pustules and minor protuberances for Sa and a smooth tegument without macroscopic specializations for Lc. The mucosal glandular content match the serous one in Lc, while the others two species had higher serous contents. That agrees with the habitat of each species, mostly associated with water bodies and terrestrial surroundings, respectively. This work will serve as a basis for future comparative studies with other anurans and for ecomorphological analyses that will deepen in the relationship between the skin properties, the way of life and habitat of species.

Key words: integument, anurans, metamorphosis, glands

(1819) **COMPARATIVE STUDY OF PRENATAL DEVELOPMENT BETWEEN *Myotis albescens* (Chiroptera: Vespertilionidae) AND *Eumops patagonicus* (Chiroptera: Molossidae).**

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The order Chiroptera comprises two suborders, Megachiroptera and Microchiroptera, the Microchiroptera is cosmopolitan, within the order, exist a great diversity of study about reproductive biology. Notwithstanding, there is still important information gaps respect to different aspects of the embryonic development of bats, The staging system proposed for *Carollia perspicillata* is, the most complete and includes the embryo sequence from fertilization until the end of development, and is used as a reference table. Moreover, the characteristics of chorionic vesicle have not been considered in previous studies. The present study aimed to characterize the embryonic development of *Myotis albacescens* and *Eumops patagonicus*, two Center and South American species, widely and commonly distributed in northern Argentina.This study was realized using 60 specimens obtaining of wildlife. There are measured and photographed, and the embryonic stage was assigned what the staging system created for *Carollia perspicillata*. We observed that the chorionic vesicle, in both, showed similarities in the disposition of the extraembryonic membranes, but they differed in characteristics of yolk sac and chorioallantoic placenta, in *E. patagonicus* was more glandular than *M. albescens*. *M. albescens* presented a discoid placenta well developed with the caudal anti-mesometrial position, but *E. patagonicus* presented a diffuse placenta which persists until the final stages of gestation and a discoid placenta in the uterus tubal junction. Respect to the embryogenesis was recognized, in both, early stages (ES), middle stages (MS) and late stages (IS). In the ES, the embryonic morphology is similar. The MS is characterized by the muzzle and pinna formation, fore and hind limb regionalization, and the formation of the patagium primordium. In the IS, occur the overall growth of the embryo, of its fore and hind limbs, patagium, and the typical craniofacial features of each species are configured.

Keywords: South American bat; Chiroptera; Vespertilionidae; Molossidae; embryogenesis; chorionic vesicle

(055) **GHRELIN AND NUCB2/NESFATIN-1: CO-LOCALIZATION IN BRAIN AND INTESTINE, POSSIBLE INTERACTION WITH DIGESTIVE ENZYMES IN THE INTESTINE OF PEJERREY (*Odontesthes bonariensis*).**

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Abstract: Ghrelin and nesfatin-1 are two metabolic peptides with opposing actions on food intake regulation. Ghrelin is a potent orexigenic, mainly synthesized in the gastro-intestinal (GI) tract of vertebrates, although is also present in central nervous system. Nesfatin-1 is an anorexigenic peptide, mainly synthesized in the hypothalamus although it gene expression was reported in the GI tract. The aim of this work was to characterize by immunohistochemistry the localization of ghrelin and nesfatin-1 in brain and intestine of pejerrey (*Odontesthes bonariensis*), a species with commercial importance in South America. Additionally, the co-localization of both peptides with enzymes involved in the digestion process such as lipoprotein lipase, aminopeptidase A, trypsin and sucrase-isomaltase in the intestine of pejerrey was studied. Results show that both ghrelin and nesfatin-1 have a similar anatomical distribution in brain, with the highest immunoreactivity detected in some hypothalamic nuclei (such as the preoptic nucleus and the posterior recess nucleus) and in the pineal gland, and lower levels observed in telencephalon, optic tectum, cerebellum and some hindbrain nuclei. Both peptides are presents and co-localize in some cells from the mucosa and submucosa layers of pejerrey intestine. Co-localization of ghrelin and nesfatin-1 with lipoprotein lipase, aminopeptidase A, trypsin and sucrase-isomaltase was found in intestine, in cells from the brush border and from the deep part of the mucosa and in the submucosa. In conclusion, in this work we present evidence for the presence and co-localization of ghrelin and nesfatin-1 in brain areas and intestine of pejerrey, which supports the role of both peptides in the food intake regulation. Additionally, the co-localization of ghrelin or nesfatin-1 with digestive enzymes suggests a role for both peptides on nutrient assimilation in pejerrey.

Keywords: digestive enzymes, food intake, ghrelin, nesfatin-1.

(299) **MALE REPRODUCTIVE SYSTEM OF THE "RED CHERRY SHRIMP" *NEOCARIDINA DAVIDI* (CARIDEA)**

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Caridea is a diverse taxon of marine, freshwater and brackish shrimps poorly studied as far as their reproductive anatomy. This study aimed to describe for the first time the functional anatomy of the male reproductive system in an Atyidae, *Neocaridina davidi, a* very popular ornamental species. For this purpose mature males were cold-anaesthetized and their reproductive systems were dissected for routine histological and histochemical tests. Male reproductive system consists of two coiled testes continuous to the vasa deferentia opening in the gonopores located in the coxae of the fifth pair of pereiopods. Testes are positioned on the dorsal side of the cephalothorax, above the hepatopancreas and below the heart. Testes comprise seminiferous tubules where spermatogenesis occurs. Each vas deferens (VD) is a long tube *dorsolaterally* positioned with respect to the *hepatopancreas,* and divided in proximal, middle and distal zones. The proximal part has a cylindrical epithelium with secretory cells while in the proximal to medial zone the vas deferens has a dorsal fold with high columnar epithelium with secretory activity, the typhlosole. This typhlosole is similar to that observed in *Macrobrachium rosembergii,* yet different from those in penaeid shrimps. The VD increases in diameter distally and the spermatophore (SPF) formation begins at the proximal part of VD. The SPF is a continuous cord with two acellular layers: the inner is thicker with high protein content, and the outermost is much thinner with low protein content, and strongly reactive to the PAS staining. The spermatozoa are arranged in clusters of 3-4 spermatozoa and immersed in an eosinophilic, and strongly PAS positive secretion. Spermatozoa have many spikes. Although further analyses are necessary, the composition of the spermatophore, and the arrangement and form of the spermatozoa appear to be novelties within Caridea. UBACYT-2014-2017-20020130100186BA,PIP-2015-2017-11220150100544, PICT 2016-0759.

Keywords: *Neocaridina davidi,* male reproductive system, spermatophore, testes, vasa deferentia.

(300) **STUDY OF SILYMARIN ACTIVITY ON BRAIN MICROSOMES AND MITOCHONDRIA**

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Production of reactive oxygen species can damage cell membranes by forming free radicals, involved in tissue diseases associated with aging processes. Antioxidant activity is the ability of a substance to inhibit oxidative degradation by reacting with free radicals. Vegetables are a source of natural antioxidants: vitamin E, vitamin C, carotenoids. The silymarin (SM) present in the seeds of the Thistle Asnal, is a known liver protector that possesses *Silybum marianum L.* (Gaertner). SM (silymarine phosphatide 16%) used in this work was kindly supplied by Vetanco Laboratories S.A.

In previous studies its antioxidant capacity on liver membranes was observed. The objective of this work was to analyze the in vitro antioxidant activity of SM on the peroxidation of microsomes and brain mitochondria of Wistar rats AH / HOK. Ascorbate-Fe^{++} -dependent non-enzymatic peroxidation assays were performed by incubating 1 mg of protein samples in 0.05 M phosphate buffer, pH 7.4 at 37 ° C. Peroxidation was initiated with ascorbate (final concentration 0.4 mM). Controls without ascorbate were used. The light emission for 180 min was quantified as counts per minute (cpm) every 10 min in a Packard 1900 TR, (Meriden, CT, USA) chemiluminescence program. To study the effect of SM, different concentrations of the product were used: 6.25; 12.5; and 25 µg per mg of mitochondrial and microsomal protein. In the peroxidation assays the protection against oxidative damage in both membranes was SM concentration dependent. Inhibition percentages were in microsomes 31.27%, 51.62% and 71.27%, and in mitochondria 27.99%, 52.92% and 63.76% with respect to the control. These results shown that SM may act as an antioxidant protecting rat brain microsomes and mitochondria from oxidative damage.

(433) **PROTEASOME INHIBITORS IMPROVE TREACHER COLLINS SYNDROME (TCS) IN A ZEBRAFISH (*Danio rerio*) MODEL.**

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TCS is a congenital disease characterized by defects in the craniofacial skeleton and absence of mental alterations. We modeled TCS in *D. rerio* embryos through the microinjection of Morpholino oligonucleotides blocking the translation of the ortholog of the causative gene (*TCOF1*). Cnbp, a protein required for proper craniofacial development, was detected in lower levels (without changes in its mRNA expression) in TCS-like embryos. As Cnbp degradation is carried out through the proteasomal pathway, we tested if proteasome inhibitors (MG132 and Bortezomib) were able to ameliorate cranial skeleton malformations in TCS.

Two-cell embryos were injected with control Morpholino (C) or *tcof1* translation blocking Morpholino (TC). At 6 hours post fertilization (hpf) embryos were exposed to MG132 (5 μM), Bortezomib (0.5 μM) or vehicle for 18 hours. Cnbp protein level was measured by western-blot in total extracts from 24 hpf specimens. Cranial cartilages measurements were performed in 4 days post-fertilization larvae stained with Alcian Blue by using the ImageJ software. Embryo viability was not affected by any treatment. Control injected embryos did not show any effect on cranial cartilages induced by MG132, Bortezomib or vehicle and were pooled together as control group (C). In arbitrary units, Meckel length: C:100.0 ±0.7, TC: 89.3±3.1*, TC+Bort: 98.7266±2.0, TC+MG132: 91.5795±2.8, Ceratohyal angle: C: 99.4±1.3, TC: 157.3±14.9*, TC+Bort: 103.0±4.6, TC+MG132: 124.3±10.7, $p<0.05$ vs. C, ANOVA. All the craniofacial parameters measured behaved similarly. The results suggest that both drugs (especially Bortezomib) partially rescued the phenotype. Cnbp protein recovered under both treatments although not to control levels.

Data suggest that proteasome inhibitors improve TCS in the zebrafish model, likely by preventing Cnbp protein proteasome degradation. This finding may have potential therapeutic implications for TCS management.

Keywords: proteasome inhibitors, therapy, zebrafish, mandibulofacial dysostosis

(1474) **SURFACE ULTRASTRUCTURE OF THE SCOLEX AND HISTOCHEMISTRY OF THE GLANDULOMUSCULAR ORGAN IN *Orygmatobothrium schmitti* (CESTODA: PHYLLOBOTHRIIDEA)**

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Cestodes are parasites of the intestinal tract of vertebrates. Attachment organs in the scolex are used to maintain their position on the intestinal wall of their hosts. *Orygmatobothrium schmitti* (Cestoda: Phyllobothriidea) is a parasite of the shark *Mustelus schmitti* (Chondrichthyes). It possesses a scolex with four bothridia, each having an apical sucker, and a central glandulomuscular organ. The objective of the present study was to describe the microtriches, glandulomuscular organ, subtegumental musculature of bothridium, sensory organs ultrastructure and the histochemical composition of the secretion of the glandulomuscular organ of *O. schmitti*. Seventeen scolices were stained with four histochemical techniques: coomassie brilliant blue, periodic acid-Schiff, toluidine blue and sudan black. Five worms were observed with scanning and transmission electron microscopy. The glandulomuscular nature of the central bothridial organ is confirmed and its ultrastructure is described in detail. Also the internal structure of the tegumental microtriches, two types of sensory organs and subtegumental muscular papillae are described for the first time. The glycoproteic nature of the secretion of the glandulomuscular organ and the apocrine mechanism of secretion are determined. All the microtriches on the apical sucker possess a more developed cap than filitriches on the bothridia, the scolex proper and the cephalic peduncle. These specializations of the scolex might be involved in adhesion and abrasion to the intestinal mucosa of the shark. The microtriches with developed caps could be involved in the attachment to the mucosa, whereas those small caps might be related to the absorption of nutrients.

Keywords: Orygmatobothrium schmitti, cestoda, scolex, glandulomuscular organ, ultrastructure.

(1489) **SURFACE ULTRASTRUCTURE AND HISTOCHEMISTRY OF THE SCOLEX GLANDS OF *Clestobothrium cristinae* (CESTODA: BOTHRIOCEPHALIDEA) PARASITE OF *Merluccius hubssi* (TELEOSTEI)**

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Clestobothrium cristinae (Cestoda: Bothriocephalidea) is an intestinal parasite of the hake *Merluccius hubssi* (Teleostei).The scolex of the tapeworms is the principal attachment organ. In *C. cristinae* it possesses an apical disk and two bothria. It is covered with capilliform filitriches and gladiate spinitriches, and possesses tumuli (frontal glands type I) in the proximal bothrial surface. In this study the internal morphology of all these structures and the nature of the secretions of the glands in the scolex are described for the first time in *Clestobothrium*. Ten scolices were stained with several histochemical techniques: coomassie brilliant blue, periodic acid-Schiff and toluidine blue. One worm was analyzed using conventional techniques for transmission electron microscopy. As a result, the internal ultrastructure of the tumuli, and the internal structure of gladiate spinitriches in this genus are described. Groups of cells PAS positive were observed in the same area where the tumuli were described using SEM. It is possible that these groups of gland cells are in fact a constitutive part of the so called tumuli; however, the internal structure is different from the tumuli described in other genera of bothriocephalideans. The secretion is based on mucopolysaccharide, which might indicate they are involved in the adhesion to the intestinal mucosa of the hake.

Keywords: *Clestobothrium cristinae*, cestoda, scolex, *Merluccius hubbsi*, ultrastructure.

NEUROSCIENCE 1

(138) **ADMINISTRATION OF NATIVE PLANTS DECOCCTIONS IN NORMAL AND STREPTOZOTOCIN-INDUCED DIABETIC RATS**

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Diabetes mellitus is a chronic disease that represents a major problem for the countries health systems. Thus the search of new alternative treatments is constant. Numerous studies indicate that 85% of world population still uses “medicinal plants” to treat its health. *Oxalis erythrorhiza* (Oe) and *Tessaria absinthioides* (Ta) are two species that growth in Argentine, in Cuyo region, and are consumed to regulate the glucose (Glu) and cholesterol (Chol) levels, even when their effects lack of scientific support. On the other hand, the liver X receptors (LXRα and LXRβ) are related with the systemic Chol clearance and the Glu metabolism in the hypothalamus (HT). Forty two days old male rats (SD), diabetics (Ed, i.p. STZ 30mg/Kg) or controls (C, i.p. vehicle) received (5 or 10% W/V) decoctions (Dcs) of Oe (EdOe and COe) or Ta (EdTa and CTa), or water (EdW and CW) as drink for 4 weeks. Glu, Chol and triglycerides (TG) were determined on weekly obtained blood samples by colorimetric kits. LXRs expression was evaluated by WB in HT. At the end of the treatment, the Glu level was lower in EdOe and EdTa (both at 10% W/V) compared to DW (36% and 37% respectively; $p<0.05$), but higher than CW (147% and 305% respectively; $p<0.05$). No significant effects were observed in Chol and TG in these groups. However, Ta 5%W/V significant reduced the LXRα expression in EdTa compared to EdW (64%; $p<0.05$) but still was higher than CW. On the other hand, in Ed groups, Oe (10%) and Ta (5% and 10%) reduced LXRβ expression compared to W (20%, 11% and 18% respectively; $p<0.05$), and also the values were higher than CW group. The Dcs did not produce any effect on the parameters evaluated into the C group. Thereby, Oe and Ta could have regulatory effects on Glu and LXRs expression. Therefore, a more extended treatment will be necessary to achive the research objective and to propose these native plants like new therapeutic tools. (PIP 0243, PIO-SECITI 2250, CICITCA UNSJ, CONICET).

Keywords: diabetes mellitus, hypoglycemic, LXR receptor, phytomedicine.

(789) **ALTERATIONS IN VASCULAR INTEGRITY AND NEURONAL FUNCTIONALITY RELATED TO EARLY STAGES OF DIABETIC RETINOPATHY IN A METABOLIC SYNDROME MOUSE MODEL**

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Diabetic retinopathy (DR) is the most serious ocular complication associated with Type 2 Diabetes Mellitus (T2DM), which is a metabolic syndrome (MS), and one of the leading causes of blindness. Thus, we proposed to analyze in a MS mouse model, markers of retinal vascular integrity and neuronal functionality, related to early stages of DR.

We used C57BL/6 (WT) and Apolipoprotein E knockout (ApoE-KO) mice either fed with a normal diet (ND) or a 10% w/v fructose diet (FD) in drinking water from 2 months of age. We demonstrated ApoE-KO after 2 month of FD, presented hypercholesterolemia, hypertriglyceridemia, hyperglycemia and hyperinsulinemia. Here, retinal functionality was assessed by scotopic ERG at 4 month of FD treatment. Extravasation of serum proteins and levels of proteins involved in neuro-glial injury were analyzed by WB. Vascular permeability was evaluated by albumin–Evans blue complex leakage and astrocyte GFAP levels on whole mounts of retina.

The ERG a-wave and the OPs amplitudes were significantly decreased in retinas of ApoE-KO after 4 month of FD vs WT DN ($p<0.05$), correlating with an increase in TUNEL positive cells. Higher vascular permeability was observed in ApoE-KO FD, evidenced by the Evans blue leakage and the albumin and α2-M extravasation. At this early stage of the DR, the GFAP expression levels were observed just in astrocytes but not in Müller glial cells (MC) demonstrating non-reactive gliosis in retinas of ApoE-KO FD, which correlated with the GS expression pointing out a normal function of MC. However, a reduction in GFAP immunoreactivity was observed in ApoE-KO FD whole mounts, which may be linked to a reduced ability to maintain BRB characteristics in ECs.

The results showed that ApoE-KO after 4 months of FD, which represent features of human MS, presented vascular dysfunction and neurodegeneration. Thus, this model could offer the opportunity to investigate DR at an early stage, whose prevalence has increased substantially worldwide.

Keywords: retinopathies, metabolic syndrome, ApoE-KO mice

(270) **CONSUMPTION OF A HIGH FAT DIET IN EARLY STAGES OF LIFE INDUCES COGNITIVE IMPAIRMENT AND HIPPOCAMPAL CHANGES IN C57BL/6 MICE.**

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Insulin resistance and obesity associated with the consumption of hyperlipidic diets are considered risk factors for the development of cognitive disorders and neurodegenerative diseases such as Alzheimer’s disease. Insulin resistance, inflammation and cognitive dysfunction are common manifestations in the context of both neurodegenerative and metabolic pathologies. Our objective is to study the effect of juvenile exposure to a moderately high fat diet (HFD), since weaning until 2 months of age, on cognitive performance and hippocampal glial and neuronal changes in C57BL/6 mice. HFD exposure induced an increase in blood glucose and peripheral inflammation shown by significantly augmented levels of seric IL1β ($p<0.05$), without changes in body weight. HFD mice showed alterations in spatial memory evidenced by the impaired performance in the novel object localization recognition test, a hippocampus-dependent task (Discrimination index= CD 33.89% vs HFD 14.11%, $p<0.01$). No significant differences were detected between groups in the elevated plus maze, a test employed to evaluate emotional behavior. Immunohistochemistry for Iba1 microglial marker allowed the analysis of morphologic alterations induced by HFD exposure. We found an enlargement in the soma size of Iba1+ cells ($p<0.01$) in response to HFD, evidencing a marked activation of this brain immune cell population. In consonance with the neuroinflammatory context and spatial memory deficits observed upon HFD, analysis of adult neurogenesis is now in progress, assessing the number and morphology of doublecortin + immature neurons in the dentate gyrus of the hippocampus. Our results indicate that juvenile consumption of a hyperlipidic diet promotes metabolic and inflammatory alterations that are associated with cognitive impairment and glial activation in the hippocampus, changes that could lead to neurological deficits in adulthood as well as in the development of neurodegenerative diseases.

Keywords: high fat diet, hippocampal neuroinflammation, cognitive impairment

(571) **INFLUENCE OF CHRONIC MILD STRESS IN METABOLIC AND BEHAVIOURAL ALTERATIONS INDUCED BY A HIGH FAT DIET. INVOLVEMENT OF NEUROTROPHINS AND CYTOKINES**

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Nowadays obesity has become a worldwide disease affecting millions of people. Clinical studies have shown an association between cognitive impairment and obesity, marking it as a risk factor for dementia development, such as Alzheimer’s disease. In a previous study we’ve gathered evidence showing that in C57BL/6J mice, a high fat diet (HFD) decreases learning and memory capacity, and desregulates sugar metabolism. Chronic Mild Stress (CMS) enhanced the metabolic desregulation while causing anxious-like behaviour.The objective of this study is to analize the role of neurotrophin and cytokines expression in behavioural alterations and their potential use as conductual disfunction markers. For this purpose

one month old male C57BL/6J mice were randomly divided in two groups receiving either HFD (35% fat w/w) or standard diet (SD, 5% fat w/w) during 6 months. Two months after starting the diet, mice under HFD were randomly subdivided: One group received CMS and the other served as control. Cytokines and neurotrophins mRNA levels were determined in hippocampus and spleen by real-time RT-PCR, using GAPDH as housekeeper. In spleen, HFD+CMS increased both IL4 and IL10 mRNA levels ($p<0.05$ vs SD) while HFD and HFD+CMS lowered BDNF expression ($p<0.05$ vs SD). In hippocampus BDNF levels diminished non-significantly with HFD and HFD+CMS ($p=0.18$). Furthermore, we found a positive correlation between discrimination index and BDNF mRNA levels in both hippocampus (coefficient=0.52) and spleen (coefficient=0.62). No relevant correlations were found between neurotrophins or cytokines levels and anxious-like behaviour. In conclusion, neurotrophin and cytokine participate in behavioural alterations induced by HFD and/or HFD+CMS. In particular BDNF mRNA levels, both peripherical and central, correlated with cognitive performance. These findings suggest that BDNF expression in spleen could be a potential marker of cognitive deficit in obesity.

Keywords: High Fat Diet, Chronic Mild Stress, Behaviour; Cytokines, Neurotrophins

(046) **LONG-TERM CHANGES IN ANXIETY-LIKE BEHAVIOR FOLLOWING SUCROSE EXPOSURE IN JUVENILE VERSUS ADULT RATS.**

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Abstract: Sucrose consumption has increased dramatically in our society. This phenomenon is mainly associated with increased obesity and diabetes, among other metabolic disorders. The youngest populations have the highest sugar consumption and, during this period of life, profound changes occur as part of the maturation of the CNS. However, little is known about the impact of overconsumption of sucrose on the developing CNS. Here we studied the long-term effects of unlimited access of sugary water during the juvenile stage (childhood-adolescence) in the open field test. The animals are allowed to freely explore a 75 cm x 75 cm x 30 cm sand for 5 min. The session was recorded and the active time, distance traveled, rearing and grooming were analyzed using the ANY-maze© software. The arena was divided into 3 zones, the central zone 1, the intermediate zone 2 and the peripheral zone 3. Although we did not find differences in the total distance traveled nor the total active time between the four groups (juvenile control-sucrose and adult control-sucrose), significant differences were found by treatment-age interaction in the number of visited zones and the distance traveled in the central zones 1+2 (all, two-way ANOVA, $p<0.05$). A deeper analysis revealed that only the animals that consumed sucrose during youth visited less number of zones, evidenced by a decrease entries to the central zones 1+2 and the distance traveled there (all, one-way ANOVA, $p<0.05$). The rearing and grooming were also compromised in animals over-exposed to sucrose in the early stages of development. The two-way ANOVA showed significance by treatment-age interaction both in the number of times and the time invested to perform these actions. Altogether these results suggest that individuals exposed to sucrose consumption during their infancy-youth, but not during adulthood, explore less in the adult stage and present an anxious profile.

Keywords: open field, childhood, adolescence, adulthood

(199) **MATERNAL CAFETERIA DIET MODIFIES THE EXPRESSION OF DOPAMINERGIC RELATED-GENES IN THE RAT OFFSPRING BY EPIGENETIC MECHANISMS.**

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The aim of the present study was to determine the effect of maternal cafeteria diet (CAF) on the expression of key genes related to the brain reward system in the offspring during the perinatal period. Female Wistar rats were fed with standard chow (CON) and/or CAF (snacks, biscuits, cookies, pudding, chocolate) from weaning. After 120 days of diet, females were randomly placed with Wistar males and vaginal smears were performed to confirm successful mating. Dams were maintained on their respective diets throughout pregnancy and lactation. Female offspring from CON and CAF dams were sacrificed on embryonic day 21 (E21) and postnatal day 10 (PND10). Offspring brains were removed and, using a micropunch technique, ventral tegmental area (VTA) and accumbens nucleus (NAc) were removed and stored at –80 °C. For mRNA analysis, real-time quantitative PCR was performed. For methylation studies, digestion with methylation-sensitive restriction enzymes followed by real-time PCR was used. Data was analyzed by two-way ANOVA and Bonferroni post-test; differences were significant at $p<0.05$. From PND1 to PND10, the body weight of pups from CAF dams was significantly lower than pups from CON dams. No changes in gene expression and methylation levels were found in the studied areas in E21. However, maternal CAF diet decreased the transcription of tyroxine hidroxylase (TH), dopamine receptor 2 (DRD2), dopamine transporter (DAT) and ghrelin receptor (GR) in VTA in the PND10. Interestingly, the changes in TH and DRD2 expression were related to the methylation status of their promoter regions. In NAc, maternal CAF diet reduced DRD1 and DRD2 expression in the offspring at PND10; although no alternations in their methylation patterns were detected. This study shows the importance of maternal nutrition during the perinatal period of their offspring and provides novel insights into the mechanisms through which maternal junk-food feeding can affect reward system in early postnatal life.

Keywords: maternal cafeteria diet; offspring; dopamine receptor; tyroxine hidroxylase; methylation.

(1534) **OPPOSING EFFECTS OF CATECHOLAMINERGIC AND SEROTONINERGIC SYSTEMS IN *C.elegans:* POTENTIAL IMPLICATIONS FOR HUMAN POSTTRAUMATIC STRESS DISORDERS**

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INIBIBB

Post-traumatic stress disorder (PTSD) is a clinical condition characterized by recurrent disrupting memories of a traumatic event, hyperarousal and anxiety. Despite it is known that PTSD patients exhibit high levels of catecholamines (CA), even in the absence of stress, the neural mechanisms underlying this condition are not understood. The selective serotonin (5-HT) reuptake inhibitors (SSRIs), paroxetine and sertraline, are the only drugs approved by the FDA for PTSD treatment. However, the role of 5-HT in PTSD and its relationship with CA is unknown and difficult to study in the complex human nervous system (NS). *C. elegans* is suited to provide insights into the crosstalk between 5-TH and CA as its NS is simple, has a defined neural wiring diagram and conserved neurotransmitter systems. Moreover, *C. elegans* coordinates stress response by releasing tyramine (TA) and octopamine (OA), which are structural and functional counterparts of CA, the mammalian “fight or flight” hormones.

We here studied parameters that, in *C. elegans*, depend on 5-HT such as egg laying, pharyngeal pumping and letargus. We exposed *tdc-1* and *tbh-1* null mutants (unable to synthetize TA and OA, respectively) to exogenous 5-HT. We found that these mutants are hypersensitive to 5-TH. Moreover, we observed a reduction in the pharyngeal pumping rate in *tph-1* null mutants (unable to synthetize 5-HT), which is partially rescued in *tph-1;tbh-1* double mutants. These results strongly suggest that 5-HT acts antagonistically to CA in *C. elegans*. These opposite actions could be conserved in mammals and explain the efficiency of SSRIs in PTSD treatment. We are now digging into the molecular and cellular underpinning of these antagonistic effects by analyzing mutants in serotonin receptors (many of them homologous to human 5-HT receptors). This study will widen the understanding of the mechanisms involved in neuronal regulation of stress response and could also provide new insights for PTDS treatment.

(278) **ATYPICAL ARRANGEMENT AND DISTRIBUTION**

OF KISSPEPTIN, NEUROKININ B AND DYNORPHIN A IN THE HYPOTHALAMUS OF VIZCACHA

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Abstract: Considering that KNDy (kisspeptin, neurokinin B and Dynorphin A) cells are localized in the arcuate nucleus (ARC) of most mammals and modulate gonadotropin-releasing hormone (GnRH) expression and secretion, the aim of this work was to identify the presence of the KNDy cells in the hypothalamus of South American plains vizcacha (*Lagostomus maximus*) and its relation with GnRH and hormone receptors involved in reproduction. Adult non-pregnant female vizcachas were captured in the Estación de Cría de Animales Silvestres (ECAS) during March (n=7). Its brains were removed and fixed. Kluwer-Barrera staining was performed to determine ARC and anteroventral periventricular hypothalamic nucleus (AVPV) localization. To study KNDy cells arrangement, immunofluorescence followed by paired confocal microscopy was developed. NeurokininB (NKB), dynorphin-A (DYN), kisspeptin (Kiss), Kiss receptor (GPR54), GnRH, estrogen receptor alpha (ERα) and progesterone receptor (PR) were labelled. A few number of NKB/Dyn immunoreactive neurons, somas and fibers, were found in ARC. Surprisingly, NKB^+/Dyn^+ neurons were more abundant in AVPV than in ARC. However, Kiss was not detected in NKB^+/Dyn^+ neurons of both ARC and AVPV but were individually found in somas of AVPV and in fibers of ARC. In addition, GPR54 colocalized with GnRH in neurons of AVPV. Both $Kiss^+$ and NKB^+/Dyn^+ cells showed colocalization of ERα and PR. This is the first description of the KNDy components distribution in the hypothalamus of the vizcacha. An atypical arrengement of KNDy system was detected in AVPV. The expression of NKB/Dyn and Kiss in separate neurons and the lack of Kiss immunoreactive somas in ARC, suggest a singular model of KNDy system in the vizcacha with physical separation but possibly with a coordinated function. This results may indicate that GnRH neurons could be directly regulated by the hormonal environment via ERα and PR, and indirectly by Kiss and NKB/Dyn in *Lagostomus maximus*.

Keywords: Sexual dimorphism, KNDy, AVPV, ERα, vizcacha

NEUROSCIENCE 2

(118) **ANALYSIS OF CEREBROSPINAL FLUID AMYLOID BETA 1-43 AS AN ALZHEIMER'S DISEASE BIOMARKER**

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Core biomarkers: amyloid beta 1-42 (Aβ42), total tau and phospho-tau have been included in the most recent diagnostic guidelines for Alzheimer's disease. New biomarkers are being intensively studied to improve diagnostic accuracy. Here, we have investigated cerebrospinal fluid amyloid beta 1-43 (Aβ43) in a group of patients with Dementia of Alzheimer's Type (DAT, n=10), Mild Cognitive Impairment (MCI, n=7) and 9 non-demented controls, using a commercially available ELISA kit. CSF Aβ43 levels were significantly decreased in DAT and MCI patients compared to controls (ANNOVA p=0.0484). Comparison of Aβ43 with the established biomarker Aβ42 revealed that both performed comparably well when analyzing receiver operating characteristic (ROC) curves between controls and DAT patients (area under the curve for Aβ43= 0.9222 vs AUC=0.9000 for Aβ42). Our results suggest that both biomarkers could be equally used as part of the diagnostic algorithm in Alzheimer's disease.

Keywords: alzheimer's disease, biomarker, diagnosis, amyloid beta

(543) **ATROGLIAL MGLU3R FUNCTIONS IN AGING ASTROCYTES AND HIPPOCAMPUS IN A MODEL OF ALZHEIMER DISEASE**

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Our laboratory recently showed that metabotropic glutamate receptors (mGlu3R) in astrocytes presents a strong neuroprotective action against different inflammatory agents like NO and Aβ. Physiological cerebral aging is characterized by an increase in oxidative stress, neuroinflammation, which among others alterations compromise cognitive functions. As an in vitro aging model, we developed primary astrocytes cultures of 3 weeks (3w) and 9 weeks (9w), treating them with the mGlu3R agonist LY379268 (LY), 0.01μM/24hr. We found that the cells reach a senescence state at 9w (β-Galactosidase assay). Furthermore, we observed decreased cell viability at 9w, MTT assay. We also observed an important decrease of SOD activity comparing 9w astrocytes to 3w, while LY treatment induced a significant ($p<0.05$) increase of SOD activity in 9w astrocytes. Intracellular and extracellular Glutathione (GSH), show a significant increase comparing 9w to 3w non-treated astrocytes ($p<0.01$ and $p<0.05$). GSH production was not modified by LY. No group shows difference in ROS production. In order to characterize the expression profile of cognition-associated genes, we performed RT-qPCR from hippocampus of wild-type (wt) and transgenic (Tg) PDAPP-J20 mice of 5, 9, 14 and 20 month (m) old. mGlu3 mRNA levels were higher ($p<0.01$) in 9m-wt mice compared with 5m-wt mice and in 20m-Tg mice compared with 9m-Tg mice ($p<0.05$). We also found a significant difference ($p<0.05$) between wt and Tg mice at 9m. Serine Racemase (SR), D-Serine synthetic enzyme involved in memory, shows lower expression in 14m-Tg mice than 5m-Tg mice ($p<0.01$), we also found a significant difference ($p<0.05$) between wt and Tg mice at 5m. This results show changes in the expression of mGlu3 and SR during aging, whereas the expression pattern is modified in Tg animals. Besides, mGlu3 during aging could modulate the oxidative stress by increasing SOD levels.

Keywords: astrocytes, mGlu3R, aging, LY379268.

(1742) **THE ER-UNFOLDED PROTEIN RESPONSE MODULATES NEUROTOXICITY INDUCED BY DEMENTIA-ASSOCIATED AMYLOID PEPTIDES**

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Alzheimer's disease (AD), the most common neurodegenerative disorder and Familiar Danish Dementia (FDD), a rare autosomal dominant disease, shares some features, including amyloid accumulation, ADan and Aβ respectively, progressive dementia and presence of neurofibrillary tangles.

Growing evidences suggest that amyloid accumulation induces ER stress in early stages of neurodegeneration. Disturbed ER homeostasis engages an adaptive reaction known as the unfolded protein response (UPR) that protects the cell against toxic misfolded proteins.

IRE1 activation, the most conserved UPR branch, includes the unconventional splicing of XBP1, a transcription factor that up-regulates genes related to protein folding (including BIP) and ER-mediated degradation.

In order to study the relation between UPR and amyloid peptides neurotoxicity we used transgenic *Drosophila melanogaster* lines.

The neuronal expression of ADan caused impairment in climbing ability in 3-day old flies, while in Aβ42 flies the impairment began at 21 days as compared to BRI2-23, a non-amyloidogenic control (ANOVA RM, $p<0.05$). To study UPR induction, we co-expressed each peptide with XBP1-GFP and found a correlation with IRE1/XBP1 activation and the onset of climbing dysfunction. In addition, HSC3/Bip levels measured in 14 day-old flies by Western blot, were higher in ADan than in Aβ42 (ANOVA $p<0.05$) These results indicate that these amyloid peptides can induce early UPR activation that correlate with the severity of their toxicity.

Modulation of XBP1 levels by down-regulation enhanced neu-

rotoxicity and the accumulation of Aβ42, while overexpression reduced Aβ42 and improved geotaxis in old flies (ANOVA p<0.01). Our results indicate that IRE1/XBP1 activation is neuroprotective and enhances Aβ42 clearance. Further analyzes with ADan expressing flies are being performed. Understading the role of UPR activation in early stages of disease may help to develop new treatments to delay the onset of dementia.

Keywords: Neurodegeneration, Aβ, Adan, Upr, Xbp1.

(413) **ROLE OF APP/GO PROTEIN Gβγ COMPLEX SIGNALING ON Aβ NEURODEGENERATION IN ALZHEIMER´S DISEASE MODELS**

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Deposition of amyloid-β peptides (Aβ) causes neurodegeneration in Alzheimer's disease (AD). Aβ is generated by regulated proteolysis of the amyloid precursor protein (APP). However, the involvement of APP, beyond its role as source of Aβ, in the mechanism of Aβ-induced degeneration remains elusive. Methods. FRET and co-immunoprecipitation were performed in primary hippocampal cultures and HEK293T in order to identify the effect of Aβ on APP and Go interaction. The physiopathological relevance of APP and Go signaling in Aβ neurodegeneration was determined by analyzing neuronal dystrophy, tau phosphorylation and neuronal death in primary hippocampal cultures and by assessment of memory performance in the 3xTgAD mice using the novel object recognition (NOR) test. Results. We found that toxic Aβ assemblies enhance APP and Go interaction ($p < 0.001$). APP overexpression renders hippocampal neurons vulnerable to Aβ-toxicity by a mechanism that requires Go-Gβγ signaling and p38-MAPK activation ($p<0.05$). We used gallein, a pharmacological inhibitor of Gβγ complex, to demonstrate that the activation of Go protein Gβγ complex mediates Aβ-induced p38-MAPK activation and neuronal degeneration ($p < 0.001$). In mature hippocampal cultures expressing endogenous proteins, treatment with gallein and PD503208 reduced neuronal degeneration, tau phosphorylation at the PHF-1 epitope induced by toxic Aβ assemblies ($p < 0.05$). Gallein also inhibited Aβ-induced synaptic loss in hippocampal cultures ($p < 0.001$). Finally, in the 3xTgAD mice, an AD model, acute application of gallein in dorsal hippocampus restores memory performance in the NOR test ($p < 0.05$). Conclusions. Our data reveal that APP/Go-Gβγ complex is a signaling hub potentially relevant for developing therapies for halting Aβ-degeneration and cognitive dysfunction in AD based on a molecular disease mechanism. Keywords. Alzheimer, Aβ, APP, Go protein, Gβγ complex, degeneration, tau phosphorylation, gallein, p38 MAPK, 3xTg-AD.

(449) **PHOSPHATIDIC ACID MEDIATES NEUROFILAMENT LOSS INDUCED BY α-SYNUCLEIN**

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In our laboratory, we have previously studied the role of phospholipase-mediated signaling in different models of neuronal injury. In this work, our aim was to characterize the effect of wild type α-synuclein (WT α-syn) overexpression on phospholipase D (PLD) pathway in a human neuroblastoma cell line. Immunocytochemistry showed a significant decrease in the neuronal marker neurofilament (NF) expression and a rise in F-actin foci in WT α-syn cells compared with control cells (pcDNA3) ($p<0.001$). Experiments of immunocytochemistry and Western blot demonstrated a diminished PLD1 expression level in WT α-syn cells ($p<0.001$).

To elucidate the implication of PLD in NF expression, we treated untransfected cells with PLD1 and PLD2 pharmacological inhibitors (EVJ and APV, respectively). We observed a decrease in NF levels only upon EVJ treatment ($p<0.001$). To confirm the inhibitor effect, we also tested PLD activity by using the transphosphatidylation assay as a phosphatidic acid (PA) production marker. We found that in untransfected cells NF loss was related with PA produced by PLD1 ($p<0.01$). Moreover, WT α-syn cells treated with EVJ showed almost complete abolishment of NF expression and increased foci of actin polymerization in the cellular periphery ($p<0.05$; $p<0.001$).

Finally, we found that constitutively active eGFP-PLD1 expression restores NF levels in WT α-syn cells but increases the apoptotic marker cleaved caspase-3 ($p<0.001$). Our results lead us to conclude that the neuronal phenotype loss, characterized by NF diminution, could be a protective mechanism mediated by PA signaling.

Keywords: α-synuclein, neurofilament, phospholipase D1, neurodegeneration

(1022) **EVALUATION OF GENES IMPLICATED IN NEURONAL PLASTICITY AS POTENTIAL PERIPHERAL MARKERS OF COGNITIVE DEFICIT.**

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Abstract: The hippocampus is a brain-structure related with learning and memory. Hippocampus is sensitive to the stress effects. In previous reports, we found that chronic mild stress (CMS) model induced cognitive deficit in mice. This was correlated with a decrease in adult neurogenesis and Th1/Th2 balance in lymphocytes. We also observed a decrease in neural nitric oxide synthase (nNOS) activity and protein levels and an increase in endothelial nitric oxide synthase (eNOS) in hippocampus of CMS mice. Moreover, other authors related the genes G protein subunit alpha q (GNAQ), cAMP responsive element binding protein (CREB) and glycogen synthase kinase 3 beta (GSK-3β) with plasticity neuronal process such as neurogenesis, neuronal differentiation, dendritic growth and in several psychiatric disorders. All these genes are expressed in lymphocytes. The aim of the present work was correlate the eNOS, nNOS, GNAQ, CREB and GSK-3β genes expression in hippocampus, lymph nodes and spleen with cognitive deficit in female BALB/c mice exposed to CMS model. The spontaneous alternation percentage was evaluated by Y-maze. We found a poor performance in the Y-maze in CMS mice respect to control mice ($p<0.01$). The mRNA levels of the all genes were analyzed by qRT-PCR. The mRNA level of eNOS ($p<0.05$), nNOS ($p<0.05$) and GNAQ ($p<0.05$) decrease in hippocampus and nNOS ($p<0.05$) level decease in lymph nodes of stressed mice respect to control. The GNAQ ($p<0.05$), GSK-3β ($p<0.01$) and CREB ($p<0.01$) expression decrease in spleen of CMS mice. The other genes did not show significant differences in the studied tissues. These findings indicate the nNOS and GNAQ genes could be potential peripheral markers of cognitive deficit. More studies are necessary to demonstrated this hypothesis.

Keywords: peripheral markers, neuronal plasticity genes, cognitive deficit, chronic stress.

(252) **GALECTIN-1 REDUCES HIPPOCAMPAL AMYLOID DEPOSITS AND CONTROLS THE NEUROVASCULAR UNIT IN A TRANSGENIC MOUSE MODEL OF ALZHEIMER DISEASE**

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Alzheimer's disease (AD) is the most common form of dementia. Although the pathogenesis of AD remains cryptic, this pathology is associated with an imbalance in the production and clearance of amyloid-β protein (Aβ). Aβ deposition and neuroinflammation are recognized hallmarks in AD, affecting mainly the brain cortex and hippocampus, both in patients and animal models. The glycan-binding protein galectin-1 (Gal1) modulates several properties on immune and endothelial cells in peripheral and central nervous system compartments, where a neuroprotective role was proposed in experimental settings of autoimmune encephalomyelitis. Here, we administered Gal1 or vehicle control (i.p. 9 injections of 100 ug/dose) during 3 weeks to 12 months-old PDAPPJ20 transgenic mice, or age-matched non-transgenic animals. The Gal1 treated group displayed a significantly improved cognitive response in the Novel Object Location Recognition test ($p<0.05$). Amyloid+ area assessed by Congo Red staining in the hippocampus was decreased by 53,5% ($p<0.05$). In addition, labelling with the tomato lectin to identify the microvasculature combined with immunofluorescence against amyloid (4G8) around vessels, revealed a strong reduction in perivascular-located deposits ($p<0.05$) but no changes in vascular morphology. Moreover, we detected a trend of reduction of the perivascular Aquaporin-4 protein in astrocytic endfeet in AD mice and a trend towards recovery of water channel protein -crucial for clearance of Aβ in the brain via lymphatic clearance and glial degradation, besides modulation of the synaptic function -after Gal1 treatment. Additional *in vitro* experiments employing endothelial and glial cells are in progress to elucidate the mechanisms by which Gal1 controls the integrity and function of the neurovascular unit in AD.

Palabras claves: Alzheimer's Desease, Neurovascular unit, endothelium, Galectin-1, amyloid-β

(447) **LIPID DROPLETS AS AN EARLY MARKER OF α-SYNUCLEIN-INDUCED NEURODEGENERATION**

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Pathological accumulation of α-synuclein (α-syn), a cytosolic protein highly expressed in the central nervous system, is a hallmark of Parkinson's disease. α-syn has the ability to bind lipids, although its involvement in lipid metabolism remains unknown. In previous work, we reported that the overexpression of A53T α-syn in dopaminergic neurons increased the cellular content of neutral lipids and fatty acids (SAIB 2014-2015). The aim of the present study was to characterize the role of α-syn in neuronal lipid metabolism. For this purpose, we worked with a human neuroblastoma cell line, IMR-32, that stably express the wild type form of α-syn (WT α-syn). We observed an increase in triacylglycerides (TAG) and cholesterol content in WT α-syn neurons when compared with control cells (pcDNA3) (**$p<0.01$ and ***$p<0.001$, respectively). In consonance with these findings, lipid droplets (LD) accumulation in WT α-syn neurons was detected. Moreover, the number and the size of LD in the presence of α-syn were increased when LD formation was induced by oleic acid (300 y 600 μM). Enhancers of α-syn toxicity such as iron, manganese and bortezomib, which increased protein aggregation, potentiated LD accumulation in WT α-syn neurons. Finally, the pharmacological inhibition of lipins (propranolol) reduced the viability of WT α-syn neurons with respect to controls (**$p<0.01$), thus suggesting that the blockage of TAG synthesis turned the neurons more vulnerable to the presence of α-syn. Our results allow us to conclude that neurons trigger the accumulation of LD as a neuroprotective mechanism against α-syn, and that this novel mechanism could be involved in the determination of neuronal fate. Sponsored by FONCyT-CONICET-UNS.

Keywords: α-synuclein, lipid metabolism, lipid droplets, neurodegeneration.

(501) **METABOTROPIC GLUTAMATE RECEPTOR 3 - SAPPα: NOVEL PATHWAY IN CLASS-A SCAVENGER RECEPTOR-MEDIATED β-AMYLOID CLEARANCE BY ASTROCYTES**

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Among metabotropic glutamate receptors (mGluR), astroglial mGlu3R subtype has particularly shown a broad range of neuroprotective functions. Our previous results demonstrated that mGlu3R activation by the synthetic agonist LY379268 promotes non-amyloidogenic APP cleavage, thereby releasing sAPPα neuroprotective fragment from astrocytes. Moreover, sAPPα mediates the antiapoptotic effect of conditioned medium from LY379268-treated astrocytes (LY-ACM) against Aβ toxicity in hippocampal neurons. We also showed that LY379268 increases Aβ uptake by astrocytes, which parallels the reduction in co-cultured neuron death. In order to elucidate the mechanism involved in Aβ uptake by astrocytes, the present study shows that LY-ACM increased HiLyte Fluor-$A\beta_{25\text{-}35}$ uptake by naïve astrocytes ($p<0.05$) and that sAPPα depletion from LY-ACM blocked this effect. Accordingly, recombinant sAPPα (1 ηM) also increased Aβ uptake ($p<0.01$). Since LY379268 (0.1 μM) increased gene expression of class-A scavenger receptor (SR-A) in astrocytes ($p<0.05$), we determined whether SR-A mediates mGlu3R-induced Aβ uptake by using astrocyte cultures derived from SR-A knockout mice. We found that LY379268 as well as sAPPα effects on Aβ uptake were abolished in SR-A-deficient astrocytes ($p<0.05$), indicating the participation of this scavenger receptor in Aβ phagocytosis by astrocytes. When Aβ was added to the wells containing LY379268 or recombinant sAPPα in wild-type astrocytes, the effect of sAPPα on Aβ uptake was abolished, whereas the effect of LY379268 was decreased by over 40%, suggesting that remaining sAPPα in the culture medium might interfere with Aβ binding to SR-A. Our results shed light on a novel neuroprotective pathway that not only prevents Aβ neurotoxicity but also promotes Aβ clearance.

Keywords: astrocytes, mGlu3R, sAPPα, β-amyloid uptake, class-A scavenger receptor

(898) **PLATELETS BIOENERGETIC AS A POTENTIAL BIOMARKER FOR ALZHEIMER'S DISEASE**

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Bioenergetic dysfunction has been suggested as an early event and as a cause for synaptic deficiency and cognitive impairment in Alzheimer disease (AD). It has been proposed that energy dysfunction can be dynamically tested in Platelets (PLTs). PLTs are good candidates to find peripheral biomarkers for AD because may reflect in periphery the inflammatory and oxidative stress that happens in the brain. We aimed to perform a time-course analysis of PLTs isolated from peripheral blood of control (CNT) and hemizygous McGill-R-Thy1-APP transgenic (Tg+/-) rats, a well characterized model of early AD. We used an extracelular flux analyzer (Seahorse XFp) and 2 different approaches: MitoStress (evaluates mitochondria functionality) and GlycoStress (asseses glycolitic performance). Animals (n= 3-6/group) from 3-12 month-old were anestethized, beheaded and blood (10 ml) recovered in sterile tubes containing Citrate Buffer/Dextrose (pH 7.4). PLTs were isolated by sequential centrifugation at room temperatura to avoid activation. Quantification was performed by hematologic analyzer (Mindray 5800). $15x10^6$ PLTs/well were seeded in XFp Cell Culture MiniPlates and centrifuged (1500g/15 min), afterthat supernatant was removed and MitoStress and GlycoStress performed. We set-up a method to evaluate mitochondrial activity in PLTs and are able to plot profiles to compare CNT vs. Tg(+/-). By contrast to synaptosomes, PLTs do not have spare respiratory capacity, it means that are not involved in process-

es that requiere extra ATP when energy demand increases, such as synaptic functionality and cognition. This result is in agreement with the phisyological role of PLTs. Preliminary data show that bioenergetic parameters of PLTs from 6 month-old Tg(+/-) are similar to CNT. Our results are in the context of a transgenic model and further validation is required in PLTs derived from blood of AD and CNT subjects to determine the relevance of PLTs bioenergetic as a peripheral biomarker for AD.

Keywords: Alzheimer; Platelets; Bioenergetic; Mitochondria; Glycolisis

CARDIOVASCULAR AND RESPIRATORY SYSTEMS 1

(1454) **ACTIVATION OF THE GPR30 RECEPTOR INHIBITS ALDOSTERONE-INDUCED CARDIAC HYPERTROPHY**

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GPR30 was first described as a membrane orphan G protein-coupled receptor. Later it was demonstrated that estrogen can act as a receptor ligand. It has been reported that the activation of GPR30 is cardioprotective. The administration of its synthetic ligand G1 reduced the infarct size in ischemia-reperfusion, attenuated heart failure and induced a decrease in perivascular fibrosis. The steroid hormone aldosterone (Ald) plays a classic role acting through mineralocorticoid receptor (MR) and its activation on the cardiovascular system generates cardiac hypertrophy, fibrosis and heart failure. It has been recently proposed that certain non-genomic effects of Ald are due to the activation of GPR30. However these results are in contraposition with the beneficial effects of GPR30 activation. The aim of this work was to evaluate the role of GPR30 activation in a model of hypertrophy induced by Ald. We cultured neonatal rat cardiomyocytes (NRCM) from 1-3 days Wistar rats and treated them for 48 hours with Ald (10nM), G1 (1uM) or the combination of both. Hypertrophy caused by Ald was attenuated by the co-treatment with G1 (cellular area % respect to control,Control:1±0.07,n =9;Ald:1.33±0.05*,n=8;Ald+G1:0.93±0.05,n=7;*p<0.05). We also studied the expression of the hypertrophic marker BNP. We found a significant increase in BNP expression in those cells treated with Ald, which was abrogated by G1 co-treatment (BNP/GAPDH expression,Control:1±0.34,n=9; Ald:2.81±0.89*,n=6,Ald+G1:1.13±0.47,n=6;*p<0.05). To analyze the involvement of MR and GPR30 in cardiac hypertrophy development, we transfected NRCM with siRNAs against them (siMR and siGPR30) during Ald treatment. We obtained preliminaries results showing that Ald-induced hypertrophy was prevented by siMR and not affected by siGPR30, indicating that MR is responsible for Ald induced hypertrophy. Overall, the present data indicate that the MR-dependent Ald-induced hypertrophy is prevented by GPR30 activation by G1, suggesting the possibility of considering this receptor a novel therapeutic target for the treatment of this pathology.

Key Words: ALDOSTERONE, GPR30, MR, HYPERTROPHY

(1846) **EFFECT OF CARVEDILOL OR LOSARTAN ON BLOOD PRESSURE VARIABILITY AND ITS IMPACT ON TARGET ORGAN DAMAGE IN SPONTANEOUSLY HYPERTENSIVE RATS**

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The increase in blood pressure variability (BPV) has been established as an independent risk factor for the development of target organ damage in hypertensive patients. The aim was to evaluate the effects of the treatment of different antihypertensive agents on central arterial pressure, BPV and target organ damage in spontaneously hypertensive rats (SHR). Male SHR (200-250 g) received vehicle (n=6), carvedilol 15 mg/kg (n=6) or losartan 10 mg/kg (n=6) for 8 weeks. A fourth group of Wistar rats were used as normotensive control (n=6). At the end of the treatment, echocardiographic evaluation, direct and indirect blood pressure (BP) and short-term variability measurements were performed. Left ventricle was removed to assess ventricular hypertrophy.

Chronic treatment with losartan, but not carvedilol, reduced indirect systolic BP. Both carvedilol and losartan diminished its intraday fluctuation compared to SHR. Also, both drugs significantly decreased carotid SBP and its short-term variability. Left ventricular mass was significantly reduced by chronic treatment with carvedilol (2.70±0.04 mg/g; P<0.05 vs. vehicle) or losartan (2.70±0.08 mg/g; P<0.05 vs. vehicle) when compared with SHR treated with vehicle (3.51±0.19 mg/g). Echocardiographic evaluations revealed that carvedilol or losartan revert diastolic anterior wall thickness, diastolic posterior wall thickness, systolic posterior wall thickness and isovolumic relaxation time. Chronic treatment with carvedilol, but not losartan, normalized systolic posterior wall thickness. In conclusion, both carvedilol and losartan are effective in the reduction of central BP and its short-term variability in SHR, resulting in an attenuation of ventricular hypertrophy evidenced by morphological and echocardiographic parameters.

Keywords: hypertension, carvedilol, losartan, echocardiography.

(1134) **EFFECT OF CHRONIC TREATMENT WITH SILDENAFIL ON CARDIAC HYPERTROPHY**

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Previously, we showed that myocardial Na^+/H^+ exchanger (NHE1) hyperactivity is responsible of cardiac hypertrophy (CH) development in spontaneously hypertensive rats (SHR), also that the increased in protein Kinase G (PKG) activity after phosphodiesterase-5A (PDE5A) inhibition (Sildenafil, "SIL") inhibits NHE1. Current study was aimed to evaluate the ability of chronic SIL treatment to reverse hypertrophy. As an initial approach, H9C2 cells were treated during 48hs with several doses of Angiotensin II (AngII) to induce growth (evaluated by measuring cell area in percent of control). A dose of 10nM AngII was then selected. A significant increase in cell area was detected after 24hs of AngII: 129±6 vs.100±5% (control), n=4, effect that was larger after 48hs: 154±8%. Addition of SIL during the last 24hs of AngII treatment promoted a significant dose-dependent decrease in cell area: 126±5% (5μM), 114±4% (10μM). To evaluate in vivo the effect of SIL on CH, 4-months old SHR rats were chronically treated (3 months) with SIL (100mg/kg/day) orally through drinking water (SHR+SIL) and compared to age-matched untreated controls (SHR). SIL reduced CH (left ventricular weight to body weight ratio: 2.69±0.04 vs. 2.52±0.05, p<0.05), myocardial interstitial fibrosis (percentage of total collagen: 1.82±0.01% vs. 1.38±0.01%, p<0.05) and myocardial stiffness (evaluated by length-tension curves in isolated papillary muscles, p<0.05, 2-wayANOVA). In addition, a decreased NHE1 activity, determined by H^+ efflux at pH_i 6.8 during the recovery from a transient acidosis, was detected in isolated papillary muscles from SHR+SIL group (1.19±0.7 mM/min) compared to non-treated controls (4.19±0.8 mM/min, p<0.05). In summary, chronic SIL treatment was able to reverse either AngII-induced H9C2 cells growth or CH in SHR. The fact that NHE1 activity was found depressed in SHR+SIL group suggests that a PKG-induced exchanger inhibition would be responsible for the antihypertrophic effect of SIL.

Key word: Sildenafil-NHE1-cardiac hypertrophy

(1132) **EFFECTS OF BIOACCESSIBLE GARLIC COMPOUNDS ON CULTURED VASCULAR SMOOTH MUSCLE**

CELLS FROM SPONTANEOUSLY HYPERTENSIVE RATS.

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Abstract: Several studies have associated a moderate consumption of garlic with improvements in health. The evidence suggests that its medicinal activities are mainly due to the organosulfur compounds (OSCs) content. Nevertheless, OSCs can have different activities/potency on different cell lines or tissues. Moreover, after garlic consumption, only the bioavailable compounds will have bioefficacy. In this regard, previous works have identified only a few OSCs in blood. In this work it is desired to evaluate the proliferation and migration of vascular smooth muscle cells (SMCs), phenomena that contribute to the remodeling of small arteries and complicate hypertension. Three bioaccessible OSCs, allyl mercaptan (AM), S-allyl cysteine (SAC) and 2-vinyl-4H-1,3-dithiin (2VD), were selected to evaluate their effects on SMCs isolated from mesenteric arteries of spontaneously hypertensive rats. Cell proliferation by MTT assay, cell migration by scrape-wound migration assay and intracellular oxidative stress by fluorometric CM-H2DCFDA assay were evaluated after cell exposure to OSCs expected quantities in plasma, 10 μg/L of AM and 2VD and 50 μg/L of SAC. The MTT results showed that 2VD significantly decreased the rate of proliferation 20% compared to the control. Migration assay showed that 2VD and AM reduced significantly cell migration, around 30%. Concerning to the intracellular ROS levels, the compounds showed different behaviors; while AM and SAC behaved as intracellular antioxidants, 2VD showed a slight tendency to increase the oxidative stress. In conclusion, expected quantities of 2VD and AM in plasma, provided by garlic intake, were able to inhibit the migration of SMCs of spontaneously hypertensive rats. These compounds could help to protect the cardiovascular system from vascular remodeling produced by hypertension. Further studies will be needed to elucidate their action mechanisms and the role of its different antioxidant effects.

Keywords: Organosulfur compounds, garlic, hypertension, vascular remodeling.

(066) **FEMALE MICE EXPOSED TO EARLY LIFE STRESS DISPLAY ANGIOTENSIN II-DEPENDENT OBESITY-INDUCED HYPERTENSION**

Analia Loria

Previously, we have shown that female mice subjected to maternal separation with early weaning (MSEW), a model of postnatal neglect, display exacerbated diet-induced obesity and high blood pressure (BP) compared with control mice. Female MSEW mice show activated renin-angiotensin system, including increased plasma renin activity and adipose tissue-derived angiotensinogen secretion. This study tested the hypothesis that augmented obesity-induced hypertension in female MSEW mice is AngII-dependent. Mouse MSEW was achieved by repeated, daily separations from the dam and weaned 4 days early. Normally reared controls (C) were weaned at postnatal day 21. Each experimental group of female weanlings was placed on high fat diet (HFD, 60% kcal from fat). After 18 weeks, mice were implanted with radiotelemetry devices for BP measurement. At week 20, average 24-hr systolic blood pressure (SBP) was 134±2 mmHg in MSEW mice and 126±2 mmHg in C ($P<0.05$). We also determined the BP sensitivity to the acute administration of AngII (1, 10 and 50 ug/kg, s.c.). AngII-induced BP changes, assessed by BP area under the curve, were similar between MSEW and C mice at all doses (50 ug/kg dose:145±10 vs. 132±15 mmHgx30 min, respectively). Chronic enalapril treatment (2.5 mg/kg/day, drinking water) was conducted for 7 days. Enalapril reduced SBP 15±2 mmHg in MSEW mice but only 6±1 mmHg in C mice ($p<0.05$). The BP response to acute AngII doses increased similarly in MSEW and C enalapril-treated mice, (50 ug/kg: 200±13 vs. 207±22 mmHgx30 min, respectively). In addition, BP and HR responses to acute injections of mecamylamine (5 mg/kg), propranolol (5 mg/kg) or atropine (1 mg/kg) were similar between untreated MSEW and C mice, suggesting that exacerbated BP in female MSEW mice is independent of sympathetic or parasympathetic dysfunction. Taken together, these data provide evidence that increased BP in female MSEW mice results from elevated circulating AngII rather than enhanced AngII sensitivity.

Keywords: Early life stress, females, obesity, hypertension, Angiotensin II

(1635) **HYPERPOLARIZING MECHANISMS AND ENDOTHELIN APPERANTLY INFLUENCE ANGIOTENSIN II RESPONSES ON AORTAS OF RATS SUBMITTED TO ADJUVANT INDUCED ARTHRITIS (AIA)**

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This study aimed to investigate the effects of adjuvant induced arthritis (AIA) on the aorta responses of rat to angiotensin (Ang II) and acetylcholine (ACh). Moreover, to determine if these changes may be influenced by a reduction of plasma testosterone. Male Wistar rats were distributed in ORQ (orchiectomy), AIA, ORQ/AIA and control. Twenty-one days after the onset of AIA aortic rings were set up in organ bath. Values of pEC50 (Log of EC_{50}) and maximal response (R_{max}; in grams), were compared by two-way ANOVA/Tukey - $P<0,05$. No changes of ACh or Ang II responses were observed due to AIA and/or ORQ, in aorta with or without endothelium. In the presence of L-NAME, the Ang II R_{max} in Control (0,44±0,09) was higher ($P<0,0001$) than in AIA animals (0,13±0,03). A similar profile of Ang II responses between groups was observed in the presence of 1400W, but without any statistical difference. In the presence of L-NAME plus depolarizing krebs solution (KCl 60mmol/L) or BQ123, the difference observed in the presence of only L-NAME were no longer observed. In the presence of Apocinin, Ang II R_{max} and pEC_{50} were higher in the ORQ than in the ORQ/AIA group (R_{max}: 0,27±0,06 and 0,09±0,02, respectively; $P<0,0007$; pEC_{50}: 8,10±0,15 and 7.54±0,21, respectively; $P<0,01$). Neither AIA nor ORQ change Ang II responses whether endothelial mechanisms are present. The inhibition of endothelial NO-related mechanisms stands out higher Ang II responses in control animals. This increase depends partially on testosterone levels since it is smaller in ORQ animals. However, this elevation of Ang II responses that may be observed only in absence of NO, can be blunted if hyperpolarizing mechanisms and ETA receptors are blocked. It suggests a cooperation between hyperpolarizing mechanisms and endothelin-1 to keep reduced the Ang II aorta responses in AIA animals.

(498) **LOSARTAN IMPAIRS HEXACHLOROBENZENE-INDUCED HYPERTENSION AND MODIFIES THE MECHANO-ENERGETIC RESPONSE OF RAT HEARTS TO ISCHEMIA/REPERFUSION INJURY**

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We have shown that the bio-accumulated organ chlorinade pesticide hexachlorobenzene (HCB) produces hypertension (Hyp) in rats, probably through an increase in arterial angiotensine II receptor 1 (AT1) expression. Also we showed an increase in post ischemic contractile recovery (PICR) and economy for contraction (Eco) in isolated hearts from HCB-treated rats, a potential adaptative response to pesticide-induced Hyp.

Here, we study the role of AT1 in HCB-induced Hyp by means of AT1 antagonist Losartan (L) treatment, focusing on heart contractile and energetic response to an ischemic insult.

Male Wistar rats were treated with 500 mg/kg HCB and with 30 mg/kg L for 45 days. Hearts were used for histological analysis or mechanical and energy assays. To this end, simultaneous mechanical and heat measurements were done in hearts arterially perfused

at 37ºC by Langendorff method, paced at 3 Hz, and exposed to 25 min ischemia followed by 45 min. reperfusion (R).

L prevented HCB-induced Hyp (110.5±8.2, vs 145.5±5.1 mmHg, p<0.05).

Hearts from HCB-treated rats showed a decrease in resting pressure (RP) during R (58.5±4.5 vs 89.2±6.3 mmHg, p<0.05). Neither L nor L+HCB altered RP vs HCB alone (66.5±6.1, 65.33±10.0% for L and L+HCB, respectively).

An improvement of PICR was observed in hearts from HCB- and L-treated rats (46.2±6.0, 39.8±7.4, 18.8±2.3%, for HCB, L and C, respectively, p<0.05 at 45 min of R). L+HCB prevented the HCB-induced increase in PICR (20.2±6.1%, p<0.05 at 45 min of R).

HCB did not alter total heat production (Ht), while L and L+HCB increased it (83.8±6.5, 79.5±5.6, 56.3±4.7% for L, L+HCB and C respectively, p< 0.05).

HCB increased Eco during R (67.8±7.2 vs. 35.0±5.1%, p<0.05 at 45 min R) and neither L nor L+HCB modified it.

Conclusion: L diminished HCB-induced Hyp. The impairment by L of cardiac HCB-induced mechanic and energetic effects suggests that HCB heart protection is a response to HCB-induced Hyp.

Keywords: Hexachlorobenzene, hypertension, Losartan, ischemia-reperfusion, cardiac energetic, dioxin-like

(1572) **PRENATAL AND POSTNATAL ZINC DEFICIENCY EXACERBATES THE CARDIOVASCULAR EFFECTS OF ANGIOTENSIN II**

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Zinc deficiency (ZD) programs cardiovascular alterations in male rats.

Objective: To evaluate the cardiovascular effects of chronic infusion with angiotensin II (AII) in male rats subjected to prenatal and postnatal ZD.

Method: Wistar rats fed during pregnancy until weaning control (C,30ppm) or zinc deficient (B,8ppm) diets. At weaning, C male offspring continued on C diet (CC) and B offspring received B or C diets for 60 days (BB, BC). At 67 days of life, subcutaneous infusions of AII (65 ng/min/rat) or 0.9% NaCl were administered to animals for 14 days using osmotic pumps (Model 2002,Alzet,Palo Alto,USA). Systolic blood pressure (SBP,tail-cuff method,mmHg); cardiac(C) and aortic(A) inflammatory markers (TNFα,IL-6;IHQ; %area marked/total tissue area); perivascular collagen/lumen area(C/L) and media/lumen area(M/L) of A and C arteries (sirius red staining) were determined at 81 days. ANOVA, Bonferroni post-hoc test(n=6/group,$^{\alpha}$p<0.05 vs CC,$^{\delta}$p<0.05 vs BB,$^{\psi}$p<0.05 vs BC)

Results: SBP(CC:120±3;CCAII:165±5$^{\alpha}$;BB:140±3$^{\alpha}$;BBAII:188±6$^{\delta}$;BC:137±1$^{\alpha}$;BCAII:193±3$^{\psi}$)

In C

C/L(CC:1,37±0,20;CCAII:2,68±0,23$^{\alpha}$;BB:2,71±0,32$^{\alpha}$;BBAII:2,30±0,66;BC:2,16±0,18$^{\alpha}$;BCAII:2,83±0,17)

TNFα(CC:3.7±0.9;CCAII:19.4±2.8$^{\alpha}$;BB:17.7±1.0$^{\alpha}$;BBAII:22.2±1.0$^{\delta}$;BC:19.6±0.9$^{\alpha}$;BCAII:22.7±1.0$^{\psi}$)

IL-6(CC:3.3±1.6;CCAII:18.1±1.7$^{\alpha}$;BB:17.3±1.8$^{\alpha}$;BBAII:21.0±1.4$^{\delta}$;

BC:17.5±1.9$^{\alpha}$;BCAII:20.5±1.3$^{\psi}$)

In A

M/L(CC:0,14±0,02;CCAII:0,28±0,02$^{\alpha}$;BB:0,23±0,01$^{\alpha}$;BBAII:0,39±0,03$^{\delta}$;BC:0,17±0,01;BCAII: 0,31±0,01$^{\psi}$)

TNFα(CC:1.5±0.5;CCAII:17.0±1.9$^{\alpha}$;BB:15.7±1.7$^{\alpha}$;BBAII:30.6±2.0$^{\delta}$;BC:15.4±1.6$^{\alpha}$;BCAII:31.2±2.3$^{\psi}$)

IL-6(CC:2.1±0.4;CCAII:22.3±2.2$^{\alpha}$;BB:20.8±1.6$^{\alpha}$;BBAII:31.7±1.7$^{\delta}$;BC:21.4±1.9$^{\alpha}$;BCAII:30.4±1.6$^{\psi}$)

C:M/L, A:C/L: No differences.

Conclusion: ZD exacerbates AII effects on SBP. AII enhanced the expression of inflammatory interleukins in C and A. This increase was lower in BBAII and BCAII animals due an exacerbated inflammatory state without treatment with AII. C arteries from BB and BC showed increased C/L. In A, BB showed higher M/L. AII increased M/L in all groups.

Key words: zinc deficiency, angiotensin II, cardiovascular system, fetal and postnatal life

(1428) **SEX DIFFERENCES IN BLOOD PRESSURE RESPONSE TO CONTINUOUS ANG II INFUSION: INVOLVEMENT OF SEX CHROMOSOME COMPLEMENT AND HORMONAL EFFECTS**

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A growing body of evidence demonstrate that the pressor response to AngII is sexually dimorphic under physiological and pathophysiological circumstances. But why do male and female show differences in rennin angiotensin system (RAS) activation and inhibition? Sex steroids can induce organizational (long-lasting or permanent) effect during critical periods of development but can also impart (temporary or reversible) activational effects. Furthermore, males and females also carry different sex chromosome complements (SCC:XY/XX) and thus are influenced throughout life by different genomes. Previous evidence demonstrates a modulating effect of SCC in RAS receptor expression (at brain and renal levels), as well as in the AngII sexually dimorphic bradycardic barroreflex and hypertensive responses.

In the present study we evaluated the involvement of SCC and the organizational hormonal effect on changes in mean arterial pressure (MAP) in a 30 min Ang II infusion protocol. We also analized in a separate group of mice the activational estradiol effect. For this purpose, we used gonadectomized mice of the “four core genotype” model, in which the effect of gonadal sex and SCC is dissociated, allowing comparisons of sexually dimorphic traits between XX and XY females as well as in XX and XY males. For hormonal replacement experiments gonadectomized mice were daily injected with ß-estradiol (2ug/g) for a 4 day period.

The statistical analysis indicated that XX-male-CON, XY-female-CON and XX-female-CON showed an increase MAP due to AngII infusion, while no changes were observed in XY-males-CON mice suggesting an interaction of SCC and organizational-sex factors {F(1.25)=7.93;p<0.01}. Furthermore, this increase was reversed by the activational effect of ß-estradiol (CON vs.E2 {F(1.59)=8.73;p<0.01}) in XY-female and XX-male E2 groups. Our results thus suggest an interaction of SCC, organizational and activational ß-estradiol effect on angiotensin mediated blood pressure regulation

Keywords: Renin angiotensin system, Sex chomosome complement, ß-estradiol, Four core genotypes mouse model

Supported: FONCyT, ISN,SECyT,Mincyt-Cba,CONICET.

(1649) **VALPROATE (VPA), A HISTONE DEACETYLASE (HDAC) INHIBITOR, DECREASES FIBROSIS IN THE HYPERTROPHYC HEART OF SHR: TRH GENE EXPRESSION**

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Cardiac TRH induces hypertrophy (LVH) and fibrosis in normal rats. Also, cardiac TRH system is overexpressed in SHR. It´s known that the histone deacetylase families, which modify the access of transcription factors to DNA, affect cardiac hypertrophy in animal models. As VPA is an inhibitor of HDACs and modulates gene expression through epigenetic alterations such as DNA methylation, we hypothesized that inhibition of HDACs with VPA might attenuate LVH and the fibrotic process in SHR by modulating cardiac TRH gene expression. 7 w-old male SHR and WKY received VPA. Blood

pressure (BP) was recorded; after 10w of treatment rats were euthanized and hearts obtained. BP, LVH index and cTRH expression were increased in SHR vs WKY. VPA slightly attenuated (ANOVA,$p<0.05$) the higher BP (mmHg) seen in untreated SHR, without effect in WKY (**WKY**= C:128±4 vs VPA:126±3 and **SHR**=C220±4 vs VPA201±4). Hypertrophic index (HW/BW*100) was reduced ($p<0.05$) only in SHR (C:0.4516±0.02 vs VPA:0.3950±0.02). By ecocardiography we found a ($p<0.05$) reduction in LVPWT(mm) only in SHR (C:0.310±0.02 vs VPA:0.242±0.021). VPA normalizes ($p<0.05$) the higher expression of BNP and type 3 collagen in the LV of SHR indicating a strong reduction in fibrosis. This effect was confirmed by Masson's Trichrome and Sirius Red stainings ($p<0.01$). The higher TRH mRNA in SHR heart was reduce in the SHR+VPA to values similar to WKY (**WKY,**C:0.61±0.7vs VPA:0.41±0.97; **SHR,**C:5.72+0.9 vs VPA:0.61+0.9, $p<0.05$). Decreased TRH level by IHQ induced by HDAC inhibition confirms this result. Offspring born from VPA-treated parents with a 2-weeks washout period before mating, and which did not receive VPA ever, had a reduction of hypertrophy, fibrosis and cardiacTRH expression showing transgenerational inheritance. We described for the first time that VPA reduces fibrosis in an independent manner of LVH, effects inherited by the next generation. Our results strongly suggest that epigenetic TRH modulation may play a role.

REPRODUCTION AND FERTILITY 1

(772) AGING-ASSOCIATED INFLAMMATION IN TESTES OF SYRIAN HAMSTERS

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Abstract: Aging constitutes a universal, multi-factorial, progressive and irreversible process. Although the aging process may vary among different tissues, it is usually associated to a chronic inflammatory condition. Relevant to male reproductive aging, we have previously shown an inverse association between longevity and testicular pro-inflammatory state in mouse models with delayed (Ames dwarf Prop1$^{-/-}$ and growth hormone releasing hormone-knockout mice) or accelerated (growth hormone-transgenic mice) aging.

In this study, we analyzed testicular aging in a physiological animal model. To this aim, testes from young adults (5m: 5 months) and aged Syrian hamsters (20m-22m: 20-22 months) were used.

Immunohistochemical studies confirmed an aged-related significant increase in total numbers of Iba1-immunoreactive testicular macrophages (MAC) in which interstitial MACs solely accounted for the rise (Total testicular MAC/mm^2, 5m: 66.28±6.67^a; 20m: 110.00±10.00^b; 22m: 120.50±14.50^b; Mean±SEM, $p<0.05$). Testicular mRNA expression of cytokine IL1β, one of the main secretory products of MACs during inflammation, was found to be significantly higher in aged testes (5m: 1.00±0.05^a; 20m: 11.29±2.58^b; 22m: 16.56±1.80^b; $p<0.05$). Moreover, mRNA expression of cyclooxygenase 2 (COX2), a key enzyme in prostaglandin synthesis and a clear marker of inflammation, was also induced in an aged-dependent manner (5m: 1.00±0.16^a; 20m: 4.74±1.04^b; 22m: 3.47±0.96^b; $p<0.05$). A similar tendency was seen when testicular protein levels of IL1β and COX2 were evaluated.

Collectively these data suggest the development of a pro-inflammatory profile during physiological reproductive aging in the hamster testis.

Keywords: testis, aging, inflammation, COX2, IL1β

(508) ENZYMES INVOLVED IN THE BIOSYNTHESIS OF THE VERY-LONG-CHAIN POLYUNSATURATED FATTY ACIDS OF RAT SPERMATOGENIC CELL SPHINGOLIPIDS

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The sphingolipids (SL) of rodent germ cells contain very-long-chain polyunsaturated fatty acids (V), in nonhydroxy (n-V) and 2-hydroxy (h-V) forms, whose biosynthesis requires the expression of several elongases (Elovl) and a fatty acid 2-hydroxylase (Fa2h). Our objective was to characterize the expression of these enzymes as a function of postnatal development and germ cell differentiation. We employed qPCR for mRNA levels, Western blot and immunofluorescence for protein expression, and [^{3}H]-labeled precursors for enzyme activity. Elovl4, involved in C_{24}-C_{34} fatty acid biosynthesis, was expressed at the mRNA, but not at the protein level, at early prepuberal ages. Such mRNA was a product of Sertoli cells. Elovl4 mRNA *and* protein were both produced by germ cells. In agreement with the relative abundance of n-V in their SL, the Elovl4 enzymatic activity was higher in the premeiotic pachytene spermatocytes than in postmeiotic round and late spermatids ($p<0.05$), and was negligible in Sertoli cells. By contrast to *Elovl4* mRNA, that of *Fa2h* was absent from Sertoli cells and from prepuberal testes ($p<0.05$). *Fa2h* mRNA *and* protein were detectable only in concomitance with the appearance of spermatids, the sperm precursors whose SL are the richest in h-V. Consistently, selective depletion of germ (but not Sertoli) cells from adult rat testes by exposures to mild hyperthermia reduced the mRNA levels of *Fa2h* to a much larger extent ($p<0.001$) than that of *Elovl4.* Among germ cells of adult testes, Elovl4 protein content was high in spermatocytes and late spermatids, while Fa2h was mostly expressed in the latter, residual bodies and spermatozoa. By using inhibitors of specific steps of the SL biosynthetic route, germ cells in culture showed ability to *de novo* synthesize SL containing n-V and h-V. Our results underscore the presence of a developmentally programmed and a cell-specific regulation of the Elovl4 and Fa2h expression and activity as germ cell differentiation proceeds.

Keywords: spermatogenic cells, sphingolipids, very-long-chain PUFA

(1600) NONYLPHENOL INDUCES CYTOSKELETAL CHANGES AND RELEASE OF PROINFLAMMATORY MEDIATORS IN RAT SERTOLI CELLS *IN VITRO*

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Nonylphenol (NP), an alkylphenol present in plasticizers, is an endocrine disrupting chemical that is potentially dangerous for male reproduction in mammals including man. Because Sertoli cells (SC) provide structural and metabolic support to germ cells, in this study the hypothesis that exposures of SC to NP affect their metabolic functions and the production of bioactive molecules was evaluated. Primary cultures of SC were preincubated with [^{3}H]arachidonic acid (AA) to label their lipids and then were treated with NP. The NP exposures resulted in increased concentrations of free AA in cells and medium, indicating that such AA was released from [^{3}H]-labeled lipids ($p<0.01$). This lipid was mostly phosphatidylinositol, acted upon after activation of a protein kinase A (PKA)/cytoplasmic phospholipase A2 (cPLA2). In NP-exposed SC, an increase of diacylglycerols (DAG) also took place in both, cells and medium ($p<0.01$). Part of such DAG may have served as second messengers, since NP-increased DAG were associated with an augmented production of PGE2 and expression (mRNA) of COX2. Since the network of vimentin intermediate filaments is important for intracellular lipid transport, the effects of NP on the structure of this network in relation to the formation of cytoplasmic lipid droplets (LD) was studied. In NP-treated SC, the vimentin network was redistributed and the LD size was increased. The NP-dependent cytoskeletal redistribution was prevented by preincubation with H89, a PKA inhibitor. The formation of large LD was prevented by preincubation with either H89 or MEP, a PLA2 inhibitor, suggesting the participation of PKA and cPLA2 in LD biogenesis. We conclude that NP is involved in

activating the proinflammatory pathway in SC, by providing the AA that is necessary for prostaglandin synthesis via PKA/PLA2 on the one hand, and by generating the DAG that is required as cofactor of the PKC-mediated activation of the NF- κβ/Cox-2 inflammatory pathway on the other.

Keywords: endocrine disruptors; (in)fertility; proinflammatory mediators; COX2

(389) **PARTICIPATION OF SIRT1 IN THE REGULATION OF SERTOLI CELL PROLIFERATION**

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Abstract: Sertoli cells (SC) provide the structural and nutritional support for germ cell development. Considering that each SC is able to support a limited number of germ cells, the final number of SC reached during the proliferative periods determines sperm production capacity in adulthood. In the rat, SC proliferate during fetal and neonatal periods and it is well known that FSH is the major SC mitogen; however, little is known about the mechanisms involved in the detention of SC proliferation, essential for the formation of blood-testis barrier and SC differentiation. Sirtuins (SIRT1-7) belong to a cellular energy sensor family of NAD^+-dependent enzymes with deacetylase activity. SIRT1, the most studied member, plays an important role in several processes ranging from cell cycle regulation to energy homeostasis. The aim of this work was to investigate whether SIRT1 activation participates in the cessation of SC proliferation. Mitotically active SC obtained from 8-day old rats were maintained under basal (B) conditions or stimulated with FSH 100 ng/ml in the absence or presence of Resveratrol (RSV, 50μM) a polyphenol that increases SIRT1 activity. BrdU incorporation and the expression of cyclins D and p21 and p27 (cell cycle inhibitors) by RT-qPCR were evaluated. Results are expressed as mean±SD (n=3, different letters indicate statistically significant differences, P<0.05). RSV decreased BrdU incorporation under basal and FSH-stimulated cultures (B: 10.9 ± 1.9^a, RSV: 3.1 ± 1.3^b, FSH: 21.4 ± 3.2^c, FSH+RSV: 2.1 ± 2.3^b; %BrdU-positive cells) and inhibited the FSH effect on cyclin D1 and cyclin D2 expression. In addition, RSV increased p21 and p27 mRNA levels (p21: RSV: 3.3±1.1*; p27: RSV: 1.9±0.3*; fold stimulation vs B, *P<0.05). Altogether, these results suggest that SIRT1 activation may be involved in the cessation of SC proliferation through the regulation of cyclins and cell cycle inhibitors expression.

Keywords: Sertoli, proliferation, Sirt1, resveratrol

(1079) **PEDF (PIGMENT EPITHELIUM DERIVED FACTOR) EXPRESION IN MEPC5 CELLS (MOUSE) AND IN MALE REPRODUCTIVE TRACT (WISTAR RATS) UNDER ANDROGEN REGULATION**

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Abstract: Pigment epithelium-derived factor (PEDF) expression has been described in many organs as showing neurotrophic, anti-angiogenic, anti-apoptotic, anti-inflammatory, anti-oxidant and pro-cell survival properties. However, references to its activity in the male reproductive system are scarce, except in prostate cancer and the regulation of sperm conjugation in rat epididymis. We aimed to characterize the expression of PEDF in MEPC5 cells (mouse epididymal proximal caput cells) and in the male reproductive tract of Wistar rats and explore their hormonal regulation. We found that PEDF is expressed in MEPC5 by Immunofluorescence and over the epididymis, prostate and seminal vesicles by immunohistochemistry, but notably not in the testes. These results agree with those obtain by semi cuantitative RT-PCR. Androgen dependence of PEDF expression was evaluated by flutamide administration during 15 days to Wistar Rats. PEDF expression diminished along the male reproductive tract. This decreased expression was reversed after 30 days without flutamide administration. The epididymis is an essential organ in sperm maturation-storage. The role of PEDF in this physiological process has not been fully elucidated. But considering that in other systems PEDF has anti-apoptotic, anti-oxidants and pro-cell survival properties, its expression along the epididymis may be related to the protection of spermatozoa while they are stored.

Keywords: PEDF, male reproductive tract, MEPC5 cells, androgens.

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(162) **POSSIBLE ROL OF TESTICULAR TRANSFERRIN IN THE HOMEOSTASIS OF SEMINAL IRON**

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Testicular Transferrin (TTf) is a glycoprotein secreted by Sértoli cells and it is involved in the transport of iron to developing germ cells. Iron homeostasis is defined by mechanisms in which the intracellular concentration of this metal is maintained at adequate levels for cellular requirement but nothing enough to cause toxic effects. The study of DNA and sperm membrane (SM) plays a fundamental role in seminal evaluation. The aim of the present work is to investigate whether there is relationship between TTf levels and SM integrity and sperm DNA. Twenty semen samples were studied: 5 fertile controls (according to WHO 2010) and 15 from patients with different andrological pathologies. The variables studied were: Concentration of TTf versus % of spermatozoa with altered membrane and Concentration of TTf versus DNA integrity, among which the Pearson Correlation Coefficient was applied. The integrity of SM was evaluated by the Hypoosmotic Test (HOST). DNA integrity was assessed by the Acridine Orange Test (AOT). Determination of TTf levels was performed using the Radial Immunodiffusion method adapted for low concentration developed by our group. The relationships were as follows: 1) a high degree of negative association (r = - 0.91) was observed between TTf Concentration (mg / dL) and SM Integrity alterations (p <0.05). 2) no significant association was observed (r = 0.03) when studying the relationship between TTf concentration (mg / dL) and DNA integrity (p = 0.48). We conclude from these results that the low levels of TTf are associated with the loss of the integrity of the SM affecting its functionality while not with the integrity of DNA. The possible role of TTf as an antioxidant protein and its stabilizing function of the sperm membrane is postulated. It is necessary to analyze a great number of samples, to incorporate other techniques for the study of DNA and to study other functional parameters of semen and its relation with TTf.

Keywords: Transferrin, Seminal plasma, Radial Immunodifussion

(782) **SPERMATOGENESIS ONSET: ROLE OF ANDROGENS IN THE REGULATION OF *ALDH1A1* IN SERTOLI CELLS**

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The onset of spermatogenesis is androgen-dependant. Because germ cells do not express the androgen receptor (AR), Sertoli cells are believed to transduce testosterone (T) signals to them. This can only happen once AR expression in Sertoli cells begins around day 4. Expression of *Aldh1a1*, a retinoic acid (RA) synthesizing enzyme, is detectable by at least day 5 in Sertoli cells. RA induces expression of *Stra8*, which is associated with spermatogonial differentiation and

meiotic onset. In the *Aldh1a1*-null mouse testis, *Stra8* expression during the first wave of spermatogenesis is delayed. We hypothesise that androgens trigger the first wave of spermatogenesis by inducing the expression of ALDH1A1 in Sertoli cells of the pre-pubertal testis.

TM4 cells were treated with T (10^{-8} M) or vehicle and *Aldh1a1* expression was evaluated by qRT-PCR (**ΔΔ**Ct method, one sample t-test against 100% control, n=3 to 6). *Aldh1a1* was upregulated by a 1 h (*P*=0.013), 2 h (*P*=0.009), 4 h (*P*=0.018) or 8 h (*P*=0.036) treatment and was expressed at four-fold baseline levels (*P*=0.031) after 24 h. Treatment with the antiandrogen Bicalutamide reversed this upregulation. Bioinformatic analysis showed two putative androgen response elements upstream of the transcription start site and two within intron 1 of the *Aldh1a1* gene. Preliminary immunofluorescence results from pre-pubertal testis of RARE-*lacZ* reporter mice show co-localization of STRA8 and β-galactosidase (evidence for RA action) and weak expression of the AR in some Sertoli cell nuclei starting at 2 days. We conclude that androgens induce upregulation of *Aldh1a1* acting through the AR and that *Aldh1a1* upregulation might occur by both genomic and non-genomic mechanisms. Thus, testosterone may be important not just for the maintenance but also for the onset of spermatogenesis, acting via upregulation of ALDH1A1 in Sertoli cells.

Keywords: Aldh1a1, Sertoli cell, androgens, spermatogenesis

(1186) **POTENCIAL PARTICIPATION OF PPARγ IN FSH REGULATION OF FATTY ACID STORAGE IN SERTOLI CELLS**

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Abstract: Sertoli cells (SC) are necessary to provide an adequate and protected environment for germ cell development. Studies of SC glucose metabolism have shown that SC actively metabolize glucose but that the vast majority is converted to lactate that represents the preferential energy substrate for germ cells. In this metabolic scenario, fatty acids (FA) have been demonstrated to be needed to sustain SC energy status. The presence of numerous lipid droplets (LD) is characteristic in SC and it is assumed that they constitute the storage of these FA. In this context, we have demonstrated that FSH –the master hormone in the regulation of SC physiology– regulates LD content and the expression of the fatty acid transporter FAT/CD36 and of proteins that regulates LD formation and stability, PLIN1 and 2. On the other hand, we have also shown that pharmacological activation of PPARγ participates in the regulation of lipid storage in this cell type, although the participation of this transcription factor in hormonal regulation has not been analyzed yet in SC. The aim of this study was to analyze the potencial participation of PPARγ in FSH regulation of the expression of proteins involved in FA transport and storage. SC from 20-day old rats were used. Cultures were treated with FSH (100 ng/ml) in presence or absence of a PPARγ antagonist (T007; 50 μM). Results are expressed as mean±SD, n=3, different letters indicate statistically significant differences, p<0.05. PPARγ antagonist inhibited FSH stimulation of FAT/CD36 and PLIN1 expression (FAT: FSH: 3.2±02a; FSH+T007: 0.7±0.3b; PLIN1: FSH: 3.3±0.3a; FSH+T007: 0.3±0.2b fold-variation vs basal) and did not modify FSH-stimulated PLIN2 mRNA levels. These results suggest that in order to ensure the availability of FA for SC energetic requirements, FSH regulates lipid storage, at least in part, through PPARγ activation.

Keywords: Sertoli, Lipid Storage, FSH, PPARγ.

(570) **BENEFITS OF MELATONIN SUPPLEMENTATION IN TESTES OF INFERTILE PATIENTS**

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Abstract: Melatonin (mel) regulates testicular function acting indirectly through the hypothalamus/pituitary but also directly at the gonads. We previously quantified mel concentrations in testicular biopsies of patients suffering from hypospermatogenesis (H) or Sertoli cell only (SCO) syndrome. We also found that mel testicular concentrations negatively correlate with the number of local macrophages (MAC) and the gonadal expression of TNFα and IL1β.

This study attempted to evaluate the impact of oral mel supplementation in infertile patients. Men presenting idiopathic infertility and non-obstructive azoospermia without an infection process were assessed and diagnosed by open testicular biopsy. Biopsies were classified according to McLachlan et al. (*Human Reprod.* 2007). From medical records data we identified a group of patients that had been treated for sleeping disorders with a daily oral dose of 5 mg of mel.

In biopsies with H and SCO, testicular mel concentrations were markedly diminished while the numbers of testicular CD68–immunoreactive MACs and toluidine blue positive mast cells (MCs) were significantly increased compared with normal biopsies (p<0.05).

Mel consumption increased its own testicular levels (p<0.05) but did not affect the amount of gonadal MCs. However, the number of total (interstitial + tubular) CD68–immunoreactive MACs in patients taking mel was significantly lower than those detected in infertile patients and similar to those determined in normal biopsies (Total testicular MACs/mm^2, normal: 77,8±9,63^a, SCO: 473,4±97,0^b, SCO+mel: 137,8±33,3^{a,d}, H: 178,6±11,1^{c,d}, H+mel: 111,2±45,7^a; Media±SEM; p<0.05). In addition, TNFα and IL1β mRNA expression significantly increased in H and SCO compared to normal biopsies (p<0.05). On the other hand, mel intake in infertile men brings TNFα and IL1β mRNA expression closer to the normal range.

These results reveal that in infertile patients, mel intake is related to a *low*-grade testicular *inflammatory state.*

Keywords: melatonin, testis, infertility, inflammation

(1054) **THE NON-CLASSICAL ANDROGEN SIGNALING PATHWAY IS INVOLVED IN THE REGULATION OF *AMH* AND *CYP26B1* IN SMAT1 PRE-PUBERTAL SERTOLI CELLS**

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Testosterone acts through the androgen receptor (AR) in Sertoli cells to induce their maturation and support spermatogenesis, but the molecular and cellular mechanisms are still poorly understood. We have previously demonstrated that, in the pre-pubertal Sertoli cell line SMAT1, androgens exert a direct effect through the AR on two genes associated with Sertoli cell maturation: *Amh* - by inhibiting its expression – and *Cyp26b1* – by upregulating its expression - (Edelsztein *et al.*, SAIC 2015, 2016). Since the AR can act through classical (genomic) or non-classical (non-genomic) pathways, we sought to elucidate whether the effect of androgens on *Amh* and *Cyp26b1* gene expression occur through the classi-

cal, the non-classical or both pathways in Sertoli cells. SMAT1 cells were co-transfected with expression vectors for mutant AR proteins that selectively activate either the classical (ARΔ372-385) or the non-classical (ARC562G) testosterone signaling pathway together with *Amh or Cyp26b1* promoter luciferase constructs. SMAT1 cells were incubated with DHT (10^{-7}M) or vehicle for 24 h. Results, expressed as percentage (mean±SEM), are compared against basal activity (theoretical value: 100%) using a one sample t-test. *Amh* expression was inhibited when the ARC562G construct was present (62.9±7.864, *P*=0.042, n=3) but not after transfection of ARΔ372-385 (82.58±11.63, *P*=0.273, n=3). *Cyp26b1* expression was upregulated by the ARC562G construct (224.2±18.59, *P*=0.001, n=6) but not by ARΔ372-385 (103.6±7.451, *P*=0.647, n=6). We conclude that the non-classical pathway participates in the regulation of both *Amh* and *Cyp26b1* in SMAT1 cells, and could be involved in the maturation of Sertoli cells around the onset of puberty.

Keywords: Amh, *Cyp26b1*, Sertoli cell, androgens, non-classical pathway

ENDOCRINOLOGY 1

(335) ANTI-ADIPOGENIC EFFECT OF THE ANTIOXIDANT N-ACETYLCYSTEINE ON 3T3-L1 ADIPOCYTES

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Reports about antioxidant effect on obesity are controversial. We showed that N-acetylcysteine (NAC) inhibits cellular lipid accumulation during 3T3-L1 adipocyte differentiation, through the inhibition of adipogenic transcription factors expression, such as PPARγ and, MAPK phosphorylation (Soto el al, Redox Rep 2016). Objectives: Here, we evaluated NAC effect on fully differentiated 3T3-L1 adipocytes (AC) and developed nanoparticles with NAC. Methods: Assays were conducted using AC, different doses of NAC (0.01 to 5 mM) were added to culture media for 5 days and, we determined: cellular content of triglycerides (Tg) and Oil-Red-O stained lipids (ORO), NAC toxicity by MTT assay, PPARγ protein expression by western blot assay, mRNA levels of PPARγ and lipid protein perilipin (Pl) by RT-qPCR analysis. NAC content in nanoparticles was determined by Raggi method. Results: Treatments with 0.01 to 5 mM NAC were not toxic on AC. 5mM NAC treatment on AC (ACN) elicited a 60% decrease in cellular Tg content (1.22±0.09 gTg/g protein [AC] vs 0.49±0.03 gTg/g protein [ACN], p<0.05). We evaluated ORO content in ACN compared to AC, which is set to 100 (100±4 [AC] vs 80±2 [ACN] arbitrary units (AU), p< 0.05), mRNA levels of Pl (Pl/ Rplp0:100±13 [AC] vs 72±9 [ACN] AU, p< 0.05) and, expression of PPARγ protein (PPARγ/GAPDH:100±6 [AC] vs 70±4 [ACN] AU; p< 0.05) and mRNA (PPARγ/Rplp0:100±7 [AC] vs 74±14 [ACN] AU, p<0.05). We developed silica hollow nanoparticles (100nm diameter, Nano) including 5mM NAC, as a better tool for NAC administration. We evaluated 5 different Nano preparations; our results showed a maximum of 13.2% incorporation of the total NAC. Conclusions: 5 mM NAC treatment on AC produced an effectively decrease in Tg content, and 20-30% decrease in ORO, Pl and PPARγ cellular contents. These results suggest that NAC could inhibit new lipid production in adipocytes. We also developed nanoparticles with NAC to use on AC.

Key words: adipocytes, antioxidant, obesity

(1588) ARTIFICIAL PANCREAS: RESULTS OF THE FIRST CLINICAL TRIAL IN LATIN AMERICA WITH AN ARGENTINEAN ALGORITHM

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An Artificial Pancreas is a system by which a Continuous Subcutaneous Insulin Infusion (CSII) and a continuous glucose monitoring (CGM) are connected by bluetooth to a smartphone in which a control algorithm is programmed. This software receives glucose values and decides the insulin infusion rate to maintain normoglycaemia, creating a closed loop (CL) system.

The first algorithm developed in Latin America -ARG Algorithm- was tested in 5 adult patients with T1DM. ARG only required that meals were announced to the system but did not need to be informed of carbohydrates quantity.The Primary Outcome was to determine the percentage of time in which the system was connected and operating adequately. Secondary outcome measures were the percentage of time in which patients achieved desirable glycemia (70-180 mg/dl) and acceptable glycemia (180-250 mg/dl), as well as number of nocturnal or severe hypoglycemia.

Patients completed a 3 days run in period in which they used a sensor-augmented insulin pump in their everyday life. In order to test the CL system, patients were admitted for 36 hours at the Hospital Italiano de Buenos Aires, in which the first 21 hours were dedicated to calibrate the system and in the remaining 15 hours the system automatically regulated blood glucose.

All 5 patients remained in CL in adequate operation for 99.6% for 15 hours. The mean percentages of time in CL with glycaemia between 70-250 mg/dl was 94.66% [83.81, 98.38]; between 70-180 mg/dl was 82.55% [69.88, 95.22]; <70 mg/dl was 4.11% [0.83, 17.95]; and <50 mg/dl was 0.22% [0.01, 3.47], all with IC 95%. One mild nocturnal hypoglycemia occurred. No severe hypoglycemia occurred. No serious adverse events were reported.

It is peremptory to empower patients with chronic diseases. Developing algorithms such as the ARG algorithm, with promising initial results, contributes to this aim. However, further trials are needed to establish the safety and efficacy of the ARG algorithm.

Key words: #ArtificialPancreas; #Algorithm; #Sensor-augmented; #Diabetes; #CSII

(689) CHRONIC ENDOGENOUS GLUCOCORTICOID EXCESS AND WHITE ADIPOSE TISSUE BROWNING

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(1) CENEXA. (2) IMBICE.

Glucocorticoids (GC) have different effects on white adipose tissue (WAT) and inhibit brown adipose tissue (BAT) thermogenesis; however, whether GC affects browning or not remains unclear. Our aim was to study the effect of chronic endogenous GC excess on browning activity of epididymal WAT (eWAT) stromal vascular fraction cells (SVFs) from 90 day-old, neonatally-treated, monosodium L-glutamate (MSG) male S-D rats. MSG and litter-mate controls (CTR) eWAT pads were dissected and processed for histological appearance and mRNA (qPCR) analysis. Then, isolated eWAT SVFs were processed for gene expression analysis or cultured up to reach confluence. Confluent cells were 3 day-differentiated with a classical pro-browning cocktail. On differentiation day 8, cells were stimulated with 10μM forskolin (4 h) or not, and cell UCP1 mRNA levels were quantified. Body weight and food intake were lower (p<0.05) in MSG rats, although eWAT mass was higher (p<0.05) and contained larger (p<0.01) adipocytes. Insulin, corticosterone (B), leptin and triglyceride plasma levels were high (p<0.01) in MSG rats. Remarkably, MSG eWAT pads showed low (p<0.05) mineralocorticoid and glucocorticoid receptor (MR and GR, respectively) gene expression levels. Interestingly, beige adipocyte markers (Cidea and PRDM16) were noticed in MSG eWAT and SVFs only. Differentiated MSG SVFs showed higher (p<0.01 vs. CTR) UCP1 expression levels before been stimulated (basal), although once stimulated with forskolin

both cells groups developed a significant ($p<0.05$ vs. basal values) increase in UCP1 mRNA levels, although being both similar among them. Altogether our data are highly indicative for a GC-resistance developed by MSG rats (decreased eWAT, MR/GR mRNAs and high plasma B levels). We conclude that the beige adipocyte lineage could be favored due to the lack of a full GC inhibitory effect on MSG SVFs. (FPREDM052015; PICT-2013-0930)

Keywords: epididymal adipose tissue, endogenous glucocorticoids, WAT browning.

(1715) **DIABETIC ENVIRONMENT INFLUENCES THE BASAL CONDITION OF DENTAL PULP PROGENITOR CELLS**

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Our objective was to characterize the basal state of the progenitor cells from dental pulp (DPPC) of control rats (C) and with diabetes mellitus (D). Adult Wistar rats injected with streptozotocin and nicotinamide (model of diabetes with partial insulin deficiency) or untreated (control) were used. The DPPCs were extracted from the dental pulp of the lower incisors of both experimental groups and were maintained in DMEM-10% fetal bovine serum at 37 °C and 5% CO_2. Once the cells reached semi-confluence, proliferation capacity (violet crystal bioassay), actin cytoskeleton distribution (Alexa fluor-phalloidin) and expression of osteogenic (Runx2), adipogenic transcription factors ($PPAR\gamma$) as well as inflammation markers (HMGB1, IL1) and the receptor RAGE by RT-PCR were evaluated. DPPC-C proliferated linearly during one week of culture, while DPPC-D showed a significant decrease in the ability to proliferate at the end of that period ($p<0.05$). On the other hand, the DPPC-D showed a reorganization of the actin filaments with thicker and isolated fibers, compared to the actin network of the DPPC-C. In addition, DPPC-D expressed approximately 16 times more $PPAR\gamma$ than DDPC-C, which in turn expressed 14 times more Runx 2 than DPPC-D. This finding was accompanied by an increase in the expression of inflammatory markers such as HMGB1, IL1 and the receptor RAGE (15, 6 and 10 times respectively) in DPPC-D. According to our results, the DPPC-D have a marked inclination to the adipocytic phenotype and reflect the inflammatory condition associated with diabetes.

We conclude that the dental pulp represents an accessible and interesting source of adult mesenchymal stem cells, in which a diabetic environment can modulate its proliferative capacity as well as its phenotypic expression.

Keywords: Dental pulp progenitor cell, Diabetes mellitus, proliferation, inflammatory markers

(1494) **EFFECTS OF MELATONIN TREATMENT ON INFLAMMATORY MARKERS AND PITUITARY DYSFUNCTION IN INSULIN RESISTANT RATS**

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Excessive consumption of diets with high sugar content has been associated with an increased incidence of insulin resistance and obesity. Several reports have shown a concomitant dysfunction of the hypothalamus pituitary adrenal (HPA) axis in these patients. We have previously reported that rats fed a sucrose rich diet (SRD, 30% sucrose in the drinking water) for 7 weeks show a significant decrease in insulin sensitivity. Analysis of HPA axis activity after 3 weeks of treatment, when changes in insulin sensitivity were still not evident, showed augmented levels of circulating ACTH and a higher pituitary expression of POMC that was accompanied by an increase in oxidative stress parameters.

Present experiments were designed to analyze the involvement of inflammation-related effects on the observed pituitary dysfunction. Animals were randomly distributed in 4 groups: 1) Control, 2) SRD, 3) Mel and 4) SRD + Mel. In groups 3 and 4, subcutaneous melatonin implants (10mg) were surgically implanted. Animals were sacrificed after 3 weeks and pituitary glands were processed to obtain protein and RNA.

Results showed an increase in mRNA levels of TNFα ($p<0.001$) and inflammasome components (ASC, NALP3, IL-1beta, $p<0.01$ *vs.* C) in the SRD group that was prevented by melatonin treatment. In addition, immunohistochemistry analysis of pituitary tissues indicated an increase in both ACTH and Iba1 positive signals in the SRD-group that were attenuated in the SRD-Mel group. Western blot studies also showed higher levels of the macrophage marker F4-80 in the SRD-group. In vitro studies, in the corticotroph AtT20 cell line, showed the induction of POMC by conditioned media obtained from peritoneal macrophages or from J774 cells stimulated with LPS.

In summary, our results suggest that changes in the synthesis and secretion of ACTH detected in SRD-treated rats could be involved the activation of inflammation-related pathways.

Key words: Corticotroph, ACTH, inflammation, macrophage, melatonin.

(572) **IMT504 INHIBITS INSULITIS AND LOWERS BLOOD GLUCOSE IN NOD/Ltj FEMALE MICE IN A SHORT-TERM TREATMENT**

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Immunomodulatory oligonucleotide IMT504 (IMT) induced a marked recovery of glycemia, glucose clearence, insulin secretion and beta cell function (by HOMA beta cell index) on a spontaneous autoimmune diabetes model (in male and female NOD/Ltj mice).

We analyzed the minimun dose at which the IMT has an early effect on glycemic control and insulitis. Diabetic female NOD/LtJ mice (two consecutive non-fasted glycemia (Gly) levels ≥ 250 mg/dl) were treated with a daily dose of IMT for five days (IMT: 2, 6 or 20 mg/kg/day, IMT2, IMT6 and IMT20) or saline as control (DC). Mice were sacrificed the day following the last injection. In fasted conditions, blood samples and pancreases were obtained for Gly and insulinemia measurment and for histological studies respectively.

We observed that 12.5% (1/8) DC mice showed spontaneous reversion of diabetic condition, whereas IMT improved Gly in 62.5% (5/8), 50% (4/8) and 75% (6/8) of mice treated with 2, 6 and 20 mg/kg/day, respectively (X^2: DC vs IMT20: $p<0.025$).

Gly did not vary with time (day 6 vs day 1) in DC mice while it significantly diminished with IMT treatment [(mg/dl):interaction, $p<0.01$, DC: Day 1: 330 ± 71 vs Day 6: 333.± 118, IMT2: Day 1: 303 ± 49 vs Day 6: 233 ± 57, IMT6: Day 1: 283 ± 22 vs Day 6: 194 ± 44, IMT20: Day 1: 313 ± 42 vs Day 6: 147 ± 42, IMT20: Day 1 vs Day 6: $p<0.02$]. Body weights did not differ among groups. Fasted glycemia also diminshed significantly with IMT20 treatment (DC vs IMT20: $p<0.04$). Since IMT20 showed the most effective response, we analyzed insulitis in this group. Treated mice showed a marked reduction in leukocyte islet infiltration (insulitis index: DC: 0.66 ± 0.05 vs IMT20: 0.48 ± 0.05, $p<0.05$).

Taken together, these results demonstrate that IMT20 treatment promotes an early, significant improvement in the diabetic condition in NOD/Ltj mice warranting further investigation of its mechanism of action.

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KEY WORDS: DIABETES,INSULITIS,GYCEMIA, OLIGONUCLEOTIDE

(264) **NARINGIN PREVENTS CHANGES IN THE MITOCHONDRIAL PHYSIOLOGY AND MORPHOLOGY OF KIDNEY IN THE EXPERIMENTAL DIABETES MELLITUS**

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We have previously demonstrated that the Diabetes mellitus (Dm.) inhibits the intestinal Ca^{2+} absorption, which was accompanied by oxidative stress. Other studies in diabetic rats have shown

enhanced glomerular filtration rate with raised urinary output and reduced Ca2+ reabsorption. Alteration of the redox state was observed in kidney mitochondria, with reduced ATP synthesis, changes in calcium homeostasis and increased biogenesis. The objective of this study was to determine if naringin (NAR), a natural antioxidant, could block the alterations in the renal morphology, mitochondrial redox state and energy metabolism from kidney in diabetic rats. Adult male Wistar rats were divided in: 1) controls, 2) streptozotocin (STZ) treated (diabetic rats) and 3) STZ + NAR treated. Mitochondria were isolated from renal tissue from each group of animals by differential centrifugation. In the mitochondrial extracts the activities of the enzymes isocitrate dehydrogenase (ICDH-NAD), malate dehydrogenase (MDH-NAD), superoxide dismutase (SOD), catalase (CAT), glutathione (GSH) and superoxide anion levels (˙O2-) were determined by spectrophotometry. Kidney morphology was analyzed by histological studies. Results were assessed by one-way ANOVA and Bonferroni multiple comparison test. The STZ treatment decreased the enzymatic activities of ICDH and MDH and the GSH content, which were avoided by NAR. STZ increased the SOD and CAT activities and the contents of ˙O2- and protein carbonyl. All these effects were blocked by NAR, except CAT activity. In addition, diabetic rats showed disorganized glomeruli, which were smaller than those from controls. The epithelial cells from distal convoluted tubules were also decreased by STZ. All these morphological changes were avoided by NAR. In conclusion, NAR abrogates morphological alterations in the diabetic kidney and changes in the energy metabolism from the renal mitochondria improving the redox state of these organelles.

Keywords: kidney, Diabetes mellitus, redox state

(029) **NARINGIN: AN ANTIOXIDANT THAT IMPROVES HEPATIC DAMAGE PRODUCED BY EXPERIMENTAL DIABETES MELLITUS**

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Hepatic injury is a major complication of Type 1 Diabetes mellitus (DM1). Since naringin (NA) is a flavonoid with antioxidant properties, it might reduce hepatic complications in DM1. The aim of this study was to know the effect of NA on hepatic oxidative/nitrosative stress and apoptosis in a model of DM1. Two-month male Wistar rats were used: a) controls, b) diabetic rats (induced by 60 mg/kg b.w. streptozotocin: STZ), and c) STZ+NA: diabetic rats treated with NA (40 mg/kg b.w.). Animals were sacrificed at 30 days post-treatment, and serum glucose, HbA_{1c}, insulin, triglycerides (TG), GOT and GPT were determined. Liver slices were stained with H&E for morphometric analyses. Total glutathione (GSH) content, superoxide anion ($^{\cdot}O_2^-$) levels and superoxide dismutase (SOD) and catalase (CAT) activities from rat liver were measured by spectrophotometry. Nitrosative stress was evaluated by nitric oxide (NO) and nitrotyrosine content and inducible nitric oxide synthase (iNOS) protein expression. Apoptosis was evaluated by counting apoptotic nuclei and Fas, FasL and Bax protein expression. Results were evaluated by ANOVA and Bonferroni test. STZ rats presented higher levels of glycemia, HbA_{1c}, TG, GOT and GPT, and lower insulin levels in relation to those from controls. Although NA treatment did not produce changes in glycemia, HbA_{1c} and insulin, it decreased the levels of TG and transaminases. The cytoplasmic and nuclear areas of the hepatocytes in the STZ rats were higher. STZ rats showed increased apoptotic nuclei and protein expression of molecules involved in apoptosis. STZ rats exhibited GSH depletion and increased $^{\cdot}O_2^-$ content and SOD and CAT activities. NA treatment normalized these parameters. NO content and iNOS protein expression were higher in STZ rats compared to controls, which were prevented by NA. In conclusion, NA would have the ability to prevent chronic liver injury triggered by DM1 due to its antioxidant, anti-nitrosative and antiapoptotic properties.

Keywords: Diabetes mellitus, liver, naringin

(153) **TYPE 2 DIABETES MELLITUS (DM2) INDUCED BY HIGH-FAT DIET (HFD) IN MICE ARE SENSITIZED TO DEN-INDUCED HEPATIC CARCINOGENESIS**

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DM2 is the most common form of DM characterized by hyperglycemia, insulin resistance, and relative insulin deficiency. DM2 results from interaction between genetic, environmental and behavioral risk factors. Epidemiological studies indicate that DM2 is associated with increased risk of developing cancer. We study the liver sensitivity of DM2 (induced by HFD) to development of hepatocellular carcinoma (HCC) Therefore we have used the carcinogenic agent diethylnitrosamine (DEN), analyzing the proliferative process as early stage of hepatic carcinogenesis. Five-week-old mice C57BL/6 were randomly divided into 4 experimental groups: Control (C, mice were fed a normal chow diet), C treated with DEN (C+DEN), HFD mice that fed a high-fat diet and HFD+DEN animals that fed a high-fat diet and received DEN at 16 weeks (n=4 in each group). Mice were euthanized at 25 weeks after DEN-injection. In liver sections we performed histological studies and quantification of hepatocytes in the different phases of the cell cycle (immunohistochemistry of PCNA). HFD+DEN showed an increase in hepatocytes in phases G1, S and M as well as proliferative index (PI) compared to C ($p<0.05$). HFD+DEN showed an increase in hepatocytes in phase G1 (+138%$^{\#}$), in phase S (+123%$^{\#}$), in phase M (+510%$^{\#}$) and PI (hepatocytes in phase G1, S, G2 and M, +173%$^{\#}$) ($^{\#}p<0.05$ vs C+DEN) which would indicate a promotion in the cell cycle with an increase in phase M.These results are in coincidence with the increase observed in the expression of markers of cell cycle progression: cyc E1 (+25%$^{\#}$), cyc D1 (+51%$^{\#}$) and cyc G1 (+52%$^{\#}$) showing a cell cycle progression toward phase M. Vascular endothelial growth factor (VEGF), signaling protein produced by cells that stimulates angiogenesis, showed an increase in HFD+DEN (+76%$^{\#}$). These results suggest that at 25 weeks after DEN administration, cell environment is modified and allowed as to analyze proteins that increase sensitivity cancer develop in the liver diabetic state.

Keywords: Diabetes Mellitus type 2, Diethylnitrosamine, Hepatocarcinoma, Proliferation, Cell cycle.

(541) **BOTH INDUCED HYPERURICAEMIA AND HIGH FRUCTOSE DIET CONDITIONS INDUCE CARDIAC MORPHOLOGICAL CHANGES ASSOCIATED TO HYPERTENSION IN MALE RATS.**

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We have demonstrated that high-fructose diet intake and induced hyperuricemia increase Systolic blood pressure (SBP) separately or together. Objective: to evaluate the effect of these metabolic conditions on cardiac morphological changes. Material and Methods: Adult male Wistar rats (*n*=7/group), were divided into four groups receiving during 5 weeks: a) Control (C): standard commercial diet and water, b) Fructose (F): control diet plus 10% fructose in the drinking water, c) Oxonic acid (OA): control diet and water plus the uricase inhibitor OA (750 mg/kg/d), d) Fructose and Oxonic Acid (FOA): control diet with 10% (w/v) fructose in the drinking water plus oxonic acid. Plasmatic creatinine, uric acid and total-cholesterol (TC), triglycerides (TG), HDL-cholesterol (HDL) and TG/HDL were measured. Myocyte morphometric analysis (H&E and PAS), was done measure cardiomyocyte volume, calculated from individual myocyte area (length (µm) x width (µm) x 7.59 x 10-3), and the study of fibrosis (Masson's trichrome). Statistical analysis by two-way ANOVA and Bonferroni post test was performed (significant $p<0.05$). Results: Plasmatic uric acid levels were significantly higher in animals treated with OA (1.28±0.14, $p<0.05$) and FOA (1.49±0.1,

p<0.01) when comparing to the control (0.97±0.04). There were no differences in all lipid profile between different experimental groups. Myocyte size was higher in FOA (59.37± 4.68 pL, p<0.001), followed by OA (52.86±3.10pL, p<0.001) and F group (42.18± 2.00 pL, p<0.05) vs C (30.15±1.47 pL) group. The percentage of cardiac fibrosis was greater in FOA (3.43±0.14 p<0,009) and in F (3.25±0.14 p<0.03) vs control (2.69 ±0.196) groups.

Conclusion: Hyperuricemia and high-fructose diet conditions, in addition to increase SBP, cause morphological changes in the cardiomyocyte translated as cellular volume and fibrosis increase, being these effects more relevant in animals with both conditions simultaneously (FOA).

Key Words: hyperuricaemia, metabolic syndrome, rats, cardiac morphology, fructose diet

NEPHROLOGY 1

(1505) AQUAPORIN-2 IN THE ADENINE-INDUCED CHRONIC KIDNEY DISEASE MODEL

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Chronic kidney disease (CKD) is one of the leading sanitary problems in Argentine. Recently, vasopressin (VP), the main regulator of Aquaporin-2 (AQP2) expression, was suggested as a contributor to the development of CKD. However, the mechanisms of VP contribution remains unknown.

The aim of this work was to characterize a new model of CKD and evaluate AQP2 expression in renal medulla.

Materials and methods: Male SD rats were treated for 2 weeks with an adenine rich diet (ERC group n=4; 0.25% adenine in standard rat chow powder). Control group received standard rat chow powder (C group n=4).

After 2 weeks, animals were kept in metabolic cages to collect 24 h urine. Blood samples were obtained, the kidneys were removed and weighed and processed for further determinations.

Determinations: Plasmatic (P) and urinary (U) urea, P and U creatinine, diuresis, AQP2 protein expression by WB and immunohistochemistry and AQP2 mRNA expression by real time PCR.

Results were expressed as media ± SEM and T-test was performed.

Renal weight/100g bw.: C: 0.35 ± 0.01 vs CKD: 0.45 ± 0.02 g (p< 0,005); P urea (mg/dl): C: 77.82 ± 1.49 vs. CKD: 110.50 ± 9.73 (p<0,05); U urea (g/L): C: 41.90 ± 1.53 vs CKD: 60.10 ± 4.73 (p<0,05); P creatinine (mg/dl): C: 0.57 ± 0.01 vs CKD: 0.89 ± 0.05 (p< 0,005); U creatinine (g/24h): C: 0.017 ± 0.001 vs CKD: 0.019 ± 0.015 (ns); creatinine clearance (ml/min): C: 2.06 ± 0.18 vs CKD: 1.50 ± 0.05 (p<0.05); Diuresis (ml/24 hs): C: 10.8 ± 3.2 vs CKD: 14.3 ± 3.8 (ns); AQP2 expression (WB): C: 1.00 ± 0.18 vs CKD: 1.74 ± 0.18 (p<0.05); mRNA AQP2 expression (fold change from C): C: 1.11 ± 0.29 vs CKD: 1.14 ± 0.22 (ns).

Adenine administration for two weeks resulted in an adequate model for CKD showing increased levels of plasmatic urea and creatinine. AQP2 expression (evaluated by WB and Immunohistochemistry) significantly increased in the renal medulla, suggesting that this could be at least one of the mechanisms by which VP is involved in the development of CKD.

Key words: chronic kidney disease, adenine, AQP2, vasopressin

(1126) CHRONIC NEPHROPATHY IN RATS SURVIVING HEMOLYTIC UREMIC SYNDROME

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Hemolytic uremic syndrome (HUS) is a thrombotic microangiopathy associated with Shiga toxin-producing E. *coli* (STEC). Its clinical features are hemolytic anemia, thrombocytopenia and renal failure. HUS is the most frequent cause of renal failure in children. After the acute episode 25% of children suffer from some degree of reduced renal function. Our aim is to characterize the physiopathological factors related with evolution of renal injury to chronicity in a HUS model in rats.

Adult male Sprague-Dawley rats (200g) were randomly divided into two groups and injected intraperitoneally with culture supernatant of recombinant E.*coli* DH5α, either expressing Stx2 (experimental group) or not expressing Stx2 (control group). In the experimental group, half of the animals received enalapril (50mg/kg) in drinking water while other half received no medication. Rats were sacrificed at 1 week and 12 weeks. Functional, histological, immune histochemical and molecular studies were performed in all groups.

The functional studies showed a decreased GFR at 1 week with no significant variation at 12 weeks while proteinuria and microalbuminuria are increased at 12 weeks. At this time we also found significant cortex and medullar fibrosis. These histological findings correlate with immunohistochemically stain of TGF-β.

In order to check the presence of the toxin in renal tissues, we performed an immunohistochemistry study and we observed the expression of Stx2 at 1 week and 12 weeks. At last time the expression was strong at medullar level. We detected the presence of bacterial Phage on kidney tissue with same pattern as the toxin, fibrosis and TGF- β expression.

Our results suggest the possibility of the internalization of phage DNA in eukaryotic cells which is capable to produce more toxin and could maintain the lesional renal mechanism to chronicity

Key words: Hemolytic Uremic Syndrome, Shiga Toxin, Phage, Chronic nephropathy

(1858) EVALUATION OF A LOW DOSE OF ANGIOTENSIN II INFUSION AS REGULATOR OF RENAL DOPAMINERGIC SYSTEM THROUGH ORGANIC CATION TRANSPORTERS

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The aim of this study was to evaluate the effects of a low dose of angiotensin II (ANG II: 1 ug/kg/hour) on renal dopaminergis system (RDS) through determination of the organic cation transporters (OCTs and OCTNs) expression in renal cortex, dopamine (DA) urinary levels, Na+,K+–ATPasa (NKA) activity and renal function. Under inhibition of DA synthesis by benserazide, anesthetized male Sprague Dawley rats were infused with isotonic saline solution during 120 minutes and randomized in nine different groups: control, pargyline plus tolcapone (P+T), ANG II, DA, ANG II+DA, D-22, ANG II+D-22, DA+D-22 and ANG II+DA+D-22. Renal functional parameters and direct blood pressure were determined and urinary DA concentration was quantified by HPLC. Expression of OCTs, OCTNs, D1-subtype receptor (D1R) and NKA in membrane preparations from renal cortex tissues were determined by Western Blot. NKA activity was determined using in vitro enzyme assay. Compared to P+T group, DA infusion significantly increased fractional excretion of sodium (FENa) and DA excretion (*: p<0,05). These effects were not

observed with ANG II infusion except in ANG II+DA group versus DA group for FENa values after 120 minutes of infusion (*). OCTs blockade by D-22, reversed DA-dependent natriuresis demonstrating that OCTs are implied in DA uptake and transport in renal tissues. The activity of NKA was greater in ANG II groups versus respective animals without ANG II (*). OCTs, D1R, NKA and phosphorilated NKA protein expression were not modified by ANG II infusion. Urinary DA concentration was not altered in ANG II+DA rats versus DA alone group. This result is consistent with the fact that total OCTNs expression was not affected by ANG II, despite obtaining significant augmentation (*) of OCTN-1 expression versus P+T rats. To conclude, ANG II modifies the activity of NKA, the final effector of DA-D1R pathway, without affecting RDS.

Keywords: angiotensin II, organic cation transporters, renal cortex, dopamine, Na^+,K^+–ATPasa

(1243) **FOCAL ADHESION ASSEMBLY/DISASSEMBLY AND TRPV4 PARTICIPATION IN AQP2-DEPENDENT RENAL CELL MIGRATION**

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We have previously demonstrated that Aquaporin 2 (AQP2) promotes renal cell migration. It is well known that migration is a process that requires continuous turnover of focal adhesions (FAs) and it was shown that AQP2 interacts with proteins forming FAs, so the aim of the present work was to study the dynamics of FAs in renal cells expressing AQP2. Moreover, since Ca^{2+} signaling is implicated in FAs turnover and results from our laboratory showed a differential activation of the Ca^{2+} channel TRPV4 influenced by AQP2, we also investigated if TRPV4 participates in the AQP2-enhanced renal cell migration. For experimental procedures two renal cell lines were used: WT-$RCCD_1$ (not expressing AQPs) and AQP2-$RCCD_1$ (transfected with AQP2). Immunofluorescence studies with paxillin were performed in scratched monolayers to visualize FAs. Images were taken with a confocal microscope, deconvolved and processed for making FAs area measurements. Cell migration in presence of TRPV4 agonists 4α-PDD (10 μM) and GSK1016790A (3 nM) was investigated with Wound Healing assay. Images were taken at different times and results were expressed as % of wound closure. Immunofluorescence studies showed punctuated paxillin labeling indicating FAs in the leading edge of migrating $RCCD_1$ cells. These FAs were found to be small in AQP2-$RCCD_1$ (1.80±0.19 mm^2, n=85) than in WT-$RCCD_1$ (5.87±1.04 mm^2, n=120, $p<0.05$). Wounding assay with TRPV4 agonists showed that the % of wound closure was diminished in AQP2-expressing cells (4α-PDD: n=15, $p<0.001$ and GSK: n=14, $p<0.001$). In summary, since actively migrating cells typically exhibit nascent small FAs and, conversely, larger FAs indicate more resistive forces to migration, our results could provide an explanation for the increased migration observed in AQP2-$RCCD_1$, revealing a rapid assembly/disassembly of FAs. On the other side, results about TRPV4 activation wouldn't be indicating, at first glance, its involvement in the AQP2-enhanced renal cell migration.

Keywords: AQP2, renal cell migration, focal adhesions, TRPV4

(1014) **IN VITRO STUDIES OF THE RENAL EXPRESSION OF CAVEOLIN 1 AND 2 IN EXTRAHEPATIC CHOLESTASIS.**

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Caveolins, Cav1 and Cav2, are integral membrane proteins present in caveolae, a subtype of lipid raft, flask-shaped and enriched of cholesterol and sphingolipids. Caveolins participate in diverse biological processes, such as signal transduction and vesicular trafficking. In addition, they act as regulators of a wide variety of proteins. So far, little is known about renal expression of Cav1 and Cav2 under pathological conditions, particularly in obstructive cholestasis. The aim of this work was to perform *in vitro* studies in order to evaluate the renal expression of Cav1 and Cav2 in extrahepatic cholestasis. Suspensions of rat renal proximal tubule cells were obtained, and incubated for 3 h with serum from Sham-operated rats (S) or from rats with bile duct ligation of 72 h (L). All incubations were performed at 37°C with constant agitation and exposition to 95% O_2–5% CO_2. Cell viability was assessed by exclusion of trypan blue. Viability of the original suspension was approximately 93%. Cell viability (%) did not change independently of the tested treatments (S=91±3 (n=5), L=89±3 (n=8)). Cell homogenates (H) and cell membranes (M) were then prepared and assayed for Cav1 and Cav2 protein expression by electrophoresis and Western Blotting. Results: mean±SEM. Cav1 (%): H: (S)=100±3 (L)=78±4* M: (S)=100±4 (L)=86±3*. Cav2 (%): H: (S)=100±3 (L)=84±4* M: (S)=100±3 (L)=82±5* (*p<0,05). Some components present in L serum may lead to reduce Cav1 and Cav2 expression through a decrease in their synthesis or an increase in their degradation. Cav1 and Cav2 have been reported to participate in the modulation of organic anions transporters (Oat) 1 and 3 located at the basolateral membrane of tubular renal cells. We have described altered expression of both Oat1 and Oat3 in extrahepatic cholestasis of 72 h. The decreased expression of caveolins observed in this study, may be influencing the expression and function of these relevant transporters in extrahepatic cholestasis.

Keywords: Caveolins, kidney, cholestasis.

(1540) **ROLE OF TRPV4 AND AQP2 EXPRESSION IN RENAL CORTICAL COLLECTING TUBULE CELL GROWTH IN ACIDOSIS.**

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As mechanisms of proliferation and intracellular pHi are associated is possible that a chronic alteration of the pH modifies cell growth. We previously showed in renal cells that aquaporin 2 water channel (AQP2) expression prevents inhibition of cell growth in acidic media. Ca^{2+} channel TRPV4 has been proposed as one possible acid sensor. Our previous work showed that TRPV4 and AQP2 interact. So, the aim of this study was to investigate whether renal cell growth in acidic media was dependent on TRPV4. To investigate this we used two renal cortical collecting duct cell lines: WT-$RCCD_1$, not expressing AQPs, and AQP2-$RCCD_1$ expressing constitutive AQP2 in the apical membrane. Cell Growth studies (CG) were performed in control and acid media in presence of vehicle or the TRPV4 inhibitor Rutenium Red (RR). At physiological pH inhibition of TRPV4 decreased both lines cell growth about 20%. .After 24h exposure to an acidic media (ΔpH = 0.4) only WT cells decreased its growth and this response was not modified by RR (%CG Acid = -32±7 vs Acid + RR= -37±9, ns n=18). On the other hand, while acid media did not alter AQP2 expressing growth acid + RR significantly decreased cell growth (%CG Acid =-3±4 vs Acid + RR= -51±15, $p<0.01$ n=17). These results demonstrate that to prevent acidic cell growth inhibition AQP2 is associated to TRPV4 channel.

KEYWORDS: KIDNEY, ACIDOSIS, TRPV4, AQP2

(1555) **EXPRESSION OF APELIN mRNA IN THE POST-ISCHEMIC RAT KIDNEY**

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The regulatory peptide apelin is the endogenous ligand for the orphan G protein-coupled receptor APJ. The apelinergic system has been demonstrated to be involved in the pathogenesis of many conditions. Apelin treatment decreased the expression of pro-inflamma-

tory cytokines and improved renal function in ischemia-reperfusion injury.

The aim of this work was to evaluate the expression of i) apelin, ii) toll-like receptor (TLR)-2, inductor of pro-inflammatory cytokines, and iii) heat shock protein 70 (HSP70), endogenous ligand for TLR-2, in the post-ischemic kidney.

Adult male Wistar rats underwent unilateral right renal ischemia for 40 minutes. Reperfusion was allowed for 24 hours (IR) before kidney removal. Sham operated controls (C) were also included in the protocol. Clearance studies and histological examination were carried out. Apelin and TLR-2 mRNA expression was analysed by qRT-PCR; and HSP70 protein expression, by Western blot.

After 24 hours of reperfusion, creatinine clearance significantly decreased and histological observation showed multifocal acute tubular necrosis, intratubular necrotic/apoptotic cells detachment and brush border diminution. Apelin mRNA in the cortex of IR kidneys was decreased (-70%, $p<0.05$) and TLR-2 mRNA was increased (+620%, $p<0.05$) compared with C. HSP70 protein levels in IR were significantly higher than in C samples (arbitary units HSP70/Coomasie blue: C= 4.5 ± 0.5; IR= 14.7 ± 1.7; $p< 0.05$).

In this model of ischemic acute kidney injury, cortical expression of mRNA encoding for apelin, the peptide whose exogenous administration was reported to exert nephroprotective actions in such conditions, is significantly reduced. Associated with the diminution in apelin expression, an increased expression of TLR-2 and its ligand, HSP70, was found. Further investigation is needed to test our hypothesis that apelin protective effects in IR are mediated by modulation of the HSP70/TLR-2/NFκB/inflammatory cytokines pathway.

Key words: acute kidney injury; ischemia/reperfusion; apelin; TLR-2; HSP70

(546) **EXPRESSION OF PROTEINS RELATED TO SODIUM BALANCE IN RENAL MEDULLA AND PERIPHERAL BLOOD LYMPHOCYTES IN A RAT MODEL OF SALT-SENSITIVE HYPERTENSION**

Abstract: The absence of female sex hormones in ovariectomized (oVx) rats alters renal medulla expression of Na^+, K^+- ATPase (NKA), Na^+ K^+ $2Cl^-$ cotransporter (NKCC2) and D1 dopamine receptor (D1DR). During high salt intake sodium excretion is deficient and rats develop salt sensitive hypertension (SSH). The immune system plays a role in arterial hypertension. In lymphocytes, NKA and NKCC2 are associated with cell growth / proliferation. Therefore, the aim of the present work was to study the expression of NKA, NKCC2 and D1DR proteins in the absence of female sex hormones in peripheral blood mononuclear cells (90% of lymphocytes, PBL) from intact female (IF) and ovariectomized (oVx) Wistar rats. The oVx was performed at 60 days of life and rats were studied 90 days post-oVx. During the last 5 days rats received normal (NS, NaCl 0.24%) or high salt intake (HS, NaCl 1%). At day 150, mean arterial pressure (MAP) was recorded and peripheral blood mononuclear cells were isolated by Ficoll gradient. The expression of D1DR, NKCC2, total NKA and NKA dephosphorylation state (NKA is more active when dephosphorylated at Ser 23) was analyzed by Western blot.

Total NKA decreased in IF with HS intake ($p <0.005$ vs IFNS). In oVx rats NS decreased ($p <0.05$ vs IFNS) while HS increased NKA ($p <0.001$ vs oVxNS). dNKA was higher in oVxHS than in IFHS ($p <0.05$). D1DR decreased in oVxNS rats relative to IFNS and increased in oVxHS ($p<0.05$ vs oVxNS). NKCC2 diminished in IFHS ($p <0.05$ vs IFNS), but NKCC2 increased in oVxHS relative to oVxNS and to IFHS ($p <0.05$). oVx rats with HS intake excreted less sodium than IFHS rats and increased MAP (138 ± 4.5 oVxHS vs 110 ± 5.2 mmHg, $p <0.05$ vs IFHS).

As in renal medulla, expression of NKA, NKCC2 and D1DR is altered in PBL cells from oVx rats compared to IF rats. These findings could be potential tools to allow the screening of SSH and to determine the role of the immune system in this pathology.

Keywords: lymphocytes, salt sensitive hypertension, Na^+, K^+- ATPase, ovariectomy

(708) **RENAL CDC42 AND CIP4 EXPRESSION IN AN ISCHEMIA-REPERFUSION INJURY MODEL**

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Acute kidney injury (AKI) is still a significant medical problem. Renal ischemia-reperfusion (IR) injury is one of the main causes of AKI. Cdc42 is a master signaling molecule, involved in the maintenance of epithelial integrity in renal tubules. Cdc42 signals the initial events of the epithelial mesenchymal transition (EMT), which is essential in the response of the injured kidney. Our aim was to contribute to the molecular characterization of the signaling pathways involved in the development of AKI by characterizing specific features of the cdc42 pathway in the IR model. Male Wistar rats (n=6 per group) were subjected to 40 min of unilateral renal ischemia followed by 1 (IR1) or 7 (IR7) days of reperfusion. Controls underwent sham operation. AKI was corroborated by histological and functional studies. Glomerular filtration rate was reduced in IR1 (-54%, $p<0.05$), and recovered in IR7 rats. Cdc42 protein levels, evaluated in renal cortex, were significantly increased in IR1 (+200%, $p<0.05$), and return to control levels in IR7. CIP4 is an effector of cdc42, which also participates in EMT. CIP4 protein levels also increase in IR1 (+1000%, $p<0.05$) and return to control levels in IR7. Cdc42 function is partially regulated by posttranslational modifications that condition its association to cellular membranes. In fact, cdc42 association to membranes is decreased in the IR model. Our previous results suggest that the isoprenyl-cistein carboximetil transferase (ICMT) could modulate cdc42 function in kidney derived epithelial cells. Our present results showed that ICMT mRNA levels, evaluated by RT-PCR, were reduced in IR1 kidneys. In conclusion, we demonstrated that in the IR model, the early stage of renal damage is associated with an abrupt increase of cdc42-CIP4 expression and a decrease in the expression of an enzyme that could regulate cdc42 association to membranes. The significance of this regulatory response in the evolution of AKI remains to be elucidated.

Keywords: renal ischemia-reperfusion; RhoGTPase; Cdc42; CIP4, ICMT

(1000) **RENAL EXPRESSION OF THE ORGANIC ANION TRANSPORTING POLYPEPTIDE 1 (Oatp1) IN RATS WITH OBSTRUCTIVE NEPHROPATHY.**

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The Organic anion transporting polypeptide 1 (Oatp1/Slco1a1) is a transporter localized in the apical membrane of the proximal tubule cells, and in others tissues like liver and brain. Oatp1 mediates the transport of bile acids, steroids, thyroid hormones, β-lactam antibiotics, cardiac glycosides, statins, among others. The purpose of this study was to evaluate the renal expression and the urinary excretion of Oatp1 in male Wistar rats with obstructive nephropathy. Bilateral ureteral occlusion (B) was induced by ligation of both ureters for 24 h (B24, n=6). The studies were performed after 24 h of ureteral releasing. In parallel a Sham group (Sh, n=11) was processed. The creatinine clearance (Cl_{Cr}) was determined by conventional clearance techniques. Renal expression in homogenates (H), membranes (M), and urinary abundance of Oatp1 were evaluated by electrophoresis and Western blotting. Results: Mean ± SEM. Statistic: t-Student (* $p<0.05$). Cl_{Cr} (mL/min/100g b.w.): Sh= 0.47 ± 0.03, B24= 0.07 ± 0.02*; $Oatp1_H$ (%): Sh= 100 ± 3, B24= 81 ± 7*; $Oatp1_M$ (%): Sh= 100 ± 4, B24= 125 ± 3*. Animals from B24 group showed a significant decrease in ClCr confirming the establishment of the pathology. In homogenates, Oatp1 expression was decreased significantly. This could be explained by an increase in degradation or a decrease in

the novo synthesis of the protein. In membranes, the Oatp1 expression was increased, perhaps as a result of an increase in the protein trafficking from the cytoplasm to the apical membrane. Urine samples from B24 group showed a defined band of 83 KDa, which coincides with the molecular weight described for Oatp1, whereas this band was not detected in the urine of Sham animals. To our knowledge, this is the first study that reports the detection of Oatp1 in urine. In obstructive nephropathy, the alterations observed in the renal expression of Oatp1 could affect the renal handling of metabolites and/or therapeutic drugs that are substrates of this protein.

Keywords: Oatp1, kidney, obstructive nephropathy.

(1924) **RENAL PROTEIN HANDLING IN FABRY DISEASE NEPHROPATHY**

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X-linked recessive disease due to alpha-galactosidase deficiency A that determines the accumulation of glucosphingolipids as intracellular globotriaosilceramide (Gb3). The prevalence of the disease is 1/40000 live births. This enzyme appears in the lysosomes of the whole organism, so the disease goes through systemic manifestations. Nephropathy is associated with the accumulation of lysosomal inclusions at the level of podocytes. The first renal manifestations are deficiencies in the capacity of urine concentration, but in adulthood renal insufficiency and proteinuria caused by the alteration in the filtration barrier appear. Megalin is an endocytic receptor type LDLR, identified at the podocyte level and apical membrane of the proximal tubule, with multiple protein ligands such as albumin. The aim of the study is to study tubular causes of proteinuria as an additional mechanism to the urinary excretion of proteins in Fabry's disease. Immunohistochemistry techniques were performed on 11 samples. Biopsies in paraffin (3) and electron microscopy (8), previously dewaxed or exposed to saturated sodium hydroxide solution respectively, for further processing for fluorescence and peroxidase-DAB immunostaining. Megalin (1/50; Abcam, USA) was used as the primary antibody. Signal was detected for megalin in the cytoplasm of the proximal tubules. Taking into account that the normal expression pattern is at the apical border, our results suggest an alteration in protein endocytosis due to lack of recycling of megalin that would indicate a mixed origin of proteinuria.

Key words: Fabry, Proteinuria, Megalin, Podocyte,Gb3.

Farmaco 1

(982) **ADVERSE EFFECTS OF OPTIONAL HPV VACCINATION ON TEENAGERS IN CÓRDOBA, ARGENTINA (2011-2015)**

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INTRODUCTION

HPV is a sexually transmitted virus which causes vagina and anal cancer as well as an important health problem in this population.

There are two vaccines (Gardasil and Cervarix) in Argentina since the year 2011.

The adverse reactions in clinical trials were local (82.9%) and fever (13%). Arthritis, arthralgia and arthropathy were informed (1.1%). Only 6% of were severe.

1- To collect information about the teenagers' level of knowledge of HPV, ways of infection and prevention.

2- To determine the percentage of vaccinated teenagers.

3- To demonstrate the ARMs related to this population.

4- To compare the adverse reactions of the surveyed group to the ones registered by the National Administration of Drugs, Food and Medical Devices (ANMAT) **MATERIALS AND METHOD:**

A survey was conducted in high school students of both sex pertaining to a group age between 15-18 years old in public and private schools in the city of Córdoba.

The adverse reactions reported by the ANMAT were investigated in order to compare them to the results showed by the survey.

RESULTS:

100 teenagers of private and public high schools of the province of Córdoba were surveyed: 50% of them were girls and the average age was 16 years old. 72% of them acknowledged HPV as a sexually transmitted virus with common lesions: verrucae (47%). 70% of the surveyed find the masculine condom as the prevention item.

Regarding vaccines, 74% of them know about their existence but, paradoxically, 75% of them are not vaccinated. Vaccination for those who did it was due to medical prescription and they do not report adverse reactions. Although these data, 65% assert they would get vaccinated.

FINDINGS:

No significant adverse reactions were showed in those vaccinated teenagers; thus the findings are in line with those registered by the ANMAT since the beginning of the vaccination

(624) **ALTERATIONS OF THE QTC INTERVAL IN PATIENTS UNDER TREATMENT WITH BETA-LACTAM ANTIBIOTICS**

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Abstract: drug-induced QTc-interval prolongation is a frequent reaction. There are no studies evaluating its frequency with beta-lactam antibiotics used for low respiratory infections. Objective: to evaluate beta-lactam effects on QT-interval prolongation in the clinical setting, frequency and risk factors. Research design and methods: medical history and comorbilities were registered for patients requiring low respiratory infections treatment. Patients receiving clarithromycin (known QTc-prolonging drug) were used as a positive control. Patients receiving ceftriaxone (no reported cases of QTc prolongation) were used as a negative control. Ionograms and ECGs were performed: baseline and intratreatment; QT was corrected with Bazzet, Fridericia, Framinghan and Hogdes formula. Results:100 patients were included (55 men) with a mean age ± SD (range) 60 ± 13 (40-84) years, weight 78.1±12.1 (51.0-96.0) kg, and body mass index 27.37±2.38 (21.23-32.15) kg/m2. No electrolytes disturbances were detected. Prior to initiation of pharmacological treatment, the patients had: QTcB 453±19 (367-480), QTcFri 425±24 (373-466), QTcFra 422±25 (357-465), and QTcH 427±16 (396-460) ms. Patients were treated with standard doses of Ceftriaxone (N=41), Clarithromycin (N=37), Ampicillin + Sulbactam (N=39), and Piperacillin + Tazobactam (N=20). Clarithromycin was coadministered with ceftriaxone (20) or ampicillin + sulbactam (17). During treatment ECG shows QTcB 431±25 (377-485), QTcFri 415±24 (357-463), QTcFra 415±23 (363-463), and QTcH 413±22 (361-462). QTc Interval prolongation was detected for clarithromycin ($p < 0.001$) and ampicillin + sulbactam ($p < 0.01$). The independent factors that correlated with QTc prolongation were: heart rate, arterial saturation, use of clarithromycin, ampicillin + sulbactam and piperacillin + tazobactam. Conclussion: beta-lactam antibiotics are associated with a small risk for QTc prolongation that may be additive to other clinical factors that must be monitored.

Keywords: antibiotics, QT-interval prolongation, pharmacovigilance, torsades de pointes

(716) **APPLICATION OF PROPOFOL BY IMMERSION AS ALTERNATIVE FOR THE MANAGEMENT OF CARP (*Cyprinus carpio*)**

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In aquaculture the use of anesthetics is frequent, due to the exposure of fish to stress that affect survival and productive performance, such as manipulation, artificial reproduction and transport. The objective of the present study was to evaluate the depressant activity

of propofol in carp (*Cyprinus carpio*), an anesthetic agonist of the inhibitory activity of GABA. Although there is a history of safety and its pharmacokinetics in cultured fish, its use as an anesthetic has not been reported in aquaculture. Twenty fishes with a total length and average weight of 45.5 ±2.6 cm and 1.5 ±0.3 kg, respectively were used. Three plastic containers of 50 liters each were used with water from the lake to maintain environmental conditions and minimize stress. The methodology of the work consisted of: 1) acclimatization of fish in a container without drug to register basal respiratory rate after adopting normal swimming behavior; 2) transfer to the second container with propofol at 5 ppm, recording times of lateral balance loss and anesthesia and respiratory rate; 3) transfer to the third container without drug registering its behavior and recovery. Statistical treatment of data was carried out with non-parametric Mann-Whitney test. As a result we found that propofol significantly reduced ($P< 0.05$) the respiratory rate from 72.0 ±8.2 to 55.6 ±13.3 opercular movements per minute. The time of loss of lateral balance and anesthesia were 49.0 ±12.1 and 87.3 ±33.0 seconds and the total recovery was 244 ± 95.3 seconds. No signs of excitation were recorded as well as dead specimens up to 96 hours after the administration of the drug. The results obtained indicate that propofol is a safe and effective anesthetic for *C. carpio* management.

Key words: propofol, *Cyprinus carpio,* sedation

(1697) **EXTRACELLULAR VESICLES SECRETED BY THE RAT SUBMANDIBULAR GLAND: ORIGIN, SIZES AND TRANSPORTED NUCLEOTIDASES**

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The rat submandibular gland (SMG) incubated *in vitro* releases nanovesicles with nucleotidase activity. The objective of this work was to verify the acinar origin of the vesicles and to identify enzymes responsible for catalyzing the hydrolysis of extracellular nucleotides. Methods: SMG fragments of 80-day male Wistar rats were washed and then incubated for 30 'at 37 ° C in physiological solution (pH 7.4). The incubation media were successively centrifuged 15 'at 2000 g and 60' at 27000 g to pellet the vesicles. Tissue sections and vesicles were analyzed by transmission electron microscopy. The vesicular diameter was measured with Image-J software. Nucleotidase activities were evaluated by measuring with a colorimetric technique the phosphate released by incubating the vesicles with 3 mM MgATP, MgADP or MgAMP. Nucleotidases were located in the vesicles using specific antibodies and immuno-labeled with colloidal gold. Results: Size distribution indicated that released vesicles could be a mixture of exosomes (50-100 nm) and plasma membrane derived vesicles (>100 nm); the lumen next to the apical side of acinar cells contained vesicles which size and morphology are compatible with isolated ones. ATP hydrolysis rate decayed 85% along incubation time ($vi= 4.10^{-4}$ μmol $Pi.min^{-1}.mg\ SMG^{-1}$, $T1/2= 1$ min), which can be attributed to NTPDase2, an ecto-ATPase that is inactivated as it hydrolyzes ATP. Incubation of vesicles in the presence of ADP or AMP gave markedly lower activities, stable over time, that could be associated to NTPDases 1 or 3 (for ADP hydrolysis) and ecto-5'nucleotidase (for AMP hydrolysis). Immunogold labelling of NTPDase1, -2 and -3, and 5'nucleotidase indicate that some vesicles carry these molecules, especially NTPDase2 and 5'nucleotidase. Conclusions: rat SMG secrete extracellular vesicles containing nucleotidases that could regulate extracellular ATP, ADP and adenosine concentrations and purinergic responses in gland ducts or oral tissues via saliva.

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Key Words: Periodontal disease, NTPDase, histamine, nanovesicles, submandibular gland

(1927) **RESVERATROL EFFECTS ON GLUCOSE, INSULIN AND LIPIDS: A SYSTEMATIC REVIEW AND META-ANALYSIS ON RANDOMIZED CONTROLLED TRIALS**

Maria Graciela Lopez Ordieres

It has suggested that resveratrol has anti-aging, anti-carcinogenic, anti-inflammatory, and anti-oxidant properties that might be relevant to chronic diseases like diabetes, obesity, metabolic syndrome. But the evidence is contradictory. The aim of this review was to evaluate the efficacy of resveratrol treatment on fast glucose control, insulin sensitivity and lipids levels in randomized controlled trials (RCTs).

The strategic search included PubMed and other databases, from January 2010 to December 2016, to identify RCTs investigating the impact of resveratrol to control fast glucose, insulin sensitivity and lipid metabolism (cholesterol, TG, HDL and LDL). Key words were resveratrol, clinical trials and humans. The size effects were calculated based on the standardized mean difference (d), heterogeneity was calculating by Q and I^2.

According inclusion and exclusion criteria 15 RCTs were selected, a total 465 of subjects were included in this meta-analysis. The size effect of fast glucose was not significant as indicated d= -0.15 (95% CI: -5.9 and -3.2, p= 0.38, n=15). Also for insulin sensitivity d=-0,02 (95% CI:-2.73 and 2.60, p=0.95,n=9); for cholesterol d=-0.15 (95% CI: -0.38 and 0.23, p=0.56, n=10); for triglycerides d= 0.04 (95% IC: -0.27 and 0.31, p= 0.88, n= 11); for HDL d=-0.19 (IC%95: -0.09 and 0.05, p= 0.63, n=10) and LDL d=0.19 (95% IC: 0.19 and 0.37, p=0.46, n=9). Forest plots were constructed with these data.

Heterogeneity was significant only for glucose with an index of 88% due to two studies with opposite results without bias of publication. The overall outcomes of the meta-analysis indicate that resveratrol daily consumption, doses 8- 3000 mg, during periods 2-3 weeks cannot significantly modify fast glucose control, insulin, cholesterol, TG, LDL and HDL. This systematic review demonstrated that there no scientific evidence to recommend resveratrol for chronic pathologies like diabetes, obesity and metabolic syndrome.

Keywords: resveratrol, systematic rewiew, diabetes, obesity, metabolic syndrome

(312) **METHOTREXATE ADD-ON EFFECT TO ANTIPSYCHOTICS: A CASE REPORT**

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Objective: Our goal is to present the case report of a patient with comorbid diagnoses of schizophrenia and psoriasis where the positive response to antipsychotics is related to the administration of immunosuppressive treatment.

Methods: A hospitalized 43 year old male with diagnosis of schizophrenia according DSM-IV criteria was followed over 15 months. His psychiatric disease lasted for over 20 years at the time of admission when also comorbid psoriasis was detected. Response to the antipsychotic treatment was evaluated by the Positive and Negative Syndrome Scale (PANSS). Treatment of psoriasis was conducted by dermatologists.

Results: After nine weeks of treatment with risperidone (4- 6 mg/day) PANSS score was 119. Clozapine (350 to 900 mg/day) associated with methylprednisone (20-40 mg/day) for the treatment of psoriasis improved both comorbid conditions (PANSS = 77), but suppression of methylprednisone was followed by a PANSS score of 105 points. After 13 weeks, clozapine was replaced by olanzapine (10-50 mg/day) and a conventional scheme of methotrexate + folic acid (10 mg/ 1 g respectively) was indicated for 2 weeks. While psoriasis improved, no changes in PANSS score (= 119) were observed. After 12 weeks, olanzapine was replaced by haloperidol (10-30 mg/day). After 10 weeks with haloperidol, a flare of psoriasis led to the reinstatement of methotrexate + folic acid for 4 weeks, and 10 weeks later (week 24^{th}), methotrexate was indicated on a chronic basis. This scheme induced a significant and persistent improvement in the psychiatric condition (PANSS = 61 points). The patient was discharged from hospital. After discharge, a scheme of risperidone (4 mg/day) and methotrexate as indicated above was accompanied by PANSS score of 51.

Conclusion: Further evidence about potential benefits of adjunctive immunosuppressant treatment to antipsychotics in resistant schizophrenia could be of interest .

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Key words: schizophrenia; immunity; drug resistant psychosis; immunosuppressant treatment; antipsychotics

(1583) **PERSISTENCE OF *Human papillomavirus* AFTER TREATMENT IN CASES OF INVASIVE CERVICAL CANCER IN NORTH OF BRAZIL**

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Abstract: In the Pará State, North of Brazil, cervical cancer is the most frequent gynecological malignancy in women; the incidence of this cancer was estimated in 820 new cases of cancer, according to the National Cancer Institute (INCA, 2016). The *Human Papillomavirus* (HPV) is a sexually transmitted infection and this virus is considered the causative agent of the cervical carcinoma; it has more than 200 different viral types, of these, some are oncogenic or high risk types. **Objectives**: To investigate the persistence of HPV-DNA in cervical samples before and after cancer treatment with radiotherapy and / or chemotherapy. **Materials and methods:** Twenty nine women attended at Ophir Loyola Hospital-Belém- PA with invasive cervical cancer were enrolled in the study. Two samples for each patient were collected (smear of the endo an ectocervix) before and after the oncological treatment (fifty-eight samples in total). The cervical samples were tested for the presence of HPV DNA using nested- polymerase chain reaction (PCR) with My9/11 and GP5/6 primers. **Results:** The presence of HPV was detected in 82% (24/29) of the samples of women before treatment and in 68% (20/29) in the samples of women that completed treatment. It was found that in 75% (18/24) of women the HPV virus persisted after treatment, 25% (6/24) cleared the infection (p=0,3). **Conclusion:** In our study the prevalence of HPV DNA detected by PCR for the entire group of women with invasive cervical cancer was 75% HPV-DNA persistence after radiotherapy and/or chemotherapy could be a predictor for disease recurrence or disease outcome.

Key words: HPV, cervical cancer, PCR, viral persistence

(493) **RISK MANAGEMENT PLAN (RMP): APPLICATION IN ARGENTINA**

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A RMP is formulated to improve the drug safety profile, minimizing risks, favoring phase IV post marketing pharmacovigilance (PVG) and minimizing risks during the drug's life cycle. The aim of this work is to analyze a temporal sample of RMPs and their corresponding Active Pharmaceutical Ingredients (API) in Argentina during 2012-2015. We reviewed data from all APIs and drug associations accompanied by their RMPs submitted to the Administración Nacional de Medicamentos y Tecnología Médica (ANMAT). Data analysis was performed using an MS Excel 2007 worksheet. From 181 APIs and 39 associations, 460 RMPs were submitted (18% biological products; 2% vaccines; 24% oncological products except biological ; 4% antivirals alone except biological; 40% other products; 12% associations). The RMP/product ratios were 2.24 and 1.38 for APIs or associations respectively, indicating the interest of pharmaceutical industry for new APIs. Of 460 RMPs; 41 (8.9%) were updated by default and 5 (1.1%) were from products withdrawn from market. Analyzing their respective risk, 6 API and 3 associations did not need their corresponding RMPs.

Drug safety is a higher interest issue because of social impacts and the burden for health system. Clinical trials may be safe-limited due to the number of patients exposed, and unfortunately, many countries included Argentina have sub-registries on spontaneous PVG. Thus, RMPs are vital to define earlier phase IV safety. Drug manufacturers or marketers, particularly if they will introduce an innovator, should present a RMP in order to evaluate its safety impact and periodically update it according to population exposed. In the case of high-cost treatments, rare diseases or special conditions treatments, the RMP updating is of major importance.

Keywords: risk management plan, pharmacovigilance, drug safety

(1197) **TEACHING ON THE MEDICINES RATIONAL USE IN AN EDUCATIVE CENTER OF ELEMENTARY AND SECONDARY LEVEL OF ARGENTINA**

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Education is an essential component of our lives, so better training will enable us to make optimal decisions. In referring practice to medicines use, the community formation involves efforts that must overcome the competence of an abundant persuasive promotion that only aims to increase consumption and, attached to a disinformation of society, reflects us complexity of the context and the tasks to be carried out. The objective of university extension project was develop educational activities aimed at school students of 6-18 years of educative center of San Luis province, due the role they play as consumers and by the multiplier effect of their training. Information on aspects related to medicines, such as their appropriate use, encouraging medical or pharmaceutical consultation and discouraging the self-medication, therapeutic non-compliance and the purchase of medicines outside authorized establishments was provided. Training lectures, distribution of educational brochures, games and surveys were prepared; furthermore, a final contest in which students developed a publicity auditory spot on medicines rational use was proposed, which were posteriorly emitted in school radio. Students were separated by educational level for the activities. A great adhesion and participation was observed, based on questions and comments made by students, evidencing a great change in the perception on the use of medicines. Education is an important implement in promoting of medicines rational use, a knowledge that must be increased, particularly in school sector due to that during their adolescence, the young's begin to become aware of the meaning of their social role as consumers. Also, it is important take into account that most consumption habits acquired in this stage will keep forever and that additionally, most of they do not have the necessary training to carry out responsible consumption.

(403) **EFFECT OF THE CALCIUM CHANNEL BLOCKERS AMLODIPINE AND DILTIAZEM ON ERYTHROPOIETIN-MEDIATED ANGIOGENESIS**

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Calcium (Ca) is an important factor involved in cell migration. Our group has previously demonstrated its participation in the promigratory effect of erythropoietin (Epo) on endothelial cells. Stemming from this, we hypothesized that Ca channel blockers (CCBs) could negatively affect angiogenesis. This is an important issue since many of these compounds are clinically used as antihypertensives.

Amlodipine (Aml), a dihydropyridine, and the benzothiazepine diltiazem (Dil) are both able to block voltage-dependent Ca channels (VDCCs) in smooth muscle cells. With an aim to determine the ability of these compounds to block Ca channels in the endothelium, we used the EA.hy926 cell line as an experimental model. In accordance to previous reports, we found that both, Aml and Dil prevented staurosporin-induced apoptosis in this cell line (Western blotting, detection of caspase-cleaved PARP-1). However, only Aml decreased

the intracellular accumulation of ROS induced by TNF-α (flow cytometry with the ROS probe DCFH-DA). In inhibition assays, cells were preincubated with Aml or Dil before exposing them to Epo. Epo (200 ng/mL) provoked a significant transient rise in intracellular Ca (<30 s, flow cytometry with Fluo-4AM), which was abrogated by both channel blockers. Wound healing assays (15 h) revealed an inhibitory effect of Aml (1 μM) on Epo-induced cell migration (Control: 25.6±1.6% *Epo: 34.8±2.0%, #Epo+Aml: 22.4±2.7%; *P<0.05 vs. Control, #P<0.01 vs Epo, Kruskal Wallis-Dunn, n=7) while Dil (5 μM) showed no activity (Control: 25.0±1.6%, *Epo: 32.4±1.5%, Epo+Dil: 33.4±2.6%; *P<0.05 vs. Control, Kruskal Wallis-Dunn, n=7). Accordingly, Aml, but not Dil, was able to inhibit Epo-induced tube formation in EA.hy926 cells.

These results not only support the much-debated presence of VDCCs in endothelial cells, but also suggest a differential effect of amlodipine and diltiazem on angiogenesis, with possible implications in their coadministration with a proangiogenic therapy.

Keywords: Amlodipine – Diltiazem – Calcium – Angiogenesis – Erythropoietin

INFECTOLOGY 1

(071) IMMUNE-ENDOCRINE MODULATION OF OSTEOCYTES RESPONSES DURING *B. abortus* INFECTION.

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INIGEM

Patients with brucellosis have an imbalance in cortisol/ DHEA ratio in plasma. Bone remodeling process is regulated by hormones. Then, we aimed to study the effect of cortisol and DHEA on osteocytes (the most abundant cells of bone) during *B. abortus* (*Ba*) infection. Cortisol treatment inhibited the expression of TNF-α, IL-6 and RANKL (the main regulator of osteoclastogenesis) induced by *Ba* infection (p<0.001, ELISA). DHEA reversed the effect of cortisol on TNF-α expression, but not on the expression of IL-6 and RANKL during *Ba* infection. *Ba* infection induced MMP-2 by osteocytes (zymography). When cortisol was added during *Ba* infection the secretion of MMP-2 was inhibited. When the infection experiments were performed in the presence of both cortisol and DHEA, DHEA could reverse the inhibitory effect of cortisol on MMP-2 production.

RANKL and TNF-α are the main cytokines involved in osteoclastogenesis in inflammatory conditions. We performed osteoclastogenesis experiments by added supernatants from osteocytes infected with *Ba* in the presence of cortisol and DHEA to osteoclast precursors (RAW 264.7) in the presence of M-CSF. Our results indicated that cortisol inhibit the osteoclastogenesis induced by supernatants from *Ba* infected osteocytes (p<0.001, TRAP staining).

It is known that cortisol regulates target genes by binding to the glucocorticoid receptor (GR). *Ba* infection inhibited GR-α and GR-β expression (p<0.01, RT-qPCR).

The levels of intracellular cortisol is not only dependent on GR expression but also a result of the activity of the isoenzymes 11β-HSD1 (cortisone to cortisol conversion), 11β-HSD2 (cortisol to cortisone). *Ba* infection increased 11**β**-HSD1 expression (p<0.05) and inhibited 11**β**-HSD2 expression by osteocytes (p<0.001, RT-qPCR).

The present study constitutes the first analysis on the immune-endocrine alterations in osteocytes during *Ba* infection and provides an initial background for studying in more detail the role of DHEA during the infection.

Keywords: *B. abortus,* osteocytes, cortisol, DHEA

(072) ADRENAL STEROIDS MODULATE OSTEOBLAST FUNCTION DURING *Brucella abortus* INFECTION

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INIGEM

Brucella abortus (Ba) induces an inflammatory response that stimulates the endocrine system resulting in the secretion of cortisol and dehydroepiandrosterone (DHEA). Osteoarticular brucellosis is the most common presentation of the active disease in humans and we have previously demonstrated that *Ba* infection inhibits osteoblast function. We aimed to evaluate the role of cortisol and DHEA on osteoblast (MC3T3-E1) during *Ba* infection. Our results indicated that DHEA treatment reversed the effect of *Ba* infection on osteoblast by increasing the proliferation (BrDU, CSFE), cell viability (MTT) and inhibiting osteoblast apoptosis (Annexin V, TUNEL & Hoechst). In contrast, cortisol increased the effect of *Ba* infection. DHEA, also, reversed the inhibitory effect induced by *Ba* infection on osteoblast matrix deposition (Alizarin & Sirius Red staining) in an estrogen receptor (antagonist fulvestrant) and ERK1/2 (specific inhibitor It is known that cortisol regulates target genes by binding to the glucocorticoid receptor (GR). *Ba* infection inhibited GR-**α** and this effect could not be reversed by cortisol or DHEA treatment. *Ba* did not induce changes in the expression of GR-**β**. Besides, the capacity of cells to respond to cortisol not only is dependent on GR expression but also on its intracellular bioavailability. The levels of intracellular cortisol is a result of the activity of the isoenzymes 11β-HSD1 (cortisone to cortisol conversion), 11β-HSD2 (cortisol to cortisone). Alterations in the expression of these isoenzymes in bone cells are associated with bone loss. Our data showed that *Ba* infection increased 11**β**-HSD1 expression. Cortisol treatment inhibited the 11**β**-HSD1 expression induced by *Ba.* DHEA treatment had no effect. *Ba* infection did not induce changes in expression of 11**β**-HSD2.

We conclude that DHEA intervention improve osteoblast function during *Ba* infection. Thus, DHEA could be considered as a new treatment against osteoarticular brucellosis.

Keywords: Brucella abortus, bone, DHEA, cortisol

(204) EGPE CELLS ARE A GOOD ANTIGEN SOURCE FOR HYDATIDOSIS DIAGNOSTIC IN SHEEP.

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Echinococcus granulosus is the causative agent of hydatidosis disease, a zoonosis with worldwide distribution. Nowadays, diagnosis of hydatidosis in livestock happens mainly in necropsies. However, an accurate serological diagnostic test would be desirable to monitor livestock in order to prevent the dissemination of the disease in non-endemic zones. A cellular model of *E. granulosus* was established from bovine protoscoleces in our laboratory and it was named EGPE. In these cells AgB4 was detected by immunohistochemistry and DCO1 (Echeverria et al, 2010) and antigen B2 subunit was detected by PCR. Purpose: to identify whether EGPE cell proteins could be recognized by infected sheep serum. Materials and Methods: EGPE cells were cultured in complete medium M199 for short (7 days) or long term culture (20 days), for protein extraction (CE). The CE were obtained in 8 mM CHAPS buffer for 2 hours and 4 freeze-thaw cycles. Serum from 7 infected sheep and 5 non-infected sheep were obtained from Chubut, Argentina and reactivity to EGPE proteins were analyzed by western blot and ELISA. An 18 μg of CE were used per lane for western blot and 1.5 μg of CE were used per well for ELISA. A 1/125 dilution for serum sample, alkaline phosphatase conjugated rabbit anti-sheep (1:5000) and BCIP/NBT (western blot) or p-nitrophenyl phosphate in diethanolamine buffer (ELISA) were used. Results: After 15% SDS-PAGE electrophoresis and immunoblotting, several bands from CE were recognized exclusively by infected sheep: 56-64 kDa and 75-83 kDa in short term culture and 44-48 kDa, 56-64 kDa and 70-74 kDa in long term culture. After ELISA, differences between control and infected sheep were detected in short (p<0.05, Student´s T-Test) and long term culture (p<0.01, Student´s T-Test). Conclusion: EGPE proteins could be used as a source of standardized antigenic material for diagnostic of hydatidosis in infected sheep.

Keywords: Hydatidosis, Serology, Sheep, EGPE cells

(436) INVOLVEMENT OF A HALOARCHAEAL RHOMBOID PROTEASE IN CELL ADHESION AND MOTILITY

REGULATION

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Rhomboids are a family of intramembrane serine proteases represented in the three Domains of Life. These enzymes participate in gene regulation and cellular signalling in eukaryotes, but little is known about their targets in prokaryotes, such as Archaea. Our previous work showed that deletion of one of the two Rhomboid protease genes (*rhoII*) has a global effect on the proteome of the haloarchaeon *Haloferax volcanii* (membrane and cytoplasm fractions). In addition, some putative RhoII targets (direct or indirect) were identified, including pilin pilA1, prepilin/preflagellin peptidase PibD and PrsW protease.

To validate these results in this work we analysed PibD and PrsW processing in *H. volcanii* wild type (H26) and ΔrhoII (MIG1). An *in vivo* assay suggested that the differences in PrsW level (29 fold reduction) previously observed in MIG1 may be due to an indirect effect of the *rhoII* mutation. On the other hand, preliminary Western blotting analysis of *H. volcanii* H26 and MIG1 membrane samples, indicated that PibD may be processed in a RhoII dependent manner. Overexpression of PibD natural substrates (pilins 1-6) in H26 and MIG1 strains showed differences in the amount of these proteins in cell extracts and culture supernatants, suggesting that they may be indirectly affected by RhoII.

With the aim of completing the initial proteomic study we also analysed and compared the secretomes of H26 and MIG1. We identified 27 differentially represented proteins, including some involved in motility and cell adhesion, such as flagellin1 and the hypothetical protein HVO_1533.

Taken together, our results suggest that RhoII regulates cell adhesion and motility, likely by processing PibD and subsequently affecting the functioning or assembly of pili and/or flagella in *H. volcanii.*

(532) **INTRANASAL IMMUNIZATION WITH THE NOVEL BtaF TRIMERIC AUTOTRANSPORTER FROM *Brucella abortus* IN A MURINE MODEL**

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Brucella spp. can be transmitted to humans through mucosae. Currently, there are no human vaccines against this infection. We have previously shown that BtaF is an adhesin involved in the attachment of Brucella spp. to biotic and abiotic surfaces, and that it is important for the dissemination and persistence of the pathogen after intra-traqueal murine infection. Based on these results, we evaluated BtaF as a potential component of an acellular vaccine against brucellosis.

Six-week-old Balb/c mice were weekly immunized for 3 weeks with BtaF (10ug) plus 3',5'-c-di-AMP (c-di-AMP,10ug), c-di-AMP alone or saline. One week after last immunization serum, saliva, feces, bronchoalveolar (BAL) and vaginal lavage (VL) fluids, lung homogenates and spleens were obtained. Levels of BtaF-specific antibodies were measured by indirect ELISA in all samples. *In vitro* production of gamma interferon (IFN-γ), interleukin-5 (IL-5) and IL-2 by spleen cells stimulated with BtaF (10 μg), ConA or RPMI was determined.

Immunized mice showed increased serum levels of total antibodies against BtaF ($p<0.0001$), and of specific IgA in saliva, feces, BAL, VL and lungs ($p<0.0001$; $p<0.0001$; $p<0.05$; $p<0.01$;$p<0.05$) compared with controls. IgA titers in serum were 12800. Serum titers of IgG2a were higher than those of IgG1 (409600 vs. 51200). The IgG1/IgG2a ratio was 0.49. In response to BtaF splenocytes from immunized mice increased their secretion of IFN-γ (495.5 pg/ml, $p<0.05$), IL-5 (454 pg/ml, $p<0.01$) and IL-2 (195.9 pg/ml, $p<0.05$). This increment in IFN-γ production was abolished when CD4+ cells were depleted ($p<0.01$) but not with CD8+ cells depletion.

Intranasal vaccination with BtaF plus c-di-AMP induced a systemic and mucosal specific immune response, which may contribute to prevent the mucosal entry of Brucella and its dissemination. The activated cellular immune response shown by high levels of IFN-γ, was dependent on CD4+ cells and may be crucial for this vaccine protective effect.

Key words: Brucellosis, mucosae, intranasal vaccine, adhesin

(857) **RHOX, A RHOMBOID PROTEASE OF *BRUCELLA ABORTUS* INVOLVED IN PATHOGENESIS**

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The intramembrane serine proteases from the rhomboid family are widespread among the different organisms and have many biological functions. Despite the lack of homology between their sequences, they all seem to cleave their substrates within the lipid bilayer by a conserved mechanism. However, little is known about the role of most of these enzymes during intracellular lifestyle of many pathogens. Our group recently identified substrates of *Brucella abortus* VirB type IV secretion system (SST4) involved in pathogenesis. One of them, RhoX (BPE275), is the only rhomboid protease present in *B. abortus* whose function is still unknown. Deletion of RhoX affects Brucella internalization. Homology sequence analysis by ClustalW revealed that despite differences in their sequences, RhoX have conserved specific regions relevant for protein structure and a catalytic dyad (Ser153-His227) responsible for the enzymatic activity. Furthermore, activity assays using *B. abortus* membrane preparations demonstrate that RhoX is an active rhomboid protease able to cleave substrates of other members in the rhomboid family such as Gurken and Spitz of *D. melanogaster*, LacY of *E. coli* and TatA of P. *stuartii.* In an attempt to identify putative substrates for Rhox among *Brucella abortus* proteome, we developed a bioinformatic screening. To validate this screening experimentally, transmembrane domains (TMD) of the putative candidates were cloned into pMal2E vectors as fusion proteins containing, as well, the sequence to encode the maltose binding protein (MBP) and both Flag and His tags. These constructions were expressed in *E.coli* MG1655, a mutant deficient for rhomboid protease, to perform activity assays. In further studies, we aim to study the function and location of BPE275 and its substrates during *Brucella abortus* pathogenesis.

Key words: Brucella, protease, rhomboid, internalization

(1061) **BIGA AND BIGB FACILITATES *Brucella* ADHESION AND INVASION TRIGGERING CYTOSKELETON REARRANGEMENTS OF HOST CELLS**

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Brucella are gram-negative intracellular bacteria that cause brucellosis, one of the most world-wide distributed zoonosis. The capacity of these bacteria to cause disease relies in their ability to invade and survive in the host cells. Adhesion to cells is the initial step in the virulence process of many pathogens and, therefore, is a central process to understand the bacterial pathogenic cycle. Our group has identified a unique gene cluster present in all *Brucella* species and important in the infectious process, participating in the adhesion to host cells. This region codes for four open reading frames: Bab1-2009, Bab1-2010, Bab1-2011 and Bab1-2012. Bab1-2009 and Bab1-2012 localize in the outer membrane and have a domain with homology to bacterial immunoglobulin-like domains (Blg-like). We found that both of these genes code for powerful adhesins that mediate attachment/adhesion to non-phagocytic host cells and for this reason we named them BigA and BigB respectively. We have shown that the addition of the recombinant Blg-like domain of BigA to HeLa and MDCK cells induced profound cytoskeletal rearrangements.

With the purpose of further understanding the role BigB could be playing in the interaction with host cells we recombinantly produced the BIg-like domain of BigB and treated HeLa cells. As with BigA, BigB induced profound cytoskeleton rearrangements that ended with the cells detaching from the plate. Additionally we tested if BigB may also cooperate or interact with BigA by treating non-phagocytic cells with both adhesins individually and in combination and assessing cell morphology as well as cytoskeleton integrity. Our results suggest that these adhesins have additive effects but need further work in order to understand at the molecular level how they enhance adhesion and invasion. Keywords: Brucella, adhesion, adhesin, cytoskeleton.

(1333) **CONTROL OF THE O-ANTIGEN CHAIN LENGTH IN *Brucella***

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Bacteria of the genus *Brucella* are gram-negative coccobacilli that cause brucellosis, a world-wide distributed zoonosis. Lipopolysaccharide (LPS) is the main component of the extracellular side of the outer membrane of the bacteria and constitutes the first physical barrier that protects them from the environment and, therefore, is central for the virulence of many pathogens. The LPS is composed of three parts, the lipid A, the oligosaccharide nucleus and the terminal polysaccharide terminal O-antigen. Although several genes that participate in the synthesis of LPS have been identified, the mechanism that regulates the length of the O-antigen is currently not known. Previous results indicated that *Brucella* actively controls the length of the O-antigen and in the search for putative genes that could play a role in this control we identified *wbkB*, a gene with no known function, present in the O-antigen synthesis cluster that codes for a protein with a putative glycosiltransferase domain. A deletion mutant in *wbkB* exhibited longer O-antigen chains measured by western blot and MALDI-TOF. Topology studies using fusions to b-galactosidase and to the alkaline phosphatase showed that the putative glycosiltransferase domain could be facing the citoplasmic side of the inner membrane strongly suggesting that this protein could be acting on the lipid-intermediate. A double mutant *pgm/wbkB* that accumulates the lipid-intermediate reinfoced this hypothesis. Additionally, infection assays with the *wbkB* mutant in cultured cells showed that the inability to control the O-antigen chain length affects virulence. In subsequent studies we will try to understand mechanistically how WbkB operates to regulate the of the O-antigen and how this fine tuned regulation affects the virulence process.

Keywords: *Brucella*, Lipopolysaccharide, O-antigen, Topology.

(981) **FOODBORNE DISEASES BY *Salmonella enterica* serovar Enteritidis DURING EARLY STAGES OF PUERPERIUM INDUCE AN ADVERSE MATERNAL OUTCOME IN THE MURINE MODEL.**

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Diarrheal disease agents, especially non-typhoidal *Salmonella enterica* (S. Enteritidis), are responsible for the majority of deaths due to foodborne diseases (FBD). Puerperium is a predisposing condition for exacerbation of infections. Our aim was to study the effect of FBD by *S.* Enteritidis during the early puerperium. To this purpose, we used an enterocolitis model. BALB/c mice received 20 mg of streptomycin 48 hs postpartum. 24 hs later they received 3,500 CFU of *S.* Enteritidis intragastrically (sub lethal dose). At day 3 post infection (day 6 postpartum) intestinal permeability was measured in vivo. Gut samples were analyzed for bacterial colonization, histological changes and tight junctions mRNA levels. Ileum and serum citoquines expression were studied by qPCR and ELISA, respectively. We found that 86% of puerperal females died within 5 days post infection whereas the mortality rate of infected virgin mice was 20%.This adverse maternal outcome is correlated with massive bacterial organs colonization. Also, gut permeability was significantly increased in infected parturient females ($p<0.05$) respect to control mice (non infected postpartum females, infected and non infected virgin females). Histology of serial cross sections showed irregular mucosa with decreased villus height, MALT hyperplasia and detriment of goblet cells versus no significant histological changes in non infected mice. Moreover, Claudin4 mRNA levels were 3 fold higher in infected parturient mice than control groups ($p<0.05$) while Zona occludens showed no significant differences. IFN-γ and IL-17 expression was up regulated in infected maternal gut and serum vs control mice ($p<0.05$). In summary, this work demonstrates that FBD by *S.* Enteritidis during early stages of puerperium cause negative effects on host survival probably by the increased gut permeability due to maternal immune response. Infected puerperal females are unable to self-limit the infection leading to fatal illness.

Key words: *Salmonella*, puerperium, enterocolitis, inflammation, foodbourne disease.

(1795) **INTESTINAL NECROTIC DAMAGE CAUSED BY *Clostridium chauvoei* IN MICE**

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The definition of the term "Sudden Death Syndrome" (SDS) often includes feedlot cattle found dead unexpectedly. The specific causes of death frequently can be determined by necropsy. Etiology and preventive measures are poorly defined. The current literature indicates that sudden death is often associated with digestive upsets. Recently SDS has been associated with specific *Clostridium perfringens* strains infections. *Clostridium chauvoei* is a Gram positive spore forming bacteria, causative agent of blackleg, a mortal myonecrotic disease that affects livestock legs muscles. Blackleg is endemic in Argentina and affects many animals around the world. The objective of this work was to characterize the endogenous infection caused by *C. chauvoei* spores administered orally. *C. chauvoei* ATCC 10092 spores were obtained in a modified clostridial medium, cultured five days at 37 ° C and 13 days at room temperature under anaerobic conditions. Groups of two Rockland mice were orally administered with 10^2, 10^4 and 10^8 spores in jelly-skin milk support during two months. The mice that showed sudden clinical manifestations such as incoordination and weakness, were immediately euthanized. The first deaths were recorded at around the 25th day of the trial. The gastrointestinal tract was collected and embedded in paraffin wax, and stained with hematoxylin-eosin. Intraperitoneal fluid was collected to look for *C. chauvoei* cells. Mice treated showed significant ($p\leq0.05$) weight loss compared to control mice. All the spores concentration assayed in this study, induced intestinal damage in mice. Macroscopic and histological analysis showed necrotic areas in large and small intestine. No *C. chauvoei* cells were found in intraperitoneal fluid. Our results suggest that *C. chauvoei* spores germinate and colonize intestine, leading poor nutrient absorption, mucosal necrotic damage, weight loss and death. Further studies will determine whether *C. chauvoei* could be associated with SDS in animals.

Key words: necrosis, clostridium chauvoei, spores.

FARMACO 2 / PHARMACOLOGY 2

(1769) **RADIOSYNTHESIS OF HYBRID NANOPARTICLES FOR BIOMEDICAL APPLICATIONS**

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IMBICE-UNQ

Surface modification of gold nanoparticles (AuNPs) with different biomolecules has been used for several decades in rapid diagnostics tests. In last decade, AuNPs have been demonstrated can be

used in therapy and imaging. This is due to their intrinsic biocompatibility and unique properties, dependent on their size, shape and coating. However, protein coating methods based on physisorption are not stable enough to prepare functional nanomaterials to be used in complex mixtures or in vivo applications.

The aim of this work is to prepare and characterize AuNPs coated with human and bovine serum albumin (Alb) multilayers by a novel radiation-induced crosslinking process. These nanostructures were called Au/Alb core/shell nanoparticles (Au/Alb NPs). Albumins from human and bovine source were added to AuNPs suspension followed by ethanol addition (30, 40, 50% v/v ethanol) to induce a dynamic protein aggregation around the AuNPs. The ethanol suspensions were irradiated at 10 kGy with a gamma source to induce a protein crosslinking according to recent findings in our working group (ref Achilli). .

The Au/Alb NPs were characterized by UV-visible and infrared spectroscopy, transmission electron microscopy (TEM) and dynamic light scattering (DLS). NPs containing 30%v/v ethanol showed a plasmon peak at about 532 nm, demonstrating the presence of non-aggregated AuNPs. Using higher ethanol concentrations, the absorbance of plasmon peak showed NP aggregation. DLS measurements were show particle population with an average diameter of about 60 nm was found. Moreover, TEM images showed that the NPs had spherical shape and the presence of a low-density halo around the metal core confirmed the presence of the protein shell.

In conclusion, we were prepared and characterized hybrid NPs by a novel radiation-induced cross-linking technique. These hybrid NPs are composed of Au core and Alb shell, which these were capable to maintain the spectroscopic properties of Alb and optical properties of AuNPs.

Keywords: radiosynthesis, gold nanoparticles, albumin,

(304) **AN ALBUMIN-BASED γ IRRADIATED NANOPARTICLE AS A POTENTIAL NANOVEHICLE FOR DRUG DELIVERY SYSTEMS: BIOPHYSICAL CHARACTERISATION, TOXICITY AND IMMUNE RESPONSE**

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A bovine serum albumin-based nanoparticle (BSA NP) was achieved by γ-irradiation of a protein ethanol solution. The first part of this study centres on the biophysical characterisation of the nanoparticle. Confirmation of the NP as an entity was stated by D.L.S and microscopic techniques (T.E.M, S.E.M. and A.F.M.). Preservation of the main structural features (α – helix content and main binding pocket) was displayed mainly through spectroscopic experiments: UV-vis, FT-IR, Fluorescence, and C.D.

The functionality of the NP as a drug delivery was study against the drug emodin (E). Fluorescence spectroscopy was used to elucidate the main binding parameters like affinity constant. The binding capacity efficiency and kinetic release profile were also studied. The toxicity of this system (BSA NPE) was tested in two different tumour cell lines: MCF-7 and PC-3. Its haemolytic activity was also tested in human blood cells.

Lastly, we studied the immune response the NP might have *in vitro* and *in vivo* in murine models (RAW BLUE cells and C57/BL6, respectively). In the *in vitro* experiments, all samples activated NF-κB and led to TNF-α secretion, nevertheless this immune response was strongly enhanced for larger BSA NPs suggesting a dependence of albumin immunostimulatory properties on particle size. Preliminary *in vivo* experiments suggested slight activation of DC cells and macrophages in the popliteal lymph node after 72 h incubation.

The BSA NP presented encouraging results to be considered as a nanovehicle for drug delivery systems. It does not only present all the albumin's main features as a drug carrier, but it improved the affinity for the hydrophobic drug together with the kinetic drug release profile.

KEYWORDS: Albumin, nanoparticle, spectroscopy, nanotechnology

(1106) **DESIGN AND DEVELOPMENT OF siRNA NANOCARRIERS USING MICROFLUIDIC TECHNOLOGY**

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Nonviral gene vectors are promising vehicles to deliver diagnostic and therapeutic agents *in vivo,* due to their low immunogenicity and biocompatibility. Within therapeutic agents, small interfering RNA (*siRNA*) is an efficient tool for gene silencing because this molecule has a very high potential effect at low doses. However, *siRNA* is manly degraded after its uptake by the endocytic/lysosomal pathway. Complexes containing *siRNA* and cationic polymers or fusogenic peptides are usually performed to overcome this barrier and to facilitate the activity of siRNA into the cytosol. Nevertheless, these kinds of polyplexes are generally obtained by manually mixing *siRNA* and polymers or peptides, generating large and heterogenic complexes with low silencing activity. The global aim of this work is to obtain small, homogenous and effective complexes by microfluidic technology for the silencing of genes associated with resistance to chemo and radiotherapies. Initially we obtained complexes using a *siRNA* against luciferase gene, a cationic polymer named polietilenimine (PEI) and the pH- sensitive influenza-derived peptide, INF7. Complexes were performed mixing manually the mentioned components (standard method, SM) and by using a hydrodynamic focused microfluidic system (MM). The complexes obtained by MM were 50-70% smaller than the ones produced by SM with a significantly reduced polydispersity index. The MM complexes were obtained continuously, fast and with a finely controlled synthesis. Likewise, the MM complexes were successful in silencing the luciferase reporter gene expression with a greater efficiency than those obtained by SM. These results allow us to infer that microfluidic technology is a promising method to obtain small and homogeneous complexes with well-defined structures and composition, to silencing efficiently target genes.

Keywords: Nanovehicles, microfluidics, siRNA, INF7

(386) **DESIGN AND IN VITRO CHARACTERIZATION OF A NOVEL MELATONIN DELIVERY SYSTEM**

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Melatonin is a hormone used for treating circadian rhythm disorders such as jet-lag and insomnia. Also, it has shown anti-tumoral properties on various types of cancers and neuroprotective effects in diseases like Alzheimer and glaucoma. Although it belongs to Class 1 according to the Biopharmaceutical Classification System (high permeability, high solubility), melatonin has low bioavailability (≈15%) due to an extensive hepatic first pass. Furthermore, it exhibits a low dissolution rate and stability problems in aqueous solutions. We hypothesize that these biopharmaceutical disadvantages can be approached through the encapsulation of the drug in muco-adhesive polymeric nanoparticles that undergo intestinal absorption or by their localized administration.

In this work, we present the encapsulation of melatonin in Eudragit® RLPO nanoparticles by a nanoprecipitation technique using acetone as solvent. The hydrodynamic diameter, determined by dynamic light scattering (DLS), ranged from 10 to 100 nm with polydispersity index of 0.118 ± 0.005 and zeta-potential of +54.7 ± +1.8 mV due to the polycationic nature of the polymeric carrier. The high value of zeta- potential predicts good physical stability. In fact, nanoparticles maintained their size and superficial charge for at least two months. Transmission electron microscopy evidenced the spherical

morphology of nanoparticles. The encapsulation efficiency was approximately 100%, as determined by UV-Vis spectrophotometry (λ = 278 nm). The muco-adhesiveness *in vitro* was characterized by mucin solution method by DLS where a significant increase in size and a significant reduction in zeta-potential were observed. Overall, these preliminary results suggest that a platform of melatonin-loaded nanoparticles could be capitalized to improve the delivery of this active molecule by a variety of mucosal routes.

Keywords: Melatonin, nanoparticles, improved delivery

(360) **NANOPARTICLES FOR CARIES LESION INFILTRATION AND POTENTIAL *IN SITU* REPAIRMENT**

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Caries disease produces serious dental damage that eventually evolves in a tooth cavity. The aim of this work was to study the application of nanomaterials to infiltrate decayed tissue and promote a biological repairment response. Zinc nanoparticles (ZnO@NP, 50nm) and gold nanoparticles synthesized and stabilized with an antibiotic (amoxicillin@AuNP, 40nm) were selected for their known antimicrobial capacity and biocompatibility. ZnO@NP produced stable suspensions during 72h in: distilled H_2O, PBS (pH=7.4), Thioglycolate broth, DMEM and Icon^R (a resin-based material mainly composed of tri-ethylene glycol dimethacrylate with high infiltration ability). The amoxicillin@AuNP stability, followed spectrophotometrically, was acceptable in DMEM and Thioglycolate broth (12.5%) for 72h, and was excellent in the resin until 72h. Agregation was noted immediately when mixing the two nanomaterials in brain-heart broth and in H_2O_2 (3%), which were alternatives for the culture of anaerobic bacteria and for infiltration, respectively. The interaction between nanomaterials and the demineralized dental surface was analyzed by SEM (Surface Electron Microscope) with coupled EDS (Energy Dispersive X-Ray Spectrometer). The load of nanomaterial was 22 times higher (Wt%: 42.9 ± 1) in dental samples treated with ZnO@NP carried in resin, than in those treated with ZnO@NP at the same concentration in PBS (Wt%: 1.9 ± 1). SEM images with mapping also showed that amoxicillin AuNP (1.5 µg/mL in PBS 50%) were attached on the dental surface, preferably in the demineralized area. Overall, best results were obtained by employing the resin as a carrier. This adhesive system based on methacrylates improved the nanomaterial adherence and recovered the dental structure by filling the porosities of the simulated lesion. Currently, their infiltration power and their effect on caries producing bacterial strains are being investigated.

KEYWORDS: nanoparticles, zinc, gold, caries, resins

(1859) **GOLD NANOPARTICLES SYNTHESIS AND FUNCTIONALIZATION FOR MITOCHONDRIAL DELIVERY**

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Melanoma is highly aggressive and its incidence increased significantly over the past decades. Despite the development of improved treatments, patients still have poor prognosis due to its resistance and high recurrence rate. Targeting cancer metabolism has emerged as a promising therapeutic strategy. In this context, multi-resistant melanoma cells have increased mitochondrial respiration and high level of reactive oxygen species. Disruption of this oxidative metabolism, in combination with other conventional therapies, could be an effective means for this highly resistant cancer. However, mitochondrial targeting of therapeutic agents is still challenging. A way to overcome this limitation is by using nanoparticles (NPs). NPs accumulate in tumors by enhanced permeability and retention effect and can be modified for selective mitochondrial targeting. In addition, gold NPs (AuNPs) possess unique electronic properties that make them excellent radiosensitizing agents. Against this background, we propose that AuNPs functionalized with the mitochondriotropic residue triphenylphosphonium (TPP), in combination with radiotherapy, can potentially be used for the radiosensitization of radioresistant melanoma cells.

Citrate-AuNPs were prepared by the Turkevich and Frens method and then functionalized with thiolated PEG polymers containing TPP (AuNPs-TPP). AuNPs-TPP were characterized by UV–Vis spectrophotometry, dynamic light scattering and transmission electron microscopy. AuNPs-TPP of 15.7±1.2 nm, highly homogeneous in morphology, with Z potential of 10±0.8 mV and extremely stable were obtained. Stability was demonstrated by no morphology change upon storage time, exposure to high ionic strength and with NPs concentration. AuNPs-TPP up to 250 µg/ml were not cytotoxic in A375 melanoma cells exposed to these NPs for 24 h. All together, these results show that these novel AuNPs functionalized for mitochondrial delivery can potentially be used as radiosensitizers.

Keywords: Gold Nanoparticles, Functionalization, Mitochondrial targeting, Melanoma, Multi-resistant cells

(1727) **HYBRIDS GOLD/PROTEIN NANOPARTICLES DECORATED WITH BIORECOGNITION PEPTIDES.**

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Functionalization of gold nanoparticles (Au-NPs) surface with different biomolecules has been used for several decades in rapid diagnostics tests. In last years, Au-NPs have been demonstrated can be used in therapy, imaging and delivery applications. This nanomaterial platform could combine those to generate theranostic nanoparticles.

In order to localize the theranostic effect onto some specific cells and focus the therapy, NPs targeting is mandatory by using specific peptides, organic molecules or proteins. In addition to that, it is necessary to reduce non-specific interactions with other proteins which strongly reduce the NPs enrichment in the target. It has been recently recognized that the surface of the NPs and their interaction with serum proteins (´*corona effect*´) has a pivotal role in the final destination in the body. Therefore, the coating process became in a strategic issue to develop functional nanomaterials.

In this context, the aim of this work is to prepare and characterize gold/albumin NPs (Au/Alb-NPs) decorated with DOTA-bombesin (DOTA-BB). The Alb coating is useful for reduction of the *corona effect* and Bombesin to target GRP receptor, present in several tumor cells, such as prostate and breast cancer. DOTA chelator will transport radionuclides.

The Au/Alb/BB-NPs were characterized by UV-visible, dynamic light scattering (DLS), atomic force microscopy (AFM). Determination of DOTA-BB onto surface NPs is performed by MALDI TOF/TOF and by ICP-AES.

Specific NPs uptake has been study by Cytometric Flow Analysis comparing NPs prepared by monolayer physisorption and our method decorated with the specific peptide onto PC-3 cell culture.

Decorated hybrids NPs have less unspecific adsorption and better receptor recognition than the monolayer coated NPs.

Keywords: nanoparticle, biorecognition, gold, albumin.

(130) **PAMAM DENDRIMERS AS AN EFFICIENT CARBAMAZEPINE DELIVERY SYSTEM: NANOTOXICOLOGICAL CHARACTERIZATION**

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Carbamazepine (CBZ) is on the market as an antiepileptic. It also has potential uses for the treatment of numerous neurodegenerative diseases. However, it has low solubility in aqueous media, inefficient pharmacokinetic profiles and multiple side effects, so it is important to avoid widespread distribution. Drugs delivery systems (DDS) have gained relevance in the field of nano- and bio-medicines.

PAMAM dendrimers G4.0 and G4.5 are optimal polymers as DDS that would increase the solubility of CBZ and the arrival to the brain.

Our objective was to characterize complexes between CBZ and dendrimers and to study their nanotoxicity. *In vitro* toxicity in human red blood cells was analyzed by hemolysis and in cell culture by colorimetric methods (MTT, crystal violet and neutral red). *In vivo* toxicity was studied in zebrafish animal model.

No hemolytic activity or morphological changes for any of the formulations were observed. As regards *in vitro* toxicity, different effects were observed for free drug, dendrimers and complexes, which allowed us to predict toxicity mechanism. *In vivo* toxicity showed no marked toxic effects between free drug and the complexes.

It has been developed a DDS based on dendrimers capable of efficiently transport the antiepileptic drug CBZ; what´s more, it is not toxic in *in vitro* and *in vivo* models.

Keywords: dendrimers, carbamazepine, cytotoxicity, zebrafish.

(375) **PROTEIN COATING DEFINES THE BIOLOGICAL IDENTITY OF NANOPARTICLES: RELEVANCE OF PROTEIN-PROTEIN INTERACTIONS.**

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Abstract: In the area of biomedicine, the use of nanoparticles (NPs) as carriers of therapeutic or theranostic agents has increased in the last years. Therefore, it is mandatory to understand the interaction between NPs and living organisms. In contact with biological fluids, the surface of NPs (synthetic identity) is covered with biomolecules (mainly proteins) that form a protein corona, which defines the biological identity. The protein corona formation is mediated by non-specific physical interactions, common to all molecular systems (van der Waals, electrostatics, etc.) as well as protein-protein interactions (PPI). This work is aimed at studying the protein corona formation on layered double hydroxides nanoparticles (LDH-NPs) previously modified with either albumin (LDH@ALB) or fetal bovine serum (LDH@FBS) and the cellular response of the biological identity. With such a purpose, LDH@ALB and LDH@FBS were dispersed in a protein containing cell culture medium, to compare the interfacial properties of the synthetic and biological identities and to characterize the protein corona. The interfacial properties were determined by dynamic light scattering measuring the size distribution and zeta potential. The molecules of the protein corona were identified by liquid chromatography coupled to mass spectrometry and the PPI network was evaluated with a novel approach, based on the bioinformatic tool STRING v.10.0 (http: //www.string-db.org/). The cellular response was evaluated through cytotoxicity and internalization experiments. We demonstrate that the protein corona formation was guided by PPI, through bioaffinity recognition, which led to different networks depending on the surface properties, defined by the protein coating. Furthermore, LDH@ALB and LDH@FBS are not cytotoxic and both present high levels of cellular internalization.

Keyword: protein corona, biological identity, PPI network.

(1218) **SYNTHESIS, CHARACTERIZATION AND BACTERIAL INACTIVATION OF GO-HYDROGELS COMPOSITES**

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The objective of this work, was synthesized, characterized and evaluated the antimicrobial activity of tetracycline (tet) loaded in hydrogel nanocomposites containing graphene oxide (GO) and GO plus carbon nanotubes (CNT). The preparation of poly(N-isopropylacrylamide) (PNIPAm) hydrogel nanocomposites containing GO and GO plus CNT in the polymer network is communicated. This one-pot preparation methods include the dispersion of GO (or GO plus CNT) in a solution of monomers and the subsequent polymerization. The texture of the nanocomposites was studied using scanning electron microscopy (SEM), where very compact surfaces are observed suggesting good dispersion of GO sheets and CNTs within the polymer matrix. In order to apply these materials for antibiotic delivery, the absorption of tetracycline (tet) is evaluated and the nanocomposites showed better absorption capability and improved antibiotic delivery. This result showed that the loading capacity to incorporate an antibiotic is around 50 % higher for the composites than for the pristine hydrogel. The *in vitro* antibacterial properties of composite hydrogels were investigated by agar diffusion tests against gram-negative *Pseudomonas aeruginosa* bacteria. Besides, the agar diffusion test demonstrates the composite antimicrobial activity against *Pseudomonas aeruginosa* of tet loaded composite hydrogels (PNIPAm with graphene oxide and tet and PNIPAm with GO and CNT and tet). The results clearly show that the inhibition zone around each loaded composite PNIPAm-GO-tet (1.60 ±0.2 cm) and PNIPAm-GO-CNT-tet (1.75±0.3 cm) are higher than the PNIPAm-GO and NIPAm-GO-CNT without the antibiotic which not present inhibition zone. These results suggest that tet loaded PNIPAm-GO and PNIPAM-GO-CNT display antimicrobial activity against the *Pseudomonas aeruginosa* turning these materials as potential candidates for biomedical applications.

Keywords: hydrogels composites, bacterial inactivation, *Pseudomonas aeruginosa.*

CELL SIGNALING 1

(277) **AUTOREGULATORY ROLE OF THE KINASE A ACTIVITY IN CHROMATIN REMODELING OF *TPK1* SUBUNIT PROMOTER IN *SACCHAROMYCES CEREVISIAE***

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Chromatin remodeling at gene promoters plays a critical role in initiation of transcription and the dynamics of chromatin structure are tightly regulated during this event. This process is performed by a large class of ATP-dependent chromatin remodeling complexes. *S. cerevisiae* PKA is composed by two catalytic subunits encoded by *TPK1*, *TPK2* and *TPK3* genes, and two regulatory subunits, encoded by *BCY1* gene. Previous results of our group indicate that a negative mechanism of isoform dependent autoregulation directs *TPKs* and *BCY1* gene expression and that *TPK1* promoter activity is positively regulated during heat shock. In order to understand the mechanism by which *TPK1* promoter is activated by PKA and its relation with heat stress, the regulation of chromatin remodeling by PKA during heat shock was investigated. First, we studied the nucleosome positioning using MNase assay, and three positioned nucleosomes were detected in *TPK1* promoter. Dramatic changes in chromatin structure were observed upon stress in wild type strain and in *tpk1^{w1}BCY1*, a strain with a very low PKA activity. The results indicate that the upregulation of *TPK1* promoter by a low PKA activity correlates with a remodeling in the promoter chromatin. PKA isoform specificity in remodeling of *TPK1* promoter was evaluated assessing: 1-TPK1 promoter activity in strains expressing only one of the three catalytic subunits using β-galactosidase reporter assay; 2-mRNA levels measurement by RT-qPCR and 3- the nucleosome positioning. The results indicate that Tpk2 isoform has the major inhibitor effect on *TPK1* promoter. Heat stress response is maintained in all strains. The results also show that yeast strains expressing only Tpk2 or only Tpk3 subunits lost the positioned nucleosome +1 on *TPK1* promoter. The recruitment of Tpk1 and Tpk2 catalytic subunits at *TPK1* promoter were assessed by ChIP assays. Our results point to a function for PKA activity in autoregulation of chromatin remodeling of *TPK1* gene

Keywords: Yeast, chromatin remodeling, transcription

(417) **POST-TRANSCRIPTIONAL REGULATION AND mRNA LOCALIZATION OF PKA SUBUNITS IN *Saccharo-***

myces cerevisiae

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In the yeast *Saccharomyces cerevisiae*, the protein kinase A (PKA) controls a variety of cellular events as a consequence of particular stimulus. The specificity of the cAMP signal transduction is maintained by several levels of control acting simultaneously. Our aim is to study the regulation of PKA subunits expression as one of such control levels, which is directed by the promoter's activity and the mRNA stability and localization. The balance among transcription, degradation and translation of individual mRNAs can be differentially regulated in response to environmental conditions such as heat stress. PKA holoenzyme from *S.cerevisiae* is composed by a dimer of regulatory subunit encoded by *BCY1* gene and two catalytic subunits encoded by *TPK1*, *TPK2* and *TPK3* genes. Previously we have shown that the promoter of each PKA subunit is differentially regulated during heat shock, particularly *TPK1* promoter is the only promoter activated. Here we demonstrate that *BCY1* mRNA half-life is longer than *TPK1* mRNA half-life and that *TPK1* and *BCY1* mRNAs are stabilized upon heat stress, as shown by Northern blot analysis. In contrast, Western blot analysis showed that Tpk1 protein abundance is not increased during heat shock. We also demonstrate that *TPKs* and *BCY1* promoter sequences can influence the levels of mRNAs abundance. *TPK1* mRNA localization was analyzed *in vivo* using the m-TAG methodology, showing the formation of ribonucleoprotein foci upon heat shock. Overall, the results suggest that in response to heat shock stress PKA subunit mRNAs increase their abundance and are concentrated in foci allowing a faster response once the environment is again favorable.

Keywords: PKA, mRNA, heat stress, localization, stability

(1217) **PKA AND HOG1 ROLE ON GENE EXPRESSION IN RESPONSE TO OSMOSTIC STRESS IN S*ACCHAROMYCES CEREVISIAE***

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Gene expression regulation by intracellular stimulus-activated protein kinases are essential for cell adaptation to environmental changes. The transcriptional induction of most genes whose transcription responds to salt stress is dependent on the association of the Hog1 kinase in stress-responsive genes that strongly correlates with chromatin remodeling and increased gene expression. Previously we described that PKA catalytic and regulatory subunits are associated with both coding regions and promoters of the several osmo-responsive genes in a stress dependent manner. Particularly, Tpk2 catalytic subunit activity regulates the chromatin remodelers Snf2 (SWI/SNF complex) and Arp8 (INO80 complex) association to promoters and coding regions to downregulate the gene expression during osmotic stress. Here, we focus on the interplay between PKA and Hog1 kinases on gene expression in response to osmotic stress. We tested for genetic interaction between *TPK2* and *HOG1* genes for cell survival to osmotic stress. While *tpk2*D mutant strain did not confer sensitivity to osmotic stress, *hog1*D mutant strain showed hypersensitivity to osmotic stress. The double mutant *hog1*D*tpk2*D displayed high tolerance to salinity suggesting that both kinase has an opposite role in cell adaptation to osmotic stress. ChIP assays showed a correlation in the *in vivo* kinetic recruitment of Tpk2 and Hog1 to *HSP42* coding region and *RPS29B* promoter region in response to osmotic stress, with a maximum at 5-10 minutes post-osmotic stress. We then analysed the nucleosome positioning at the promoter and coding region in *the RPS29B gene* by Micrococcal Nuclease (MNase) digestion of chromatin before and after stress in wild type. We found that osmotic stress, promotes an increase of compactness of the chromatin that correlated to downregulation of *RPS29B* gene expression. Our results suggest that transient stress adaptive response could be regulated by opposite roles of Hog1 and PKA.

Keywords: yeast, osmostress, transcription, PKA, Hog1

(1269) **PROTEIN KINASE A: BEING IN THE RIGHT PLACE AT THE RIGHT TIME**

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Capacitation is the process by which mammalian sperm acquire the ability to fertilize and can be mimic *in vitro*. Once completed, sperm obtain acrosomal responsiveness and a hypeactivated flagellar movement.

When sperm are exposed to HCO^{3-}, there is rapid intracellular increase in cAMP, that activates PKA, which acts as a key player during capacitation. Its regulation has been mainly studied focusing on its **catalytic activity**. However, biological activity is also dependent on its **subcellular localization**, which is mediated by interaction with scaffolding AKAP proteins.

Here, we study the role of PKA anchoring **during** capacitation murine sperm. The binding and anchoring of PKA to AKAP was affected by the peptide st-HT31, which disrupts PKA-AKAP interaction without affecting catalytic activity.

When PKA anchoring was impaired ***throughout*** capacitation, sperm neither acquired acrosome responsiveness (assessed by PNA-staining of the acrosome), nor hyperactivated motility. Phosphorylation of PKA substrates as well as tyr phosphorylation were also blocked, as evaluated by western-blot.

Conversely, when PKA de-anchoring was induced in capacitated sperm, AR was triggered by a mechanism that involved 1) **membrane depolarization**, and 2) **Ca^{2+} influx**, seemingly through CatSper channel. These events were assessed by single-cell microscopy and population fluorimetric measures, using specific-sensitive dyes.

Addition of the inhibiting peptide st-HT31 induced AR only in capacitated sperm but not in non-capacitated cells, showing there are capacitation-associated events that prime the sperm for this response to PKA de-anchoring. In addition, AR needed active PKA activity, since inhibition of PKA right before st-HT31 exposure impaired AR. Moreover, an inactive analogue of st-HT31, named st-HT31p, did not show any effect.

Overall, we show that PKA anchoring is essential for its biological activity in sperm processes.

Keywords: PKA, sperm capacitation, AKAP, Acrosome reaction

(1237) **PROTEOME WIDE ANALYSIS OF GRANULAR PROTEIN COMPLEXES EVOKED BY DIFFERENTIAL HEAT STRESS STRIGENCY IN *SACCHAROMYCES CEREVISIAE***

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Cellular responses to stress comprise a variety of different mechanisms, including translation arrest and the relocation of translationally repressed mRNAs to ribonucleic particles (mRNPs) like stress granules (SGs) and processing bodies (PBs). Given recent evidence on the role of liquid phase transition in signalling and cytosolic mRNPs formation, it is possible that SGs might represent regions where certain processes or the activity of enzymes are concentrated. Our results from biochemical approaches and microscopy analysis show that under mild heat stress the catalytic subunit of PKA isoform Tpk3 aggregates and promotes aggregation of eIF4G, Pab1 and eIF4E, whereas severe heat stress leads to the formation of PBs and SGs that contain the Tpk2 isoform and a larger 48S translation initiation complex. PKA affects the translational response to heat stress, where each Tpk catalytic isoform appears to have a different role, with Tpk2 and Tpk3 playing negative and positive roles in the translational response, respectively. We favour a model where depending on the severity of an external stimulus, such as

heat stress, each catalytic isoform of PKA interacts with a complex network of distinct protein factors and potential substrates.

Our ongoing studies are focused on a global characterization of protein complexes under different heat stress conditions. To this end, we performed a label-free quantitative proteomic analysis of granular enriched fraction from mild and severe heat stress using a QExactive. We identified proteins exclusively enriched in mild and severe heat stress, as well as proteins common to both groups. Gene Onthology analysis showed annotations associated with several GO biological processes, such as structural constituent of ribosome, RNA binding and translation factor activity. Networks built from these results will let us start defining how different degrees of severity of the same stress evoke a specific response on RNP assembly.

Keywords: yeast, proteomics, heat-stress, mRNP granules.

(1069) **A NOVEL PHOSPHATASE GOVERNS STRESS GRANULES' DYNAMICS**

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Fundacion Instituto Leloir

Stress granules (SGs) are macromolecular aggregates of mRNAs and proteins that belong to a growing family of "liquid organelles". SGs are specific to the cellular stress response and form transiently upon acute stress.SGs are dynamic and exposure to polysome-stabilizing drugs such as cycloheximide induces SG disassembly, by "trapping" messengers in polysomes. We performed a high-throughput RNAi screen in Drosophila cells to identify novel signaling pathways that regulate SG dynamics. Here, we focus on the role of a novel phosphatase (NP) that showed a high score in the primary screen. We used fly and mammalian cell lines, specifically HEK293T and U2OS,exposed to arsenite, a well-known model of oxidative stress, in combination with imaging and RNAi. We found that NP KD increases SGs formation in both Drosophila and mammalian cells ($p<0.05$). Conversely, the overexpression of NP impairs SGs assembly ($p<0.05$).In addition, SGs formed in NP KD cells are resistant to cycloheximide-induced dissolution ($p<0.05$). We investigated the subcellular localization of NP in U2OS cells, and found that NP is mostly nuclear, and concentrate in discrete domains. NP is not present in speckles but SF2 -a speckle marker- partially colocalizes with NP nuclear domains. Polyadenylated RNA stained by FISH is excluded from NP domains. We wonder whether NP changes localization upon stress and/or if it is recruited to SGs. We found that the nuclear staining becomes homogeneous and the NP domains vanish. Also, we found that NP partially mobilizes to the cytosol, and is not significantly recruited to SGs. Finally, western blot assays revealed that NP levels rapidly decline immediately after the oxidative stress stimulus (60% of basal levels; $p<0.05$). We hypothesize that the degradation of NP triggered by stress facilitates SGs formation and, accordingly, the KD potentiates the response. Relevant NP targets are currently being investigated. We thank ANPCyT, CONICET and UBA, Argentina for funding.

Keywords: Stress granules, cellular stress response, phosphatase, protein decay

(399) **PRE-EQUILIBRIUM PROPERTIES OF SIGNALING DOSE-RESPONSE CURVES ALLOW DISCRIMINATION BETWEEN NEGATIVE COOPERATIVITY AND INDEPENDENT BINDING**

Federico Sevlever, Juan Pablo Di-Bella, Alejandro Colman-Lerner, Alejandra Cristina Ventura
IFIBYNE

Previous works in cellular signaling propose to study pre-equilibrium properties in order to distinguish between negative cooperativity (NC) and independent binding (IB). We have already presented the existence of a mechanism known as Pre-Equilibrium Sensing and Signaling (PRESS), which allows cells to discriminate between signaling levels that saturate receptors in equilibrium.

In this work, we combine analytical-computational studies of two toy models of a ligand-receptor binding with two binding sites, one model presenting NC and the other, IB. Using statistical analysis of the parameters space, we have discovered Hill coefficient pre-equilibrium general properties whichallows to design new protocols of discrimination between NC and IB. We have tested the efficiency of these protocols for simulated data from both models and its dependency with noise from chemical reactions stochasticity.

We found that pre-equilibrium Hill coefficient adds information and improves previous discrimination protocols.

Keywords: Signaling, cooperativity, Hill coefficient.

(1377) **BIOCHEMICAL TRANSDUCTION MEDIATED BY AN AMPEROMETRIC SIGNAL: HORSE RADISH PEROXIDASE IMMOBILIZED IN A LAPONITE/POLYMER HYDROGEL**

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A device composed by a biological receptor and a transducer is defined as a biosensor, where a biomolecule detects a substance and the transducer, interprets and "translates" the biological recognition reaction into a quantifiable signal. Both components are responsible for biosensor sensitivity and selectivity, as well as give rise the ability of direct and real time analysis. This work proposes the design and development of an electrochemical biosensor including the enzyme horseradish peroxidase (HRP) into matrices of mineral nanoparticles (Laponita®), gold nanoparticles (AuNP) and polymers of vinylbenzyl thymine and vinylbenzyl ammonium units (VBT-VBA). Hydrogels were prepared mixing laponite and polymer with different amounts of HRP and AuNP, deposited on a glassy carbon electrode surface. The biosensor experimental variables were optimized and the analytical characteristics were determined. The analytical signal was obtained by amperometry. The stability and the functionality of the hydrogel film are influenced by a mass/charge ratio resulting in a balance between the components involved. The optimal composition of the hidrogel was: 30 µg laponite + 15 µg polymer + 15 µg HRP and 2.5 µl AuNP solution. The immobilization of the biomolecule is fundamental in the design and development of a biosensor and the laponite demonstrated optimal qualities in this sense. On the other hand, the thymine biopolymers make possible the stabilization of these structures and the AuNP improve the current signal increasing the conductivity of the hydrogel, this produce suitable microenvironments in which the biomolecules can retain their biological and biocatalytic activity. The developed biosensor showed outstanding characteristics, reason why it is potentially applicable to the analysis of real samples.

Keywords: biochemical transduction, amperometric signal, horse radish peroxidase, hydrogel.

(1571) **BIOENGENEERING OF THERMOSENSORS**

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Living organisms are exposed to changes in their environment and the ability to adapt to such changes is crucial for survival. Adaptation to temperature changes has particular importance in poikilothermic organisms such as bacteria, plant and fish. Two-component systems (TCS) are tools that bacteria employ to detect changes in their environments and execute an adaptive response. Understanding the molecular and biophysical bases of bacterial signal transduction is key for drug design and biotechnology. DesK / DesR TCS from *B. subtilis* is involved in adaptation to temperature changes. Using this system as a model we study the mechanism of temperature perception of the histidine kinase DesK containing five transmembrane segments (TMS). To address this challenge, we constructed a chimerical protein that presents a single TMS but retains the ability to detect temperature and membrane fluidity. We focus on responding: What mechanism uses DesK to control gene expression? What is the difference between a sensor TMS and a structural TMS? Do

regions of the protein that are not involved in the thermodetection respond to any other stimulus? We have identified three determinants of thermodetection that allow to activate the catalytic domain of DesK. We retuned these determinant of thermodetection to improve the sensing capacity and incorporated them into an inert poly-valine scaffold and recovered activity. The significance of this work is that the designed sensors function as a modular reversible system, which can be incorporated into different genetic circuits to control the expression of genes in the study of cells. This is relevant in medicine and industry, because the temperature is an important tool to manipulate gene expression, which allows cells to be stimulated reversibly and non-invasively to generate responses of interest.

Keywords: Bioengeneering, Thermosensors.

(330) **INVOLVEMENT OF WNT/ β-CATENIN INHIBITION IN OVARIAN CANCER CELL LINES PROLIFERATION AND APOPTOSIS**

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Wnt/β-catenin is a highly conserved pathway which regulates proliferation, apoptosis and differentiation. While it has been demonstrated to participate in cell growth of different biological systems, no studies have been performed in ovarian cancer cell growth regulation. To evaluate the involvement of Wnt/β-catenin pathway in ovarian cancer cell proliferation and apoptosis, two human epithelial ovarian cancer cell lines (IGROV-1 and SKOV-3) were incubated in the presence of a Wnt inhibitor ICG-001). The cells were incubated in the presence of ICG-001: 1, 10, 20 and 50 μM or with no stimulus (control). After 48hs of incubation, we harvested the cells to count them and we performed protein extracts for western blot technique. We also determined apoptosis by citometry: cells were double-stained with Annexin V-fluorescein isothiocyanate (FITC) and propidium iodide (PI).

Our results showed a substantial and dose dependent decrease in proliferation when both cell lines were incubated in the presence of ICG-001. We also observed a decrease in protein levels of Wnt/β-catenin and CyclinD1, when cells were treated with the inhibitor (with all the concentration used). Regarding to apoptosis, we observed a low percentage of apoptotic cells (<5%) among cells treated with 1μM ICG-001 and the control group. The percentage of apoptotic cells notably increased (> 25%) in cells incubated with 20 and 50 μM ICG-001.

In conclusion, we demonstrate that Wnt/β-catenin signalling regulates ovarian cancer cell lines proliferation and apoptosis.

Keywords: ovarian cancer, Wnt/β-catenin, proliferation, apoptosis

BIOPHYSICS 1

(956) **A VIEW ON PROTEIN CONFORMATION BY GENERAL METHYLATION OF SURFACES. AN NMR APPROACH.**

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Abstract: The solvent accessible surface area (SASA) of the polypeptide chain plays a key role in protein folding and interactions. However, this fundamental parameter eludes direct analysis. Our approach involves the reaction of the minimal photochemical reagent diazirine (DZN) with polypeptides. Because of its size, DZN (i) mimics water, and (ii) shows narrow chemical selectivity, due to the extreme reactivity of methylene carbene (MC). Detection of products by NMR benefits from the fact that it does not demand cleavage of the polypeptide. The extent of MC reaction at various sites across the surface of E. coli thioredoxin (TRX) was assessed. The preservation of conformation and stability of the labeled samples was evaluated by circular dichroism (CD) and fluorescence emission. The dominant phenomenon involves methylation of amino acid side-chains, as attested by the enrichment of the aliphatic region in ^{1}H-NMR spectra. In the unfolded state, an enhanced and broad methylation profile points to extensive solvent exposure. ^{1}H-^{13}C-HSQC spectra of native TRX reacted with ^{13}C-DZN reveal new cross-peaks corresponding to water-exposed methyl groups. ^{1}H-^{15}N-HSQC spectra reveal the different impact of the reaction on backbone amide environments. The relative intensity of CHα spots is indicative of the extent of methylation at individual amino acid residues. Moreover, CHβ, CHγ and CHδ cross-peaks pinpoint details of side-chain modification. A fully consistent pattern emerges from both HN and HC profiles. Outer as well as inner (cavity) surface components become prime targets of methylation. Because of its mild reaction conditions and strong emphasis on side-chain modification, the MC labeling approach emerges as a distinctive footprinting method. All in all, the newly gathered spectral data highlights a rich panorama on protein conformation.

Keywords: protein conformation, solvent accessible surface area, footprinting technique, NMR, thioredoxin.

(489) **CONFORMATION AND STABILITY OF A RECOMBINANT VARIANT OF HUMAN FRATAXIN PRECURSOR**

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Abstract: Friedreich´s Ataxia is a disease caused by a decrease in the expression frataxin (FXN) and loss of FXN functionality. The outcome of the lack of FXN function is an inefficient iron-sulfur cluster assembly, with widespread enzymatic deficit and oxidative damage. FXN is translated in the cytoplasm as a precursor of 210 residues (FXN1-210). Then, this protein is imported into the mitochondrial matrix and processed to the mature form (FXN81-210). One outstanding strategy to increase the concentration of active FXN inside mitochondria involves the production of recombinant variants with the capability of crossing cell membranes, thus yielding an active and stable form of the protein.

We investigated the conformation and folding of the recombinant precursor variant His6-TAT-FXN1-210 that includes a cell-penetrating peptide (TAT) in the N-terminal region (NTR) and a His tag. We studied the thermal unfolding process by circular dichroism (CD). Results showed unfolding is not reversible unless a given GmdCl concentration is added to the protein sample. In this case, the process is completely reversible. Neither urea nor NaCl exhibit the complete effect observed for GdmCl, but a combination of both does it. These results suggest that (i) the NTR establishes interactions favoring FXN aggregation; (ii) aggregation is enhanced at high temperatures but may be attenuated by the presence of additives; and (iii) NTR-mediated interactions are of both sorts: ionic and apolar.

His6-TAT-FXN1-210 was studied by light scattering. It is mainly monomeric and, at subdenaturing concentration of GdmCl, it exhibits a R_h larger than the expected for fully-folded protein. According to this, Far-UV CD spectra and secondary structure predictions suggest that the NTR is disordered. To know whether His6-TAT-FXN1-210 is active, we performed a NFS1 activation assays. Results suggest that FXN function is conserved in this variant.

Keywords: Frataxin, conformation, folding process, Friedreich´s Ataxia, protein replacement therapy

(1713) **LINKING THE OLIGOMERIC STATE TO FUNCTION IN Trypanosoma cruzi HPRT**

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Hypoxanthine phosphoribosyltransferase (HPRT) is the key enzy-

me of the purine salvage pathway. It is essential for the survival of trypanosomatids, organisms that -unlike humans- cannot benefit from de novo nucleotide synthesis. As such, this enzyme has been proposed as a potential target for drugs aimed at treating parasitic diseases. This study underscores the effect of different ligands on the structure, stability and function of T. cruzi HPRT (TcHPRT). Light scattering experiments indicate that the protein adopts a tetrameric arrangement. Interestingly, proteolytic removal of the C-terminal region (CTR) yields a well-defined dimer showing somewhat increased activity. Considering that TcHPRT is imported to glycosomes, one can hypothesize that once inside the organelle, the CTR -which includes the import signal- could be excised giving rise to the dimer. The activating effect observed at low concentrations of olpadronate and ibandronate, bisphosphonates (BPs) analogues of the substrate phosphoribosyl pyrophosphate, is more evident for the tetramer than for the dimer. Correspondingly, their inhibitory action at high concentrations is less marked for the former. These facts fall into place in our proposed cooperative model for the activity of this oligomeric enzyme. Strikingly, the inhibitory effect is essentially null when assayed against human HPRT. To assess the action of these small molecules within a cellular environment, we exposed cultures of epimastigotes to selected BPs, demonstrating significant growth inhibition. To visualize the enzyme forms that might be interacting with those ligands, we expressed both proteins in Leishmania tarentolae as fusion constructs with GFP. Western blot analyses reveal that TcHPRT is not processed to a shorter form in this system. This new molecular and cellular knowledge becomes most relevant for the design of innovative parasite-targeted therapeutics.

Keywords: T. cruzi, oligomeric state, enzymatic activity, maturation

(820) **MONITORING METHIONINE SULFOXIDE REDUCTASE ACTIVITY IN NATIVE ENVIRONMENTS BY NMR**

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Abstract: Oxidation of methionine side chains, which has been traditionally perceived as damage derived from oxidative stress, is now emerging as a post-translational modification capable of regulating protein activity and cellular processes such as the activation of Ca^{2+}/Calmodulin dependent kinase II or remodeling of the acting cytoskeleton. Methionine oxidation leads to two diastereomers known as the R and S forms of methionine sulfoxide (MetOx). In vivo, MetOx is reduced back to methionine by two families of stereospecific and conserved methionine sulfoxide reductases, MSRA and MSRB, which specifically target protein-bound MetOx-R and -S isomers, respectively. Methionine oxidation is amongst the most difficult-to-study post-translational protein modification in vivo because sulfoxides are not easily detected with chemical probes or antibodies and because they spontaneously form during sample preparation for mass spectrometry. By contrast, MetOx display unique NMR signals, including different sets of resonances for its R and S isomers that are readily identified in the NMR spectra. In order to assess the role of MSRs in cellular function we ought to monitor their activities in vivo with high resolution. In this work we designed an NMR based assay to simultaneously delineate MSRA and MSRB reductase activity in vitro and in cell extracts and validated its use for further in vivo studies using In-cell NMR methodologies. Our results showed that MSRA is more active than MSRB in E. coli cells and those bacteria have free MetOx reduction activity, in line with previous reports. We expect that our results will open new research paths to expand the repertoire of MetOx-regulated signaling pathways and to further understand the role of MSRs in physiology and pathology.

Keywords: methionine oxidation, methionine sulfoxide reductase, In-cell NMR, cell signaling

(474) **NEW INSIGHTS ON ICA512 AND INSULIN INTERACTION**

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Abstract: ICA512/IA–2/PTPRN is a decoy receptor protein tyrosine phosphatase enriched in secretory granules (SGs) of the pancreatic β-cells and other neuroendocrine cells. Previous studies imply its involvement in the biogenesis, trafficking and exocytosis of insulin SGs, as well as in β-cell proliferation. Its regulated endocrine-specific protein 18 homologous domain (RESP18HD) is necessary and sufficient for the sorting of ICA512 to SGs of rat insulinoma INS-1 cells and its binding with high-affinity to insulin and proinsulin. In previous studies we observed the formation of complexes between insulin and RESP18HD 35-131 by co-aggregation in vitro. We now show by EM negative staining that complexes of insulin and RESP18HD are amorphous, in contrast to RESP18HD alone, which instead self-aggregates in a moderately ordered fashion. ICA512-RESP18HD consists at least two distinct regions, including a N-terminal Cys-rich motif (aa 35-90) and an intrinsically disordered region in the C-terminal part of the domain (aa 91-131). Fluorescence microscopy of RESP18HD-TQ2 tagged fusion protein variants in INS-1 cells shows that the region encompassing the signal peptide (aa 1-34) followed by the Cys-rich motif of the RESP18HD (aa 35-90) contains sufficient information for SG targeting. Moreover, co-expression of different RESP18HD-TQ2 variants with Insulin-Venus fusion to assess Fluorescence lifetime imaging-fluorescence resonance energy transfer (FLIM-FRET), revealed moderate interaction (FRET) of these proteins also in the cells. These results further underscore the enabling role of ICA512-RESP18HD in targeting insulin and possibly other cargoes to the SGs.

Keywords: aggregation, insulin, SG targeting

(1179) **SOLUBLE LIKE STRUCTURE-FUNCTION OF β-GAL DESORBED FROM INCLUSION BODIES**

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Bacterial inclusion bodies (IBs) are mesoscopic protein aggregates commonly observed in transformed bacteria, primarily formed by recombinant proteins. Historically, IBs have been considered a hurdle for the production of soluble protein species, and many genetic and process-based strategies have been developed to minimize their formation. However, recent research has described the use of IBs as functional materials usefull as reusable catalysts, drug delivery systems, and functional topographies in tissue engineering [1]. We have proved that β-galactosidase (β-Gal) IBs ($IBs_{\beta\text{-}Gal}$) can be found in an amyloidal form which holds in non-amyloidal functional proteins with some particular stability properties. Also, we have demostrated that β-Gal desorbes spontenously from IBs in low osmotic pressure media (achieve by successive dilutions). In this work we study the structure/function relationship of the desorbed β-Gal ($\beta\text{-}Gal_D$). By means of intrinsic fluorescence, infrared spectroscopy (IR) and enzymatic activity experiments we have got evidence that $\beta\text{-}Gal_D$ retains the structural/functional properties of the soluble protein. Furthermore, by means of IR and DLS experiments in real time, we have found that while $\beta\text{-}Gal_D$ is spontaneously released from the aggregates a reorganization of IBs occurs. This results demonstrate that the IBs isolation and the concomitant dilution steps of this sample is a simple and a proper methodology to obtain active protein. Hence, the nature of the recombinant protein and the conditions for IBs formation and isolation determine the success of the desorption of the protein in a soluble-like conformation.1.Rinas, U., et al., Bacterial Inclusion Bodies: Discovering Their Better Half.Trends in

BiochemicalSciences, 2017.

Keyworks: inclusión bodies, β-galactosidase, structure-funtion, desorption

(497) **STUDY OF INTERDOMAIN INTERACTIONS THROUGH THE USE OF PARAMAGNETIC TAGS**

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Micro-RNAs (miRNAs) are a class of small endogenous RNAs that negatively regulate the expression of mRNAs through the recognition of complementary sequences. Proteins with double stranded RNA binding domains (dsRBD) are essential in miRNA biogenesis. These proteins recognize pri-miRNA precursors in plants lacking conserved sequences. However, this process is not well characterized. With the aim of understanding the process of recognition of this type of domains, we decided to carry out a structural study of the two dsRBDs domains of HYL1 from Arabidospsis thaliana. The N-terminal portion of HYL 1 containing two dsRBD domains connected by a native loop is sufficient to complement the hyl1 mutant phenotype. We generated constructs with 14 residue lanthanide-binding tags (LBTs) at each end of either domain, and performed PRE (Paramagnetic Relaxation Enhancement) experiments by measuring the signal intensity for each residue in the protein loaded with either Lu(III) as diamagnetic control, or Gd(III), a paramagnetic metal. Unpaired electrons in Gd(III) cause an increase in the relaxation velocity of nearby nuclei as a function of the distance to the metal. Based on the results obtained in each domain, we modeled the dynamics allowed by the loop that connects them. We observed that the movement of the domains is not completely random, and that there is a predisposition for forms connecting the N-terminal end to the second dsRBD domain. Once free system is fully characterized, the complex between these proteins and miRNA precursors will be further studied, in order to better understand how these domains recognize their substrate and thus precisely position the active site of the complex in the precursor.

Keywords: PRE (Paramagnetic Relaxation Enhancement) LBT (Lanthanide Binding Ta) dsRBD (Double-stranded RNA-binding domain)

(1227) **SURFACE EVALUATION TECHNIQUES TO CHARACTERIZE "DE NOVO" DESIGN ANTIMICROBIAL PEPTIDES**

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Conventional antibiotics are facing strong microbial resistance that has recently reached critical levels. This situation is leading to a reduced therapeutic potential of a huge proportion of antimicrobial agents currently used in the clinic. Antimicrobial peptides (AMPs) could provide the medical community with an alternative strategy to traditional antibiotics for combating microbial resistance. However, fully understand their mechanisms of action and promote the reduction of the unwanted toxicity remains as a critical step to consider these compounds to be a therapeutic promise and overcome clinical setbacks. In this context, this work focused on unravel the way of action of de novo designed AMPs by using surface evaluation techniques as Surface Pressure, Surface Plasmon Resonance (SPR) and Atomic Force Microscopy (AFM). Surface pressure results obtained well correlate the highest affinity toward negatively charged membranes with the lowest CIM values. Whereas SPR technique that allows to obtain association (ka) and dissociation (kd) constants and calculate the fraction of the peptide that remains bound to the lipid membranes after dissociation (Rb), allows to dissect the kind of interactions. SPR results showed that beside the importance of a high membrane affinity, that allow the peptides reach the membrane, this Rb parameter complements the analysis and helps to clarify misinterpretations of the Kd data, confirming that Is also important that peptides remained strongly bound to membranes (i.e. high Rb). Finally, by AFM we were able to visualize the effects of AMPs directly on the bacterial cells. Error and height images showed that after 1h of peptide incubation, bacteria surface was severely damaged and leakage of intracellular media was also observed.

Overall, our studies focus on understanding possible relations between AMP–membrane interactions and the efficiency of these drugs, offering a rational basis for the improvement of these compounds.

(1416) **TFE UNVEILS THE EXISTENCE OF INTERMEDIATE SPECIES IN INTESTINAL FATTY ACID BINDING PROTEIN (IFABP), A CLASSICALLY RECOGNIZED TWO STATE PROTEIN MODEL**

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IFABP is a 15 kDa protein exhibiting a β-barrel fold that resembles a clamshell consisting of two perpendicular five-stranded β-sheets with an intervening helix-turn-helix motif between strands A and B. Despite the fact that naturally occurring β-sheet proteins avoid aggregation through a variety of strategies, the addition of high concentrations of a structure-promoting cosolvent such as 2,2,2-trifluoroethanol (~ 25% v/v TFE) induces conformational rearrangements that trigger the onset of amyloid-like aggregation. Previous work from our group revealed that, in equilibrium, low concentrations of TFE (up to 15 % v/v) foster conformational changes akin to those leading to aggregation-prone species. Most significantly, the protein remains functional as attested by its capacity to bind fatty acids. As retaining function is a signature of the native state, it can be concluded that this level of co-solvent favors the population of alternative conformations scarcely explored in water. In view of this fact, we decided to evaluate which were the most relevant conformational rearrangements.

Hardly any structural change in IFABP is observed by the full preservation of secondary structure and the minimal perturbations in tertiary structure, evidenced by the circular dichroism (CD) spectra and the unaffected λmax of fluorescence emission. Strikingly, even TFE concentrations as low as 2.5% turn the protein susceptible to thermal irreversible unfolding. Interestingly, data derived from guanidinium chloride-induced equilibrium unfolding transitions reveal the population of an intermediate species. This result demonstrates for the first time that IFABP does not follow a simple two-step folding behavior in equilibrium. Full characterization of this hidden intermediate might shed light on the genesis of aggregation-prone species.

Keywords: β-barrel; TFE; protein aggregation; protein folding intermediates; amyloids

(1667) **FATTY ACIDS EXCHANGE ON CONFINED PROTEIN. X-RAY EVIDENCE AND MD EXPLANATION.**

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The fatty-acid-binding proteins (FABPs) act as intracellular lipid chaperones. The structure is a beta barrel surrounding an inner cavity that accommodates fatty acids, enabling their transport in an aqueous media inside the cell. But the mechanism of lipid binding and release is still the topic of many studies. This exchange involves at least three stages: opening a portal at the protein surface, ligand entry/release, and portal closure. It is challenging to obtain experimental evidence on the functionality of the portal without modifying the amino acid sequence or the protein conformation. Studying these processes in the crystallized protein opens a unique possibility, because when confined in the crystalline network the conformational changes are limited by the crystal packing. We soaked a FABP-Palmitic acid crystal with Br-palmitic acid, using the heavy Br atom as a marker. After solving the structure at 1Å resolution, we observed

Br-palmitic acid inside the cavity, proving that ligand exchange does take place in the crystal. However, the crystal structure does not differ significantly from the already resolved structures, and does not show details of the ligand exchange. Since the accuracy of our structure provides a reliable starting point for Molecular Dynamics simulations, we build a model of 2 unit cells (8 proteins), and properly choosing the simulation box, we model the whole crystal by means of the periodic boundary conditions. After 2 μs of simulation at constant volume (NVT ensemble), the computational results correctly describe experimental behavior. MD simulation shows details of possible pathways for lipid release that are not visible by diffraction techniques, independently of their quality. Therefore, this simulation provides a complementary methodology to fill in the gaps left by the crystallographic images of the conformational average. The joint use of diffraction techniques at subatomic resolution, and molecular dynamics from reliable models, are consequently confirmed here as a powerful set of tools.

Keywords: FABPs, Crystal, X-ray, Molecular Dynamics.

STRUCTURAL AND FUNCTIONAL BIOCHEMISTRY 1

(752) CHARACTERIZATION OF MALATE METABOLISM ENZYMES INDUCED DURING SOYBEAN SEED FILLING

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Seeds are of great economic interest due to the value of their organic composition. The metabolic pathways leading to the deposition of triacylglycerols and proteins in seeds are complex in terms of the amount of enzymes involved, regulatory context and variability between species. Previous studies of our group indicated that NAD- and NADP-dependent malic enzymes (ME) participate in the provision of reducing power and citrate or acetyl-CoA, respectively, to the pathways of reserve biosynthesis during soybean maturation. In this context, the aim of this work was to analyze the biochemical and regulatory properties of the enzymes belonging to this family. For this, the different ME isoforms were obtained through cloning, heterologous expression in *Escherichia coli* and subsequent purification. Our results indicated that the only plastidic NADP-ME detected in seeds has a high affinity for its substrates malate and NADP. Furthermore, it is activated by glutamine, the main nitrogen compound that is imported to the seed to support the synthesis of reserves. On the other hand, the mitochondrial NAD-ME isoforms also showed an important positive regulation by metabolites present in the seed, being NAD-ME2.4 (the product of the most abundant transcript throughout the filling) the most regulated. The activation by fructose-1,6-bisphosphate and phosphoenolpyruvate, both produced during irreversible steps of glycolysis, indicates a coordination between this pathway and the malate metabolism. In turn, the activation of NAD-ME proteins by CoA and acetyl-CoA suggests their involvement in fatty acid biosynthesis. These findings provide new insights into intermediary metabolism in oilseeds and nominate ME as new biotechnological tools to be used in breeding programs.

Keywords: intermediate metabolism, malic enzyme, oilseeds, proteins, lipids

(816) MUTATIONAL APPROCHES TO OBTAIN LOCKED CONFORMATIONS OF THE BACTEROIPHYTOCHROME FROM THE PLANT PATHOGEN *XANTHOMONAS CAMPESTRIS*

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Light modulates the host-microbe interaction and several virulence-related phenotypes in the plant pathogen *Xantomonas campestris*. This is mediated by its bacteriophytochrome, XccBphP.

Bacteriophytochromes are photoreceptors found in bacteria that bind biliverdin (BV) as their chromophore. They typically present a red-absorbing form (Pr) and a far-red absorbing form (Pfr).

XccBphP is a bathy-like phytochrome. This means that it exhibits a dark conversion from Pr to Pfr, reaching an equilibrium Pfr:Pr ratio of 85:15 (pH 7.7) with a half-life of 7.45 h.

XccBphP presents at the N-terminus a PAS2-GAF-PHY domain triad as its photosensory module, and a PAS9 domain as its output module. In order to get insight into the light-dependent activation mechanisms, the crystal structure of the full version of XccBphP was recently solved by our group. The solved structure shows a parallel homodimer arrangement along a central helical spine in the Pr state.

With the aim of obtaining XccBphP versions locked in Pfr or Pr states to perform molecular, structural and physiological studies, we are carrying out rational and random mutagenesis approaches in several key positions likely to affect its photochemical behavior. The analysis is accomplished by UV-VIS spectroscopy.

By this means, we have identified the mutant G454E, which exhibits a 100% Pfr conversion in the dark. This is reminiscent of the monomeric ΔPAS9 mutation. Hence, we performed a static light scattering determination of its molecular weight. The results show that the oligomeric state of the mutant is a dimer. Thus, we have obtained a full-length dimeric variant with a stable (but not locked) Pfr state.

Currently, a crystallogenesis screening of XccBphP G454E in dark conditions is being performed. We hope to obtain a crystal structure in the Pfr state to compare it to the already solved structure and elucidate the light-driven conformational changes.

Keywords: Xanthomonas campestris; bacteriophytochrome, biliverdin

(836) DISSECTING THE MOLECULAR PROCESS OF N-END RULE SUBSTRATE RECOGNITION AND DELIVERY TO CHAPERONES BY THE CHLOROPLASTIC N-RECOGNIN ATCLPS1

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Proteolysis is one of the main strategies for proteostasis maintenance. In chloroplasts of *Arabidopsis thaliana*, the caseinolytic protease (Clp) is the main proteolytic system. It is composed by the protease ClpPR (a complex of 9 different proteins), the Hsp100 unfoldases ClpC1/2 and AtClpS1, an N-recognin who mediate substrate recognition. N-recognins identify structural features in the N-terminal end of the target, a process known as the N-end rule. Bacterial ClpS is extensively characterized, mainly due to the possibility of reconstituting the bacterial Clp system *in vitro* and evaluating the degradation of fluorescent targets, a strategy that could not be replicated for the chloroplast system. To unravel the specificity of the plant proteolytic machinery and to exploit the usefulness of the *in vitro* activity of the bacterial system, we rationally designed five chimeric proteins using the AtClpS1 backbone, exchanging different pieces of its sequence with the corresponding part of bacterial ClpS. We focused specially on the parts that mediate binding to the bacterial chaperone with the ultimate goal of obtaining a fully functional bacterial system but with plant specificity. The five chimeras were successfully obtained in recombinant form and purified. Preliminary results show that the N-terminal end of AtClpS1 is essential for substrate delivery, as the chimera NtEcClpS::Nt-AtClpS1 greatly inhibited the degradation of fluorescent substrates. Yet in another approach, we designed an AtClpS1 protein, where the predicted AtClpS1 amino acids involved in Hsp100 interaction were mutated. Pull down experiments showed that these amino acids are crucial for AtClpS1 and ClpC2 interaction. This mutant will be used to fish out substrates from the chloroplast stroma and sequence their N-terminal end. Our work provides new molecular tools to unravel the N-end rule in chloroplasts.

Keywords: proteolysis; N-end rule; N-recognin; chloroplasts

(841) PROBING THE SPECIFICITY OF THE CHLOROPLASTIC N-RECOGNIN ATCLPS1 WITH FLUORESCENT PROBES BEARING DIFFERENT N-TERMINAL ENDS

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Proteolysis is a highly regulated process as useful proteins must not be inadvertently eliminated. One of the mechanisms of target selection for degradation is recognition of N-degrons by an N-recognin. An N-degron is structural feature of the N-terminal end which is recognized by proteins (N-recognins) that deliver the target to a protease o mark it for degradation. In bacteria, the N-recognin ClpS recognizes destabilizing amino acids in the N-terminus, like Phe, Tyr, Trp and Leu, binds to the substrate and presents it to the ClpAP protease. Binding of ClpS is also enhanced by positive charges in second position; proteins starting with Phe-Arg (FR) are degraded within seconds. In chloroplasts of *Arabidopsis thaliana*, a homolog of bacterial ClpS has been found (AtClpS1) so it is believed that proteolysis of chloroplastic proteins is similar, although experimental evidence is lacking. Moreover, previous results by our group have shown that the specificity of AtClpS1 is different from bacterial ClpS as positive charges in second position seem to be detrimental for recognition. We created fluorescent probes to survey the specificity of AtClpS1, as it was done for bacterial ClpS. The N-terminal end of the green fluorescent protein was mutated to FR/FE/EE/EL and SA, being the last two N-terminal ends from two proposed natural substrates. The fluorescent probes were obtained in recombinant form and purified to homogeneity. By pull down assays and fluorescence anisotropy experiments, we show that AtClpS1 can bind to substrates with negative charges in the first and second position. This is the first experimental evidence of an N-recognin of the ClpS family with predilection for negative charges at or near the N-terminal end. Our results will help elucidating the sequence determinants of substrate recognition for degradation of chloroplastic proteins.

Keywords: proteolysis; N-degron; N-recognin; chloroplasts

(1271) **THE EFFECT OF TRICHODIENE SYNTHASE INHIBITION IN *Fusarium GRAMINEARUM* VOLATILES.**

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Fusarium graminearum is the main fungal pathogen associated with Fusarium head blight (FHB). The trichothecene (TCH) deoxynivalenol (DON) is one of the major mycotoxins produced contaminating agricultural products. Trichodiene synthase catalyze the synthesis of trichodiene (TRI) intermediate in the biosynthesis of TCHs, coded by the *TRI5* gene. During trichothecene metabolism several volatile organic compounds (VOC), including TRI, are produced. Our aims were to obtain a non TCH-producing strain of *F. graminearum,* and to test the effect of synthetic analogues of plant-derived chemicals on the production of TRI by *F. graminearum*. TCH non-producing mutants were obtained using a split marker recombination approach; *TRI5* was replaced with a marker gene (*hph*, which confers resistance to hygromicin B). Seventeen transformants were obtained, 76% of them were -*TRI5* mutants confirmed by PCR, qRT-PCR, aggressiveness tests (F = 23.28) and inability to produce DON. Volatiles were analyzed by head space-solid phase microextraction (HS-SPME) coupled to capillary gas cromatography-mass spectrometry (CGC-MS). This technique is useful for early detection of *F. graminearum* before FHB development. In -*TRI5* mutants no sesquiterpene volatile compounds were detected. Wheat cultivars were inoculated with TRI synthase inhibitors in field assays, one of the -*TRI5* mutants was used as control. Of the chemicals tested, apocynin and eugenol produced a significant inhibition of TRI amounts 2 days post inoculation (dpi). However, TRI production was recovered at later stages (> 7dpi) due to continue fungal growth. Further experiments are necessary to determine the appropriate inhibitor concentrations and/or inoculation period to prevent TCH formation throughout the critical period (15 days) of FHB development. These studies are of interest in FHB control.

Keywords: Fusarium, Trichodiene, HS-SPME, GC-MS

(1535) **CHARACTERIZATION OF CASEINOLYTIC ACTIVITY OF TWO *S. tuberosum* ASPARTIC PROTEASES (*St*APs)**

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Worldwide increasing cheese demand has led to the search of novel proteases with milk clotting activity. Plants are used as source of proteases due to their easy availability, efficient purification processes and isolation of natural coagulant.

*St*AP1 and *St*AP3 are two aspartic proteases (APs) from potato tubers and leaves with milk-clotting and caseinolytic activity. The aim of this work was to optimize the caseinolytic activity of *St*AP1 y *St*AP3 and to test the specific activity on bovine casein subunits.

Box-Behnken design with three factors at three different levels was used to optimize the caseinolytic activity. Second order models were used to generate response surface contours (RSC). Optimum condition determined for caseinolytic activity were: pH 8,20, temperature 43,19ºC and concentration $5{,}20 \times 10^{-7}$ M for StAP1, and pH 8,21, temperature 42,68ºC and concentration $5{,}16 \times 10^{-7}$ M for StAP3. These conditions are compatible with ones required for industrial cheese making process.

Additionally, we evaluated the specific activity of *St*AP1 and *St*AP3 on casein subunits (α, β and κ). In order to do that, we incubate each *St*AP with casein subunits for 2 h and the degradation patterns were analyzed by SDS-PAGE. Densitometric analysis were performed using Scion Image. Results obtained shown that β-casein was degraded by both APs in a major proportion than others casein subunits (34% for StAP1 and 66% for StAP3). The percentages of degradation to α-casein were 25% and 36%, to *St*AP1 and *St*AP3, respectively. Finally, κ-casein was degraded 18% for *St*AP1 and 23% for *St*AP3. These results suggest that *St*AP1 y *St*AP3 degrade mainly β-casein. However, the minor degradation of κ-casein is enough to inducing milk clotting. Based on this results *St*AP1 y *St*AP3 might be considered a promising alternative of natural calf rennet for the coagulation of milk leading to new dairy products.

Keywords: proteolytic enzymes, milk-clotting activity, plant proteases, cheese making.

(1541) **CHARACTERIZATION OF THE ALTERNATIVE SPLICING VARIANT OF THE PIP SUBFAMILY IN *Medicago Truncatula:* THE TRUNCATED PROTEINS VARIANTS AS NEW ELEMENTS OF HETERO-OLIGOMERIZATION REGULATION**

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PIPs are transmembrane proteins of great biological importance given the large number of representatives found in plants and the specific ability to transport different small compounds (water, CO_2, H_2O_2, ions). The functional diversity and localization of this protein family is usually attributed to three possible mechanisms: a) transcriptional regulation and mRNA processing; B) interaction of different monomers forming hetero-oligomers and c) the presence of regulatory subunits coupled to the structure of the transmembrane proteins. To date, in this family, important advances have been made in understanding the post-translational regulation mechanisms such as hetero-oligomerization and gating by pH, calcium, and / or phos-

phorylation. In contrast, the mechanisms of transcriptional regulation and RNA processing have not yet been addressed in depth. In the field of biology, co-transcriptional modifications occurring on mRNAs by splicing and alternative splicing are crucial in the protein diversity present in eukaryotic organisms. Our preliminary results in *Medicago truncatula* suggest that splicing variants would determine the translation of truncated protein variants. Unlike prior reports for other plant Membrane Intrinsic Proteins (MIPs), these protein variants lack two transmembrane helix and one extracellular loop. The existence of these variants open new questions about the role they could play in PIP regulation in particular but in plant metabolism in general.

(1551) **CLPB3 FROM *ARABIDOPSIS THALIANA* DISPLAYS CHAPERONE ACTIVITY *PER SE***

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Abstract: All cells have developed a protein network involved in maintaining the proteome homeostasis, particularly after periods of stress. When massive protein aggregation occurs due to heat exposure, cell survival relies on the ClpB/Hsp100 subfamily of molecular chaperones which promote the solubilization and reactivation of protein aggregates. The bacterial chaperone ClpB and its homologue in yeast, Hsp104 are the most extensively studied disaggregases. Like many members of the AAA+ superfamily, ClpB/Hsp104 protomers form ring-like homohexameric complexes.The mechanical energy necessary to disentangle protein aggregates is provided by ATP hydrolysis at the two nucleotide-binding domains of each monomer. ClpB and Hsp104 collaborate with the DnaK or Hsp70 chaperone system, respectively, to dissolve protein aggregates both *in vivo* and *in vitro*, with DnaK/Hsp70 playing a main function in the regulation of ClpB/Hsp104 activity. In the present study, we have explored the substrate recognition by ClpB from *A. thaliana* chloroplasts (*At*ClpB3) and the role of its cpHsp70 chaperones in this recognition. We demonstrated that *At*ClpB3 ATPase activity is stimulated by caseins, poly-lysine and glucose-6-phosphate dehydrogenase (G6PDH) aggregates, but not by luciferase aggregates. In addition, cpHsp70s did not enhance *At*ClpB3 activity, albeit the presence of caseins. Likewise, *At*ClpB3 could mediate G6PDH disaggregation independently from cpHsp70 chaperones, recovering the activity of the denatured enzyme up to a 65 %. Besides, it has distinct properties not previously reported for Hsp104 or ClpB, as it interacts more efficiently with the aggregated G6PDH in the presence of the non-hydrolysable ATP analog AMP-PNP rather than ATPγS. This is the first evidence that a ClpB disaggregating activity can be exerted without the participation of other proteins and establishes a first approach to understanding *At*ClpB3 substrate preferences.
Keywords: ClpB, Hsp100 chaperone, disaggregases, Hsp70, *Arabidopsis thaliana*

(1673) **THREE NEW CYSTEINE PEPTIDASES CLONED AND SEQUENCED FROM FRUITS OF *Bromelia hieronymi* MEZ (BROMELIACEAE)**

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Fruits of *Bromelia hieronymi* Mez (Bromeliaceae) contain high amounts of cysteine peptidases (CPs), which have been characterized from the biochemical and kinetic point of view. These peptidases are capable to hydrolyze food proteins, releasing bioactive peptides with both antioxidant and antihypertensive activities. The aim of the present work included cloning and sequencing of several CPs from RNA obtained from unripe fruits. The total RNA was extracted from 0.2 g of fruit crushed with liquid nitrogen. Retrotranscription reaction was carried out to synthesize the cDNA. Then, a specific primer designed from conserved N-terminal sequences of CPs was used in a PCR in order to amplify and select peptidase cDNAs. The amplified products about of 990 bp were cloned into pGEM-T Easy vector and transformed into competent Top 10 *Escherichia coli* cells. Ten clones were selected, and sequences corresponding to three new peptidases were obtained and named Bh-CP 3, 4, and 5. Their deduced amino-acid sequences encoded three proteins of 230 residues each one, with molecular masses of 24781.82, 24787.92, and 24793.88 kDa, respectively. The predicted pI was a unique value (8.41), and the aliphatic index presented values of 71.65, 74.22, and 73.35, respectively. All sequences displayed the characteristic primary structure of plant CPs (including four residues of the catalytic site, and six Cys residues involved in disulfide bonds), and did not present possible N-glycosylation sites. Bh-cp 3, 4, and 5 showed 97, 99, and 97% of identity with Bh-CP1, other peptidase sequenced from *B. hieronymi*, and all of them showed 87 % identity with fruit bromelain from *Ananas comosus*. This work is the first step to express these enzymes, which might be promising biocatalysts for food and pharmaceutical industries.
Keywords: Bromelia hieronymi, cysteine peptidase, cloning, sequencing

(155) **RECOMBINANT PRODUCTION OF THE ARABIDOPSIS MITOCHONDRIAL ENZYMES PROLINE DEHYDROGENASE AND P5C DEHYDROGENASE**

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The aim of our work is to determine the activity and oligomeric state of the Arabidopsis enzymes proline (Pro) dehydrogenase (ProDH) and P5C dehydrogenase (P5CDH) that participate from Pro catabolism in the matrix of plant mitochondria. We also need to evaluate if there is physical interaction between these proteins and, if so, the protein domains involved. To address the above-mentioned issues *in vitro* we expressed the proteins as recombinants in *E. coli*. Arabidopsis cDNA was used as template and adequate primers designed to clone the full length open reading frame of the genes *ProDH1*, *ProDH2* y *P5CDH* without the mitochondrial transit peptide, as well as mutant versions of ProDHs lacking the N-terminal or P5CDH without the C- terminal into the pMAL-p2p vector. This expression plasmid allows N-terminal fusions to the Maltose Binding Protein (MBP), which, as we confirmed, increases the fraction of soluble fusion proteins obtained. It also contains a cleavage site for the Prescission protease to detach the protein of interest from the tag. We used *E. coli* BL21DE to test different conditions to optimize the soluble expression of the proteins (temperature, time and inducer concentration –IPTG-). Soluble fusion proteins were purified by affinity chromatography with amylose resin and we could verify that either tagged or untagged these were properly recognized by homemade polyclonal antibodies developed in the laboratory against peptides of ProDH and P5CDH. The activity of ProDH1 and ProDH2 were determined by using spectrophotometry monitoring the reduction of the absorbance at 600nm of the acceptor DCPIP. Details about experiments designed to evaluate oligomerization state and interaction will be presented.
Keywords: ProDH, P5CDH, Proline, Arabidopsis, Recombinant proteins.

ONCOLOGY-ONCOIMMUNOLOGY 3

(441) **ROLE OF GLUCOCORTICOIDS IN MYELOID LEUKEMIA CELL DIFFERENTIATION INDUCED BY RETINOIC ACID**

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The leukemias are malignant diseases of hematopoietic cells in which the proper balance between proliferation, differentiation and apoptosis is no longer operative. Synthetic glucocorticoids like dexametasone (Dex) are frequently used in the treatment of hematopoietic diseases due to its pro-apoptotic properties. On the other hand, in many clinical trials the differentiation inducer retinoic acid (RA) resulted not encouraging in most myeloid patients. In this sense, our hypothesis suggests that a combination of steroid hormones and RA

could represent an alternative and promising therapy. The main goal of this project is to study the role of glucocorticoids in RA-induced human promyelocytic leukemia cell differentiation. Undifferentiated NB4 cells were treated with RA in the presence or absence of Dex over 72h. Our results showed that Dex markedly enhances a RA-induced cell differentiation response, observed as a potentiated expression of the cell surface marker CD11c by flow cytometry analysis (control: (0±1)% RA: (19±2)% RA+Dex: (54±3)% - p≤0.01). To gain functional insights into this differentiation process, the expression of *hox* genes, *pscd4*, *meis2, gr* and *rarβ* was monitored in RT-qPCR assays. Notably, upon 48h, the addition of Dex potentiates RA-induced expression of *hoxA3* (p≤0.02) and *pscd4* (p≤0.05) genes. Finally, PML-RARα and RARα protein down-regulation upon RA stimulation was confirmed by western blot, and remained unchanged upon combined treatment with Dex. Overall, our data reveals the existence of a synergistic effect of Dex and RA on NB4 cell differentiation. Further characterization of this molecular context could aid to identify attractive targets for therapeutic strategies in myeloid leukemia.

Key words: Glucocorticoids-Retinoic Acid-Gene Expression-Leukemia

(706) **AUTOANTIBODIES FROM BREAST CANCER PATIENTS UP-REGULATE PRO-TUMOR MEDIATORS IN HUMAN DENDRITIC CELLS**

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Abstract: Systemic humoral response against different antigens in tumor bearers has been reported. We documented that immunoglobulin G (IgG) from breast cancer (BC) patients in T1N0Mx (tumor size<2 cm, without lymph node metastasis) promotes tumor progression by activation of cholinergic receptors express in tumor cells. We also reported that non-neuronal cholinergic system is present in human dendritic cells (DC), and T1N0Mx-IgG modulates the expression of maturation markers and the production of cytokines by these cells, reinforcing the tolerogenic/immunosuppressive profile in these patients. Here, we analyzed the effect of T1N0Mx-IgG on vascular endothelial growth factor-A (VEGF-A) and matrix metalloproteinase-9 (MMP-9) production by DC as promoters of tumor progression. DC were obtained from human monocytes cultured with IL-4 and GM-CSF for 5 days. Then, cells were cultured with or without LPS to obtain mature DC (mDC) or inmature (iDC). By Western blot, we demonstrated that iDC and mDC express VEGF-A. The addition of T1N0Mx-IgG (10^{-8}M) for 1 h increased VEGF-A expression only in mDC (2.19±0.38; p≤0.01 vs. control). The preincubation of mDC with cholinergic antagonists reduced this effect. Moreover, we found that both iDC and mDC express the pro-MMP9 and MMP-9 active forms (by zimography), and T1N0Mx-IgG increased MMP-9 activity in both cell populations (iDC:1.67±0.01; p≤0,01; vs. control iDC; mDC:1.77±0.44; p≤0,001 vs. control mDC). This effect was also reduced by the preincubation of cells with cholinergic antagonists. In conclusion, the interaction between DC and T1N0Mx-IgG through cholinergic receptors up-regulated VEGF-A and MMP-9 production by DC; these mediators could not only promote tumor progression but also the immunosuppressive profile of patients with BC.

Keywords: autoantibodies, breast cancer, dendritic cells, cholinergic system.

(507) **BEXAROTENE TREATMENT FOR CUTANEOUS T CELL LYMPHOMA (CTCL): IMPLICATIONS OF THYROID HORMONE (TH) REPLACEMENT IN THE ANTI-LYMPHOMA ACTIVITY AND IN THE ANTITUMORAL IMMUNE RESPONSE.**

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Bexarotene (Bex) is used for the treatment of CTCL and is associated with hypothyroidism, so patients concomitantly received TH administration. We found that physiological levels of TH increase the proliferation of CTCL by activating TH membrane receptor, integrin αVβ3 (mTR). Here, we determined the influence of TH replacement therapy on the anti-lymphoma activity of Bex.

In standard conditions, Bex decreases the proliferation and viability of HuT78 and MJ CTCL cells. To study this mechanism we conducted RNA-sequencing in Bex-treated HuT78 cells (vs. vehicle) and found that Bex up-regulates genes related to *cell proliferation and differentiation* (*REL, CCND1*) and to *immunity* (*TBX21, IFNG*). This suggests that Bex treatment could impact in antitumor immunity. In TH-depleted culture conditions we observed a higher effect of Bex on CTCL viability. As hypothyroidism is associated with marked immunosuppression, thus favouring CTCL progression, we determined the impact of TH replacement on Bex using a murine TCL model of EL4 cells. Once tumors developed, we treated mice with vehicle (Veh), Bex (Bex) and Bex with TH replacement (BexT4+). Bex administration decreased tumor growth being the effect significantly higher in the absence of T4 replacement (p<0.05 vs Bex). Mice with Bex showed a significant decrease of $CD8^+CD44^+$ T-cells (p<0.05 vs. BexT4+) and an increase of myeloid-derived suppressor cells in lymph nodes, indicating that Bex should be administered with T4 to avoid a negative immune microenvironment.

Then, we analyzed if the inhibition of the mTR would impair the pro-survival effect of TH. TCL tumor-bearing mice were treated with Veh or BexT4+ in combination (BexT4+Cil) or not with cilengitide 40mg/kg. Atthe end of treatment, BexT4+Cil mice showed significantly smaller tumors than Veh and BexT4+ mice (p<0.001 and p<0.05, respectively). Our data indicates that the inhibition of the integrin αVβ3 could be an effective strategy to improve Bex-based treatments in CTCL.

Bexarotene, Cutaneous Lymphoma, Thyroid Hormone, Integrin Alphav Beta3

(1785) **BREAST CANCER SHAPES THE B CELL COMPARTMENT TOWARDS IMMATURE DEVELOPMENTAL STAGES**

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Unlike T cells, that have been broadly studied in anti-tumor immunity, the role of B cells in cancer progression remains a matter of controversy. Using different murine tumor models (4T1, LM3 and CT26) we sought to understand how the B cell compartment is phenotypically and functionally shaped by the presence of tumors, altering the host's immune response. For this, we analyzed B cell ontogeny by determining the frequency and number of B cell subsets in the bone marrow and spleen of tumor-free and tumor-bearing mice. We found that mice with 4T1 and LM3 mammary tumors had a dramatic reduction in the number of bone marrow B cells B220+ CD19+ (p<0.05), when compared to BALB/c mice without tumor. In addition, we found an altered frequency of the different subsets of B cells in the bone marrow, including decreased mature recirculating B cells (p<0.001) and increased pre- and pro- B cells (p<0.05). Changes in B cell development were also observed in the following steps of B cell maturation, with an increased frequency of splenic transitional 1 B cells in mice with 4T1 tumor compared to tumor-free controls (p<0.001). Transitional 2 (p<0.05), follicular (p<0.001) and marginal zone B cells (p<0.0001) were fewer in frequency in the presence of 4T1 tumor. In line with this evidence, we also found an elevated proportion of immature CD93+ B cells inside 4T1 tumors. On the contrary, the presence of a CT26 colon tumor did not alter the B cell compartment suggesting that the observed effect was a feature of breast tumors. As immature B cells display immune suppressive activity it is likely that, once these cells are recruited to the tumor, they

modulate anti-tumor immunity favoring tumor progression. Further studies on the mechanism underlying tumor-driven modulation of the B cell compartment will unveil novel targets for cancer immunotherapy.

Keywords: B cell compartment, breast cancer, immature B cells, anti-tumor immunity.

(1735) **CHARACTERIZATION OF THE NEWLY DEVELOPED TLR3-GFP MICE: NOVEL REPORTER TOOL FOR STUDYING THE EXPRESSION OF TLR3**

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TLR3, an endosomal receptor, is able to recognize dsRNA present in the abnormal location of the endocytic pathway. For some year now, the lack of a properly working antibody against TLR3 has been a methodological barrier for a more profound study of the receptor. Our collaborators at Institut Curie have developed a TLR3-GFP knock-in mice where the gene for GFP was introduced downstream the TLR3 promoter in order to generate a fusion protein to properly follow TLR3. In this work, we first characterized the expression of TLR3 on different cell subsets purified from harvested organs of TLR3-GFP mice (spleen, ingLNs, mesenteric LNs, bone marrow, lung, gut), and as expected from data banks we detected expression of TLR3 on different subsets of dendritic cells present in these organs, CD8α+ DCs being the main one. To note, the proportion of TLR3+ cells varied from organ to organ detecting TLR3 expression in CD8α+DCs and a subpopulation of B cells in the spleen. We then took one group of reporter mice receiving i.p. injections of 200μg of poly I:C (pIC), a well-known TLR3 agonist, and a control group receiving vehicle; and evaluated the activation state of these TLR3+ populations 24h post-injection. When we analysed CD86 and MHCII we did not observe any significant changes on TLR3-GFP mice treated and non-treated with pIC while we did observe activation upon pIC on littermate controls. This suggested an incomplete capacity of TLR3 to respond to its ligand on the reporter mice. To further characterise these mice we took an *in vitro* approach and obtained BMDMs (Bone Marrow-Derived Macrophages) from TLR3-GFP and littermate controls and stimulated them with pIC and poly A:U, both well-established TLR3 agonists, and after 24h we evaluated activation markers, observing that littermate controls responded by upregulating activation markers while TLR3-GFP failed to do so. These results suggest that TLR3-GFP mice are a useful tool to follow TLR3 but fail to respond to its stimuli.

Keywords: TRL3-GFP, Dendritic Cells, BMDMs, Poly I:C

(1051) **CHRONIC LYMPHOCYTIC LEUKEMIA: ANTI-CD20 THERAPEUTIC ANTIBODIES AND BCR KINASE INHIBITORS OVERCOME LEUKEMIC CELL RESISTANCE TO VENETOCLAX (ABT-199)**

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Leukemic B cells from CLL patients survive and proliferate within lymphoid tissues in contact with activated T cells and myeloid cells and receiving signals through the BCR. Effective therapy should target both, leukemic cells from peripheral blood and from the protective microenvironment. We previously reported that ABT-199, a potent BCL2 inhibitor, is highly cytotoxic against unstimulated CLL cells but it is much less effective against CLL cells that received survival signals from activated T cells, suggesting that leukemic cells from the supportive microenvironment might not be properly targeted by the drug. The aim of this study was to overcome the ABT-199 resistance using combined therapies with anti-CD20 MoAbs and/or BCR-associated kinase inhibitors (BCR-KI). To this aim peripheral blood mononuclear cells from CLL patients were cultured with anti-CD3 (aCD3) to activate autologous T cells with or without ABT-199 and the BCR-KI GS-9973. CLL cell survival was evaluated by flow cytometry; the expression of BCL2 family proteins by western blot and phagocytosis of CFSE-labeled CLL cells coated with anti-CD20, Rituximab (Rx) by flow cytometry and confocal microscopy. We confirmed that autologous T cell activation induced ABT-199 resistance on CLL cells (n=18, $p<0.01$) which was associated with the upregulation of MCL1 and BCL-XL on the leukemic clone (n=6). GS-9973, which impairs T cell activation (Colado A, Cancer Immunol Immunother. 2016), overcame aCD3-mediated resistance of CLL cells to ABT-199 (n=14, $p<0.05$). Finally, we found that ABT-199 allowed a more successful phagocytosis of Rx-coated CLL cells (n=18, $p<0.05$).

Our results encourage the combination of ABT-199 with BCR kinase inhibitors, such as GS-9973 which overcame the resistance induced by activated T cells. Combination with CD20 MoAbs could also be useful because ABT-199-enhanced the phagocytosis of Rx-coated-CLL cells.

Key words: CLL, ABT-199, microenvironment, GS-9973, rituximab.

(414) **CHRONIC PROSTATE INFLAMMATION INDUCED BY *E. coli* INFECTION LEADS TO TISSUE LESIONS ASSOCIATED TO PROSTATE CANCER DEVELOPMENT**

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Prostate cancer (CaP) is the second most frequent type of cancer in men. Prostate carcinogenesis is multifactorial and several environmental and genetic factors are involved. In this set, chronic inflammation was suggested as an important risk factor by several epidemiological studies. Herein, and using an animal model, we analyzed if chronic prostate inflammation caused by *Escherichia coli* infection may have a role in prostate carcinogenesis.

Male C57BL/6 mice were transurethrally inoculated with $2x10^8$ CFU of uropathogenic *E. coli* 1677 (infected) or saline (controls), euthanized at 5 days (dpi), 12 and 26 weeks (wpi) post infection, and the immune response, prostate infiltrating leukocytes and histopathology analyzed.

Inoculated animals developed an ascending infection early after infection (5 dpi) that persisted along time (12 and 26 wpi). Infection induced inflammation that was characterized by significantly increased circulating IL17+ and IFNg+ T cells when compared with controls ($p<0,05$), either at early (5 dpi) or late times after inoculation (12 and 26 wpi). The peripheral immune response was accompanied by histological lesions in the prostate from infected mice. Several acute inflammation foci, mainly composed of Gr1+ cell infiltrates, hemorrhage, necrotic debris, abundant epithelial shedding and tissue disorganization were shown at 5 dpi. Later on, prominent chronic inflammation was evident by dense infiltrates in the stroma, mostly composed of CD3+ and CD11c+ cells (12 and 26 dpi). Strikingly, sites of intense inflammation were associated with shedding of epithelial cells, papillomatosis, and varying degrees of atypical hyperplasia and dysplastic changes mimicking high grade prostate intraepithelial neoplasia.

Our results indicate that chronic bacterial infection of the prostate induces chronic inflammation associated in close proximity with tissue changes similar to neoplastic lesions, which could constitute a potential precursor of prostate adenocarcinoma.

Keywords: Prostate cancer, chronic inflammation, infection, prostatitis.

(1407) **DEFICIENCY IN THE IL-17RA/IL-17 PATHWAY AFFECTS PROTECTIVE CD8+ T CELL IMMUNITY IN A MODEL OF TUMOR VACCINATION**

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The role of IL-17 cytokines in cancer remains controversial as anti- and pro-tumoral effects have been described. We and others demonstrated that IL-17 family plays a central role for the induction of NK and CD8+ T cell (CTL) responses. As these subsets are critical for cancer resistance, we evaluated the role of IL-17/IL-17R in modulating anti-tumor immunity and tumor progression. To this end, B6 (WT), IL-17RA KO (RKO) and IL-17A/F double KO (DKO) mice were injected with tumor cell lines exhibiting progressor (B16-SIY and MCA101-OVA) and regressor (MC57-SIY) growth patterns. Previously, we showed that volumes of B16 and MC57, but not MCA101, tumors were increased in RKO and DKO mice compared to WT mice at several days post injection (dpi). Analysis of the primary immune response against MC57 demonstrated that compared to WT controls, DKO mice presented reduced numbers of SIY-specific CTL in draining-lymph nodes at d12pi ($p<0.05$). Also, tumor-specific CTL displayed decreased frequency of cells with memory phenotype (CD62L+CD127+). As the memory CTL response developed in MC57-SIY-immunized WT mice is critical to host protection against challenge with B16-SIY tumors, we aimed at evaluating whether RKO and DKO mice were also able to generate protective SIY-specific CTL. Then, we challenged MC57-SIY immunized mice with B16-SIY tumor cells and determined tumor growth until day 32 post challenge (pc). While most (75%) of immunized WT mice rejected B16 tumor, DKO and RKO mice were less protected as only few mice (around 20%) were tumor free at d32pc ($p<0.05$). Accordingly, blood and tumors of DKO mice showed reduced frequency of SIY-specific CTL ($p<0.05$) as well as decreased tumor-specific cells with a memory effector phenotype ($CD44^+CD62L^-$; $p<0.001$) in comparison to WT controls at day 8pc. Altogether, our results suggest that the IL-17/IL-17RA pathway modulates primary and secondary antitumor CTL responses and may influence tumor progression in certain cancers.

Keywords: IL-17, CTL, vaccination, tumor immunity.

(1206) **DEVELOPMENT OF A MIMETIC PEPTIDE OF TUMOR-ASSOCIATED GLYCAN GD2 AS A PROMISING CANDIDATE FOR ACTIVE IMMUNOTHERAPY**

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Immunotherapy has been gaining importance in cancer treatment. In particular, some vaccination strategies focus on the presence of carbohydrate structures associated with tumor cells. Aberrant glycosylation is a phenomenon described in the malignant transformation and includes loss or excessive expression of certain glycans and appearance of unfinished and novel structures. The ganglioside GD2 is a tumor-associated glycan, highly expressed on cancer cells of neuroectodermal origin, including neuroblastoma, melanoma, brain tumors and small cell lung cancer. Taking into account that carbohydrates are T-cell-independent antigens and thus generate poor immune responses; the aim of this work was to develop a peptide compound that mimicsGD2, for its future use as the active compound in a cancer vaccine. We have selected a series of peptide candidates that mimic the structure of GD2, using the Ph.D.12 Phage Display Peptide Library Kit (NEB) and a monoclonal antibody (Ab) specific for human GD2 (clone 14G2a, Biolegend) as screening Ab. Four candidates were selected after a sequence alignment and taking into account their hydrophilicity. Candidates were able to bind to the screening Ab in an ELISA assay. Moreover, when pre-incubated with the screening Ab, they were able to interfere in its binding to GD2-positive tumor cells in a flow cytometry assay. Based on these results and their antigenic mimetic capacity, we selected the most promising candidate and fused its sequence with the bacterial carrier BLS (Brucella lumazine synthase). This highly immunogenic platform allows the production of chimeric proteins decorated with ten copies of the desired peptide. The anti-GD2 Ab was able to recognize the resulting chimera in a Western Blot. Our results demonstrate the experimental approach used is appropriate for selecting peptides with glycan antigen-mimicry and the promise of becoming potential active compounds in an immunotherapy against GD2-expressing tumors.

Palabras Clave: GD2, mimetic-peptide, cancer, immunotherapy, aberrant glycosylation

(067) **INTRINSIC ROLE OF GALECTIN-1 IN THE CONTROL OF THE FUNCTIONAL PROPERTIES OF IMMUNE CELLS IN A PROSTATE CANCER CONTEXT**

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The identification of effective new therapies for prostate cancer requires a better understanding of the multiple molecular interactions between tumor cells and their associated stroma. In this context, Galectin-1 (Gal-1) plays a major role determining the properties of the prostatic carcinoma microenvironment. The aim of this study was to elucidate whether Gal-1, in addition to promote tumor neoangiogenesis and immune regulations, plays an additional role as an intrinsic regulator of $CD8^+$ T cell function.

To address this, we used two approaches: an *in vitro* T cell polyclonal activation model, combining different cell types (purified by cell sorting) in a prostate tumor microenvironment and an adoptive transfer of Gal-1 deficient *Lgals1*$^{-/-}$ (KO) and wild type (WT) cells into immunodeficient mice.

The *in vitro* combination of KO and WT cells, allowed us to elucidate how the endogenous Gal-1 of each cellular compartment impacts on the $CD8^+$ T cell proliferation and citotoxicity. The absence of Gal-1 in antigen presenting cells did not significantly modify any function of $CD8^+$T cells. Conversely, the absence of Gal-1 in $CD4^+$ T cells induced a 1.21 fold increase in the proliferation of CD8+T cells. However, the most significant difference in the proliferation was obtained by absence of intrinsic Gal-1 in the $CD8^+$ T cells themselves (2.12). We further evaluated whether Gal-1 is relevant for effector function. Our results demonstrated that upon activation, KO T cells have increased their ability to degranulate (evaluated as % (1.87 fold) and the content of granules (2.48 fold increase) ($p<0.05$, t test Student).

We then compared the *in vivo* tumor growth in nude mice adoptively reconstituted with Gal1 KO or WT immune systems: the presence of KO immune cells increased for 24.1 days the lag time and doubled the tumor duplication time. These results demonstrate that Gal-1 modulates the intrinsic function of lymphocytes in a prostate cancer context.

Key words: Prostate Cancer, Galectin-1, Proliferation, Immunooncology

ONCOLOGY-ONCOIMMUNOLOGY 4

(709) **COFILIN-1 LEVELS AND INTRACELLULAR LOCALIZATION ARE ASSOCIATED WITH PROGNOSIS OF MELANOMA**

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Melanoma is an aggressive cancer with highly metastatic ability. We propose cofilin-1, a key protein in the regulation of actin dy-

namics and migration, as a prognostic marker. We determined cofilin-1 levels in a retrospective cohort of patients with melanomas and benign lesions of melanocytes (nevi) by immunohistochemistry. Results show a significant association between high levels of cofilin-1 and tumor malignant features, such as advanced stages of malignant melanoma (MM) ($p<0.001$), increased number of mitosis per field ($p<0.05$) and Breslow index (BI) ($p<0.05$). Higher cofilin-1 levels were found in MM with BI>2 vs MM with BI<2 ($p<0.05$) and vs melanoma in situ (MIS) and nevi ($p<0.001$). MM with metastasis also showed increased cofilin-1 levels vs MM without detected metastasis ($p<0.01$). Kaplan-Maier survival curves were performed, clustering patients according either to the type of melanocytic lesions (nevus, MIS, MM BI<2 and MM BI>2) and to the cofilin-1 level. Significant differences among the different types of lesions were found ($p<0.05$) and those patients with higher cofilin-1 levels exhibited lower survival rate at 5 years ($p<0.0001$). Moreover, a significant negative correlation between survival percentage and cofilin-1 levels (Spearman's Rho: R=-0.73, p=0) was found. Therefore, the levels of cofilin-1 can be associated with melanoma outcome in this patient cohort. Supporting these results, TCGA database analysis of a melanoma cohort also showed low survival in patients with upregulated cofilin-1 mRNA. As cofilin-1 has a dual function depending on its intracellular localization, we evaluated nuclear and cytoplasmic levels of cofilin-1 by immunofluorescence. An increase in nuclear/cytoplasmic cofilin-1 mean fluorescence ratio was observed in MM with BI>2 vs MM with BI<2, MIS and nevi. In conclusion, our study suggests that not only the higher levels of cofilin-1, but also its nuclear localization can be markers of worse outcome of patients with melanoma.

Keywords: Cofilin-1, Melanoma, Breslow Index, Metastasis, Nuclear localization.

(983) **COMPREHENSIVE CLINICAL, PATHOLOGICAL AND MOLECULAR CHARACTERIZATION OF A COHORT OF LOCALLY ADVANCED RECTAL CANCER PATIENTS: TOWARDS AN INTEGRATIVE CLASSIFICATION FOR RECTAL CANCER MANAGEMENT**

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Introduction: Locally Advanced Rectal Cancer (LARC) response to preoperative chemoradiotherapy (CRT) is very heterogeneous. Current prognosis-related factors lack robustness to individualize therapeutic decisions. Four Consensus Molecular Subtypes (CMS) represent the current best description of colorectal cancer heterogeneity at the gene expression level. We aim to integrate CMS predictions with available clinical data from an Argentinian LARC cohort in search of biomarkers to improve patient management. **Methods:** We analyzed gene expression in baseline tumor biopsies of the first 24 LARC patients from an ongoing recruiting translational research trial. Mutations in RAS and BRAF were measured by PCR Entrogen panel. DNA repair proteins(MLH1,MSH2,MSH6,PMS2) were measured by Cell Marque monoclonal primary antibodies. Gene expression data was obtained using Agilent Agi4x44K Whole Human Genome microarrays, and CMS-classified with the R package 'CMSclassifier' using a similarity-to-centroid approach. **Results:** Subtype classification showed CMS2-Canonical subtype patients (79%) of which one showed MSH2 gene and protein expression deficit, and one carried KRAS G12A mutation; CMS3-Metabolic (13%) with one KRAS-mut patient; CMS1-MSI immune (4%) diagnosed Lynch Syndrome confirmed by a germinal MSH2 mutation; CMS4-Mesenchymal (4%) showing higher stromal gene expression. **Conclusion:** To our knowledge, this is the first classification of local LARC patients into descripted colorectal molecular subtypes combining prospective clinical and genomic data to enrich patient characterization. We expect that future analyzed cases from this ongoing trial might help us establish further molecular features to correlate with CRT response.

(787) **CYCLOOXYGENASE-2 REGULATES THE SENSITIVITY OF LUNG ADENOCARCINOMA CELLS TO IONIZING RADIATION.**

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Abstract: Radioresistance is defined as a cell's capacity to survive after exposure to high energy radiation, a fact that may limit therapeutic options by reducing the efficacy of radiotherapy (Rt). Numerous tumor cells show a high expression of cyclooxygenase-2 (COX-2), a fact that has been linked to more aggresive tumor phenotypes. Also, the COX-2 inhibitor celecoxib (CXB) has been proposed as a possible Rt enhancer. We aim here to investigate whether COX-2 activity and expression regulate radiosensibility in a radioresistant lung adenocarcinoma-derived cell line (A549). Cells were exposed to varying doses (0-10 Gy) of ^{60}Co ionizing radiation (IR) in the presence or the absence of 7.5 mM CXB and radiosensitivity was determined using a standard clonogenic assay, cell cycle was studied by flow cytometry after propidium iodide staining and MAPKs activation and COX-2 expression by western blot using specific antibodies.

IR produced a significant and dose-dependent reduction of cell viability showing an a/b ratio of 14.2 when calculated using the linear quadratic model, an effect that was significantly ($p<0.05$) enhanced by CXB. Rt (2.5 Gy) induced a clear G0/G1 phase accumulation and a reduction of G2/M phase when compared to control (79.0 vs 68.0 and 9.0 vs 15.5% for Rt and control, respectively), whereas CXB had no effect on cell cycle. CXB produced a significant ($p<0.05$) and rapid activation of p38 and JNK (5 and 10 min after exposure) but had no effect on COX-2 expression for up to 120 min as determined by Western blot.

Our results show that inhibition of COX-2 activity increases the effect of IR in A549 cells without affecting cell cycle progression although activating p38 and JNK. We conclude that COX-2 plays a role in the regulation of radiosensitivity of tumor cells in a mechanism that includes the participation of MAPKs.

Keywords: cyclooxygenase-2, radiosensitivity, celecoxib, p38, JNK.

(281) **DEVELOPMENT OF AN HYDROXYMETHYLGLUTHARYL-COENZYMEA REDUCTASE (HMGCR) OVEREXPRESSION SYSTEM WITH CRISPR-ON TECHNOLOGY FOR THE STUDY OF METABOLIC REPROGRAMMING TO STEM-LIKE STATES IN BREAST CANCER.**

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HydroxymethylglutharyI-coenzymeA reductase is deregulated in tumors, increasing the synthesis *de novo* of cholesterol, critical for the regulation of cell survival and proliferation signals. Therefore, we aimed to induce an HMGCR-on phenotype in the breast cancer (BC)-derived cell line MCF-7, to evaluate whether this phenotype involves the acquisition of stem-like traits in BC. With this

purpose, we developed an HMGCR overexpression model taking advantage of a CRISPR-on system (dCas9-VP160), which includes expression plasmids for guide RNAs (pSPgRNAs) and a plasmid carrying the sequence coding for the CRISPR-on. Five guide RNAs (gRNAs) targeted to the promoter of the human HMGCR gene were designed with the informatics tools Genome Engineering Toolbox from the Zhang Lab (MIT, Cambridge, MA) and CRISPR-ERA. The gRNAs and the CRISPR-on were then co-transfected into MCF-7 cells, and the levels of total HMGCR and its two isoforms (FL-Full Length and DL13-deletion of exon 13) mRNAs were assessed by qRT-PCR at 2 days postransfection. The CRISPR-dCAS9 system increased HMGCR total levels in MCF-7 cells (MCF-7/HMGCR-on) by a 2,26±0,03-fold (X±SD; p<0,05) when compared to transfection controls (MCF-7/CT), due to an increase in the levels of both isoforms (FL: 2,2±0,03-fold and DL13: 1,56±0,03-fold; X±SD p<0,05). Treatment with the statin Simvastatin (SIM), decreased HMGCR levels a 0,33±0,06-fold in MCF-7/HMGCR-on cells (X±DS; p<0,05), whereas MCF-7/TC cells remained unaffected. Interestingly, changes in HMGCR levels in MCF-7/HMGCR-on and MCF-7/TC cells in the presence of SIM correlated with corresponding changes in the frequency of stem cells (R^2=1), as measured by mammosphere formation assay by limiting dilution and statistical analysis with a specialized software (http://bioinf.wehi.edu.au/software/elda). These data suggest that cellular models expressing a HMGCR-on phenotype may offer useful tools for the study of metabolic phenotypes prone to acquire stem-like traits in BC.

Keywords: HMGCR, CRISPR-CAS9, BREAST CANCER, CANCER STEM CELLS.

(1089) **EVALUATION OF ZNPC AND ONE AMINO DERIVATIVE EFFECTIVENESS FOR PDT ON GLIOBLASTOMA CELLS**

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Glioblastoma multiforme is a type of tumor of the central nervous system (CNS), known for being one of the worst and fast-growing brain tumor. This type of cancer presents a poor prognosis after diagnosis with a survival rate of approximately one year. The current protocol for glioblastoma treatment consists in the surgical excision of the primary tumor followed by radio and chemotherapy. Photodynamic therapy (PDT) is a well-known therapeutic approach that combine two components (light and a photosensitizer (PS)) to induce cell death triggered by singlet oxygen ($^1O_2$) and/or reactive oxygen species (ROS) as a result of photochemical reactions. Phthalocyanines (Pcs), are excellent second generation photosensitizers for PDT. They present a far red wavelength absorption (>670 nm), long triplet lifetime (~1 ms), and high quantum yields of singlet oxygen generation (>0.2). In this work, we compared the capacity of ZnPc and one of its derivatives (TAZnPc) to photo inactivate glioblastoma cells *in vitro*. We analyzed the photochemical properties of both PS, their incorporation, subcellular localization and we observed a relation with the mechanism of cell death induced after illumination: ZnPc associate more to mitochondria compared to the TAZnPc which present a markedly lysosomal localization. We compared the clonogenic capacity after PDT, the type of cell death triggered, the molecular signals associated to them and the cellular organelles damaged after PDT. Also, both Pcs were vehiculizated into DPPC-cholesterol liposomes and measured the PDT capacity of both PS compared to the DMF administration. In summary, ZnPc results more efficient to photoinactivate glioblastoma cells *in vitro*, perhaps due to its subcellular localization and singlet oxygen generation, however, TAZnPc absorb in a longer wavelength allowing the irradiation with greater wavelength assuring a deep action in tissue by the PS (application in vivo).

Keywords: Glioblastoma- PDT- Cancer – Phtalocyanines-

(1577) **EXPRESSION AND FUNCTION OF PROLACTIN AND ITS RECEPTOR IN GLIOBLASTOMA CELLS**

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Glioblastoma multiforme (GBM) is the most common and aggressive primary brain cancer in adults. In spite of improved standard of care (surgery, chemotherapy and radiation) the median survival of GBM patients remains dismal. Prolactin (PRL) has been recently associated to the development of several hormone-dependent cancers. However, its role in the pathogenesis of GBM remains unknown. The expression of PRL has been detected in human GBM biopsies and over 30% of brain cancer patients exhibit hyperprolactinemia, which has positive correlation with the proliferation index and the vascular density of brain tumors. We have previously observed that GBM cells secrete PRL and that the PRL receptor (RPRL) antagonist Δ1–9-G129R-hPRL reduces their proliferation rate. Now we aimed to assess the expression of RPRL in human GBM cells and the effect of PRL on the sensitivity of these cells to chemotherapeutic agents. Expression of RPRL was detected by immunofluorescence in human U251 GBM cells. Various isoforms of RPRL have been reported, which are produced by alternative splicing and differ in the length of their intracellular domain, which in turn determines the characteristics of their signaling. We assessed the expression of these isoforms by Western blot and detected abundant expression of short RPRL isoform in U251 GBM cells. We next evaluated the effect of recombinant PRL and Δ1–9-G129R-hPRL on the sensitivity of human GBM cells to chemotherapeutic drugs. We found that addition of recombinant PRL (100-500 ng/ml) reduced the cytotoxic effect of Cisplatin (5 μM) and Temozolomide (15 μM) in U251 GBM cells (p<0.05), as assessed by MTT assay. On the other hand, Δ1–9-G129R-hPRL (5 μg/ml) reduced the viability and increased the cytotoxic effect of Cisplatin in human U251 and U87 GBM cells (p<0.05). Our findings suggest that locally synthesized PRL and its receptor could constitute a therapeutic target to improve the sensitivity of GBM cells to antineoplastic agents.

(1816) **EXTRACELLULAR VESICLES, MEDIATORS OF TUMOR STROMAL CROSSTALK, IN THYROID TUMOR MICROENVIRONMENT.**

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The tumor microenvironment (TME) comprises multiple cellular and non-cellular components that converge to promote tumorigenesis in a variety of solid malignancies. Extracellular vesicles (EVs) have been implicated in cell-cell communication in tumor and distant microenvironments. In preliminary studies, we found that thyroid tumor cell-fibroblast interactions promoted the secretion and activation of matrix metalloproteinases (MMPs) and a migratory phenotype in tumor cells. CD147, a transmembrane glycoprotein implicated in MMPs expression, has been related to thyroid tumor progression. Our goal was to identify EVs production and their CD147 expression as mediators of malignant progression in thyroid TME. As an *in vitro* tumor-stroma cell interaction model, non-tumor cells (N-Thy-Ori), thyroid papillary carcinoma cells (TPC-1) and thyroid anaplastic cells (8505c) were co-cultured with normal fibroblasts (Fb). Cellular CD147 expression was analysed by FACS in isolated and co-cultured cells. EVs secretion and size, in cell and cell-Fb conditioned media (CMs), were characterised by Transmission Electronic Microscopy (TEM) and Dynamic Light Scattering. Isolated EVs were analysed for differential total protein expression by SDS-PAGE and Coomasie-Blue staining, and for CD147 expression by western blot-

ting and gold-immunocytochemistry. CD147 expression showed no differences in thyroid isolated and co-cultured cells while increased in co-cultured Fb, irrespective of the thyroid cell used. Isolated EVs from CMs ranged between 50-600nm. In SDS-PAGE, an extra protein band was detected in EVs from TPC-1-Fb and N-ThyOri-Fb co-cultures. CD147 expression in EVs increased in TPC-1-Fb and 8505c-Fb co-cultures, without significant changes in N-ThyOri-Fb co-cultures. The results suggest that EVs-CD147 could play an active role in intercellular communication events in thyroid TME, stimulating the release of MMPs and the consequent migration and cellular invasion.

Keywords: extracellular vesicles, CD147, tumor microenvironment, thyroid.

(1413) **FUNCTIONAL ABILITIES ACQUIRED BY METASTASIC CELLS ARE REFLECTED IN A DIFFERENTIAL INTERACTION WITH MESENCHYMAL STEM CELLS**

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Lung metastasis is a therapeutic challenge during osteosarcoma (OS) progression (15–30% survival rate with pulmonary metastasis at diagnosis). Niche establishment is critical for metastasis; we aimed at determining molecular and functional events that could favor a suitable OS metastatic niche. Tumor stroma plays a key role in modulating tumor progression through an interplay between tumor and stromal cells. Through proteomic run and raw data analysis we demonstrated differential gene expression related to biological processes in an OS cell line selected by enhanced lung metastatic ability (LM7). Molecular differences were reflected in different functional behavior relevant in LM7 cells as compared to non-metastatic OS cells. Interestingly, mesenchymal stem cells (MSC) cells had significance in these functional differences. We demonstrated that MSC had higher migratory response to the non-metastatic cell line SAOS2 (1.5 fold higher compared to LM7; 994.1±82.72 no. cel/field), and the metastatic cell line LM7 was more responsive to MSC' secretome (2 fold increase in migration; 752.73±83.01 no. cel/field) suggesting a prime recruitment of MSC to the primary tumor site and later a prime incorporation of metastatic cells into a niche colonized by MSC. Active metalloproteinases (MMP) were expressed only by LM7 cells. Further, MMP2 expression related with diminished metastasis free survival rate and increased overall survival pointing at a role in the creation of a functional metastatic niche (Genomics Analysis & Visualization Platform, Acad Med Center, Amsterdam). Further, proteomic analysis and qPCR validation pointed at Fas associated factors as differentially expressed proteins in metastatic OS cells probably contributing to the signaling network in the lungs niche and as a mechanism of cell selection associated to apoptosis.

Keywords: osteosarcoma; mesenchymal stem cell; metastasis

(863) **GLUCOSE RESTRICTION COMBINED WITH ALPHA-LIPOIC ACID DECREASES CELL SURVIVAL AND MIGRATION OF HEPATOCELLULAR CARCINOMA DERIVED CELLS**

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Hepatocellular carcinoma (HCC) is the second most lethal cancer, which is in part due to its rapid development of intra and extra hepatic metastasis. a-Lipoic acid (LA) is clinically used as an antioxidant. Recent studies suggest the use of LA as an anti-cancer agent, associated with activation of AMPK. However, direct evidence of LA contribution to the treatment of HCC has not been elucidated. In recent studies we have demonstrated that AMPK activation signals the survival decrease in HCC cells subjected to glucose starvation (Gs). In this study, we evaluate the effects of LA and Gs on HCC cell lines (HepG2/C3A and HuH-7) proliferation, migration and differentiation, and the putative involvement of AMPK signaling pathway. First, we demonstrated that AMPK is activated by LA, and that this activation was enhanced when the cells were incubated with Gs. LA 0.5 (LA0.5) and 1(LA1) mM decreased the number of viable cells from 48h, and this effect was enhanced in Gs (HuH-7:LA1:67±2*, LA1Gs:45±8*#; C3A:LA1: 66±5*, LA1Gs:46±3*# % vs C). Treatment with LA for a longer period of time, drastically reduced clonogenic capacity in HuH-7 cells. In addition, LA increased the percentage of these cells in G0/G1 by 20% vs C. Wound-healing assay showed that AMPK activation by 0.5mM LA reduced cell migration, and Gs increased this result (HuH-7:C:339±7*, LA0.5:191±12*, LA0.5Gs:120±15*#; C3A:C:94±4*, LA0.5:62±5*, LA0.5Gs:35±2*# μm). Cell differentiation was evaluated in C3A cells as the capacity of generating canalicular structures typical of epithelial hepatocytic cells. In fact, the number of canalicular structures significantly increased by LA. In conclusion we found that LA reduces cell proliferation and migration in HCC cells, what parallels the activation of AMPK, and these antitumor effects are increased by Gs. These findings support further studies of LA as a potential therapeutic agent for HCC treatment, alone and combined with metabolic interventions.*p<0.05 vs C, #p<0.05 vs Gs.

Keys words: Hepatocarcinoma, AMPK, a-Lipoic Acid, Glucose Restriction.

(40) **METABOLIC SYNDROME INCREASES CTBP1 EXPRESSION AND PROSTATE CANCER TUMOR GROWTH IN NON-IMMUNODEFICIENT MICE**

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Abstract: Metabolic syndrome (MeS) is a patophysiological disorder that increases prostate cancer (PCa) risk. C-Terminal Binding Protein (CtBP1), a transcriptional co-repressor of many tumor suppressor genes, is considered a molecular metabolic sensor because it needs to bind NADH to become active. Previously, in our lab we have established two mice models of MeS and PCa that allowed us to investigate CtBP1 role on tumor growth and progression from localized to metastatic disease. Both models share the same flaw; tumors were generated on immunodeficient mice. Given the importance of the immune system on all the aspects of cancer progression and the association between, cancer, inflammation and obesity; our aim was to investigate the interaction between PCa and MeS on non-immunodeficient mice. We worked with male C57BL/6J mice that were fed with control diet (CD) or high fat diet (HFD) by 15 weeks. PCa TRAMP-C1 cells were injected subcutaneously in the right flank of the animals. Tumor size was measured 3 times a week during 30 days after inoculation. Animals were weighted once a week along the whole experiment. After 10 weeks, HFD fed mice were significantly overweight compared to CD fed mice. These mice also showed higher glucose and cholesterol levels on serum samples compared to CD fed mice. Regarding tumor growth, we found a significant increase in tumor volume on MeS group. Sixty days after cell inoculation we sacrificed the animals and collected tumors, lungs and liver tissue for RNA isolation and histopathological analysis. We found that tumors generated on HFD fed mice showed higher expression of CtBP1 and lower expression of E-cadherin (CDH1), a known tumor suppressor and CtBP1 target. Histological analysis showed no sign of micro- or macro-metastasis in soft tissues (the lungs and liver). Altogether, these results reinforced CtBP1 role as a crucial link that associates PCa development and MeS in mice independently of immune system.

Key words: Metabolic syndrome, CtBP1, Prostate Cancer.

GENETICS AND MOLECULAR BIOLOGY 3

(812) **OMEGA-3 FATTY ACIDS MEDIATE IMMUNE REGULATION ANTICANCER MECHANISMS BY IL-6, 5 (S)-HETE AND ROS GENERATION IN PANCREATIC HUMAN**

CELLS

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Abstract: Pancreatic carcinogenesis is characterized by the activation of inflammatory signaling pathways. Numerous studies have demonstrated the role of essential fatty acids (EFAs) in pancreatic cancer initiation and progression, but the underlying molecular mechanisms have not been completely elucidated. Here, we studied the effects of two omega-3 EFAs, the eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and the omega-6 fatty acid, the arachidonic acid (AA) on a human pancreatic cell line (PANC-1). We determined the release of interleukin-6 (IL-6) as inflammatory marker in supernatants from PANC-1 cultures using ELISA, as well as their gene expression by qRT-PCR. Cell viability (Rezasurin) and reactive oxygen species (ROS) production (DCFH-DA assay kit) by fluorimetry. Eicosanoids generation and lipid cell profile by High pressure liquid and Gas chromatography (HPLC and GC).We observed a significant reduction in cell viability in DHA and EPA compared to AA and control (ethanol) treatments ($P<0.05$). Moreover, we demonstrated an increased apoptotic levels in DHA and EPA treated cells in comparison with the control and AA treatment, probably mediated by ROS production ($P<0.05$). A diminution of IL-6 gene expression ($P< 0.01$) and liberation ($p< 0.02$) resulted after DHA and EPA treated cells. The release of proinflammatory eicosanoids: 13-HODE and 5(S)-HETE decreased on EPA and DHA treated cells compared with AA and control ($p<0.05$).Therefore, we demonstrated that omega-3 EFAs may reduce the growth of PANC-1 by several mechanisms that include significant increase of ROS production, diminution of lipid peroxides and down regulation of gene expression and secretion of inflammatory cytokines such as IL-6.

Keywords: pancreatic cancer, essential fatty acids, eicosanoids.

(1590) **ANTIPROLIFERATIVE EFFECT OF ESSENTIAL OIL OF *Artemisia mendozana* IN TUMOR CELLS B16F0.**

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The basic pathophysiology of cancer comprises aberrations at different points in the cell cycle. Due to the increasing incidence of cancer worldwide, there is an intense search for new therapeutic strategies to treat this disease. In this area, important efforts have been oriented to exploring the action of compounds of plant origin. *Artemisia mendozana var mendozana* (ajenjo), is a plant belonging to the Asteraceae family that growth in the piedmont of Mendoza province and it is used as a medicinal plant with antispasmodic and antifungal properties. The essential oil of ajenjo (EO) contains 28 compounds of which the higher concentrated are: artemisia alcohol 4.8%, α-thujone 5.1%, borneol 11.2% and bornyl acetate 43.7%. In this project the effect of EO was analyzed in the *in vitro* proliferation of B16F0 murine melanoma cells. For the assay the cells were cultured with vehicle DMSO (control) or 0.1- 0.25 µl / mL EO dissolved in DMSO for 72 h. The growth rate (GI) ± SE was calculated from 3 independent experiments. At 72 h of culture, the GI of the control was 6.37 ± 1.07 and with EO was: 4.57 ± 1.07 (A); 1.37 ± 0.18 (B) and 0.43 ± 0.03 (C) for 0.13, 0.2 and 0.25 µL / mL respectively. GI of B and C were significantly different to the control ($p\leq0.001$). These results show that EO, at concentrations, is a powerful inhibitor of the in vitro proliferation of B16 F0 cells.

General, Cellular and Molecular Biology (BM).

(301) **EFFECT OF FETAL BOVINE SERUM ON DELTA AMINOLEVULINIC ACID SYNTHASE EXPRESSION IN C3A HEPATOMA CELLS**

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Starvation or reduction of fetal bovine serum (FBS) in culture medium arrest cells in a quiescent state or in G_0 phase. In these cells, addition of serum induced numerous genes and proteins phosphorylation. Hepatic delta aminolevulinic synthase (ALAS1), the first and regulatory enzyme of heme pathway, is inducible at transcriptional and/or post-transcriptional levels through processes involving participation of tyrosines phosphorylation. FBS in chicken embryo hepatocytes culture medium affected ALAS1 expression forcing medium modifications to obtain a reproducible model.

The aim of this study was to investigate the effect of serum on ALAS1 expression. In the *in vitro* assays C3A cells were incubated in DMEM medium with different concentrations of FBS. mRNA and protein levels were determined at different time points by qPCR and western blot respectively.

FBS fasting (18h) provoked a significant drop in ALAS1 protein levels that increased when FBS was restored. Different concentrations of FBS (1, 2, 5 and 10%; 2h) increased ALAS1 protein levels progressively reaching an 800% increment at FBS 10% compared to control (C) group, nevertheless, mRNA levels were significantly lower than those observed in group C. Time course of FBS effect (FBS 2%; 5, 15, 30, 60 and 120min) showed increased ALAS1 protein levels up to 300% at 2h, whereas treatment with FBS+cycloheximide (0.1mM) didn´t modify ALAS1 levels respect to time 0. Pretreatment with a tyrosine phosphatases inhibitor, phenylarsine oxide (PAO) (5 and 40 uM; 30 min), and subsequent addition of FBS 2%, 2 h resulted in a decrease of ALAS1 35% and 80% at both PAO concentrations respectively.

These results suggest that FBS affected ALAS1 content due to an increase in protein translation and tyrosine phosphatases are involved in this induction

Keywords: delta aminolevulinic synthase 1, phenylarsine oxide, serum,

(420) **ISOLATION OF EXOSOMES FROM BREAST CANCER CELLS AND ITS ROLE IN THE INTERACTION WITH TUMOR ASSOCIATED MACROPHAGES.**

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Worldwide, breast cancer is the most frequently diagnosed malignant cancer and the main cause of mortality in women. Despite the clinical benefits that the endocrine therapies offer to breast cancer patients, a significant rate develops resistance to those therapies. It is known that tumor associated macrophages (TAMs) promote tumor growth; however it is unknown if they play a key role in the development of endocrine resistance. Lately, there has been a greater interest in the nanovesicles called exosomes that are secreted by almost all cellular lines. These exosomes play an important role in cellular communication, growth of the microenvironment and tumor progression since it would allow the transmission of proteins, miRNA, RNA between cells; yet their mechanism and biology are still unclear. Here, we study different isolation techniques of the exosomes of the MCF-7 breast cancer cell-line and macrophagues KG-1 trying to understand if TAMs induce endocrine resistance *in vitro*. We used two different methods: ultracentrifugation and the commercial reagent “Isolation Exosomes Reagent (Invitrogen)”. MCF-7 cells and macrophagues were cultured until reached 70-80% of confluency and then, medium depleted of SFB exosomes was added and cultured for 48 h. Following the protocols of both methods, the supernatant was collected and the intact exosomes were obtained. To confirm the presence of the exosomes, we performed Electron Microscopy, and concordant images were obtained as previously described in bibliography. Additionally, the exosomes were detected by Western Blot with anti CD63 (a tetraspanin used for the detection of nanovesicles). Moreover, it was not observed the presence of Golgi markers, assuming that there is not contamination with other cellular vesicles. Exosomes of MCF-7 cells and macrophagues were

successfully isolated by both methods and we found that the commercial reagent is simpler, faster and its results are as good as one obtained by ultracentrifugation.

Keywords: Exosomes; TAMs; microenvironment; breast cancer.

(1580) **EVALUATION OF CIRCULATING TUMOR DNA AS A POTENTIAL LIQUID BIOPSY IN HEAD AND NECK TUMORS**

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The squamous cells carcinoma of the head and neck (SCCHN) affects the squamous epithelium of the oral cavity, tongue, oropharynx and larynx. In the world it represents the sixth most frequent cause of cancer. The main risk factors for the development SCCHN are smoking, alcohol, chronic inflammation and HPV. More than 70% of SCCHN are associated with mutations in the gene *TP53*. Circulating tumor DNA (ctDNA) are small fragments of nucleic acid originated by tumor cell turnover. CtDNA can be assessed in plasma, and offers the potential of a sensitive and specific biomarker for a host of applications including diagnosis or early detection of tumors, prognostic or overall survival, and predictive information on resistance and probability of lack of response to treatment.

The aim of the present study was to develop a laboratory workflow that allowed us evaluate the potential use of ctDNA can be used as a biomarker in head and neck cancers. Biopsy of primary tumor, blood and saliva were collected from 30 patients of SCCHN. DNA was extracted from tumoral tissue by Qiagen DNA Mini Kit. To obtain ctDNA isolation was performed from plasma and saliva obtained maximum 4 hours after blood collections. We tested several protocols and conditions to improve ctDNA levels. DNA concentration was measured on Bioanalyzer to obtain quality and Quantity. PCR of *TP53* exons were performed on tDNA and ctDNA. NGS libraries were done using Qiagen library kit. Tumor samples were sequenced by Sanger sequencing and ctDNA was sequenced using the PGM system at an average of 5000X. We improved ctDNA level carrying out a second plasma centrifugation at 6000 rpm. We were not able to obtain enough ctDNA from saliva. At the moment, two patients were evaluated by comparing sequencing results of both tDNA and ctDNA and we were able to detect same genetics variants. In conclusion, this preliminary results suggest that it is feasible to detect mutations in circulating free DNA in HNSCC.

Keywords: NGS, HnN, *TP53*, ctDNA, liquid biopsy.

(73) **IDENTIFICATION OF A NOVEL INDUCER OF SYNTHETHIC LETHALITY IN A HOMOLOGOUS RECOMBINATION BRCA1/2 DEFICIENT CELLULAR MODEL**

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The identification of compounds with selective tumor cytotoxicity can improve anticancer therapies. A promising strategy which takes advantage of the frequent impairment of DNA repair pathways in tumor cells is synthetic lethality (SL). For example, the loss of the Homologous Recombination Repair (HRR) capacity is frequent in breast and ovarian cancer. In those patients normal cells are HRR proficient and therefore, the accumulation of HRR substrates should selectively cause cell death in HRR defective cancer cells but not normal cells. Such hypothesis has been validated using Poly(ADP-ribose) polymerase inhibitors (PARPi), therefore prompting the systematic search for better and more specific SL inducers.

HRR loss is frequently triggered by defective or null expression of tumor suppressors BRCA1 and BRCA2. We designed a screening method to evaluate the specific ability of 684 kinase inhibitors to kill cancer cells with defects in BRCA1 and BRCA2 expression. Unexpectedly, the inhibition of one of those kinases caused the selective killing of BRCA2 defective cells with a much more modest effect in BRCA1 deficient cells. Such observations were validated in different cell lines and using commercial inhibitors of the same kinase (Ki), ruling out off- target effects. When treated with Ki, genomic aberrations and 53BP1 foci formation increased in BRCA2- but not BRCA1- depleted cells, suggesting that replication stress precedes cell death in Ki treated BRCA2-depleted samples. Finally, when combining sub-lethal concentrations of PARPi and Ki, SL was observed in BRCA2 (but not BRCA1) cells, suggesting that the treatments are not epistatic. Since the Ki sensitizes BRCA2-defective cells to sub-lethal doses of PARPs, the combined use of low doses of Ki and PARPi may improve the specific killing of BRCA2 defective tumor cells, sparing normal cells from unwanted collateral effects.

Keywords: homologous recombination, BRCA1, BRCA2, synthetic lethality, genomic instability

(573) **STUDY OF MECHANISMS UNDERLYING THERMOTOLERANCE ACTIVITY INDUCED BY CHLOROGENIC ACID IN C elegans**

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Chlorogenic acid (CGA) is a polyphenolic compound present in foods associated to different beneficial properties. In previous studies we demonstrated that CGA confers thermal stress resistance in *C.elegans* exposed to lethal temperature (37ºC). Its role in the stress resistance is not fully understood. Autophagy is thought to promote cell survival by providing fundamental building blocks to maintain energy homeostasis during starvation or stressful situations. On the other hand, hypoxia-inducible-factor (HIF)-1, a master transcriptional regulator of adaptation to hypoxia, plays a central role in stress resistance. In these sense, in the present study we have proposed to investigate the relationship between CGA, HIF-1 and autophagy. Results showed that CGA was unable to confer resistance to thermal stress in worms with loss of function of HIF-1 (ZG31) or with autophagy defects (cdc-48.2(tm659)). Moreover, preincubation of N2 worm with CGA before to be expose to thermal stress showed an increment in autophagy flux and HIF-1 activity. Finally, interactions between HIF-1 and autophagy underlying in CGA activity were explored by RNAi assay. Here, we demonstrated that autophagy and HIF-1 are mechanisms necessaries for CGA activity. All together, these preliminary results tend to explain the molecular mechanism that CGA activates in C elegans and evidence a possible hormetic response.

Keywords: C elegans, chlorogenic acid, autophagy, HIF-1, thermal stress.

(1267) **PRODUCTION AND CHARACTERIZATION OF A POLYCLONAL ANTIBODY AGAINST 14-3-3AcK49 TO STUDY 14-3-3 REGULATION.**

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Post-translational modifications (PTMs) of proteins affect almost all cellular processes. Among them, acetylation of lysines (K) has emerged recently as a reversible and dynamic PTM with important biological functions. The 14-3-3 proteins regulate the function and subcellular localization of thousands of proteins, through interaction with specific phospho- serine and threonine residues. The acetylation of a specific K (K49) which is part of the 14-3-3 binding pocket and is essential for its function, results in an inactive 14-3-3. To study its regulation and the crosstalk mechanism between acetylation-phosphorylation, we produced an antibody to recognize the above-mentioned AcK49 in 14-3-3. A chemically synthetized 15 amino acid peptide, including the acetylated K49, and conserved in all 14-3-3 paralogs, was coupled to Bovine Serum Albumin (BSA)

as carrier. This antigen was used to immunize rabbits in a pathogen-free animal facility (ISAL, UNL-CONICET). After 4 immunizations, blood was drawn from the animals, and the serum obtained by centrifugation. The later was tested by Dot- and Western blot against different 14-3-3 constructions. It recognized all the acetylated peptide or complete 14-3-3 forms (the antigen, an unconjugated synthetic peptide and a recombinant peptide coupled to Histone H3, all corresponding to the same sequence in 14-3-3, and *in vitro* acetylated 14-3-3), but not the non-acetylated recombinant 14-3-3. The serum did not recognize the recombinant peptide-H3 when blocked with the synthetic peptide. As expected, the preimmune serum did not recognize the antigen. This was the first step in a project to study 14-3-3 regulation by acetylation, which means regulation of regulators. We produced and characterized a polyclonal antibody that recognizes site-specifically the acetylation of 14-3-3 on its K49, which at the best of our knowledge is not commercially available.

Keywords: post-translational modification, 14-3-3 acetylation, regulation of regulators, crosstalk acetylation-phosphorylation

(88) **TUMOR SPECIFIC PROTEIN MAGEB2 REGULATES RIBOSOMAL BIOGENESIS**

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MageB2 is a tumor specific protein belonging to MAGE family. Previously, we reported MageB2 confers proliferative advantages to tumoral cells and localized in the cytoplasm, nucleus and also in the nucleolus, where the ribosome synthesis takes place. Here we study the relationship between MageB2 and the ribosomal biogenesis. In line with its nucleolar localization, immunofluorescence performed in an U2Os cell line inducible for MageB2 expression showed a higher number of nucleolus per cell (5.69 in control vs 6.93 in MageB2 expressing cells), suggesting an increase in nucleolar activities due to MageB2 expression. Ribosomal RNA (rRNA) gene expression is linked with cell growth and compromised in cancer cells. rRNA is synthesized in the nucleolus as a 47S precursor (pre-rRNA). We quantified the level of pre-rRNA by RT-qPCR and observed it was higher under MageB2 overexpression conditions. To complement this observation, we generated the HCT116 MageB2 KO cell line, using CRISPR/Cas9 technology. As expected due to MageB2 pro-proliferative role, cell proliferation was affected in the HCT116 MageB2 KO cell line and cell cycle analysis performed by flow cytometry showed a reduction in S phase percentages in comparison with its parental cell line (8.63% in HCT116 wt vs 4.02% in HCT116 MageB2 KO). Besides, we observed a 30% reduction in the pre-rRNA levels in the HCT116 MageB2 KO in comparison with HCT116 wt cell line.

rRNA precursor is transcribed by RNA pol I and controlled by multiple mechanisms. Activators as UBF and SL1 and repressors as HDACs and DNMTs regulate rRNA transcription to maintain optimal levels of cellular rRNA. In particular, HDAC1 is a known repressor of RNA pol I transcription. By performing immuneprecipitation assays we observed MageB2 strongly interacts with HDAC1. All together, our results suggest MageB2 could modulate ribosomal biogenesis, explaining, at least partially, the basis of it proliferative potential.

Keywords: MageB2, nucleolus, ribosomal biogenesis, pre-RNA synthesis

(95) **REGULATION OF MAGE TUMOR PROTEINS BY ONCOGENES AND ONCOSUPPRESSORS**

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MAGE-I genes expression is restricted to tumor and male germ cells. In the last years our group and others characterized different members of the Mage-I family, showing low functional redundancy, despite their high sequence homology.

Here we demonstrate that similarly to we reported for MageA2, MageC2 represses p53 transcriptional activity (3.0 fold) in a HDAC-dependent manner through trichostatin A (TSA) treatment.

We also identified two potential candidates able to regulate MAGE function: p14ARF tumor-suppressor and the oncogene RASV12. We observed that p14ARF expression significantly reverted both MageC2 and MageA2 repressive function of ectopically expressed p53 using DKO cells (murine embryonic fibroblasts $p53^{-/-}$, $mdm2^{-/-}$) and therefore in an Mdm2-independent fashion. Western Blot assay showed that MageC2 is degraded by p14ARF expression depending on proteasome activity (60% decrease reverted by MG132, a proteasome inhibitor). On the other side, immunofluorescence showed that MageA2 is relocalized to the nucleoli by p14ARF but no significant degradation was observed. On the contrary, RasV12 expression caused an increase in MageA2 and MageC2 protein levels. The effect of RASV12 on MageC2 protein was reflected in a greater inhibition of p53 when MageC2 is co-expressed with RasV12. Furthermore, the stabilization of MageC2 by RASV12 strongly prevents its degradation by p14ARF. Our observations suggest for the first time that MAGE-I tumor proteins could be targeted by oncogenes and tumor-suppressors. However, as demonstrated here, RASV12 oncogene can protect MageC2 from p14ARF degradation thus enhancing MageC2 ability in repressing p53 tumor-suppressor function. Altogether, our results uncover a novel notion where oncogenes could enhance their tumor-prone functions through MAGE proteins.

MAGE-I, p53, p14ARF, RasV12, stability.

GENETICS AND MOLECULAR BIOLOGY 4

(942) **CHARACTERIZATION OF THE PERFORIN-LIKE PROTEIN FAMILY IN *BABESIA* SP.**

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Bovine babesiosis is a tick-borne disease caused by protozoan parasites of the *Babesia* genus that affects livestock production worldwide. The study of proteins involved in their virulence is of special interest to understand the host-pathogen interaction and develop strategies to control the disease. The aim of this study was to characterize the Perforin-like protein (PLP) family of *Babesia* sp. since these pore-forming proteins are critical for pathogenesis and cell invasion. The PLP family in *Babesia* sp. has not been characterized yet.

Here we identified the *plp* genes in genomes of different *Babesia* species through detection of conserved motifs and domains using bioinformatics tools. All strains of *B. bovis* (n=2) and *B. bigemina* (n=4) had 6 and 8 genes respectively. Phylogenetic analyses were conducted on nucleotide and aminoacid sequences and showed that protein domains are highly conserved among *Babesia* species. Analysis of the secondary and tertiary structure of the functional domain of the PLP proteins showed that the conformation is conserved with the other proteins of the family suggesting that the *Babesia* proteins perform similar functions to the orthologous in other related organisms. Gene expression analysis in *B. bovis* merozoites showed that all the members of PLP family are expressed. Previously reported transcriptome analysis of *B. bovis* suggests that *plp1* has higher expression levels that the other PLP genes. Therefore, we selected this gene to further characterize it by expressing its functional domain in *E. coli*. Antibodies against recombinant PLP1 will allow to confirm its presence in merozoite lysates. Erythrocyte hemolysis assays will also be performed to evaluate the protein's lytic activity.

By combining bioinformatics as well as molecular and biochemical studies we expect to fully characterize the family of PLP proteins in *Babesia* and gain deeper knowledge of its role in this pathogen.

(1279) **IDENTIFICATION OF NOVEL PARTNERS OF THE SR-RELATED PROTEIN TbRRM1 IN *Trypanosoma brucei***

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Previously, we have demonstrated that *TbRRM1* silencing leads to cell cycle block, apoptotic-like death and abnormal cell elongation in the procyclic stage of *T. brucei*.

TbRRM1 has three RNA binding motif, two zinc fingers and one small C-terminal SR domain potentially involved in protein-protein interaction. Recently, TbRRM1 has been shown to retain numerous RNAs in nucleus avoiding their translation. In addition, TbRRM1 was also associated with a few protein partners including core histones and several hot spot proteins. However, these results were obtained from an in-gel digestion assay, leading to a partial identification of the associated proteins.

Therefore, to obtain a more complete view of this interaction map we addressed the identification of TbRRM1 partners by liquid chromatography tandem-mass spectrometry approach (LC-MS/MS). For that purpose, we carried out the N-terminal tagging of one allele of the *TbRRM1* gene in RNAi-TbRRM1 procyclic cells. It is worth mentioning that parasites conditionally express the RNAi which corresponds to the 5´UTR fragment of the *TbRRM1* gene present only in the wild type allele, therefore allowing the unaffected expression of the tagged version. The tagged protein rescued the lethal phenotype caused by the depletion of the TbRRM1 protein, since no differences were observed in the growth curve of the induced parasites relative to the uninduced cultures. Moreover, immunofluorescence analysis with anti-flag monoclonal antibody and anti-TbRRM1 serum evidenced the correct localization of the tagged protein in parasites from TET+ cultures. Altogether, these results indicate that the tagging does not interfere with either protein localization or TbRRM1 function. Thus, pull down of TbRRM1 was carried out by anti-flag antibody and samples were subjected to LC-MS/MS. Partners identified in this work, will contribute to elucidate the function of TbRRM1 protein to gain a better understanding about Trypanosoma biology.

Keywords: RNA binding protein, protein interaction, gene expression regulation.

(165) **TbVPS15 PLAYS A ROLE IN VESICULAR TRANSPORT AND CYTOKINESIS IN Trypanosoma brucei**

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The class III phosphatidylinositol 3-kinase (PI3K) Vps34 is an important regulator of key cellular functions, including cell growth, survival, intracellular trafficking, autophagy and nutrient sensing. In yeast, Vps34 is associated with the putative serine/threonine protein kinase Vps15, however, its role in signaling has not been deeply evaluated. Previously, we have reported the cloning and characterization of both TcVps34 and TcVps15 in *Trypanosoma cruzi* and demonstrated that in this parasite these proteins function as a complex. In addition, we showed that TcVps34 plays a prominent role in vital processes for *T. cruzi* survival such as osmoregulation, acidification, endocytosis and vesicular trafficking, and that both TcVps34 and TcVps15 are involved in autophagy in this parasite. Here, we have identified the Vps15 orthologue in *Trypanosoma brucei*, named TbVps15. Knockdown of TbVps15 expression by interference RNA resulted in inhibition of cell growth and blockage of cytokinesis. Scanning electron microscopy revealed a variety of morphological abnormalities, with enlarged parasites and dividing cells that often exhibited a detached flagellum. Transmission electron microscopy analysis of TbVps15 RNAi cells showed an increase in intracellular vacuoles of the endomembrane system and some cells displayed an enlargement of the flagellar pocket, a common feature of cells defective in endocytosis. Uptake of dextran, transferrin and Concanavalin A were impaired. Finally, TbVps15 silencing affected both general kinase activity and PI3K activity, supporting the hypothesis that both proteins are conforming a complex as occurs in other organisms. In summary, we propose that *T. brucei* TbVps15 has a role in the maintenance of cytokinesis, endocytosis and intracellular trafficking in this parasite.

Keywords: *Trypanosoma brucei*, kinase TbVps15, cell cycle, endocytosis

(1280) **TbRRM1 SILENCING INDUCES CELL DEATH BY AN APOPTOTIC-LIKE MECHANISM IN THE BLOODSTREAM FORM STAGE OF *Trypanosoma brucei***

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Since transcription in trypanosomatids is polycistronic, the regulation of gene expression occurs mainly at the post-transcriptional level by RNA binding proteins. In our lab we study the gene expression regulation in *T. brucei*, particularly we focus on elucidating the function of the SR-related protein, TbRRM1.

Previously we have demonstrated that TbRRM1 is essential for the cell survival since its silencing produces cell cycle block, morphological alterations and transcriptional elongation impairment in procyclic form parasites. In the bloodstream form stage, silencing of *TbRRM1* by RNAi altered the growth curve with parasites displaying abnormal nucleus and kinetoplast configurations. Microscopic analysis of TbRRM1 depleted parasites, also showed detachment of the flagellum and increased proportion of linked parasites.

To further characterize the cell death process, we analyzed the mitochondrial membrane potential by Rho123 incorporation after *TbRRM1* silencing. Results demonstrated that TbRRM1 depletion decreased both the proportion of viable parasites and the cell population positive for Rho123. In addition, dual staining with FITC-Annexin-V and propidium iodide assays showed an increment of both early and late apoptotic population after RNAi induction. Moreover, cell cycle distribution analysis by flow cytometry evidenced an increase of the hypodiploid population after *TbRRM1* silencing.

Altogether, these results demonstrate that TbRRM1 depletion led to cell cycle death compatible with an apoptotic-like process. Interestingly, our results also showed that the apoptotic-like events began after the cell growth curve was altered, suggesting some kind of quiescence or lethargy after TbRRM1 depletion. Keywords: RNA binding protein, apoptotis, annexin-V, mitochondrial membrane potential, hipodiploidy.

(126) **CHARACTERIZATION OF THE CEST MOTIF (CHAPERONE FOR THE SECRETION OF E. COLI FROM TIR) IN TRYPANOSOMATIDS**

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Abstract: Using a bioinformatic approach we identified a small family of proteins restricted to trypanosomatids that present a 'CEST' domain, as it shows structural homology with CesT proteins (Chaperone for *E. coli* secretion of Tir). These proteins form a family of chaperones involved in the secretion of virulence factors through the type III secretion system (TIIISS) in enteropathogenic bacteria. Since CEST has a structural homology with protein templates from *Salmonella sp.*, we selected these bacteria as a biological model. We aimed to characterize the different CEST motifs of trypanosomatids by making a functional panning of the spectrum of motifs using a functional complementation model in *Salmonella sp*. We started by characterizing TCLP1 (Trypanosomatid CesT-like Protein 1) which accumulates in the flagellar pocket (unique site of surface protein export and nutrient uptake) of *T. cruzi* replicative forms and may be involved in nutrient uptake. Through biochemical, biological and immuno-cytochemical techniques, we verified that the expression of this parasite protein reverts the effects of the deletion of the homologous chaperone SicP of *Salmonella thyphimurium* on the TIIISS secretion dependent to the SptP effector. SptP presence was observed in supernatants from secretion assays. Infection assays followed by fluorescence microscopy demonstrated that complemented bacteria produce normal alterations of the cytoskeleton of host cells during the infection process. These results demonstrate that TCLP1 not

only has structural homology with bacterial chaperones but is also able to reverse the original phenotype of KO bacteria for its own chaperone. Given the socio-economic relevance of the diseases produced by trypanosomatids, the strategic location of TCLP1 and the importance of the CEST motif in enterobacteria, we hope that the results obtained may help to better understand the basic biology of these parasites.

Keywords: trypanosomatids, CEST, *Salmonella*

(923) **MULTIGENE TcTASV FAMILY: CHARACTERIZATION OF THE SUBFAMILIES TcTASV-B AND TcTASV-W IN DIFFERENT *T.cruzi* STRAINS.**

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TcTASV is a *Trypanosoma cruzi* multigene family comprising about ~40 members and without orthologs in other trypanosomatids. All protein products of the TcTASV family have conserved amino and carboxy-termini, and a central variable core that allows partitioning TcTASV into four subfamilies: A, B, C and W. TcTASV-A and TcTASV-C subfamilies are the most numerous and were already characterized by our group (García, et al 2010; Bernabó et al 2013; Floridia et al 2016) but little is known about the less numerous TcTASV-B (2-10 genes) and TcTASV-W (2-4 genes) subfamilies. Our own previous results on sequences of the TcTASV family (Garcia et al, 2010) and the detailed inspection of more recently sequenced *T. cruzi* genomes, showed TcTASV-B genes in DTU-VI but not in DTU-I strains. This analysis was expanded here for a higher number of strains, by PCR and sequencing. On the other hand, we identified TcTASV-W genes in DTU-I, II, V and VI, by PCR and sequencing. TcTASV-B proteins are detected in trypomastigotes and extracellular amastigotes, while TcTASV-W is only expressed in trypomastigotes. Signals for surface localization and GPI anchoring are predicted *in silico* for both subfamilies. We determined that TcTASV-B is attached to the parasite membrane by a GPI anchor (but is not spontaneously secreted) in trypomastigotes, while TcTASV-W is not secreted nor attached to the parasite membrane by GPI, at least in the studied conditions. TcTASV-B is observed -by fluorescence microscopy- as discrete points on the membrane of both trypomastigotes and extracellular amastigotes. Interestingly, TcTASV-B and TcTASV-W proteins have divergent carboxy-termini domains that could explain the differences in protein location. Our final goal is to define which sequences determine the differential expression, traffic, cellular localization and, eventually, the function of the different TcTASV subfamilies. Supported by PICT 2014-1151 y PIP-2015-186.

*Keywords: T. cruzi,*TcTASV

(1135) **TRAFFICKING AND ASSORTMENT OF GPI-ANCHORED PROTEINS IN *TRYPANOSOMA CRUZI* CELL SURFACE.**

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At variance with mammals, trypanosomatids have a high percentage of proteins anchored by GPI in their plasma membrane. *T. cruzi* GPI-anchored virulence factors, such as *trans*-sialidase (TS) and mucins, are transported to the membrane by a non-conventional transport involving the contractile vacuole complex (CVC) prior to reaching the flagellar pocket and then the plasma membrane. Both proteins are only detectable in the CVC during the differentiation to the infective stage of trypomastigote. Once on the membrane, they are ordered in mutually exclusive domains: mucins are found in *"lipid rafts-like"* domains, whereas TS is not. Also, it is known that each protein has different GPI-lipidic composition. There is only scarce information concerning the signals that determine this arrangement on the plasma membrane or about the role of CVC on this novel transport pathway. Herein, we assessed the involvement of the lipid nature of the GPI anchor in this pathway and in the final membrane assortment. Recombinant *MucII* genes were constructed with either their native GPI or the TS-GPI signal, and cloned into the inducible expression vector *pTcIndex*. We found that protein intracellular trafficking was not modified. Using this regulated system with the recombinant *MucII* genes, we observed that both proteins were localized on the membrane with no accumulation in the CVC at low levels of expression. However, at higher expression levels constant protein accumulation was observed only in the CVC from 24h to 96h post-induction and not in other organelles of the secretory pathway. This suggests that CVC could function as a "bottle-neck". Then, the membrane arrangement of these proteins was analyzed. We have observed that the TS GPI-signal clearly changes the surface protein domain pattern both in size and distribution as compared to the native GPI-signal, thus highlighting the relevance of the GPI anchor in the final destination of surface proteins.

Keywords: Glycoproteins, *Trypanosoma cruzi,* GPI-signal.

(1226) **MONOCLONAL ANTIBODIES AGAINST THE C-TERMINAL REGION OF *TRYPANOSOMA CRUZI* RIBOSOMAL P0 PROTEIN EXERT ONLY B1-ADRENERGIC BUT NOT MUSCARINIC FUNCTIONAL ACTIVITY**

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The chronic Chagas heart disease (cChHD) is the most frequent and severe manifestation of *T. cruzi* infection. Its pathogenesis is currently explained by two, non-mutually exclusive hypotheses: the immune response associated with parasite persistence and the molecular mimicry between host and parasite proteins. In this regard, we have previously demonstrated the presence of high antibody (Ab) levels against *T. cruzi* ribosomal P proteins, TcP0 and TcP2β proteins, which bear similarities with cardiac receptors β1 adrenergic (β1-AR) and muscarinic (M2-R), in cChHD patients´ sera. Since immunization of mice with TcP0 induced Abs against its C-terminal region (P015 peptide) that bind to and stimulate both types of cardiac receptors, we decided to explore its functional activity by the generation of anti-P015 monoclonal Abs (mAbs). After immunization of BALB/c and autoimmune-prone mice with P015-BSA, three anti-P015 mAbs were obtained. All of them recognized P015 peptide and P0 protein, as well as the other Tc ribosomal P proteins in ELISA and Western blot. Immunofluorescence assay showed a positive staining in the cytoplasm of the different *T. cruzi* stages. The functional activity of these mAbs was measured by pharmacological assay based on primary cultures of neonatal rat cardiomyocytes. Results showed that the mAbs induced a positive chronotropic effect ($p<0.0001$) which decreased in the presence of β1-blocker bisoprolol ($p<0.0001$) and H26R peptide (second extracellular loop of β1-AR) ($p<0.0001$), but not with M2-R peptide. These data clearly demonstrate that anti-P015 mAbs exert a specific β1-AR stimulatory effect. In agreement with these results, the confocal immunofluorescence of murine HL-1 cardiac cells showed a plasma membrane staining compatible with the β1-AR localization. Our results strengthen the role of antibodies against *T. cruzi* ribosomal P proteins in the pathophysiology of cChHD.

Keywords: Chagas disease, *Trypanosoma cruzi*, cardiac receptors, molecular mimicry, cardiomyopathy

(1936) **POLARIZED LOCALIZATION OF SUBCELLULAR COMPONENTS IN PERITUBULAR MYOID CELLS**

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In rat testes, peritubular myoid cells (PM cells) form the outer monolayer of the seminiferous tubule (ST). PM cells have characteristics of smooth muscle cells and participate in ST contraction. The study of MP cells has been carried out in crushed TS, so the 3D shape and distribution of cellular components are unknown. The objective of this work was to construct a 3D model of MP cells and determine

microfilaments, lipid microdomains and caveolae location using optical planes (OP) (36-40 cross sections of 0.4 µm). To achieve this objective, subcellular structures of the MP cells (alpha-actin filaments, plasma membrane, nucleus, lipid microdomains and caveolae) were identified by immunofluorescence. The OP were processed with Image-J sofware. 3D reconstruction of the OP allowed us to determine that MP cells has an irregular hexagon shape with a cleft in its apical face. Remarkably, MP cell has marked apical-basal polarization. In the apical face, most of actin filaments were distributed longitudinally (LF) (71% ± 0.9) and a few ones transversally (TF) (29% ± 0.9). In the basal face TF were the majority (72% ± 2.2) and LF (28% ± 2.2). In other hands, caveolae (identified with anti-caveolin-1), were located following actin filaments distribution. They are identified as isolated dots arranged in longitudinal lines (LL) or transversal lines (TL). In the apical face most of caveolae are in LL (n=9) in a number of 58 ± 1.5 dots/line. and in a few TL (n=5) in a number of 25 ± 1.5 dots/field. In the basal face most of caveolae are in TL (n=10) in a number of 60 ± 2.5 dots/line. and in a few LL (n=6) in a number of 28 ± 1.2 dots/line. Also the lipid microdomains are distributed throughout the cell surface, but show a higher concentration on the basal surface. These results indicate that MP cells are organized in an apical-basal polarity similarly to epithelial cells. The question is ¿Are MP cells in contact with two different environments?

IMMUNOLOGY (INNATE IMMUNITY) 2

(371) ROLE OF INNATE CD8⁺ T CELLS IN CANCER

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Innate CD8⁺ T cells were discovered about 10 years ago. These cells have particular phenotypic features (CD44^{hi} CD122^{hi} CD49d^{hi}) and exert cytotoxic activity mainly through NKG2D expression without specific antigen recognition. Innate CD8⁺ T cells express high levels of the transcription factor Eomesodermin and low expression of T-bet. They rapidly produce IFNγ after IL-12 and IL-18 stimulation due to constitutive expression of their receptors. Moreover, an antitumor role of innate CD8⁺ cells has been recently postulated both in mice and human. Our experiments using murine tumor cell lines demonstrate that systemic expression of these 2 cytokines (by hydrodynamic injection of their cDNAs) significantly attenuate tumor growth in WT mice compared to control group (injected with an empty cDNA) ($p<0.05$). Phenotypic analysis (based on the mentioned markers) of splenocytes and tumor-draining lymph node showed an increase of innate CD8⁺ cells in 12+18-treated mice compared to control group in both WT and OT-I mice ($p<0.05$).

Several chemokines and their receptors have been described to participate in the homing of different immune cells to tumors. Between them, CCR5 seems to be quite important for T cell extravasation. When we analyzed the expression of CCR5, we observed an increment in CCR5⁺ CD8⁺ but not in CD4⁺ T cells (by flow cytometry) only in mice treated with IL-12+IL-18 cDNAs compared to control mice ($p<0.05$). Accordingly, preliminary flow cytometry data revealed an increase in Percoll purified-infiltrating innate CCR5⁺ CD8⁺ T cells into B16 tumors of 12+18-treated mice compared to control group that correlates with an increase of total CD45⁺ cells infiltrating B16 tumor of 12+18-treated mice (microscopic analysis by immune fluorescence).

All together this data suggest that innate CD8⁺ cells may have a critical role in the control of tumor growth that could be important when TCR specific immune response is bypassed by tumors that down-regulates MHC-I expression.

Keywords: Cancer, Innate CD8⁺ T cell, tumor infiltration, IL-12 e IL-18, CCR5.

(666) A NEW PLATFORM TO DETECT INNATE IMMUNE RESPONSE MODULATING IMPURITIES (IIRMIS) IN THERAPEUTIC PROTEINS BASED ON THE USE OF MONOCYTE-DERIVED MACROPHAGES AND DENDRITIC CELLS

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Biotherapeutics are agents produced through complex multi-step manufacturing methods. However, these products can contain contaminants derived from the host cell and manufacturing process that can trigger innate immune receptors, leading to the activation of immune cells and the secretion of cytokines and chemokines that could ultimately lead to the generation of anti-drug antibodies. To date numerous experimental approaches have been proposed to assess the risk of product immunogenicity and are mainly based on peripheral blood mononuclear cells (PBMC). However, macrophages and dendritic cells, which are rich in pattern recognition receptors (PRR) are present at very low frequency or absent in peripheral blood (PB). To address this, we evaluated the use of monocyte-derived macrophages (MØ) and dendritic cells (mo-DC) as screening tools to detect IIRMIs. Using purified PRR Agonists (PRRAgs) as model IIRMI and human IL-6 and IL-8 mRNA and protein as biomarkers for cell activation, we show that both primary cultures are more sensitive than PBMC in detecting IIRMI ($p\leq0.05$). Interestingly, MØ and mo-DC showed different limits of detection (LLOD) for individual PRRAgs. In addition, PRRAgs induced increased expression of a set of pro-inflammatory genes and the profile of genes induced varied with the TLR agonist and concentration. Finally, we tested the capability of human PBMC, MØ and mo-DC for detecting impurities in a commercially available product for the treatment of autoimmune inflammatory diseases. While PBMC samples were not activated by impurities present in the product, MØ and mo-DC showed a robust activation ($p\leq0.05$), even when low amount of product where added to the cell culture. In conclusion MØ and mo-DC constitute a suitable cell platform for testing impurities in therapeutic products, especially when these entities are present at such low levels not even detected by PBMC.

Keywords: Biotherapeutics, immunogenicity, impurities, cell-based assays.

(35) *Brucella abortus*-STIMULATED PLATELETS ACTIVATE BRAIN MICROVASCULAR ENDOTHELIAL CELLS

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Central nervous system invasion by bacteria of the genus *Brucella* results in an inflammatory disorder called neurobrucellosis. Blood-brain barrier activation is a common feature of human neurobrucellosis, but the underlying mechanisms are largely unknown. The aim of this work was to investigate the influence of *B. abortus* (*B.a.*)-activated platelets (PTL) on human brain microvascular endothelial cells (HBMEC). For this, supernatants (SN) from *B.a.*-stimulated human PTL were used to stimulate HBMEC. Expression of ICAM-1 (CD54) and secretion of IL-6, IL-8 and MCP-1 were evaluated to determine HBMEC´ activation. SN from *B.a.*-stimulated PLT induced a significant secretion of IL-6, IL-8, MCP-1 and the up-regulation of CD54 expression in a dose-dependent manner compared with SN from unstimulated PLT ($p<0.001$).To avoid the possible implication of *B.a.* outer membrane vesicles (OMV) we ultra-centrifuged the SN (to eliminate OMV). HBMEC were stimulated with ultra-centrifuge SN and we observed no differences on HBMEC' activation compared with non-centrifuge SN ($p>0.05$), demonstrating that a soluble factor released from PLT was implicated on HBMEC' activation. SN from heat-killed *B.a.*-stimulated PTL also activated HBMEC indicating that B.a.' viability was not necessary in activating PLT. Soluble CD40L was not implicated on HBMEC' activation, as an anti-CD40L antibody could not block cytokine secretion or ICAM-1 up-regulation. Treatment of SN with trypsin eliminate the effect ($p<0.0005$), demonstrating that the PLT effector is a protein. These results

demonstrate that *B.a.*-stimulated PTL can activate brain endothelial cells, and this could be implicated on the entry to B.a. to the central nervous system.

Brucella abortus – platelets - brain microvascular endothelial cells - Neurobrucellosis

(43) **ROLE OF HUMAN LUNG FIBROBLASTS IN THE *Brucella abortus* INFECTION BY INHALATION**

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Inhalation of contaminated aerosols is one of the most common forms for human infection by *Brucella abortus*. Together with epithelial and endothelial cells, fibroblasts are an important component of the alveolar respiratory barrier. While the interaction of *Brucella* with human lung epithelial cells has been studied, its interaction with human lung fibroblasts remains unknown. In this work, we investigated the ability of *B. abortus* 2308 to infect and induce inflammatory mediators in a cell line of normal human lung fibroblasts (MRC-5). The cells were infected for 2 h and then incubated with gentamicin to kill extracellular bacteria (time 0 p.i.). At 2, 24 and 48 h p.i., cells were lysed to determine CFU of intracellular bacteria, and culture supernatants were collected at 48 h p.i. to measure cytokines. Results showed that *B. abortus* infects and replicates in lung fibroblasts and that its survival depends on a functional *virB* operon. The infection induced the secretion of interleukin-8 (IL-8) ($p<0.0001$), which secretion was mediated by p38 MAPK and NF-κB pathways, and monocyte chemotactic protein 1 (MCP-1) ($p<0.0001$), which secretion depended on p38 MAPK, NF-κB and PI3K pathways. Furthermore, the infection increased the gelatinase/collagenase activity ($p<0.0001$). The cytokine secretion did not depend on bacterial viability since heat-killed *B. abortus* and *B. abortus* lipopolysaccharide were also able to induce IL-6, IL-8 and MCP-1 secretion. In addition, the secretion of IL-6 and MCP-1 by fibroblasts increased significantly upon stimulation with conditioned medium (CM) from *B. abortus*-infected macrophages as compared to stimulation with CM from non-infected cells. These results suggest that human lung fibroblasts respond to *B. abortus* infection producing chemokines either directly or by stimulation with soluble factors secreted by infected macrophages.

Keywords: *Brucella*, human lung fibroblasts, cytokines, chemokines

(1012) **CATHEPSIN L3 FROM Fasciola hepatica induces IL-1β AND IL-18 SECRETION IN DENDRITC CELLS AND PROMOTES AN IN VIVO IFN-γ RESPONSE**

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Cathepsin L3 (CL3) is expressed in the juvenile stage of *F.hepatica* and has collagenolytic activity, however there is no information about its interaction with the immune system. The aim of this work was to study the ability of CL3 to modulate dendritic cells (DC) activation and its effect on adaptive immune response. DC were differentiated from bone marrow cells of C57BL/6 (WT) or NLRP3 KO mice with GM-CSF and cultured at different times with CL3. DC were pulsed with recombinant CL3, an inactive variant recombinant (rvCL3) or LPS/ATP. IL-1β and IL-18 secretion were quantified in supernatants by ELISA. Moreover, to study the ability of CL3 to induce a specific immune response, WT mice were intraperitoneally immunized with CL3 plus MPLA, three times at 48 h intervals. After 7 days of last immunization, lymph node cells (LNC) were recovered, re-stimulated with CL3 and IFN-γ production was studied. Statistical analyses were performed by ANOVA. To determine the uptake of CL3 by DC, we analyzed the presence of labeled CL3 into DC by confocal microscopy. Although after treatment, there was some colocalization between CL3 with lysosome, the presence of labeled CL3 in the cytoplasm of DC was evident. Besides, CL3 promotes IL-1β and IL-18 production in WT DC, at similar levels than those secreted by LPS/ATP-stimulated DC and this effect was not observed when DC were treated with rvCL3. However, when NLRP3KO DC were treated with CL3, IL-1β and IL-18 secretion were significantly diminished ($p\leq0,05$). On the other hand, increased levels of IFN-γ in CL3 re-stimulated LNC from CL3-MPLA immunized mice were observed ($p\leq0,01$).

Our results suggest that CL3 up-taking by DC induces IL-1β and IL-18 production-dependent on NLRP3, which in turn was dependent on CL3 enzymatic activity. This event could be related to the uptake of CL3 that promote the inflammasome activation. The up-regulation of these cytokines may be involved in IFN-γ production.

(1080) **AKAP350 REGULATES NATURAL KILLER CYTOTOXICITY BY CONDITIONING CELL POLARIZATION AT THE IMMUNE SYNAPSE**

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The immunological synapse (IS) defines the interface between an immune cell and the cell with which it interacts. For natural killer (NK) cells, the formation of an IS is essential for cell activation and for directed cell secretion of lytic granules content into the target cell. The development of the IS in NK cells involves, after an initial interaction with the target cell, the formation of an actin ring at the IS, and the repositioning of the microtubule organizing center (MTOC) towards this site, which conditions the specificity of the final lytic response. AKAP350 is an A-kinase anchoring protein with a main role in the regulation of microtubule dynamics, which, in T-cells, participates in integrin LFA-1 clustering. The aim of our work was to analyze AKAP350 participation in the development of the IS in NK cells. We first characterized AKAP350 expression and localization at the MTOC in NK YTS cells. Control YTS cells and YTS cells with decreased expression of AKAP350 (AKAP350KD) were exposed to erythroleukemia derived KT-86 cells (10:1 ratio) for 3 h and their lytic response analyzed by flow cytometry. AKAP350KD cells lytic activity against their specific targets was significantly decreased (-50%*). Flow cytometry analysis indicated that AKAP350KD cells did not show any alteration at the initial YTS-KT86 conjugate formation or final YTS degranulation. Nevertheless, analysis by confocal microscopy showed that LFA-1 recruitment at the IS was inhibited (-41%*) in AKAP350KD cells. Furthermore, MTOC translocation towards the IS, analyzed as the distance between the YTS centroid and the IS (d_c) minus the distance between the MTOC and the IS (d_{MTOC}), was severely impaired by the decrease in AKAP350 expression. Our results indicate that AKAP350 conditions NK lytic capacity by modulating LFA -1 clustering and MTOC translocation towards the IS. Considering previous results, AKAP350 could modulate these processes by facilitating microtubule/actin cytoskeleton dynamics.

Keywords: NK cells, AKAP350, MTOC, LFA-1, Cytotoxicity

(1149) **ASSAYS FOR STUDYING CYTOSOLIC C23/NCL IN MACROPHAGES AS A SIGNALLING HUB INTEGRATING CASPASE, KINASE, mRNA AND INNATE IMMUNE PATHWAYS**

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Abstract: During a systematic screening of the cytoplasmic proteome of bacterial-infected human macrophages (n=19) we detected a 110 KDa protein, identified by MS, antibodies and pI range as C23/NCL, which was down-regulated after infection (51.7 % at day 4, p=0.05). The triggering of the effect did not depend on bacterial viability suggesting an innate response to surface bacterial components. Little is known about the roles in the cytosol during infection of this major nucleolar phospho-protein. To understand this down-regulation, we developed novel biochemical and proteomic

assays including, protein kinase determinations. We followed putative upstream protein candidates in a NCL pathway studying kinases and other candidates. Several sensitive assays were developed to monitor its level and *in vivo* phosphorylation state. The down-regulation was not due to affected kinases regulating its phosphorylation state or to enzymes modulating its isomerization. Instead, it was due to increased partial cleavage of the full-length protein generating smaller fragmented forms. The cleavage was detected with higher sensitivity and more quantitatively with our assays compared to immunoblots. To understand the cleavage, we studied caspase activation. The results suggest that the cleavage is correlated with transient caspase activation and it could be a biomarker of apoptotic versus inflammatory signaling balance in the cytosol. It will be interesting to further study C23/NCL as a signaling hub integrating apoptotic, proliferation, nucleolar and immune/inflammatory signals both in infected and non-infected macrophages. Our sensitive assays will help studies in other cell types and cell compartments since the multitasking, acidic, RNA-binding and histone-binding C23/NCL is considered in cell cycle, gene expression and mRNA half-life studies and also as target for anti-cancer and anti-angiogenesis treatments. It is also a cell surface receptor for microbes and other ligands.

Keywords: nucleolar, cytosolic

(1718) **ANTIGEN PRESENTING CELLS AND IMMUNOMODULATION MEDIATED BY DIFFERENT INFECTIVE STAGES OF *T. cruzi***

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Dendritic cells (DCs) are key players mediating the innate and the adaptive immune response. Previously, we have reported that blood (b) trypomastigotes (Tp), negatively regulate the activity and function of bone-marrow derived DCs (CD-MO) *in vitro*. In the present work we compared the effect of bTp and metacyclic (m)Tp from culture, *in vitro* in two different subsets of DCs, CD-MO and XS106, a cell line derived from epidemic DCs (Mohan, 2005). Furthermore, we studied the extent of these results *in vivo* in the experimental model of infection after *T. cruzi* intradermal inoculation.

By flow cytometry, we better characterized the phenotype and functional properties of the XS106 cell line. We found F4/80, Ly6C, CD207, CD11b, CD11c, MHCII, CD40 and CD86 surface expression, confirming the myeloid origin of these cells in addition to a basal activation state, not affected by Tp (bTp or mTp). Moreover, by ELISA we detected TNF-α, and IL-10 downregulation in the presence of LPS+Tp, contrary to previous results reported in CD-MO. Co-culture of XS106 or CD-MO with bTp or mTp demonstrate that bTp presented enhanced infectivity in XS106 compared to mTp and to CD-MO.

The experimental model of infection with bTp and mTp showed differences in the recruitment of leukocytes into the site of infection. Animals inoculated with mTp displayed 100% of survival and no parasite detected in blood, while bTp inoculation induced 80% of mortality in addition to high parasitemia. Infection with mTp was confirmed by parasite detection at the site of infection in the skin and in the spleen by PCR. The challenge of mTp-infected animals by intraperitoneal infection with a lethal doses of bTp two weeks after the initial inoculation showed high activation of splenic DCs and control of the parasite load in blood and tissues. These results suggest that mTp obtained *in vitro* from epimastigotes and bTp triggered different early immune response.

(1751) **CASPASE-1/11 SIGNALLING IS CRITICAL FOR INDUCING CYTOTOXIC CD8+ T CELLS AGAINST *Trypanosoma cruzi* ACUTE INFECTION**

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NLRP3 inflammasome has been reported as a protection mechanism to control *T. cruzi* infection during the innate immune response. Once it gets assembled, active caspase-1 cleaves the pro- IL-1β and IL-18 cytokines into its active forms. We previously demonstrated that infected *nlrp3-/-* in a similar way to C57BL/6 WT mice, exhibited similar parasitemias associated with Th1 and Th17 phenotype. Furthermore, high levels of transaminases and pro-inflammatory cytokines IL-1β and IL-6 were found in plasma of WT and *nlrp3-/-* mice. Here, we studied the hepatic parasite load and its relation with the liver histopathological status from WT, *nlrp3-/-* and *casp-1/11-/-* mice. Additionally, we comparatively analyzed the expression of IL-1R and IL-18R on CD8+ T cells and the expansion of IFNγ-, IL-17- and IL-10-producing CD8+ T cells during acute *T. cruzi* infection. Male C57BL/6 WT, *nlrp3-/-* and *casp1/11-/-* mice were infected with *T. cruzi* (Tulahuén).

The parasitic load was increased in both knock-out (KO) strains ($p<0.001$), presenting the *casp1/11-/-* mice the highest parasitism linked to a parasitemia peak at 21 dpi ($p<0.05$). Both KO strains presented minor hepatic leukocyte infiltration ($p<0.0001$) with augmented F4/80+ ($p<0.01$) and reduced CD8+ T cell numbers ($p<0.001$) compared to WT mice. As expected, the number of IL1R+ ($p<0.0001$) and IL18R+ ($p<0.001$) CD8+ T lymphocytes was strongly diminished in *casp1/11-/-* mice. Interestingly, IL-17+CD8+ T cells were only present in WT mice ($p<0.0001$) while IFN-γ- and IL10+CD8+ cells were detected in WT and *nlrp3-/-* ($p<0.001$). Last, plasmatic IL-18 quantification revealed that *casp1/11-/-* animals were not capable of increasing its levels, unlike WT and *nlrp3-/-* mice. These results clearly show that although NLRP3 partially participates in the response against the parasite, casp-1/11 play a critical role in the induction of adaptive immunity mediated by cytotoxic CD8+ T cells although probably also contribute to tissue damage.

Keywords: Caspase, Adaptive immunity, Trypanosoma cruzi.

(1796) **ANTI-INFLAMMATORY EFFECT OF RHIZOMES OF AN ARGENTINIAN MEDICINAL PLANT (*Smilax campestris)* MEDIATED BY THE INHIBITION OF THE NFkB TRANSCRIPTION PATHWAY**

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Smilax campestris (*S. campestris*) is a medicinal plant wildly distributed in northern Argentina. It has been used in folk medicine for its anti-inflammatory and antioxidant effect. Activated macrophages produce pro-inflammatory mediators and reactive oxygen species (ROS) promoting the establishment of an inflammatory state. The NF-kB transcription factor pathway has a crucial role as a modulator of the inflammatory response. Previously we showed an anti-inflammatory effect of *S. campestris* aqueous extract on the production of pro-inflammatory cytokines (IL-1β, IL-6, IL-8), metalloproteinase-9 activity (MMP-9) and superoxide anion levels in LPS-activated THP-1 macrophages without exerting cytotoxic effects. In this study, we evaluated the effect of *S. campestris* aqueous extract on the production of the chemokine MCP-1 (ELISA) and the activation of IκB/NFκB transcription pathway (western blot) in a human monocyte-macrophage cell line, THP-1, differentiated with PMA. Cells were stimulated with *S. campestris* aqueous extract (10, 100, 1.000 and 10.000 ng/ml) in absence or presence of the inflammatory stimulus, lipopolysaccharide (LPS). The aqueous extract of rhizomes of *S. campestris* did not affect MCP-1 levels in absence of LPS. But the extract significantly decreased the production of the latter chemokine in human LPS-activated THP-1 macrophages ($p<0.05$). In addition, *S. campestris* extract (10.000 ng/ml) decreased NFκB-p65 levels in nuclear extracts ($p<0.01$), suggesting NFκB pathway is involved in the anti-inflammatory effect of *S. campestris* extract on THP-1 macrophages. In conclusion, we suggest an anti-inflammatory effect of *S. campestris* aqueous extract mediated by the inhibition of the NFkB-p65 transcription pathway affecting the production of

pro-inflammatory mediators, in human LPS-activated THP-1 macrophages. Our results provide evidence for the potential use of *S. campestris* aqueous extract as a therapeutic agent against several inflammatory diseases.

Keywords: Smilax plant extract, NFkB pathway, cytokines, THP-1 macrophages

IMMUNOLOGY (CLINICAL IMMUNOLOGY) 1

(539) **TRISOMY 21 CAUSES GLOBAL CHANGES IN GENE EXPRESSION IN REGULATORY T CELLS.**

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Trisomy 21 (T21) causes Down syndrome, due to the presence of an extra copy of chromosome 21 in some or all of an individual's cells. Individuals with T21 have alterations in disease incidence: these individuals have a higher predisposition of Alzheimer's disease, leukemia and autoimmune disorders, but a decrease susceptibility to solid tumors compared to euploid individuals (disomic, D21). Also, individuals with T21 present a constant activation of interferon signaling. Recently, we found that regulatory T cells (Treg) from individuals with T21 show an impaired suppressive capacity *in vitro* with no significant changes in the Treg phenotypic signature. In order to understand why T21 Treg are dysfunctional, we sorted CD4+ CD25hi CD127lo from peripheral blood of T21 and age-matched D21 individuals (n=6) and performed transcriptomic analysis by RNA-seq (using Clontech SMARTer Ultra Low Input RNA kit v4 followed by the Nextera XT kit to create the libraries that were sequenced on the HiSeq 2500). The comparison of T21 versus D21 Treg cells identified 134 consistent differentially expressed genes (DEGs, padj<0.1), with a disproportionate number of them being upregulated in T21 cells (91 up and 43 down). When we evaluated where the DEGs were located across the genome, we found, as expected, that many chr21 genes were overexpressed. However, ~73% of DEGs were located on other chromosomes, with no obvious contiguous domains of up- or downregulation. Next, we subjected T21 DEGs to upstream regulator analysis using Ingenuity Pathway Analysis (IPA) to identify putative factors contributing to consistent changes in gene expression (pval<0.05). IPA effectively identified canonical pathways important for maintenance of the expression of Foxp3 and therefore its functionality. Further functional studies should be performed to test these signaling pathways in T21 Treg to relate them to the diminished suppression capacity observed in Treg from individuals with T21.

Keywords: Trisomy 21, Regulatory T cells, suppressive function,

(271) **CHARACTERIZATION OF IGG ISOTYPES AGAINST SEVERAL *Trypanosoma cruzi* ANTIGENS AND THEIR PROFILES ALONG CHAGAS HEART DISEASE STAGES**

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In murine infections, the inflammatory or regulatory profile of the immune response is associated with different IgG, but such association is less evident in humans. In Chagas heart disease (CCHD), several studies have corroborated the association of increased inflammatory cytokines levels in patients with advanced degrees of chronic disease but the profile of subclases of IgG has been scarcely reported. Tanking in mind that IgG1 is an inflammatory subclass while IgG2 and IgG4 have regulatory profiles, we hypothesized that these antibodies should be detected in different levels in asintomatic vs sintomatic patients. Then, we evaluated whether the severity of myocardiopathy is associated with an antibody subclass expression profile and if different antigens triggers different profiles.

A cross-sectional study involving 60 individuals who underwent complete clinical examination was performed. Patients were classified into three stages according to classification proposed by Storino et al.: CCHD stage I (n = 18), CCHD stage II (n = 23), CCHD stage III (n = 19). Serum samples were obtained for indirect ELISA assays that were performed to assess antibody levels for IgG subclasses (IgG1, IgG2 and IgG4) against two mimetic antigens (P2B, B13), a constitutive one (FRA) and total *T. cruzi* homogenate. For P2B, FRA and *T. cruzi* homogenates the higher reactivity was obtained with IgG2, followed by IgG1, and IgG4, respectively. For B13 the order of reactivity was IgG1> IgG2> IgG4. Along severity stages of CCHD, no significant differences in the levels of the different subclasses were detected for any of the antigens studied (p>0.05).

Although it is necessary to increase the number of patients in the sample, the homogeneity of the different antibodies subclasses levels against a heterogeneous group of antigens allow to infer that there is no association between subclass of antibodies and degrees of heart disease in humans.

Keywords: Chagas heart disease, *Trypanosoma cruzi*, anti-T antibodies *cruzi*

(1675) **COMPLEMENT FACTORS C3, C4 AND C5A IN MYASTHENIA GRAVIS: LOOKING FOR A POTENTIAL DISEASE ACTIVITY MARKER.**

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Myasthenia Gravis (MG) is an autoantibody-mediated pathology. Many of these autoantibodies bind to the acetylcholine receptor in the neuromuscular junction, activating the complement system, thus generating damage and muscle weakness. We aimed to measure C3, C4 and C5a concentration in MG patients, and to analyze their potential as disease activity markers. We collected 63 samples from MG diagnosed patients (17 exacerbated) and normal matching controls (nc). There were 11 cases of paired samples. Disease status (remission: rem or exacerbation: ex) was determined by neurologic evaluation. We quantified C5a using a home-setup ELISA (expecting increases). C3 and C4 were measured with commercial radial immunodifussion plates (expecting decreases). There were no statistically significant (ss) differences in C3 and C4 mean values between groups (rem, ex, nc). But there was a ss correlation between C3 and C4 values in the 63 samples. This correlation was strong (r^2=0.9) in the group of patients in an ex period (p<0.0001). Among all MG patients, 10/17 (59%) showed high C5a values during an ex period. On the other hand, 27/46 (59%) showed low C5a values during a rem period (vs. mean value of nc). There was a ss increment in C5a values (p<0.05) in the ex group vs. nc, when analyzing the group of 11 paired samples. We couldn't find a ss mean difference or correlation when analyzing paired samples themselves. In conclusion, C3 and C4 were not good markers at differentiating disease status in MG patients, mainly due to the wide range of normal values they have. Alternatively, these two values have a very good correlation in ex MG patients, indicating that the two factors are being consumed simultaneously due to complement system activation by autoantibodies. The increment in C5a values in ex MG patients seems to be a promising disease activity marker, however, more patients need to be evaluated in order to achieve ss differences.

Keywords: Myasthenia Gravis, complement, remission, exacerbation, activity marker

(683) **CHRONIC LYMPHOCYTIC LEUKEMIA CELLS IN-**

CREASE NEUTROPHIL SURVIVAL AND PROMOTE THEIR DIFFERENTIATION INTO CD16^{HIGH} CD62L^{DIM} SUBSET

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Abstract: Chronic lymphocytic leukemia (CLL) is characterized by the progressive accumulation of clonal B lymphocytes in blood, lymph nodes, spleen and bone marrow. Within lymphoid organs, CLL cells foster a protective microenvironment where they survive and proliferate. While almost every cell type in lymphoid tissues was reported to be modified by the leukemic clone, there is scarce information regarding the interaction of CLL cells with neutrophils. Tumor associated neutrophils (TANs), described in multiple types of solid tumors, facilitate cancer progression. Our aim was to determine if CLL cells were capable of modifying neutrophil phenotype and survival. To that purpose, neutrophils from healthy donor (HD) were co-cultured with purified leukemic cells from CLL patients (ratio 1:1) and apoptosis was assessed by flow cytometry using Annexin-V. Our results showed that CLL cells delayed neutrophil apoptosis at 24-72 h (n=15, p<0.05) and this effect was associated with the up-regulation of the anti-apoptotic protein Bfl-1. Protection of neutrophil apoptosis was similarly induced by CLL-conditionated media (CLL-CM) or transwell culture. Although there is not a unique phenotype for TANs, the downregulation of CD62L is a key feature of reprogrammation of neutrophils by tumor cells. Therefore we compared the percentage of CD16^{high}CD62L^{dim} subset in HD neutrophils cultured with or without CLL-CM for 24 h. We found an increase in the proportion of this subset induced by CLL-CM (n=10, p<0.05). Given that IL-10 and TGF-β have been implicated in TANs reprogrammation, we evaluated their role in the cross-talk between CLL cells and neutrophils. Our results showed that depletion of both cytokines from CLL-CM did not impair its anti-apoptotic effect but did impair its capacity to downregulate CD16 expression. Altogether these data show that CLL cells modify the survival and phenotype of neutrophils through the release of soluble factors.

Keywords: chronic lymphocytic leukemia, apoptosis, tumor-associated neutrophils, microenvironment

(883) **A CORNEAL INJURY IN ONE EYE LEADS TO DISRUPTION OF MUCOSAL TOLERANCE IN THE OPPOSITE OCULAR SURFACE THROUGH A TRPV1- AND SUBSTANCE P-DEPENDENT NEUROINFLAMMATORY CIRCUIT**

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Both ocular surfaces are considered functionally independent, thus an injury to one cornea is not expected to affect the opposite eye. However, we have observed that the tolerogenic immune response observed in mice after exposing one ocular surface to a foreign antigen is prevented if the opposite cornea is injured. Here we explored the underlying mechanisms for such inter-eye influence. First, we observed an increase in NFκB signaling in the conjunctival epithelium of the opposite eye (p<0.001), as measured by p65 intracellular localization by confocal microscopy 48 h after a corneal burn. At the same time point we also detected increased numbers of dendritic cells (p<0.05) and more activated T cells cells (p<0.05) in the contralateral lymph node. The fast kinetics of these changes and lack of lymphatic cross drainage from the eyes suggested that an inter-eye neuroinflammatory reflex could be involved. TRPV1 activation is an afferent signal for local neuroinflammation, whereas substance P (SP) released from the same sensitive terminals serves as an effector signal. In line with this hypothesis, instillation of TRPV1 antagonists on one eye before the corneal burn precluded the disruption of the fellow eye's tolerogenic response (p<0.05). Conversely, instillation of a SP receptor antagonist onto the fellow eye also precluded the inter-eye disruption (p<0.05). Moreover, a TRPV1 agonist instilled on one eye recapitulated the contralateral conjunctival NFκB activation and immune disruption (p<0.05). Remarkably, a clean 180º conjunctival incision, which severs most nerve endings, in one eye had a similar contralateral effect (p<0.05), whereas subconjunctival injection of complete Freund's adjuvant, which is a strong inflammatory stimulus but does not directly affect nerve terminals, did not (p>0.05). These findings suggests that nerve damage in one ocular surface leads to a protective change in the immune stance of the opposite eye that is mediated by TRPV1 and SP.

Keywords: ocular surface, mucosal tolerance, neuroinflammation, substance P

(1114) **IL-6 INDUCED BY SPONDYLOARTHRITIS SYNOVIAL FLUIDS MODULATES METALLOPROTEINASE-9 ACTIVITY IN SYNOVIAL FIBROBLASTS**

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Spondyloarthritis (SpA) is the second most frequent chronic inflammatory arthritis. Fibroblast-like synoviocytes (FLS) are involved in SpA pathogenesis. The cytokine IL-6 has emerged as an important therapeutic target for rheumatic disorders. However, the IL-6-mediated mechanisms in chronic arthropathies are not fully understood. Metalloproteinase (MMP)-9 is associated with chronic inflammatory diseases. In previous studies, we demonstrated that synovial fluids (SF) of SpA patients induce early IL-6 production by FLS. The IL-6 blockade decreased MMP-9 activity of FLS. In the present work, we explored the role of IL-6 induced by SpA SF in regulating MMP-9 activity in FLS. The SF of SpA patients were pooled (n=9). Primary FLS or SW982 cell line were stimulated with SpA SF pool at dilution 1:10 in culture medium. The IL-6 function was blocked by the anti-IL-6 receptor antagonist tocilizumab(TCZ) (200ug/ml). Because IL-6 can activate JAK pathway, JAK-selective small molecule tofacitinib (1000 nM) was used to inhibit this pathway. The supernatants (SN) were collected after 48 h. The MMP-9 activity was determined by zymography. Cytokine levels in the SN were measured by ELISA. We found that in both primary FLS and SW982 cell line, TCZ decreased significantly MMP-9 activity induced by SpA SF (p<0.01, compared with FLS stimulated in absence of TCZ). Unexpectedly, JAK inhibition enhanced MMP-9 activity (p<0.001).To explore the mechanism of this paradoxical increase, SW982 cells were stimulated with recombinant human IL-6. We found that IL-6 induces MMP-9 activity in a dose-dependent manner. Moreover, IL-6 augmented IL-10 production, which decreased significantly by JAK inhibition (p<0.05). The results indicate that SpA SF recreate a microenvironment inducing IL-6 in FLS. Consequently, IL-6 provokes MMP-9 activity, but also it might drive a JAK-dependent mechanism, inducing IL-10 to control MMP-9 activity. These findings contribute to link IL-6 to the pathogenesis of SpA.

Keywords: spondyloarthritis, synovial fibroblasts, IL-6, MMP-9, synovial fluid

(1626) **B CELL RECONSTITUTION AFTER BONE MARROW TRANSPLANTATION IN PRIMARY IMMUNODEFICIENCIES**

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Background: Hematopoietic stem cell transplantation (HSCT) provides a curative therapy for primary immunodeficiencies (PID). While

the timing and extent of Tcell reconstitution following transplant for PID has been studied in depth, less is known about the kinetics of B-cell development and long-term restoration of humoral functions. Materials and methods: We retrospectively analyzed immune reconstitution after HSCT in 11 paediatric patients with PID from our centre, with a follow-up ranging from 1 to 5 years. Lymphocyte subset populations (T $CD3^+$, $CD3^+/CD4^+$, $CD3^+/CD8^+$, $CD19^+$, $CD56^+$ lymphocytes) and B subsets (naïve, total memory, pre switch, post switch, transitional) were determined by flow cytometry and compared with reference values (RV). Serum IgA and IgM were analyzed by kinetic nephelometry and functional responses to tetanus toxoid (TT) and pneumococcal (Pn) vaccines by ELISA. Patients were followed from 1 to 5 years post HSCT. Results: B-cells counts normalized 6m post HSCT, median% (p25-p75): CD19:19% (10.5-33.75). Serum IgM and IgA normalized between 12-18m: median IgM: 61mg/dl (RV: 63-177); IgA: 88.5 mg/dl (RV: 52-150). Switched memory B-cells ($CD19^+CD27^+IgD^-IgM^-$) were restored early and reached normal values for age after 2 years (median 1/2/3/>4 yr: 3.2/5.2/8.7/9.0% respectively) RV: 5.5-14.9%. IgM memory B-cells ($CD19^+CD27^+IgD^+IgM^+$), remained significantly reduced long-term (median1/2/3/>4yr: 3.2/2.9/3.2/6.0%) RV: 9.5-17.2. B transitional decreased over time (median 1/2/3/>4 yr: 22.4/16.2/9.9/8.9%) RV: 5.1-8.7%. 7/11 suspended immunoglobulin replacement therapy (IgRT) and 6/7 had good TT response and 4/7 responded to Pn. Conclusion: Monitoring of phenotypic and functional changes on B-cells following HSCT may prove clinically relevant to tailor patients' care. In particular the analysis of IgM memory and switched memory B-cells are recommended before IgRT discontinuation.

Keywords: B cell reconstitution, PID

(1702) **CHANGES IN ANCA PATTERN POST SALT TREATMENT**

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Neutrophil anti-cytoplasmic antibodies (ANCA) associated with vasculitis are characterized by being directed against constituents of cytoplasmic granules of neutrophils. Previous studies have shown that treatment with high salt concentration prior to Indirect immunofluorescence (IFI) implies a decrease or loss of the P-ANCA pattern by removal of the cationic antigens to which they are directed, such as lactoferrin and mieloperoxidase (MPO). The aim of this work was to determine the effect of salt treatment on the ANCA patterns analyzed by IFI. Sera from three patients (1 PR3 / C-ANCA, patient with C-ANCA vasculitis under treatment, 1 MPO / P-ANCA, no clinical information and 1 PR3 / P-ANCA,vasculitis) The homemade slides were prepared by adsorption of a drop of blood, incubated 2 hours in a humid chamber at 37 ° C and fixed for 5 minutes with ethanol. For treatments with high salt concentration, the ethanol-fixed slides were incubated with TrisClH 50 mmol/l, magnesium sulphate 1 mol/l. IFI results on ethanol-fixed homemade slides showed that PR3 / P-ANCA serum (IFI titre: 1: 160) after saline treatment changed to C-ANCA (titre: 1:20), while in the commercial it remained as P-ANCA but with low title (titer: 1:20). Patient PR3 / C-ANCA maintained the same pattern and titer after the treatment in both slides and the serum that presented MPO / P-ANCA (titer IFI: 1: 640), in the homemade slides was negativized but in the commercial showed a P -ANCA titer of 1:40. The results obtained in the patient PR3 PANCA after saline treatment show a decrease in antibody titer or a change of pattern to CANCA possibly linked to different antigenic characteristics to which those antibodies are directed. We concluded that the PR3 PANCA antibodies could be directed against PR3 antigens with different characteristics or a mixture of antibodies directed against different specificities. New studies that address other antigenic specificities are needed.

(1705) **ANTICONVULSANT HYPERSENSITIVITY SYNDROME: A CASE REPORT**

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A 25-year-old female presented a week evolution generalized, pruritic exanthema, which had begun on the neck with edema in eyelids and lips. She took cefadroxil 1 g / day for 24 hours after a biopsy due to a scleroatrophic lesion on the left leg. It switches to clavulanic amoxicillin for 24 hours, then it was discontinued and desloratadine and betamethasone was established.

After a week, she reported a fever episode of 39.5°C, and whitish vomiting. During the examination, mild dehydration, temperature increase, morbilliform exanthema, blistering scars on the anterior arm (three weeks of evolution), upper and lower limb edema were evidenced.

She had epilepsy, medicated with levetiracetam 1 g / day for a year, decreased to 500 mg / day 15 days prior to consultation; and oxcarbazepine 300 mg / day for a month, increased to 600 mg / day 15 prior.

Hepatitis was demonstrated in laboratory results, AST 413 ALT 367, leukopenia 3400 (47/0/0/44/9), platelet count normal, VDRL/ HIV/ HBV/ HCV serologies were negative.

Patient was hospitalized. Oxcarbazepine was discontinued, while dexamethasone and diphenhydramine treatment was instated.

She was discharged after 5 days of treatment. Fexofenadine and meprednisone were given ambulatory.

Key words: Hypersensitivity, Anticonvulsants, Epilepsy, Oxcarbazepine

(1869) **COCAINE SELF-ADMINISTRATION MODULATES PERIPHERAL IMMUNE CELLS LEVELS AND THEIR CB2 RECEPTOR EXPRESSION**

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The immune system (IS) is able to modulate several central nervous system (CNS) functions (including learning, memory, neural plasticity and neurogenesis) by complex interactions among glia, neurons, neural precursor cells and peripheral immune cells (particularly T-cells and macrophages). In parallel, CNS acting drugs, such as psychostimulants, are able to modulate the IS. CB_2 cannabinoid receptors are expressed both in CNS and IS, and have been associated with cocaine- induced effects. The aim of the present work was to study the levels of peripheral T- and B-cells and macrophages, as well as their CB_2 receptor surface expression after cocaine treatment. Lewis rats were trained during 3 weeks in cocaine (1 mg/kg/infusion, i.v.) or vehicle self-administration. Twenty-four hours later, the rats were sacrificed and the spleens were removed in order to obtain spleen mononuclear cells (SMC). In cocaine self-administered rats, we observed a macroscopic splenomegaly reflected in augmented relative spleen weight ($p<0.001$). We consistently found increased levels in the number of SMC/spleen ($p<0.001$) after cocaine self-administration, so that the SMC/splenic index ratio was also increased ($p<0.05$). Flow cytometry (FCM) analysis of SMC subpopulations revealed that T-cells percentage was not modified after cocaine, although the absolute number showed a remarkable elevation ($p<0.001$). Conversely the B-cells percentage significantly decreased ($p<0.05$), while the absolute levels had also increased after cocaine ($p<0.001$). Thus, the T-/B-cells ratio showed an increment following cocaine ($p<0.05$). Macrophages levels, both relative ($p<0.05$) and absolute ($p<0.001$), were elevated after cocaine self-administration. Finally, after cocaine self-administration, CB_2 expression increased on T-cells and macrophages ($p<0.05$ and $p<0.01$) but not on B-cells. These results open new research perspectives concerning the influence of cocaine on the immune system.

Keywords: cocaine – self-administration – spleen mononuclear cells – CB2 receptors – flow cytometry
Biologia animal 2

(1519) **EARLY BACKGROUND ADAPTATION IN THE MEDAKA *Oryzias latipes***

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Background colour adaptation is both an endocrine and a physiological response to the surrounding environment that many vertebrates are able to display owing to changes in specialized cells called chromatophores present in the skin. However, it is not known if such a process of adaptation may take place during early developmental stages and how they can influence other aspects of development such as growth. The aim of this work was to evaluate if background color adaptation occurs during early developmental stages in the medaka and its possible effects on growth. Thus, we analyzed the number of melanophores, a type of chromatophore, in the head, from medakas adapted to 18, 21, 25, 40 and 60 days post fertilization (dpf) to white or black background. To do this, medaka eggs were taken from 2 to 3 spawnings of different male and females medaka pairs for each timepoint and randomly assigned to black or white backgrounds, reared in Petri dish until hatching and then transferred to tanks of the same background till sampling. Additionally, total length and bodyweight were registered. Data were analyzed by two way ANOVA followed by post hoc comparison. P-values were corrected to avoid type I error. Black adapted medaka larvae presented more head melanophores than white adapted ones ($p=0.0001$). This difference is not present at 18dpf ($p=0.118$) but begins at 21dpf ($p=0.0292$) and increases at 25dpf ($p<0.0001$), 40dpf ($p<0.0001$) and 60dpf ($p=0.0107$). No differences in total length and bodyweight were detected between black or white adapted medaka larvae ($p>0.05$). These results showed that background adaptation occurs early during development and the biological processes by which this takes place must be present at least as early as 21dpf.

Keywords: Background adaptation; melanophores, development, medaka

(602) **EFFECT OF SUPPLEMENTATION WITH GIBERELIC ACID (GA3) IN *APIS MELLIFERA* COLONIES ASSOCIATED TO BEE QUEEN CELLS PRODUCTION**

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Abstract: The general objective of this work was to evaluate the effect of gibberellic acid (GA) supplementation on *Apis mellifera* colonies over the production of queen bee cells and hypopharyngeal glands development from nurse bees. We worked with 2 starter colonies (Langsthrot hives) subjected to the vertical semi-orphaned method of production of bee queen cells. Each starter colony received a specific treatment: colony 1 (i1) was the control group and fed with 200 ml of 66% sugar syrup only; colony 2 (i2) received 25 uM GA in 200 ml of 66% sugar syrup. Each beehive received 10 applications of their respective treatment every 3 days. For each colony, 24 larvae were transferred to the upper body the day after the first treatment was applied. Five days after, the total number of accepted larvae was recorded, defined as royal cell that had been sealed. After the 5th application, 250 nurse bees were collected from royal cells to measure the weight of their heads (indirect quality estimator of the hypopharyngeal glands development). In the laboratory, 50 bees were randomly selected from each treatment, the heads were dissected and their weights were recorded by analytical balance. Wilcoxon test and t-test were used to determine if there were differences in the production of cells and head weights respectively. In the i2 colony, higher percentages were obtained in the efficiency of royal cells ($p <0.05$ - Wilcoxon Rank sum test, $W = 0$, $P = 0.01091$) and in the weight of the bees' heads (Sample t-test, $t = -3.1241$, $df = 100.98$, $P = 0.002328$) compared to the control colony i1.

Keywords: *Apis mellifera*, brood method, dietary supplement

(239) **EFFECT OF THE EXTRACT OF TOMATO (*Lycopene)* IN THE PEROXIDATION OF RAT LIVER MICROSOMES**

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Lycopene is the important pigment responsible for the characteristic red color of tomato; it has attracted attention due to its biological and physicochemical properties, especially related to its effects as a natural antioxidant. The objective of this study was to investigate the antioxidant effect of the lycopenes obtained from the extract of tomato on the peroxidation of hepatic microsomes membranes. Rat liver microsomes where incubated with different concentrations of extract (2, 4, 6, 8 μg) in an *in vitro* non-enzymatic ascorbic acid-Fe^{+2} system in order to determine the oxidative effect on membranes and to quantify peroxidation level in standardized conditions. Peroxidation was quantified in a liquid scintillation counter Packard 1900 TR by chemiluminescence in cpm (counts per minute). Microsomal membranes without extract were used as controls. Analyzing the effect of lycopene (tomato extract), was observed that the total cpm/mg protein originated from light emission: chemiluminescence, was statistically lower in samples obtained from lycopene group than in the control group (without lycopene), the antioxidant effect found was not dependent concentration.

(52) **EFFECTS OF DIETARY PROTEIN:LIPID RATIO ON GENES IMPLICATED IN SOMATIC GROWTH, LIPID METABOLISM AND FOOD INTAKE IN PEJERREY FRY (*Odontesthes bonariensis*)**

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Abstract: Pejerrey is a freshwater fish from south America with potential as an aquaculture specie. With the major goal to develop its aquaculture, the aim of this study was to determine the best protein:lipid ratio of diets to achieve higher growth rates in first stages of development. To do this, pejerrey fry (30 days after hatching) were divided in three groups, each one fed per 60 days with an experimental diet containing one of the following protein (P):lipid (L) ratios: 40% P – 12% L (Diet 40P12L); 40% P – 20% L (Diet 40P20L); 50% P – 12% L (Diet 50P12L) and 50% P – 20% L (Diet 50P20L). After trial, RNA from head, trunk and tail were collected to analyze the gene expression of components from the GH-IGF axis. Also, the gene expression from the enzyme Δ6-desaturase (involved in lipid metabolism) and from the anorexigenic peptide nesfatin-1 were measured. Data were analyzed by two-way ANOVA, followed by post hoc Holm-Sidak test at a significance level of $P < 0.05$. Fry fed with diets 40P12L and 50P12L showed the highest somatic growth ($p<0.05$) and highest growth hormone (*gh*) gene expression ($p<0.05$). The total amount of PUFAs from the serie n-6 was higher in group fed on diet 50P20L ($p<0.05$). The *Δ6-desaturase* gene expression was higher in head of those fry fed on diet 40P12L. The expression levels

of *nucb2/nesfatin-1* and *gh* in head, and of *nucb2/nesfatin-1* and the *ghr-I* and *ghr-II* in body follow the same patterns. In conclusion, diets with 12% of lipids produces the higher fry growth compared to those diets containing 20%. Comparing diets with 12% of lipid content, the higher percent of proteins (50%) generates the higher growth-rates. In these groups, mRNA expression from GH-IGF axis could be related with the promotion of somatic growth. The different expression patterns of *nucb2/nesfatin-1* in head and body of pejerrey fry seems to indicate some effect not only related to food intake regulation.

Keywords: Δ6-desaturase, growth hormone, lipid, nesfatin-1, protein.

(1364) **EFFECTS OF EXTRACTS OF BROWN SEAWEEDS AS DIETARY ADDITIVE ON THE METABOLIC PROFILE AND FEED DIGESTIBILITY IN *Artemesia longinaris***

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A study was conducted to evaluate polysaccharide extracts of brown seaweeds as dietary additive for prawns (5.9±1.30g). The aim of this study was to compare zootechnical parameters, metabolic profile and apparent digestibility of *Artemesia longinaris* fed diets with 0; 1 or 2g of polysaccharide extracts of *Undaria pinnatifida* or *Macrocystis pyrifera* (C, U1, U2, M1, and M2, respectively), and with 0.5% Cr_2O_3 as inert marker. To determine apparent digestibility, feces were collected during the last two weeks of the trial, and to measure metabolic variables (glucose, triglycerides, cholesterol, total proteins), haemolymph was extracted at the end of the experiment. After 30 days of feeding in all treatments, the survival was superior to that considered as acceptable in commercial culture, percentages varied between 60% and 79.2% and no significant differences were found between treatments. Percentage of increment in mean weight of prawns under different treatments varied between 8.5 and 16.7%, with higher values in animals fed with *U. pinnatifida* extract (U1 and U2). Apparent digestibility decreases significantly with the addition of 2g of both extracts, although this effect was lower in those animals feed U1 and U2. Regarding the determination of metabolites, no significant differences were found in levels of plasma metabolites in animals feed with *M. pyrifera* but a significant increase was observed in total protein and cholesterol concentrations in animals feed with diet supplemented with 1g of *U. pinnatifida*. These results suggest that, the use of polysaccharide extracts of *U. pinnatifida* is feasible as additive in diets for the prawn *A. longinaris*. The optimal level of inclusion may vary depending on the algal species, for culture this species, the recommended concentration should be 1g, since it is the one that produces increase in mean weight, without interfering with apparent digestibility.

Keywords: *Artemesia longinaris*, seaweeds, apparent digestibility, metabolic profile.

(688) **ROLE OF DP53 IN METABOLIC HOMEOSTASIS AND AUTOPHAGY UNDER NUTRIENT STRESS**

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The ability of an organism to adapt to nutritional stress is crucial for its survival. We have previously shown that the transcription factor Dp53 plays a central role in metabolic remodeling in Drosophila. Depletion of Dp53 activity specifically in the adipose tissue accelerates the consumption of main energy stores, reduces the levels of sugars in the animal, and compromises organismal survival upon fasting. In this work, we aim to investigate in more detail the role of Dp53 in response to nutrient deprivation. By using a combination of transgenic reporter lines and fluorescent dyes we found that Dp53 is required for the induction of autophagy, a conserved catabolic process which is central in the nutrient stress response. This function of Dp53 appears to be tissue specific as depletion of Dp53 protein levels and/or activity in the fatbody strongly reduced starvation-induced autophagy. Interestingly, the role of Dp53 in modulating autophagy appears to be a systemic function rather than through cell-autonomous mechanisms. Insulin-like peptides (dIlps) regulate physiological and metabolic responses to nutrients in Drosophila and, consistently, we observed differences in dIlps expression levels and insulin signaling when Dp53 activity was compromised in the fatbody. From these results, we propose that Dp53 is required in the fatbody to remotely modulate insulin secretion and/or production in the brain therefore providing mechanisms for rapid adaptation to starvation conditions.

Keywords: Dp53, Drosophila, autophagy

(606) **ACARICIDAL ACTIVITY ON *VARROA DESTRUCTOR* OF TWO ESSENTIAL OILS OF *CYMBOPOGON NARDUS* FROM TWO DIFFERENT COUNTRIES AND THEIR CITRONELLA MONOTERPENOID**

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Varroosis caused by *Varroa destructor* is considered the greatest disease of the honeybee. Mite control is mainly based on the application of synthetic acaricides but its continuous use over time leads to a selection pressure causing several foci of resistance and contamination of bee products in the entire world. A range of essential oils have been found to exhibit acaricide activity against *V. destructor.* The main objective of the present work was to evaluate the acaricidal activity on *V. destructor* of the essential oil *Cymbopogon nardus* obtained from Argentina and India, as well as the activity of the major component of this oil, the Citronelal monoterpenoid. The LC_{50} of both essential oils and the compound was estimated by using a full exposure method. Oils and component concentrations between 0 (control) to 40 µl/ml were applied in the inner surface of Petri dishes. Alcohol was used as solvent, after itsevaporation, 5 adult worker bees and 5 mites were placed with a feeder containing water and candy in each Petri dish. Capsules were incubated at 30°C and 70% RH. The mortality of mites and bees at 24, 48 and 72 hours was recorded for each treatment. By probit analysis the LC_{50} was estimated for each essential oil and citronelal compound, expressed in µl of compound/ml. A selectivity ratio, calculated as *A. mellifera* LC_{50}*/V. destructor* LC_{50}, was determined for each treatment at each observation. The essential oil from India and the Citronelal compound were the most toxic against mites and bees (LC_{50} 2.38 µl/ml and <2.5 ul/ml, and 2.48 ul/ml and 3.61 ul/ml respectively). *C. nardus* oil from Argentina presented low toxicity in adult bees (>10µl/ml) and promising acaricide effect on mites (4.26 ul/ml). These results show that the essential oil of *C. nardus* from Argentina could be a succesfull alternative in the control of Varroosis.

Keywords: *Varroa destructor, Apis mellifera*, Essential Oils, Acaricidal Activity.

(1442) **INTESTINAL MICROBIOTAMODULATION INDUCED BY ANTIBIOTICS AND TANNINS-BASED FEED ADDITIVES UNDER EXPERIMENTAL AND COMMERCIAL CONDITIONS IN POULTRY**

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Antibiotic growth promoters (AGPs) have been used for decades in animal production as a means to maintain animal health and improve feed efficiency. Global concern about development and transference of antimicrobial resistance is rising and so the development of alternatives to AGPs is urgently needed. Information about the effects of phytogenic compounds on chicken intestinal microbiota is required to understand the potential of these bioactive natural products as alternatives to antibiotics. The aim of this study was to assess the impact of a blend of chestnut and quebracho tannins in the microbiota of chickens by high-throughput sequencing (HTS) of 16S rRNA gene amplicons.

Cecal contents were obtained at 21 and 40 days of age from chickens raised under experimental or commercial conditions. In both environments, two treatments groups were compared: tannins and bacitracin supplemented birds. DNA was extracted from each

pooled cecal sample and the V3–V4 region of the 16S rRNA gene was amplified. HTS was performed in the Illumina MiSeq platform. Bioinformatics analysis was done with QIIME and STAMP software.

A significantly different microbial profile was found between experimental and commercial samples and also between the two sampling ages analyzed. Significant differences in the diversity metrics across dietary treatments were detected in both environments. Tannins increased *Firmicutes* to *Bacteroidetes* ratio, a parameter linked to feed conversion efficiency, in both experimental and productive conditions. This was mainly due to the growth of members of order *Clostridiales* and genus *Lactobacillus* and the decrease of genus *Bacteroides*. Tannins also increased the relative abundance of other potentially probiotic genera as *Bifidobacterium* and *Enterococcus*. Further studies using whole shotgun sequencing are required to go in deep in investigating the effects of alternative feed additives and AGPs on intestinal microbiota of poultry.

Keywords: feed additives, tannins, poultry, high-throughput sequencing, intestinal microbiota.

(1734) **PREDICT AREAS OF OCCURRENCE OF *Lonomia obliqua* WALKER 1855 (SATURNIIDAE, HEMILEUCINAE) IN THE PROVINCE OF MISIONES, ARGENTINA**

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The *Lonomia obliqua* moth is a species of broad medical interest because its larvae are etiological agents of lonomism, a form of erucism which can lead the patient to death. Accidents have been common in Brazil since the end of the 80s, appearing in the province of Misiones (Argentina) by the beginning of this millennium. In order to find areas of high suitability for *L. obliqua* in the province of Misiones, *Species Distribution Modeling* algorithms were used: Bioclim, Gower, Mahalanobis, Maxent and SVM. For the model, 19 biogeoclimatic variables, the mean annual solar radiation and 9 soil variables were used. In order to avoid multicollinearity of the variables, a *Principal Component Analysis* was performed to create new orthogonal variables, resulting in seven new variables (cumulative variance> 90%). For the extraction of the niche information, 38 points of occurrence from Misiones and Brazil were selected and partitioned by the *jackknife n-1* technique. The background contemplated the geographic extension that refers to the biomes where the presences of *L. obliqua* were obtained: *Pampa*, *Atlantic forest*, *Caatinga* and *Cerrado*. From it, 10% of the information was sampled randomly to be considered as pseudo-absences in the model. The *Ensemble* technique was also applied from the frequency for the construction of a map that shows the appropriate areas in concordance among the models. The *Ensemble* took place from the threshold for the minimal presence of the species. The model was adequate (sensitivity=99%) and better than a null model ($p<0.05$). The final map shows the potential regions of occurrence of *L. obliqua* cut for the province of Misiones-AR. From the final map it is seen that the north and east of Misiones presents high suitability for the presence of *L. obliqua*. Therefore, public policy efforts need to be specially addressed in both regions in order to prevent cases of lonomism in this province.

Key words: venomous caterpillar, niche modelling, specie distribution, Brasil, Argentina.

(1692) **SUCCESSFUL MANAGEMENT OF PARASITIC INFECTIONS IN THE SOUTHERN GREEN PARROT SNAKE *Leptophis ahaetulla marginatus* (SERPENTES: COLUBRIDAE)**

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There are only a few examples of parasitism affecting snakes in Argentina, and there is a limited understanding of endoparasites in wild caught snakes. The current study summarizes the parasitic infections detected in some specimens of *Leptophis ahaetulla marginatus* (Lam) collected in the Iguazu National Park (Misiones, Argentina), in accordance with authorization from the National Park Administration (APN) N° 335, and transported to the serpentarium of the National Institute of Tropical Medicine (INMeT) (Argentina). Ecto and Endoparasite examinations were carried out by skin scrapping and parasitological analysis of fecal samples by both sedimentation and flotation concentration techniques. Four samples (each corresponding to different specimens) were positive for presence of Ascarididae-type eggs (Lam 1), Rhabdiasidae-type eggs and larvae (Lam 2), Eimeria-type oocysts (Lam 3) and Digenean trematode eggs (Lam 4). Treatments were done with administration of: 10% fenbendazole suspension orally at a dose of 40 mg/kg, which was repeated after 6 days (for Lam 1 and 2); Trimethoprim/Sulfamethoxazole 25 mg/kg IM per day for 2 days and then one dose every two days for another 10 days (for Lam 3); Praziquantel orally at a dose of 7.5 mg/kg which was repeated after 15 days (for Lam 4). Fecal sample re-examinations were carried out after fifteen days of each treatment and they were found negative. Thus, this study provides relevant information about the parasitic fauna of wild specimens of a colubrid snake commonly found in northeastern Argentina, and gives insight into their corresponding treatment.

Keywords: Antiparasitic drugs, Endoparasites, Colubrid snake.
neuroc 3

(687) **A CASPASE-INDEPENDENT PATHWAY IS INVOLVED IN MANGANESE-INDUCED APOPTOSIS IN BV-2 MICROGLIAL CELLS**

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Manganese (Mn) overexposure causes a neurodegenerative disease known as Manganism. Several mechanisms have been implicated in Mn neurotoxicity including oxidative stress (OS) and inflammation. Microglia are activated in response to Mn and release various pro-inflammatory cytokines (IL-1β, IL-6 and TNF-α) and soluble factors, includingreactive oxygen and nitrogen species, which could promote neuronal death. However, little is known about Mn toxicity in microglia. We previously demonstrated that BV-2 microglial cells exposed to Mn (250 and750*μ*M; 24h) exhibit apoptotic features such as the activation of caspases involved in intrinsic and extrinsic cell death pathways. In addition, we reported that OS and lisosomal membrane permeabilization were involved in cell death. In the present work we continue unraveling the molecular pathways involved in Mn-induced toxicity in BV-2 cells.To determine the role of caspases, we performed cell viability assays (MTT) in the presence ofcaspase-8(Ac-LEHD-CMK), caspase-9 (Z-IETD-FMK) and pancaspase (Z-VAD-FMK) inhibitors. Interestingly, these inhibitors did not prevent Mn-induced cell death. In accordance, Mn increased a 68% ($p<0.001$) the nuclear translocation of AIF (WB).AnnexinV/PI analysis (FACS) showed that 51% of Mn-exposed cells were necrotic/late apoptotic ($p<0.001$). In this context, Necrostatin-1, an inhibitor of RIP1-dependentnecroptosis had no effect on Mn-induced cell death (MTT). On the other hand, we demonstrated that Mn induces DNA damaged revealed by increased expression levels of γH2Ax in nuclear fractions ($p<0.001$)(WB) and a 30% reduction in procaspase-2 levels ($p<0.01$).In summary, our results suggest that Mn exerts genotoxicity in BV2 cells. In this scenario, caspases could be activated to induce microglial activation while cell death is governed by a caspase-independent apoptotic mechanism.

Keywords: manganese, manganism, microglia, cell death

(223) **ANTI-INFLAMMATORY EFFECT OF OXYTOCIN DURING AN LPS CHALLENGE**

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The hypothalamic-neurohypophysial system has recently emerged as an important component of neuroendocrine–immune network, wherein the oxytocin (OXT)-secreting system, consisting of OXT magnocellular neurons in the paraventricular (PVN) and supraoptic nuclei (SON), plays an essential role. Lipopolysaccharide (LPS) administration activates the immune response and neurohypophysial hormones secretion. Our previous studies provided direct evidence for the enhancement of OXT release from hypothalamus during LPS-induced inflammation (J Neuroimmunol 221, 2010). Moreover, a cross-talk between OXT neurons and adjacent astrocytes determines the neuroimmunoendocrine final response. These findings reveal that OXTnergic system could be a relevant component of the modulatory network of immune processes. Our objective was to evaluate the possible anti-inflammatory role of OXT during an LPS challenge. Adult male Sprague-Dawley rats were treated with OXT via sc (10, 100 and 1000 mg/kg) or via icv (1mg/kg) or vehicle (CTROL) and after 15 min were injected with LPS (5mg/kg, ip). Additionally, microglial tumoral cells (BV-2) and primary astrocytes cultures were pre-treated with OXT (0.1, 1, and 10 μM) for 15 min and then incubated with LPS (500 ng/ml) for 30 and 60 min. TNF-α concentration in plasma and medium were determined by ELISA. ANOVA analysis indicated OXT immunomarcation of magnocellular neurons at PVN and SON was increased by LPS, suggesting the activation of these neurons with an accumulation of OXT vesicles in the hypothalamus. OXT (100 and 1000 mg/kg, sc) significantly ($P<0.05$) prevented LPS increased in TNF-α plasma levels. Conversely, OXT (1ug/kg, icv) centrally injected did not modify TNF-α plasma levels. However, pre-treatment of BV-2 microglial cells and primary astrocytes cultures with OXT significantly ($P<0.05$) prevented LPS-increased TNF-α levels in culture media. In conclusion, our data suggest an anti-inflammatory effect of OXT during an immune challenge.

Key Words: Oxytocin, LPS, glial cells, TNF-α, immunohistochemistry

(194) **CEREBELLAR MICROVASCULAR AND GLIAL ALTERATIONS DURING THE ENCEPHALOPATHY PRODUCED BY SHIGA TOXIN 2 (Stx2) FROM ENTEROHEMORRHAGIC *Escherichia coli* (EHEC)**

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Hemolytic uremic syndrome is a triad of events that includes thrombocytopenia, microangiopathic hemolytic anemia and acute renal failure caused by Stx2 from enterohemorrhagic *Escherichia coli* (EHEC). It has been observed that Stx2 produces deficits of coordination and equilibrium in patients which suggest cerebellar impairment. The aims of this study were to determine whether Stx2: i. passed through the blood-cerebrospinal fluid barrier into cerebellar parenchyma and ii. caused cell damage. Male NIH mice (n=4) were injected intravenously with 1ηg of Stx2 per mice or 100 μl of saline solution (control group). After 4 days of treatment, fixed brains were subjected to immunofluorescence with lectins to determine the microvasculature profile, anti-Stx2 to identify the presence of the toxin in cerebellum tissue, and anti-GFAP (Glial Fibrillary Acidic Protein) to determine reactive astrocytes. Micrographs were taken in a confocal microscope and obtained images were analyzed through the software ImageJ. Stx2 was found in parenchyma cells of the cerebellum. The statistical results analyzed by Student's t-test showed that Stx2 decreased the area occupied by the microvasculature (18.78 ±0.67 μm^2 control versus 9.37 ±0.42 μm^2 Stx2) and increased the expression levels of GFAP in comparison to the control (20.32 ±0.41 AU control versus 29.05 ±0.48 AU Stx2, in IOD) $p<0.01$. As shown in lectin histofluorescence binding essay this study demonstrated that, after 4 days of treatment, Stx2 was able to reach the Purkinje and granular cerebellar layers and damaged the endothelial cells. Furthermore, the astrocyte reaction is an evidence of cerebellar parenchyma injury, probably through Gb3 neuronal receptor or tissular mediators.

Key Words: Astrocyte, Microvasculature, Damage, Purkinje, neurons

(400) **DAMAGE ASSOCIATED PROTEIN HMGB1 PLAY A KEY ROLE IN THE NEUROINFLAMMATION AND NEURODEGENERATION THAT FOLLOWS STATUS EPILEPTICUS**

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IBCN

High-mobility group box-1 (HMGB1) is a DAMP released after brain injury that activates local microglia and astrocytes but also behaves as long-range cytokine acting on the professional immune organs. Activated (reactive) astrocytes, can secrete molecules such as Thrombospondin-1 (TSP-1) that induce excitatory synapses and thus could have a major role in the altered excitatory/inhibitory balance that is the biological substrate of epileptogenesis. However, HMGB1 role in epileptogenesis is still unclear. For that purpose, status epilepticus (SE) was induced on 300 g Wistar rats by lithium chloride plus pilocarpine treatment (127 mg/kg and 30 mg/kg respectively) and stopped with 20 mg/kg diazepam after 20 min of SE. Then, we blocked the signalling of HMGB-1 applying glycyrrhizin (GLY) 333 mg/kg (i.p) twice a day for four days. To evaluate the expression of synaptogenic molecule TSP-1, i*n vitro* studies were performed on glial mix cultures or enriched astrocytes cultures. Both types of culture were stimulated with recombinant HMGB-1 (500 ng/ml) and TSP-1 expression was determined by immunofluorescence. *In vivo*, we found that GLY treatment significantly reduced both reactive gliosis and neuronal death in cortex and hippocampus (one-way ANOVA $p<0.05$). Furthermore, GLY treatment also reduced the typical SE-induced infarct zone in the pyriform cortex by proximately 10 times (t-test $p< 0.0001$) and 25% of rats were infarct free. *In vitro* studies showed that astrocytes as well as microglia stimulated with HMGB-1 expressed TSP-1, on a microglia-dependent manner (one-way ANOVA $p<0.01$). We conclude that HMGB-1 may play a main role in the mechanism of the epileptogenesis by promoting the microglial and astroglial activation and their subsequent expression of TSP-I that could be responsible for altered the excitatory/inhibitory synaptic balance in the epileptic brain. Supported by grants: PICT 2014-2178; PICT 2015-1451; UBACYT

(425) **EFFECTS OF ESTROGEN MEMBRANE RECEPTOR GPR30 AGONISTS ON HIPPOCAMPAL ASTROGLIOSIS AND MICROGLIOSIS OF SPONTANEOUSLY HYPERTENSIVE RATS**

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Hippocampal neuropathology is a recognized feature of the spontaneously hypertensive rat (SHR). Previous studies have found that SHR present abnormalities in the hippocampus consisting of decreased cell proliferation in the dentate gyrus (DG), astroglial and microglial reactivity and decreased neuronal density in the hilus of the DG. These abnormalities are reversed by exogenous administration of estradiol, an active neuroprotective and hypotensive factor. We recently demonstrate that both types of estradiol classical receptors (ERα and ERβ) are involved in the neuroprotective action of estradiol. GPR30 is a membrane estradiol receptor which is expressed in hippocampus and it is known to be involved in neuroprotective actions. To elucidate if this receptor is involved in the protective effects in SHR we used the GPR30 agonist G1 given s.c. during 2 weeks at 150 μg/kg/day to 4 month old male SHR. We measured the expression of GFAP and IBA1 in the CA1 and hilus of the hippocampus by immunocytochemistry. We found that hypertensive animals have increased expression of GFAP+ astrocytes in both regions of the hippocampus ($p<0.01$) and treatment with G1 reduced the number of positive astrocytes ($p<0.05$). The number of IBA1+ microglia is increased in hippocampal CA1 area of SHR and G1 treatment

reduced this number (p<0.001).

These data indicate that GPR30 membrane estradiol receptor is involved in the reduction of astroglial and microglial reactivity. Given the undesirable effects due to estradiol treatment, much effort is made to look for alternative treatments that mimic estradiol neuroprotective actions avoiding the undesirable effects. These effects of G1 open an interesting possibility in this direction.

Hypertension-Neuroprotection-Estradiol Receptor

(530) **MIGRATION OF RETINA GLIAL CELLS REQUIRES THE SYNTHESIS OF CERAMIDE-1-PHOSPHATE**

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Retina proliferative diseases are major causes of visual dysfunction. Müller glial cells (MGC),the major glial cell type in the retina, and retinal pigment epithelial (RPE) cells play a key role in these diseases. Injuries to the retina exacerbate their proliferation and migration that contribute to visual loss. The molecular cues involved in these processes are still ill defined. We demonstrated that a sphingolipid, sphingosine-1-phosphate (S1P), promotes glial migration. We now investigated whether ceramide-1-phosphate (C1P), which controls proliferation and migration in certain cell types,participates in glial and RPE cell migration.

We evaluated migration in primary glial cultures,prepared from newborn rat retinas, and in a RPE cell line (ARPE19), by the wound repair assay. Addition of 10 μM C1P doubled MGC migration. To investigate whether C1P synthesis was required forglial migration, we first established by PCR that MGC expressed ceramide kinase (CerK), the enzyme catalyzing C1P synthesis. NVP231, a CerK inhibitor, completely prevented glial migration, which was not restored by C1P addition. Noteworthy, ARPE19 cell migration was also inhibited by NVP231. We then investigated the signaling pathways involved in C1P effect. Inhibiting the PI3K and the ERK/MAPK pathways, with LY294002 and U0126, respectively, decreased glial migration, both in control and C1P-treated cultures. SP6000, a Jun K inhibitor, also blocked C1P-induced glial migration. C1P activates a cytoplasmic phospholipase A2 (cPLA2) in different cell types. Pre-treatment with ATK, a cPLA2 inhibitor, markedly reduced glial migration in control and C1P-treated cultures.

These results show that C1P promotesglial migration through the activation of cPLA2 and the Jun K, the PI3K and the ERK/MAPK pathways. They also imply that exogenous C1P promotes C1P endogenous synthesis to stimulate glial and epithelial cell migration, supporting C1P as a central cue for regulating migration in these cells.

(492) **NEUROINFLAMATORY-RELATED PROCESES AND COGNITIVE DEFITIS INDUCED BY AN ANMIMAL MODEL OF TRAUMATIC BRAIN INJURY: CHARACTERIZATION OF TEMPORAL COURSE**.

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Brain damage caused by acute trauma (TBI) is associated with high morbidity and mortality. In Argentina is considered the leading cause of disability in children and young adults.TBI is a complex disorder caused by primary and secondary injury mechanisms. Primary mechanisms are the result of mechanical damage. Secondary injury progresses (SI) begin after minutes, or even months after the initial trauma as a result of neurochemical, metabolic and cellular changes. These secondary events are believed to underlie the development of many neurological deficits.The aim of the present investigation is to elucidate the temporal course of SI related to neuroinflamation and cognitive deficits induced in an animal model of TBI, to find a suitable window time for pharmacological intervention. For this purpose we used a closed head impact controlled by weight dropas an animal model of TBI. Animals were evaluated clinically immediately post TBI (hart and respiratory rate and apnea), and 60 min, 24 h and 7 days after TBI, were sacrificed to measure glia inflammation labeling GFAP (astrocytes) and CD11b (microglia) by immunohistochemistry (IHC). Also, Barnes Maze was performed 24 h post TBI to evaluate spatial memory. Our results showed increased glial activation profiles in hippocampus at 60 min and 24 h, returning to SHAM values at 7 days post TBI. No differences between SHAM and TBI groups were observed in behavioral performance. IHC and behavioral data were analyzed by one way ANOVA or t-test respectively. In conclusion, our data suggest that the neuroinflamation observed early after TBI did not affect a hippocampal-dependent memory acquisition and retrieval, at the timeevaluated. As described by other authors, these deficits could be observed long term after TBI as a consequence of early neuroinflamation. Further studies need to be performed to evaluate this possibility and to complete the neuroinflamatory profile.

Keywords: Traumatic brain injury (TBI), neuroinflamation, spatial memory, glia.

(228) **PARTICIPATION OF THE MICROGLIA IN HIPPOCAMPAL DYSFUNCTION BY SHIGA TOXIN 2 (STX2) FROM ENTEROHEMORRHAGIC *Escherichia coli* (EHEC) PRODUCING HEMOLYTIC UREMIC SYNDROME**

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Stx2 causes bloody colitis, HUS and neurological dysfunctions. Hippocampal cognitive deterioration is observed. EHEC secretes Stx2 and releases LPS. The aim was to determine whether sub-lethal Stx2 (S), LPS (L) or Stx2 co-administered with LPS (S+L) altered the hippocampal neurovascular unit by microglial activation. Male NIH mice (n=4) were injected iv. with: saline (control, C); 800ηg of L; 1ηg of S or 1ηg of S with 800ηg of L (S+L). Fixed brains were subjected to immunofluorescence with lectins to determine the microvasculature profile, anti-GFAP, anti-NeuN and anti-Iba1 to identify reactive astrocytes, neuronal damage and the microglial state respectively from 2 to 20 days post injection. Primary microglial cultures were incubated with either DMEM (C), 1 and 100 ηg/ml of S, or 1 and 100 ηg/ml of S with 800 mg/ml of L (S+L), following heat shock incubation to test microglial activation. Anti-Iba1 and anti-Stx2 immunofluorescences were used to identify microglia cells and intracellular Stx2 respectively. The deepest hippocampal deterioration was observed after 2 days of S+L treatment. S+L and S treatments resulted to decrease the area occupied by microvasculature (7.35 ±0.25 C; 5.25 ±0.38 L; 2.75 ±0.15 S; 1.4 ±0.21 S+L), increased the expression levels of GFAP (0.19 ±0.02 C; 0.36 ±0.04 L; 0.52 ±0.02 S; 0.60 ±0.02 S+L) and decreased the thickness of pyramidal layer (59 ±1.3 C; 48 ±1.44 L; 42 ±1.15 S; 36 ±1.5 S+L) $p<0.05$. The maximal expression of Iba1 were found at 100 ηg/ml of S co-incubated with 800 mg/ml of L, and at 100 ηg/ml of S on microglial primary culture cells: 74 ±2.31 C; 85 ±1.8 L; 143 ±2.21 S; 189 ±2.57 S+L; accordingly, Stx2 was found maximally intracellular in the microglia in S+L: 71 ±1.63 S+L, 34 ±2.81 S, in IOD, $p<0.05$. Stx2 damaged the microvasculature, affected the astrocytic state and caused neurodegeneration. LPS aggravated Stx2 deleterious effects. The microglia, being reactive to Stx2, could play a pivotal role in inflammatory mechanisms.

Keywords: microglial cultures, microvasculature, astrocyte, LPS

(627) **TOLL-LIKE RECEPTORS IN THE PROPAGATION OF REACTIVE GLIOSIS IN THE INJURED BRAIN**

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Following brain injury astrocytes undergo phenotypic changes collectively known as reactive gliosis. Detrimental effects of reactive gliosis for neuronal survival occur when astrocytes polarize to the

A2 phenotype expressing proinflammatory genes. We previously shown that Toll-like receptor 4 (TLR4) and NF-kB signaling are required the A2 polarization (Rosciszewski et al., 2017). However, it is still unknown how reactive gliosis propagates to reach distal brain regions. It is proposed that damage associated proteins released by dying neurons acting on TLR are probably involved. To address this question, we here performed a penetrating traumatic brain injury in rodents by stab wound (2 mm lateral; 2 mm Bregma, 2 mm depth) in wild type, TLR4KO and TLR2KO animals. In vitro, we studied the downstream signaling pathways involved in the reactive gliosis propagation by using the scratch wound healing assay. Stab-wounded animals showed a gradient of reactive gliosis analyzed by GFAP immunostaining morphometry that was present at 3-7 days post-injury (3-7 DPI), and then at 14 DPI, the gradient was lost and reactive gliosis propagated by the entire hemisphere. At 7 DPI, the TLR4KO, but not TLR2KO, animals showed a decreased gradient of reactive gliosis ($p<0.05$, two way ANOVA). In vitro, scratch wounds also produced a gradient of reactive gliosis either in purified astrocytic culture or in mixed microglial-astroglial culture. In astroglial-enriched cultures, but not in mixed culture, the gradient was prevented by the NFkB chemical blocker BAY117082 (10 μM), by Ca2+ chelating agent EGTA (20 μM) or by the gap-junction blocker MFA (100 μM). On the other hand, the exposure to the TLR4 agonist LPS exacerbated the reactive gliosis gradient. Taken together these results show that reactive gliosis propagation is a phenomenon that involves both astrocytes and microglia and seems to require TLR4. Supported by PICT 2015-1451; UBACYT.

Keywords: glia, TLR, brain ischemia, neuroinflammation.

NEUROSCIENCE 4

(1251) **ALCOHOL HANGOVER INDUCES ALTERATIONS IN MITOCHONDRIAL FUNCTION AND NITRIC OXIDE METABOLISM IN MOUSE BRAIN CORTEX SYNAPTOSOMES AND NON-SYNAPTIC MITOCHONDRIA**

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Alcohol hangover (AH) is the transient state that occurs after acute ethanol consumption and begins when blood alcohol concentration is zero. The aim of the present work was to study mitochondrial bioenergetics and NO metabolism at the beginning of AH in brain cortex synaptosomes (S) and in non-synaptic mitochondria (NSm). Swiss male mice were treated with a single i.p. injection of saline (control group) or ethanol (3.8 g/kg; AH group) and the biochemical assays were performed at AH onset. Results showed a significant decrease in respiratory function in both subcellular fractions ($p<0.05$). The enzymatic activity of complex I-III and II-III was 21% and 33% decreased respectively in S while no changes were observed in NSm. Complex IV activity was 48% and 42% decreased in S and NSm respectively. Transmembrane potential was 20% decreased in S and 15% decreased in NSm ($p<0.05$). The expression of the uncoupling protein UCP-2 was 49% increased in S and 31% decreased in NSm. ATP basal content was 51% and 64% reduced in S and NSm respectively ($p<0.01$). Moreover, ATP production was 26% decreased in S. In NSm, malate-glutamate ATP production was 28% decreased while a 37% decrease was observed in succinate ATP production. On the other hand, NOS expression was 19% and 10% decreased in S and NSm respectively. Together with this, NOS activity was 36% decreased in S and remained unchanged in NSm. In line with this, total content of NO was 30% decreased in S while no changes were observed in NSm. Related to NO metabolism, the expression of the post-synaptic density 95 protein was found to be 16% decreased due to AH in S fractions ($p<0.05$). It could be concluded that AH induced a significant mitochondrial dysfunction and alterations in NO metabolism. AH-induced oxidative stress previously observed in S could be associated with the induction of UCP-2, which would exert a regulatory action on oxygen radical generation and mitochondrial depolarization.

Keywords: alcohol hangover, synaptosomes, mitochondria, nitric oxide

(1360) **BRAIN ANGIOTENSIN II INVOLVEMENT IN THE DEVELOPMENT OF LONG LASTING AMPHETAMINE-INDUCED NEUROINFLAMATION RESPONSES IN PRELIMBIC PREFRONTAL CORTEX RELATED TO WORKING MEMORY DEFICIT**

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Amphetamine (Amph) promotes enduring alteration over neural circuits that can be observed long after. These persistent neuroadaptations also extend to the development of central inflammatory related pathologies, such as glial activation and vascular damage. Angiotensin II, through AT_1 receptors (AT_1-R) activation, modulates dopamine synthesis and release over limbic areas, and participates in reward and learning responses. Moreover, its activity has a greater extend as it modulates several stages of the inflammatory process in neurons and glia. This work aimed to study AT_1-R involvement in neuroinflammatory alterations induced by Amph repeated exposure. Neuroinflammation was evaluated by astrocyte and microglia reactivity at different brain areas, whereas working memory was asses to identify cortical alterations.

Male Wistar rats (250-300g) were used. To study the participation of AT_1-R in long–term Amph effects, the AT_1-R blocker Candesartan (CV 3mg/kg p.o.) was administered for 5 days prior the daily Amph administration (2.5mg/kg i.p x 5 days). On weeks 1 or 3 of Amph withdrawal working memory performance was evaluated using Y-maze test, 24h later the animals were perfused and the brains prepared for GFAP and CD11b immunohistochemistry. The results were analyzed using 2-way ANOVA followed by Bonferroni test. It was found that, at both times analyzed, Amph increased GFAP and CD11B expression only at prelimbic prefrontal cortex concomitant with working memory deficit. These structural and functional alterations were prevented by AT_1-R blockade. We conclude that AT_1-R activation is a key mediator in the development of Amph-induced neuroinflammatory responses underpinning prelimbic prefrontal functional alterations.

Keywords: Angiotensin II, neuroinflammation, amphetamine, working memory, prefrontal cortex.

(1876) **MORPHINE WITHDRAWAL SYNDROME: INVOLVEMENT OF THE CANNABINOID SYSTEM IN ADOLESCENT MALE AND FEMALE MICE**

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Previous studies suggest the existence of an interaction between the opioid and cannabinoid systems during morphine (MOR) withdrawal syndrome. CB_1 cannabinoid receptor deficient (CB1 KO) mice showed lower intensity in naloxone (NAL)-precipitated MOR withdrawal syndrome than their wild-type (WT) littermates. On the other hand, previous studies from our laboratory have shown sex differences in the expression of NAL-precipitated MOR withdrawal syndrome in WT Swiss Webster Albino mice. The aim of the present study was: I) to explore the presence of sex differences in the expression of MOR withdrawal syndrome in CB1 KO mice and their WT littermates, II) to evaluate the c-Fos expression of certain brain areas in CB1 KO and WT mice during MOR withdrawal syndrome.

CB1 KO and WT mice from day 25 postnatal forward, were treated for 9 days with MOR (2 mg/kg, i.p.) or saline (SAL) twice daily. On the tenth day, animals received NAL (6 mg/kg, i.p.) or SAL 60 min after the last injection in order to precipitate the withdrawal syndrome, and immediately after somatic sings were counted during 30 minutes. At the end of the behavioral test, mice were transcardially perfused with 4% paraformaldehyde solution. Brains were removed

and postfixed for 2 h in the same fixative, and coronal frozen sections were made at 30 µm on a freezing microtome to perform the c-Fos immunohistochemistry.

No sex differences were observed during MOR abstinence syndrome in any of the genotypes (WT and CB_1 KO mice). In addition, It was observed an increase in the number of c-Fos positive nuclei in the Nucleus Accumbens Core, Habenula Lateral ($p<0.01$) and Caudate Putamen ($p<0.05$) of WT, but not in CB1 KO mice during MOR withdrawal syndrome.

These results suggest that the lower intensity previously observed during NAL-precipitated MOR withdrawal syndrome in mice lacking CB_1 receptors, could be related to the lack of changes in c-Fos expression.

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Key words: morphine, CB_1 receptor, withdrawal, c-Fos

(1260) **THE COMBINED ADMINISTRATION OF ALCOHOL AND TAURINE INDUCES MITOCHONDRIAL DYSFUNCTION AT THE BEGINNING OF ALCOHOL HANGOVER IN MOUSE BRAIN CORTEX**

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The combination of alcohol intake and energy drinks is often used as a possible attenuator for hangover symptoms. Alcohol hangover (AH) is the transient state that occurs after an acute ethanol consumption episode and begins when blood alcohol concentration is zero. Particularly, taurine is one of the major components of energy drinks. Therefore, the aim of this work was to evaluate if the combined administration of taurine and alcohol induces brain cortex mitochondrial dysfunction, through determinations of oxygen consumption, mitochondrial membrane potential and hydrogen peroxide production at the beginning of alcohol hangover. Swiss male mice were divided into four experimental groups: control (saline i.p.), taurine (70 mg/kg i.p.), alcohol (3.8 g/kg i.p.) and taurine-alcohol. Data were analyzed by one-way ANOVA. Alcohol hangover group showed 28% and 20% decreases in malate-glutamate state 3 respiration and respiratory control respectively ($p<0.05$). In taurine-treated animals, a 24% decrease was observed in malate-glutamate state 4 respiratory rate, leading to a 24% increase in respiratory control ($p<0.05$). The combined administration of alcohol and taurine significantly reduced state 4 and state 3 respiratory rates in 35% and 42% respectively at the hangover onset ($p<0.05$). Mitochondrial membrane potential was 11% and 20% decreased in alcohol and taurine-treated groups respectively ($p<0.05$). Hydrogen peroxide production was increased by 30-35% in alcohol, taurine and alcohol-taurine groups using malate-glutamate as substrates ($p<0.01$). Moreover, hydrogen peroxide production using succinate as substrate was 54%, 100% and 30% increased in alcohol, taurine and alcohol-taurine groups respectively. Altogether, the results allow concluding that the combined administration of alcohol and taurine not only did not prevent mitochondrial dysfunction induced by alcohol hangover but also aggravate it and induced active oxygen species generation.

Keywords: alcohol hangover, taurine, mitochondria, oxidative stress

(1897) **BRIEF ETHANOL DOSES MODIFIES 5HT LEVELS AT RAPHE OBSCURUS IN NEONATE RATS**

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Ethanol is the most commonly abused substance that impacts the humans during early ontogeny (gestation and lactation). Ethanol effects include alterations in brain development, learning and memory, cognitive abilities and neurovegetative functions such as breathing. A major target of ethanol in the central nervous system is the serotonin (5HT) neurons involved on the respiratory regulation. Preclinical studies employing chronic and severe ethanol consumption models during pregnancy and lactation, showed a decrease in the number of 5HTneurons and 5HT levels in the raphe system. However, the status of the serotonergic system in animal models with moderate and neonatal ethanol exposure are understudied. Thus, the main goal of this study was to evaluate 5HT levels in the raphe obscurus (ROb) nucleus in neonate rats, as a function of moderate ethanol or vehicle exposure (2.0 or 0.0g/kg, intragastrically) at postnatal days (PD) 3, 5 and 7. At PD 9, pups were intoxicated or vehicle administrated. Ninety minutes after, all pups were sacrificed and 5HT levels were immunodetected at the ROb. A significant reduction of 5HT levels was observed in pups exposed to ethanol for the first time at PD 9 and vehicle-pretreated at PDs 3, 5 & 7. Interestingly, a similar decrease in 5HT levels was also found in ethanol-pretreated pups evaluated under sobriety at PD 9. These results indicate a profound effect of ethanol upon on 5HT levels at ROb, nucleus specifically involved in breathing regulation. These data indicate that perinatal ethanol exposure is enough to alter 5HT levels in the Rob. These results became important when associating the function of the ROb on respiratory response and breathing disturbances commonly observed in neonate rats and humans, such as Sudden Infant Death Syndrome.

(1772) **IMPAIRMENT OF NITRIC OXIDE METABOLISM AT CENTRAL SYNAPSES BY LEVOCABASTINE, AN ANTAGONIST FOR NEUROTENSINERGIC NTS2 RECEPTOR**

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Neurotensin is able to modulate ionic gradient equilibria through neuronal membranes because it inhibits the activity of the sodium pump. Some properties of Na^+, K^+-ATPase are modified by the administration of L-NAME (Nω-nitro-L-arginine methyl ester), an inhibitor of nitric oxide synthase (NOS), and by levocabastine, an antagonist for neurotensinergic NTS2 receptor. In the search of a relationship between the activity of neurotensin NTS2 receptor and nitric oxide (NO) synthesis, levocabastine was administered to rats and the activity and expression of NOS were evaluated. Wistar rats injected (i.p.) with levocabastine (50 µg/kg) or saline solution (controls) were decapitated 30 min, 18 or 36 hours later. Cerebral cortices were processed by differential and sucrose gradient centrifugation to obtain synaptosomal membrane fractions. Nitric oxide production by NOS was measured following the oxidation of oxyhemoglobin to methemoglobin at 577-591 nm in a double-beam dual-wavelength spectrophotometer. Neuronal and inducible NOS expression were evaluated by Western blot assays. In synaptosomal membrane fractions NOS activity decreased 46% and 74% at 30 min and 18 hours after levocabastine administration ($p<0.05$). These changes were reversible in time. *In vitro* incubation of control synaptosomal membranes with 1 µM levocabastine also decreased the activity of NOS. At 30 min and 18 hours after levocabastine administration the expression of neuronal NOS protein decreased by 18% and 56% ($p<0.05$). These changes did not reverse in time. Concomitantly, an enhancement of inducible NOS expression was recorded after levocabastine treatment. Altogether, the results allow us to suggest that the NTS2 antagonist levocabastine markedly modified NOS activity and expression at CNS synapses, showing a possible interrelationship between the activities of neurotensinergic and nitrergic systems.

Keywords: cerebral cortex, nitric oxide synthase, synaptosomal membranes, neurotensin receptor, levocabastine

(951) **KETAMINE EFFECT ON CALCIUM CONCENTRATIONS AND CELL METABOLISM IN PC12 CELLS**

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Ketamine an inhibitor of NMDAR; has coupled ion channels highly permeable to calcium ions. Changes in intracellular [Ca^{2+}], are essential for neurophysiological processes, being indicators of physiological functions in neurons. This work evaluates the changes in [Ca^{2+}] induced by depolarization after 24 h exposure of PC12 cells to 100, 500 and 1000 μM ketamine. Ketamine effects on mitochondrial membrane potential ($\Delta\Psi m$) and nitric oxide (NO) levels were also analyzed. Methods using, Fluo-4 AM, epifluorescent microscopy, multiple-label counter and flow cytometry were used. Micrographies quantification showed that ketamine 100 μM and 500 μM decreased the number of positive fluorescent cells. Meanwhile, 1000 μM ketamine showed highest fluorescence intensity, with highest values of positive fluorescent cells, being significantly different from control cells ($p<0.05$). The results obtained by multilabel counter Glo-Max®-Multi Detection System, and by flow cytometry showed that low ketamine concentrations decreased the calcium fluorescent signal significantly ($p<0.05$) as compared with control, and similar differences were recorded with both methods, being in absolute intracellular [Ca^{2+}], for control, 100 and 500 μM ketamine: 612±60 nM, 239±15 nM and 258±8 nM versus 567±33 nM, 99±10 and 118±7 nM respectively for each method. With 1000 μM ketamine the absolute intracellular [Ca^{2+}] was significantly ($p<0.05$) different as compared with control, being 1035±234 nM and 2175±443 nM for the two methods respectively, and was accompanied by cellular morphology typical of apoptotic death as predicted by SSC and FSC. Metabolic alterations, with a significant decrease ($p<0.05$) in $\Delta\Psi m$ and NO levels were observed after all ketamine doses as compared with control. We conclude that ketamine has consequences on intracellular calcium homeostasis, probably mediated by NMDARs blockage, inducing drastic changes in neurons.Keywords: ketamine, calcium concentration, mitochondria and nitric oxide.

(219) **EARLY ANALYSIS OF CEREBELLAR MICROVASCULAR AND ASTROCYTIC DAMAGE BY SHIGA TOXIN 2**

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Shiga Toxin 2 (Stx2) is secreted by enterohaemorragic *Escherichia coli* (EHEC) and produces damage in several organs of the human body, including the cerebellum. The toxin travels through the blood stream and reaches several organs. The objective of this work is to observe whether Stx2 reaches the parenchyma of the cerebellum, and once in, if it can produce cell damage. For this work, NIH male mice were used. They were divided and injected intravenously in two groups (n=4): 1ηg/mice of Stx2 or saline solution (control group). Two days after the toxin was injected, they were sacrificed and their brains were fixed with PFA 4%. Then, their cerebellums were extracted and cut in slices 20μm thick. They were then subjected to histofluorescence technique with lectins to determine the microvasculature profile, anti-GFAP (glial fibrillary acidic protein) to identify reactive astrocytes and anti-Stx2 to identify immunopositive Stx2 cells. The group treated with Stx2 showed a decrease in the area occupied by the microvasculature (18.27 ±0.57 AU control and 5.77 ±0.2 AU Stx2, in IOD) along with an increase in the expression levels of GFAP (20.27 ±0.54 AU control and 36.35 ±0.95 AU Stx2, in IOD) $p<0,01$. The presence of Stx2 was detected in the Stx2 treated group and not in the control one, in the cerebellar cortex. This work proved that Stx2 damages the microvasculature of the cerebellum, and it produces astrocytary reaction (hiperthrophy and hyperplasia), which suggests a disruption on the Blood Brain Barrier of the cerebellum, and damages its parenchyma.

Key Words: Purkinje, Gfap, Damage, Cns, Immunofluorescence

(669) **NEURODEGENERATIVE EFFECTS OF THE CYANOTOXIN** B**-N METHYLAMINO-L-ALANINE** (**BMAA) ON RETINAL NEURONS**

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The non-proteic aminoacid BMAA is a cyanotoxin released by many cyanobacteria occurring in most dams and water resources around the world. Human chronic intake of this toxin has been linked with the development of neurodegenerative diseases, like Amyotrophic Lateral Sclerosis (ALS); Parkinson and Alzheimer Disease. Multiple studies have shown the effects of BMAA on live animals; however, its effects at the cellular or molecular levelsía are still mostly unknown. We here investigated the effects of BMAA on retinal amacrine and photoreceptor neurons and Muller glial cells (MGC) in vitro. We incubated pure neuronal cultures and mixed neuro-glial cultures obtained from newborn rat retinas, with 400 nM BMAA for 2 days. We then evaluated cell death and apoptosis by Propidium Iodide (PI) TUNEL assays, and DAPI staining. Mitochondrial activity was assessed by using Mitotracker and cytoskeleton integrity and axonal outgrowth were analyzed by immunocytochemical methods. In `pure neuronal cultures, BMAA increased the percentage of apoptotic amacrine and photoreceptor neurons, from 21.6% to 64.5% and from 29.3% to 65.7%, respectively, in controls and BMAA-treated cultures. Noteworthy, functional mitochondria decreased from 55% to 35% in photoreceptors but only slightly in amacrine neurons. In addition, BMAA treatment disrupted the organized assembly of tubulin in axons. BMAA addition to mixed neuro-glial cultures induced lamellipodia retraction and loss of mitochondrial membrane potential in MGC, but did not increase their cell death. Moreover, MGC partially prevented neuronal death.

These results suggest that BMAA induces subcellular changes in both neurons and glial cells, and markedly affects the viability of retinal neurons, confirming its threat to human health as a potential inducer of neurodegenerative damages.

Keywords: BMAA, cyanotoxins, apoptosis; retina neurons; glial cells

(206) **PHOTODAMAGE INDUCES MITOCHONDRIAL QUALITY CONTROL DYSREGULATION IN RETINAL PIGMENT EPITHELIUM.**
IMPLICATIONS FOR AGE-RELATED MACULAR DEGENERATION

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Age-related macular degeneration (AMD) is a late-onset neurodegenerative retinal disease. Accumulated lipofuscin fluorophore A2E in retinal pigment epithelium (RPE) confers susceptibility to blue light-mediated damage, a risk factor for AMD. Cells have developed well-coordinated Mitochondrial Quality Control (MQC) mechanisms in order to limit mitochondrial injury induced by oxidative stress, ensure mitochondrial integrity and preserve cellular homeostasis. RPE dysfunction is a critical event in AMD. Thus, our study was designed to evaluate MQC status, including mitochondrial dynamics (fusion/fission) and mitophagy, under photooxidative damage conditions in RPE.

Light-emitting diodes irradiation (λ=445nm; 1.7mW/cm^2, 30min) significantly reduced the viability of both non-pigmented and 10 or 25µM A2E-containing human RPE cells (MTT assay: 52%, 46%, 31%, respectively) as well as increased mitochondrial anion superoxide production (MitoSOX). A2E (25μM), along with blue light, induced apoptosis measured by Annexin V/PI-flow cytometry (6-fold, $p<0.001$) and by procaspase-3 levels (38%, $p<0.01$). Photodamage produced fusion/fission imbalance towards mitochondrial network

fragmentation in non-pigmented cells (72%) and 25μM A2E-fed cells (92%) ($p<0.01$) which correlates with deregulation of mitochondria-shaping proteins levels. We detected a dramatic rise in the expression of fission protein DRP1 and a decrease in the mitochondrial fusion and anti-apoptotic OPA1 protein levels. Mitochondrial Δφm loss activates OMA1, a MQC protease that cleaves OPA1 to inhibit fusion and facilitate apoptosis. Particularly, A2E and blue light stimuli lead to OMA1 activation, revealed by it self-cleavage (5-fold, $p<0.01$). Finally, TOM20 and LC3 ICC analysis suggested an altered mitophagy. Our work reveals for the first time, that photodamage cause RPE dysfunction through MQC dysregulation which may contribute to AMD pathology. Moreover, the possibility to develop MQC-based therapies is outlined.

Keywords: AGE-RELATED macular degeneration, A2E, blue light, mitochondrial quality control, retinal pigment epithelial cells

CARDIOVASCULAR AND RESPIRATORY SYSTEMS 2

(1205) HEART MITOCHONDRIAL FUNCTION IN RATS WITH THYROID DISORDER AND HYPOVOLEMIA

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Cardiovascular system regulation would be crucial in pathological situations such as hypovolemia. The actions of thyroid hormones would be relevant during this condition considering its actions on cellular respiration and mitochondrial function. The aim of the present work was to examine the effect of thyroid state on rat heart mitochondria function during hypovolemia. Sprague-Dawley rats aged 2 months old treated with T3 (hyper, 20 μg/100 g body weight) or 0.02% methimazole (hypo, w/v) during 28 days. Hypovolemic state was induced by a loss of 20% of blood volume during 2 minutes. Hearts were removed for mitochondria isolation and determination of O2 uptake, enzyme activity of complex I and protein levels (mt-NOS, akt T and akt P). The malate-glutamate-supported state 3 respiration decreased and increased in hypo and Hyper rats. Hemorrhage did not change this parameter. Malate-glutamate-supported state 4 did not change. No differences in succinate-supported state 4 and 3 respiration were observed in all groups. Hypothyroidism increased nNOS protein levels. This protein levels did not change in Hyper animals. Withdrawal decreased and increased nNOS protein levels in hypo and Hyper rats, respectively. Hyper increased complex I activity and hemorrhage did not change this activity. No differences were observed between Eut and hypo rats. Hemorrhage only increased complex I activity in hypo group. Thyroid disorders increased aktT protein levels. Hemorrhage did not change this parameter. Hyper increased akt P protein levels meanwhile hemorrhage induced a decreased of this protein levels. In hypothyroidism the lowest NO production would be responsible for increasing cellular respiration and guaranteeing the supply of oxygen to the tissues. The opposite would happen in the hyperthyroid where high levels of NO try to decrease the oxygen consumption as in the euthyroid. Alterations of complex I activity as well as AKT pathway could mediate these effects.

(615) INFLUENCE OF NITRIC OXIDE AND ADRENERGIC STIMULATION ON THE CARDIOPROTECTION OF HYPOTHYROIDISM IN RAT HEARTS EXPOSED TO ISCHEMIA-REPERFUSION

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Hypothyroidism (HypoT) reduced the stunning in severe ischemia-reperfusion (I/R), but increased the sensitivity of mitochondria to the Ca^{2+} overload-induced dysfuntion (SAFE 2016). The aims of this work were to evaluate whether HypoT cardioprotection was due to nitric oxide (NO) release, and if it remains under more physiological adrenergic conditions. HypoT was induced by drinking methimazole (0.02%) for 15 days. Isolated hearts were perfused inside a calorimeter at 37°C to measure left ventricular pressure (LVP, in mmHg) and total heat rate (Ht, in $mW.g^{-1}$) during the exposition to severe I/R (30 min/ 45min R). The role of NO was evaluated by perfusing HypoT hearts with 30 μM L-NAME (to inhibit NO-synthase) before I/R. L-NAME improved the postischemic contractile recovery (PICR) to 84.8 ± 6.7% of initial pressure (Pi) ($p<0.05$ vs 53.9 ± 4.7% in non treated C-HypoT, n=8-5) and total muscle economy (Eco=P/Ht) to 4.0 ± 0.7 $mmHg.g.mW^{-1}$ (vs 2.8 ± 0.3 $mmHg.g.mW^{-1}$) without changing the diastolic tone. Nevertheless, the treatment with 10 μM nitroprussate (NO-donor) did not change the behavior of HypoT hearts on stunning (n=5). Moreover, perfusing 50 nM adrenaline before I/R reduced PICR to 20.0 ± 7.9 % of Pi (vs 53.9 ± 4.7 % in HypoT-C, $p<0.05$, n=6-5) and Eco to 1.2 ±0.6 $mmHg.g.mW^{-1}$ (vs 2.85 ± 0.3 in C-HypoT) with diastolic contracture (ΔLVEDP = 7.8 ± 0.5 mmHg, $p<0.05$ vs 0). Results suggest that: a) The NO release is not a cause of the cardioprotection of HypoT; b) There would be a threshold NO level to contribute to stunning; c) Under the physiological adrenergic conditions, HypoT was not cardioprotective as in its abscence, in agreement to that reported for clinical situations. *Grant: UNLP X-795*.

Keywords: heart, hypothyroidism, ischemia-reperfusion, nitric oxide, adrenaline

(946) METALLOPROTEINASE ACTIVITY IN HEART IS INCREASED IN MICE TREATED WITH A LOW DOSE OF CORTICOSTERONE

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Continuous increase of glucocorticoids due to chronic activation of the pituitary axis is one of the consequences of chronic stress. The knowledge of its effects on plaque vulnerability markers and cardiovascular disease will help to elucidate different pathophysiological mechanisms. With this aim, we studied 15 C57BL/6j mice treated (TM) for 4 weeks with 35 μg/ml corticosterone and 15 control mice (CM). After euthanasia, heart, liver, adrenal glands, epididymal and intestinal adipose tissue (AT) were weighed. Serum lipid profile was evaluated. We performed a zymography of homogenized cardiac tissue and epididymal AT for measuring metalloprotease activity (MMPs). In isolated and isovolumetric hearts perfunded with Langendorff technique, we measured the left ventricle develop pressure (LVDP) and its response to ß adrenergic stimulus (Isoproterenol (iso) 1 μM). Results: At the end of treatment, adrenal gland weight was lower in TM (0.002 (0.0002) vs 0.003 (0.0004) gr, p=0.03). Weight gain was observed in TM vs CM (5.0 (1.5-9.5) vs 3.3 (2.0-7.0) gr, p=0.007), at expense of epididymal AT (0.73 (0.06) vs. 0.33 (0.04) g, $p<0.001$), without differences in heart, liver or abdominal AT (p= ns). In TM, a higher concentration of triglycerides (98 (12) vs 63 (7) mg/dl, p=0.02) and total cholesterol (116 (11) vs 78 (4) mg/dl, p=0.002) was observed. In TM increased activity of MMP-9 (0.48 (0.42-0.49) vs 0.37 (0.34-0.40) relative units (RU), p = 0.01) in heart tissue and lower MMP-2 activity (1.03 (0.04) vs 1.26 (0.07) RU, p=0.01) in AT was observed. Under baseline conditions, no differences in LVDP between TM and CT were observed. However, the response to Iso was lower in TM compared with CM (ΔLVDP: 14.9 vs 47.6 mmHg, p=0.02). Conclusions: After 4 weeks of treatment, we observed certain typical metabolic consequences effects of glucocorticoids. In addition, the impact on heart evidenced through an increase in MMP activity and a decrease in contractile reserve.

Keywords: Glucocorticoids; Metalloproteinases; Contractile reserve

(944) **REGULATION OF ANGIOGENESIS AND VASCULAR CALCIFICATION BY PROGESTERONE (Pg) AND ACETATE OF MEDROXYPROGESTERONE (MPA)**

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Hormone replacement therapy including natural or synthetic progestins is a therapeutic alternative to prevent cardiovascular disease, although the risk/benefit of its use is controversial. The cellular/molecular mechanisms of Pg and MPA displayed at vascular level are not fully understood. Vascular calcification (VCa) within atherosclerotic plaque involves vascular smooth muscle cells (VSMC) transdifferentiation to osteogenic lineage. Furthermore, angiogenesis represents a survival option for damaged tissue. The aim of this work was to investigate the role of Pg and MPA on: a) metabolic/phenotypic changes of VSMC in a pro-calcifying environment, and b) angiogenesis (capillary tube formation and VEGF synthesis). VSMC were cultured in DMEM for 21 days with 4 mM $CaCl_2$ and 10 mM β-glycerolphosphate to induce VCa. Calcium content in the extracellular matrix and alkaline phosphatase activity (ALP) were selected as markers of osteoblastic transdifferentiation. Long treatment with 10 nM Pg showed a significant decrease in ALP activity (239.9±21; 203.6±2.9 x10^3 IU, control; Pg, $p<0.02$) and calcium levels (365.1±38.2; 248.2±20.3 μg/mg protein, control; Pg, $p<0.02$). Similar results were obtained when VSMC were exposed to 1 nM MPA (24%; 20% below control, ALP activity; calcium content, $p<0.02$). In order to investigate the effect of agonists on capillary tube formation, the total number of vessel segments was quantified after Giemsa staining using optical microscopy and ImageJ software. The results indicate that both progestins significantly enhanced capillary tube formation (44%; 38% above control, 10 nM Pg; 10 nM MPA respectively, $p<0.05$). VEGF production was measured using an immunoassay. Treatment with 50 nM Pg markedly increased VEGF synthesis (43% above control, $p<0.05$). In contrast, MPA did not affect growth factor synthesis. In summary, both progestins exhibit a favorable action on vascular remodeling through the inhibition of VCa and the stimulation of angiogenesis.

Keywords: progesterone, acetate of medroxyprogesterone, vascular calcification, angiogenesis

(1517) **ROLE OF NLRP3/CASP1/IL-1B AXIS IN RENAL ISCHEMIA/REPERFUSION-INDUCED CARDIAC ELECTRICAL DISTURBANCES**

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Patients with chronic renal ischemia have a high risk to develop cardiovascular diseases. Therefore, understanding the pathogenic links between renal ischemia and cardiovascular disease is of utmost importance. Previous work of our group showed a systemic peak of IL-1βon the day 8after renal ischemia-reperfusion (IR) in mice. This finding was associated with prolongedQJ on the EKG, an arrhythmogenic marker, sustained up to day 15. We have also demonstrated the important role played by resident heart macrophages in IL-1β release upon NLRP3 inflammasome activation andin cardiac electrical remodeling. Thus, in this work we tested the hypothesis that the activation of NLRP3/Casp1/IL-1β axis is involved in cardiac electrical disturbances promoted by renal IR. In order to investigate the role of NLRP3 inflammasome in cardiac electrical disturbances, *Nlrp3*$^{-/-}$ and *Caspase-1*$^{-/-}$ mice induced with renal IR or Sham were used. Cardiac electrical function was assessing by *in vivo* electrocardiogram (EKG) 15 days post- renal IR. Our data showed that the genetic ablation of NLRP3 or Caspase-1 preventedeither the QJ prolongation ($p<0.01$ and $p<0.001$) and the renal IR-induced systemic peak ofIL-1βon day 8 (measured by ELISA). In fact, renal IR failed to prolong the QJ interval in IL-1r$^{-/-}$ mice. The depletion of macrophages (by Clodronate Liposomes) on day 7 post-renal IR was able to prevent the IR-induced longer QJ observed at day 15 post-IR($p<0.05$). Finally, the daily treatment with IL-1r-antagonist (Anakirna) from day 8 to day 15 reverted the IR-induced longer QJ ($p<0.01$).The data presented here demonstrates that NLRP3/Casp1/IL-1β axis is involved on the induction of prolonged cardiac QJ by renal ischemia/reperfusion. Additionally, the block of the IL-1r was found to be a potential therapeutic approach to revert the IR-induces cardiac changes.

Keysword: renal ischemia-reperfusion, cardiac electrical alterations, NLRP3 inflammasome, IL-1β.

(1880) **LACK OF GALECTIN 3 ALTERS MACROPHAGES POLARIZATION AND CYTOKINES PROFILE DURING THE WOUND HEALING OF MYOCARDIAL INFARCTION IN MICE**

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We previously showed that lack of Galectin 3 (Gal3) reduced the macrophages (MØ) infiltration and fibrosis during the early wound healing after myocardial infarction (MI) leading to adverse cardiac remodeling in mice. However, whether Gal3 alters MØ polarization and cytokines profile during the evolution of wound healing after MI has not been previously investigated. Here we aimed to study whether lack of Gal3 modify the polarization of MØ and changes the proinflammatory and antiinflammatory expression of cytokines during the evolution of wound healing process in mice with MI. Adult male C57 and Gal3KO mice were subjected to permanent coronary artery ligature. After 1 and 4 weeks (w) post-MI echocardiography was performed. LV end-diastolic diameter (LVEDD) was measured and ejection fraction (EF, %) calculated. Then, animals were euthanized and the hearts from animals of 1w were harvested and store (-80ºC). IL10, TNFα and Mannose receptors (MR) were quantified by qPCR. At 4w MI size and fibrosis were quantified in cardiac slices stained with Masson's Trichrome and Picrosirius Red. Results**:** X±SEM; * $p<0.05$ C57 vs Gal3KO. At 7 days post-MI the MØ polarization and cytokine expression were affected in by lacking Gal3. MR were increased from 0.5±0.2 (C57) to 1.8±0.4* (Gal3KO), while IL-10 from 0.7±0.0 (C57) to 2.8±0.3* (Gal3KO). At the same time, TNFα was reduced from 26±0.3 (C57) to 0.4±0.1* (Gal3KO). The lack of Gal3 increased the LVEDD from 4.4±0.1 (C57) to 4.8±0.2* (Gal3KO) and from 4.9±0.2 (C57) to 5.7±0.3* (Gal3KO) simultaneously reduced the EF from 47±2 (C57) to 37±3* (Gal3KO) and from 48±6 (C57) to 39±5 (Gal3KO) at 1 and 4w postMI respectively. Fibrosis at the MI zone was reduced from 91±6 (C57) to 61±3* (Gal3KO). In summary, the lack of Gal3 affected the MØ polarization and the pro and antiinflammatory cytokine profile. Those effects unfavorably modified the evolution of wound healing as observed by reduced fibrosis in the MI zone at 4w and adverse LV remodeling.

Keywords: Myocardial infarction, Healing, Remodeling, Galectin 3, Inflammation

(950) **LUNG PRE-FIBROTIC STATUS IN A MOUSE MODEL OF METABOLIC SYNDROME: IMPLICATION OF OXIDATIVE STRESS AND INFLAMMATION.**

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During the last decades there was a sustained increase in morbidity and mortality due to respiratory and metabolic diseases associated with obesity, such as the metabolic syndrome (MS). Components of MS, such as dyslipidemia, hyperglycemia, hypertension

and obesity were independently associated with lung function, but from all of them, obesity is clearly implicated. Herein we aimed at determining the impact of the MS on redox/inflammatory status and potential consequences in the lung using a previously developed mouse model of MS. To achieve this goal, 6 week-old male C57BL / 6J mice were fed for 16 weeks with a low fat- (LFD) or a high fat-diet (HFD, chicken fat) and water supplemented with 10% fructose (F). Thus, the experimental design included 2 groups: LFD (control) HFD+F (MS). At the end of the treatment, lung of the HFD + F group compared to the LFD group had an increase in NOX-2 ($p < 0.05$) and iNOS ($p < 0.05$) expression, generating an imbalance between oxidants and antioxidants. This imbalance produced local oxidative / inflammatory damage, with a consequent decrease in the activity of antioxidant systems (PAT, GSH / GSSG, CAT, SOD and GPx) and an increase of proinflammatory cytokines (TNF-α and IL-6). Oxidative damage increased neutrophil chemotaxis, and the HFD + F group had neutrophils higher retention (ICAM-1 $p < 0.01$) and activation (MPO $p < 0.01$) in lung parenchyma, which led to an increase of connective tissue in the HFD + F group compared to the control. In this study, we report that mice model with MS exhibit lung inflammatory symptoms with an imbalance of the oxidant / antioxidant system towards oxidative stress and inflammation leading to a profibrotic state.

Keywords: metabolic syndrome, lung, Inflammation, oxidative stress, connective tissue, fibrosis

(520) **MECHANISMS OF STUNNING DUE TO ISCHEMIA/ REPERFUSION IN AGED RAT HEARTS AND MITOCHONDRIAL MECHANISMS OF CARDIOPROTECTION: ENERGETICAL STUDY.**

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Age is a cardiac risk, and previous results showed that hearts from >20 months aged female rats (AgF) had less postischaemic contractile recovery (PICR) and muscle economy than young female (YF) and male (YM) rats exposed to moderate I/R (I/Rm, 20 min I/45 min R) but similar in severe I/R (I/Rs, 30 min I/45min R) (SAFE 2016). The aim of this work was to evaluate the underlying mechanisms of stunning in the AgF. Isolated hearts were perfused inside a calorimeter at 37°C to measure left ventricular pressure (LVP, in mmHg) and total heat rate (Ht, in mW) during exposition to I/Rs. To evaluate whether the SR Ca^{2+} content and release was altered by age, AgF hearts were exposed to 30 min I and R with Krebs-10 mM caffeine-2 mM Ca-36 mM Na, and the consequent contracture (ΔLVP) and ΔHt were measured as well as the respectives area-under-curves (AUC) over the ischemic line. The AgF hearts developed similar AUC-LVP (2834.9±647.9 mmHg.s) and AUC-Ht (419.3±32.7 $mJ.g^{-1}$) than Y hearts (2152.6±492.8 mmHg.s and 441.2±62.5 $mJ.g^{-1}$, respectively). In order to evaluate the role of mitochondrial transporters in the stunning, AgF hearts were perfused with 10 μM clonazepam (Clzp) to block the mNCX before I/Rs. Clzp increased PICR from 13.3±3.0% to 42.7±5.8% ($p<0.05$) during R, as well as muscle economy (P/Ht) from 1.0±0.2 to 2.2±0.3 $mmHg.mW^{-1}.g$), while reduced diastolic contracture (+ΔLVEDP from 74.9±9.1 to 25.5±7.2 mmHg). To evaluate the role of mKATP channels in AgF, hearts were perfused with 30 μM diazoxide (Dzx) before I/Rs. The PICR was increased up to 62.6±7.3 % at the end of R ($p<0.05$) as well as P/Ht (to 3.2±0.8) while reduced ΔLVEDP. Results suggest that: a) the low cardioprotection associated to the age was not due to a low SR Ca^{2+} store and release, b) mitochondrial $[Ca^{2+}]$ of AgF has influence on stunning, since PICR, could be improved by reducing the mitochondrial Ca^{2+} extrusion through mNCX, c) as in Y hearts, activation of mKATP channels improves PICR of AgF. *UNLP-X-795.*

Keywords: cardiac energetics, age, ischemia/reperfusion, mitochondria, calcium

REPRODUCTION AND FERTILITY 2

(913) **A DUAL DEATH/SURVIVAL ROLE OF AUTOPHAGY IN THE ADULT OVARY OF *LAGOSTOMUS MAXIMUS* (MAMMALIA- RODENTIA).**

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The follicle reserve in the mammalian ovary is lost from the fetal/perinatal period and throughout post-natal lifespan mainly through apoptosis-driven follicular atresia. However, other cell death mechanisms such as autophagy, seem to be also involved. The South American plain vizcacha, *Lagostomus maximus* (*L.m),* shows massive ovulation and a very low or suppressed apoptosis-dependent follicular atresia. Here we investigated autophagy markers in the adult ovary of *L.m* aimed to discern a possible role of autophagy proteins in germ cell death or survival. A total of 14 adult female ovaries were screened for the expression of BECLIN1, LC3B, LAMP1, *cleaved CASPASE-3* and BCL2 by immunohistochemistry (IHQ), western blot (WB) and protein co-expression by confocal microscopy. The presence of autophagosomes (AP) and autolysosomes (AL) in follicles and corpora lutea (CL) was analyzed by electron microscopy (EM). Ovary explants were incubated in culture starvation media to determine whether autophagy was triggered by a stress stimulus. Ovary explants incubated in enriched culture media were used as controls. Strong BECLIN1, LC3Band LAMP1 staining was observed in atretic follicles (AF) and degenerating CL that also expressed nuclear cleaved-CASPASE-3 . Non-atretic follicles showed a slight expression of autophagy proteins but a strong expression of BCL2 and negative expression of cleaved CASPASE-3. In cultured ovaries, BECLIN1, LC3B-II and LAMP1 were significantly increased in starvation condition ($p<0,05$). EM revealed a high formation of AP and AL in AF and CL and a low number of autophagic vesicles in non atretic follicles. The co-expression of LC3B-BECLIN1, LC3B-LAMP1 and LC3B-CASPASE-3 was only detected in AF and CL, while co-expression of BCL2-BECLIN1 was only observed in non-atretic follicles. These results suggest a dual role of autophagy in the ovary of *L.m.* inducing cell death in AF and CL and promoting cell survival in non atretic follicles.

Keywords: autophagy, ovary, germ cell, vizcacha.

(1432) **ENDOMETRIOSIS ALTERS THE EXPRESSION OF STEROIDOGENIC PROTEINS IN THE OVARY AND THE RELEASE OF STEROID HORMONES IN A RAT MODEL**

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Objetive: To study the expression of steroidogenic proteins, the release of estradiol and progesterone, the ovulation and luteal size in the ovary of rats with experimentally induced endometriosis

Materials and Methods: Endometriosis was surgically induced in Sprague Dawley rats by autotransplantation of uterine horn pieces to the bowel mesothelium. Sham animals were used as controls. Rats were sacrificed one month after surgery at different stages of menstrual cycle (proestrus, estrous and diestrus). Luteal size was measured in ovary sections stained with hematoxylin-eosin. Aromatase P450, P450scc, 3β-HSD and StAR expression was assessed by western blot in isolated follicles and corpora lutea (CL). Estradiol and progesterone serum levels were evaluated by RIA. Ovulated oocytes were counted in the ampulla in the morning of estrous.

Results: StAR expression decreased in follicles from rats with endometriosis in all estrous cycle stages ($p<0,001$) and increased in CL during estrus ($p<0,05$). P450scc expression decreased in

follicles during proestrus (p<0,05) and increased in CL during estrus (p<0,001) in rats with endometriosis compared to sham rats. 3β-HSD expression diminished in CL from rats with endometriosis during proestrus (p<0,05). Aromatase P450 expression decreased in CL (p<0,001) and follicles (p<0,01) in diestrus and increased in CL during estrus (p<0,01) in rats with endometriosis. Serum levels of estradiol were decreased (p<0,01) and progesterone serum levels were increased (p<0,01) in rats with endometriosis in all estrus cycle stages. Related to these results, CL size was increased (p<0,05) in diestrus and the number of ovulated oocytes was diminished (p<0,05) in rats with endometriosis compared to the sham ones.

Conclusion: Our results suggest that experimental endometriosis in rats alters steroidogenic proteins expression in ovary leading to endocrine and ovulatory changes and a delay in structural luteolysis.

Keywords: Endometriosis, steroidogenesis, ovulation, luteolysis

(1680) **REGULATION OF THYROID HORMONE RECEPTORS BY SUCKLING IN RATS WITH NORMAL OR DEFICIENT LACTATION.**

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During lactation, sustained hyperprolactinemia is maintained by the suckling stimulus and decreased hypothalamic sensitivity to prolactin (PRL) negative feedback, that maintains a reduced dopaminergic tone. OFA (hr/hr) rats, derived from the Sprague Dawley (SD) strain have deficient lactation, high hypothalamic dopaminergic tone and a blockade in suckling induced PRL release. Hyperthyroid rats also have deficient lactation, caused by increased hypothalamic sensitivity to PRL negative feedback that leads to a partial blockade in suckling induced PRL release. Thus, both groups show elevated dopaminergic tone responsible for the decreased PRL release and lactation failure. This evidence suggests that thyroid hormones may be also involved in prolactin regulation during lactation. We investigated the changes in hypothalamic thyroid hormone receptors (TR) expression in response to the suckling stimulus in OFA rats compared to SD rats (normal lactation). Using real time quantitative RT-PCR, we measured the changes in hypothalamic mRNA expression of TRβ isoforms 1 and 2 and TRα isoforms 1 and 2, in mid-lactating OFA and SD rats, continuously suckled (CL), separated from their pups for 12 h (Sep), or separated and subsequently suckled during 2 (S2) or 4 (S4) h. We also determined serum PRL levels by RIA and litter weight gain. After suckling, PRL release and litter weight gain were higher (p<0.05) in SD than OFA rats. In SD rats, separation had no effect, but S2 and S4 induced increases in all TR isoforms mRNAs (p<0.01). OFA CL and Sep rats had higher values of all TR isoforms compared with SD rats (p<0.05), and they decreased after suckling to lower values (p<0.05) than in SD rats for TRβ1, TRα1 and TRα2. These results show that lactating OFA rats have differential hypothalamic TRs expression patterns in response to suckling, suggesting that they may be involved in the regulation of the negative PRL feedback mechanism present in neuroendocrine dopaminergic neurons.

Keywords: thyroid hormone receptors, lactation, hypothalamus, suckling.

(1026) **HUMANIN PEPTIDES AS NOVEL CYTOPROTECTIVE FACTORS IN THE OVARIAN CELLS**

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Humanin (HN) peptides have cytoprotective action in several cell types such as neurons, spermatogonias and Leydig cells. Our previous results showed that HN peptides are expressed in normal ovarian cells, being localized in granulose cells, theca cells and oocytes. Also, we observed that HN is expressed in the human granulosa-like tumor cell line (KGN). In addition, we showed that HN increases the viability of KGN cells. The aim of present study was to explore the function of HN peptides in the ovary from adult rats and KGN cells. To evaluate the effect of endogenous HN, we used a baculovirus encoding a shRNA to inhibit HN expression (BV shHN). We injected BV shHN and control baculovirus (BV control) into the ovarian bursa of adult rats. In ovarian sections stained with hematoxylin-eosin, we observed that BV shHN injection reduced the cortex area whereas increased the stroma area. The inhibition of HN expression increased the percentage of atretic follicles (BV control: 15%; BV shRNA: 78%, $p < 0.01$, $\chi 2$). In addition, we studied the effect of silencing HN expression on the apoptosis in follicles by TUNEL. BV shRNA increased the percentage of follicles with apoptosis (BV control: 11%; BV shHN: 31%, $p < 0.01$, $\chi 2$). Also, to evaluate the function of endogenous HN in KGN cells, they were transduced with BV shHN or BV control. The inhibition of HN expression changed the morphology of KGN cells, which become elongated and fibroblast-like, and increased the percentage of apoptosis of KGN cells (BV control: 0.3%; BV shRNA: 4%, $p < 0.01$, $\chi 2$). Considering that the inhibition of endogenous HN modifies the morphology of KGN cells, the architecture of the ovary and increases the percentage of apoptotic KGN cells and follicles with apoptosis, our results suggest that HN peptides exert a cytoprotective action in normal and tumor ovarian cells. HN arises as a novel cytoprotective factor that could play a role in ovarian physiology and pathology.

Keywords: ovary, humanin, follicle, KGN, baculovirus

(1342) **NEONATAL EXPOSURE OF EWE LAMBS TO A GLYPHOSATE-BASED HERBICIDE ADVERSELY AFFECTS OVARIAN FOLLICULAR DEVELOPMENT INDEPENDENTLY OF ADMINISTRATION ROUTE**

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Glyphosate-based herbicides (GBH) are widely used around the world. Previous studies suggest that GBH may act as endocrine disruptor. Our hypothesis suggests that low doses of GBH affect ovarian ovine development. Ewe lambs were exposed from postnatal day (PND) 1 to PND14 to sc (n: 13) or orally (n: 5) environmentally relevant doses of GBH (2 mg/Kg/day, EPA reference dose) and controls (n: 13) with saline solution. On PND 45 ovaries were weighted and paraffin-embedded, sectioned in 4 adjacent 5 μm -serial sections taken 200 μm apart. Follicular dynamic was established by histomorphological features on picrosirious-hematoxilin-stained sections (8 sections/ovary). Follicles of different types and multioocyte follicles were expressed as a percentage. Immunohistochemistry of androgen receptor (AR) and Ki (proliferation marker) were evaluated. Proliferation was measured in granulose (GC) and theca cells (TC) of antral follicles. GBH treatment did not alter ovarian weight, total number of follicles or AR expression. Lambs exposed to GBH showed a reduction of primordial follicles (C=87.9±2.7; scGBH=57.8±3.9; oGBH=62.7±5.4) together with an increase of transitory (C=6.8±1.7; scGBH=36.2±3.3; oGBH=30.7±4.6) and primary (C=1.4±0.2; scGBH=4.4±0.9; oGBH=5.2±1) follicles. In addition, GBH treatment increased the percentage of atretic follicles (C=29.0±3.7; scGBH=44.3±4.0; oGBH=44.3±2.9) and induced higher proliferation of GC (C=26.3±5.5; scGBH=43.3±5.1; oGBH=52.8±11.1) and TC (C=4.9±0.9; scGBH=11.1±1.6; oGBH=28.7±4.8) in antral follicles. Moreover, an increased incidence of multioocyte follicles (C=0.1±0.1; scGBH=1.3±0.4; oGBH=1.1±0.3) was observed. Our results demonstrated that neonatal exposure to low doses of GBH disrupts the ovine follicular development independently of administration route, showing no differences between sc and oral treatment. Further studies with the same GBH protocol will define whether the described effects have consequences in the adult ovarian function.

Keywords: glyphosate, neonatal exposure, ovary

(668) **RESVERATROL REDUCES DOXORUBICIN-INDUCED OVARIAN DAMAGE IN MICE OVARY**

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IByME

Premature Ovarian Failure (POF) is characterized by the disappearance of ovarian follicles in young women, which may be caused by chemotherapy. POF treatments such as hormone therapies are not completely effective. We evaluated whether Resveratrol (Rs) can prevent gonadotoxicity caused by doxorubicin (Dx) in a mouse model. F1 mice (BalbC x C57 / BL6) (8 weeks) were given either an single intraperitoneal injection of Dx (10mg/kg) or saline solution. Control and Dx mice underwent sham surgery and received an intrabursal injection of saline solution, while Dx+Rs groups received 1μM or 10μM of Rs intrabursa, 1h prior to Dx administration. Animals were sacrificed 2 weeks after surgery (n=5-8/ group) and ovaries were removed, one for Western Blot (WB) and the other for histological analysis. Paraffin-embedded ovarian slides were stained in H&E for morphological study or immunostained for DDX4 (germ-line cell specific marker) and AMH (ovarian reserve marker). Results were evaluated by ANOVA followed by Dunnet's test.

Dx reduced the % of primary follicles compared to control ($p<0,05$) while Dx+Rs10μM increased it ($p<0,05$). Dx increased the % of atretic follicles compared to control ($p<0,05$) but Rs10μM restored these values. Dx decreased the number of primordial follicles compared to control ($p<0,05$), but Dx+Rs1μM maintained control values, as studied by DDX4 immunostaining. By WB, Dx decreased protein expression of AMH compared to control ($p<0,05$), while Dx+Rs10uM increased its expression compared to Dx alone ($p<0.01$), which was corroborated by IHC. Since Rs induces the expression of ovarian sirtuin 1 (SIRT1) and modulates follicular development, we evaluated this protein. Dx reduced SIRT1 expression ($p<0,05$) but Rs (1-10μM) restored it to control values.

Our results suggest that Rs can protect the ovary from gonadotoxic injury by Dx, as shown by its effects on follicular dynamics and ovarian reserve. Rs could be a novel strategy for fertility preservation in cancer patients.

(646) **STEM CELL MARKERS OCT3/4, SOX2 AND SOX17 IN ADULT OVARY OF *Lagostomus maximus***

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Lagostomus maximus (Rodentia: Chinchillidae) does not show apoptosis as a mechanism of intra-ovarian elimination of germ cells (GCs); moreover, it shows a continuous production of GCs suggesting *de novo* GC generation in the adult ovary. We investigated the expression of pluripotent markers in ovaries of non-pregnant, early-, mid- and term-pregnant vizcachas. We semi-quantified the different follicular stages (primordial, primary, secondary, pre-antral, antral and corpora lutea) in the ovaries of each experimental group and examined the expressions of Oct3/4, Sox2 and Sox17 in ovary cross-sections by immunohistochemistry. Although ovaries from all the experimental groups exhibited all follicular structures, the markers analyzed showed a distinctive expression pattern: neither Sox2 nor Sox17 were detected in any ovarian structure at any of the reproductive stages studied. Yet, Oct3/4 was observed in nuclei and/or cytoplasm of the oocyte in primary, secondary, pre-antral and antral follicles. Cytoplasm Oct3/4 expression is related with the survival of GCs during its maturation. Oct3/4 appears to be up-regulated in oocytes of both primary and growing (secondary and antral) follicles of adult females. This up-regulation would be occurring at the completion of the prophase I of meiotic division which would suggest a specific involvement of this transcription factor in oocyte growth.

Key words: stem cells, adult ovary, *Lagostomus maximus*

(334) **PROTEIN EXPRESSION AND CELLULAR LOCALIZATION OF ESTROGEN RECEPTORS ALPHA AND BETA IN ENDOMETRIOSIS-ASSOCIATED EPITHELIAL OVARIAN CANCER CELL LINES**

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The potential role of the estradiol secreted by endometriotic lesions on the pathophysiological mechanisms of endometriosis-associated epithelial ovarian cancer (EOC), such as endometrioid and clear-cell epithelial ovarian tumors, is not yet entirely known. So, the main objective of this study was to evaluate the protein expression and cellular localization of Estrogen Receptors alpha (ERα) and beta (ERβ) in cell lines derived from human endometriosis-associated EOCs. Human TOV-112D cell line derived from an endometrioid-type ovarian carcinoma and human TOV-21G cell line derived from a clear-cell ovarian carcinoma were cultured under standard conditions until confluence to further analysis of ERα and ERβ protein expression and cellular localization, in 4 independent experiments, by Western blot and indirect immunofluorescence respectively. We found that both endometriosis-associated ovarian cancer cell lines expressed the canonical steroid receptor proteins, being ERα expression stronger than ERβ in both cell lines. In addition, ERα was mainly localized into the cellular nucleus, whereas ERβ was mostly localized into cytoplasm. These results suggest that these cell lines are endowed with the necessary molecular machinery to respond to estradiol, becoming them as a suitable *in vitro* model to study the potential role of steroid hormones secreted by endometriotic lesions on the onset and development of endometriosis-associated EOCs. This is an initial descriptive study, so functional studies will be addressed to fully understand the role of the estradiol secreted by endometriotic lesion on the pathophysiological mechanisms underlying the etiology of endometriosis-associated EOCs.

Keywords: Estrogen Receptors, Endometriosis, Epithelial Ovarian Cancer, Pathophysiology.

(435) **ANDROGEN EXCESS DURING PRENATAL LIFE AFFECTS HEPATIC LIPOGENESIS IN A MURINE PCOS MODEL AT ADULT LIFE**

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Prenatal androgen excess is considered as one of the main factors contributing to the development of Polycystic Ovary Syndrome (PCOS). Most of PCOS patients present hyperinsulinemia or Insulin Resistance (IR) which are associated with different metabolic disorders such as nonalcoholic fatty liver disease (NAFLD) and steatosis. In previous results, at puberal stage (60 days of age), we have shown that prenatal hyperandrogenism affects hepatic lipogenesis, thus leading to a high risk of developing steatosis.

This study aimed to evaluate the effect of prenatal hyperandrogenism on the regulation of lipogenesis in the liver at adult life (90 days of age).

Pregnant rats were hyperandrogenized with testosterone and a control group was obtained by the injection of vehicle. The prenatally hyperandrogenized (PH) female offspring (N=150) and control offspring (C, N=96) were characterized according to the estrous cycle as ovulatory (PHov) and anovulatory (PHanov) phenotypes at adult age. The gene expression of PPARg, SREBP and ChREBP (lipogenic enzyme's modulators), PPARa (involved in b-oxidation) and chemerin (adipokine associated with IR and NAFLD disorders) were quantified by qPCR. In addition, the hepatic triglyceride content was quantified by an enzymatic kit.

We found that PPARg, SREBP and ChREBP mRNA levels were lower in both phenotypes (PHov and PHanov) than in the control group ($p<0.01$). On the other hand, PPARa and chemerin mRNA levels were not affected in the PH groups and no difference was found in hepatic triglyceride content relative to the control ($p>0.05$).

We conclude that the alterations promoting lipogenesis observed at puberal age are not maintained at long term. Instead, at adult age, the inhibition in this pathway may be preventing the possible accumulation of hepatic triglycerides. Nevertheless further research is needed as they might be involved in energy imbalance processes.

Keywords: PCOS, lipogenesis, hyperandrogenism, liver, developmental programming.

(291) **STROMAL-DERIVED FACTOR-1 (SDF-1) DIRECTLY PROMOTES GENES EXPRESSED WITHIN THE OVULATORY CASCADE**

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SDF1, a chemokine that is expressed in multiple tissue types including the ovary, serves as the ligand to chemokine CXC-motif receptor-4 (CXCR4). While LH upregulates follicular CXCR4, the role that SDF1/CXCR4 signaling might play in the COC (cumulus-oocyte complex) was unknown. We hypothesized that the SDF1/CXCR4 system was present in feline COCs and that COCs culture with SDF1 would directly upregulate genes expressed in the ovulatory cascade (HAS2, TSG6, AREG, and GDF9). To address this hypothesis, a COC culture was established. Ovaries (n= 50) were obtained from adult domestic cats during breeding season and COCs were mechanically recovered from antral follicles. The culture environment was optimized by testing different conditions (media, serums, serum concentrations and pretreatments). During culture optimization, COCs (n=24) were cultured at different time-points (3, 12 and 24h) with and without gonadotropins (GNT). Then, the effects of 25ng/ml SDF1 and CXCR-4 inhibitor were examined in the COC at all time-points (n=48) and gene expression was assessed by real-time PCR. CXCR4 and SDF1 immunofluorescence was also performed. MEM-hepes with 1% of charcoal stripped-FBS was the optimum medium for this culture, as seen by the expansion of the COC at 24h. The mRNA expression of HAS2, TSG6, and AREG peaked at 3h in the GNT group as compared to GNT treatment in the 12 and 24h groups, and as compared to negative controls ($p<0.05$). COCs cultured with 25ng/ml SDF1 showed increased HAS2 and TSG6 mRNA expression at 3h as compared to negative controls and the CXCR4 inhibitor group. CXCR4 and SDF1 immunostaining was present in both cumulus cells and the oocyte. In summary, these results suggest that this model can be used to examine molecular processes within the COC. In addition, the SDF1-CXCR4 pathway may extend its function beyond chemoattractant, and may play a role in cumulus cells via upregulation of key ovulatory genes concomitant with GNT stimulation.

Keywords: ovary, feline, chemokines, cumulus oocyte complex.

ENDOCRINOLOGY 2

(1143) **EFFECTS OF IL-1**B **ON ADRENAL STEROIDOGENESIS AND MIRNA EXPRESSION BY *IN VITRO* STUDIES IN THE NCI-H295R HUMAN CELL LINE.**

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The adrenal gland is a key component of the hypothalamus–pituitary–adrenal (HPA) axis, playing a crucial role in the adaptation of organism to stressors. Adrenal steroidogenesis is a vital process widely regulated at several stages. Immune mediators released during infections are known to influence adrenal steroidogenesis, as is the case of tuberculosis (TB), in which the prolonged presence of the pathogen together with an exacerbated immune response is likely to alter the endocrine profile. IL-1β which is mainly produced by macrophages is a key mediator in TB. Beyond its immunological effects, IL-1β stimulates the production of ACTH and the ensuing cortisol release. However it is not well known whether this cytokine exerts a local effect on the adrenal gland itself. As such, we decided to study the effect of different concentrations of IL-1β, at 6, 12, 24 and 48 h, in the human cell line NCI-H295R, derived from a carcinoma of adrenal cortex, stimulated with Forskolin (Fk), or not. Treatment with IL-1β 1.25 pg/ml + Fk during 24 h resulted in an increased cortisol and DHEA production ($p<0.05$) in comparison to cultures only stimulated with Fk or no treatment (Basal). Given that MicroRNAs are endogenous small noncoding RNAs that decrease the expression levels of specific genes by translational repression, sequestration, and degradation of their mRNAs; we next explored eventual changes in the microRNAs (miRNAs) profile of cells exposed to IL-1β 1.25 pg/ml + Fk during 24 h. Six out of 84 analyzed miRNAs showed a differential expression, depending on treatment conditions. MiRNAS 17-5p, 26a-5p, 93, 484 and 499a-5p were augmented in cultures treated with IL-1β + Fk ($p<0.05$, vs. Fk only) while MiRNA 206 was diminished in Fsk-treated or Basal cultures ($p<0.05$). The IL-1β-induced changes in hormonal secretion by NCI-H295R cells coexist with certain modifications in the miRNA profile which may bear relation with the regulatory mechanisms underlying adrenal steroid production.

(36) **EXPRESSION OF STEROIDOGENIC ENZYMES IN PERIPHERAL BLOOD MONONUCLEAR CELLS (PBMC) OF HEALTHY INDIVIDUALS AND ITS ASSOCIATION WITH AGE AND SEX.**

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There is strong evidence that cells of the immune system can secrete a wide variety of hormones, however little is known about their function, or its association with certain pathologies. Our goal was to study the expression of different enzymes related to steroid synthesis in PBMC from healthy individuals. We separated PBMC from males and females of ages 25 – 90 using a Ficoll gradient, RNA was obtained with Trizol reagent, and reverse transcribed to obtain cDNA. We designed primers for aromatase (Aro), 5α-reductase (5aR) 1, 2 and 3, and 3β hydroxysteroid dehydrogenase (3bHSD). Real-Time PCR was performed using L19 as a housekeeping gene. Statistical analysis were carried out using R software. We found that 5aR1, 5aR3 and 3bHSD were expressed on PBMCs, but not Aro or 5aR2. Moreover, 5aR1 showed correlation with age in men (P=0,049), with a significantly higher expression in men older than 50 years (P=0,0257). When comparing both sexes, we found higher values of 5aR1 in men vs women older than 50 years (P= 0,0262). Concerning 5aR3, we found a higher expression in women older than 40 vs younger than 40 years (P=0,0399), and when we compared men and women, we found higher levels of enzyme expression in males older than 50 years (P=0,0371). These findings demonstrate that PBMCs have the required machinery to produce several sex-related hormones, and that these effects are more noticeable at a higher age, when hormone production from endocrine organs decreases. Future studies will compare these findings with the expression of steroidogenic enzymes in Chronic Lymphocytic Leukemia (CLL) samples, taking into account that CLL is more frequent in people older than 50 years, and has a sex dimorphism, being men more susceptible than women.

Keywords: steroid synthesis; peripheral blood mononuclear cells.

(1453) **INTRACRINOLOGY: VASCULAR ENDOTHELIUM AS TARGECT OF DEHYDROEPIANDROSTENEDIONE ACTION**

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According to intracrinology, active sex steroid hormones can be locally synthesized from their precursor Dehydroepiandrostenedione (DHEA). In vascular system sex hormones regulate cell migration, proliferation, production of nitric oxide (NO) and adhesion of plate-

lets (PT) and monocytes to endothelial cells (EC). We evaluated the effect of DHEA on these events and evaluated if they were assessed by DHEA, or if conversion to other sex steroids is required.

Primary cultures of rat aortic EC were obtained from Wistar rats and exposed to DHEA (20nM-2mM 0.5-48h). DHEA stimulated NO production (Griess reaction 0.17±0.02; 0.24±0.04; nmols NO/mg prot, Cont; DHEA p<0.01), effect that was not affected by pretreatment with androgen or estrogen receptor antagonists (flutamide or ICI182780). In contrast, when DHEA conversion to androstenedione was blocked by trilostane (TRI) an inhibition of NO synthesis was observed (55-65% 95%CI).

PT adhesion to endothelium was significantly reduced by DHEA, effect that was suppressed by pretreatment of EC with the NOS inhibitor L-NAME (35±6, 25±5, 34±6, 33±7 adhered PT/field cont, DHEA, L-NAME, DHEA+L-NAME p<0.05) suggesting that NO production is required. Monocytes adhesion to EC was also prevented by DHEA (55-65% below cont 95% CI), effect that was significantly inhibited by TRI.

Angiogenic process requires EC proliferation and migration. EC proliferation (neutral red uptake) was enhanced by DHEA (15-22% above cont 95% CI). Migration assays (wound healing) showed that DHEA also induced EC migration, effect that was significantly inhibited by TRI (10±2, 42±8, 12±2, 25±9 Cont, DHEA, TRI, DHEA+TRI migrating cells/field p<0.01). Capillary tube formation in fibrin gells was evaluated. A twofold increase of basal effect was observed with DHEA 0.2 and 2mM p<0.01.

Our results provide evidence of a direct action of DHEA on vessel cells that is mediated by intracrinology mechanisms and may be of physiological relevance in vascular homeostasis.

Keywords: DHEA, intracrinology, vascular endothelium

(1241) **PRO-INFLAMMATORY CYTOKINES AND EPAC2 AS POSSIBLE MODULATORS OF ADRENAL STEROIDOGENESIS DURING EXPERIMENTAL *TRYPANOSOMA CRUZI* INFECTION**

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During inflammatory or infectious stress, the HPA axis is activated by immune mediators. Adrenal steroidogenesis is a dynamic process, under the stimulation of ACTH, this process, depends on the activation of signaling pathways particularly mediated by PKA. However, glucocorticoid (GC) synthesis can be stimulated by cytokines and Exchange Protein directly Activated by cAMP 2 (Epac2) troughs PKA-independent pathway. Previous studies in mice infected with *Trypanosoma cruzi* (Tc) showed increased circulating levels of GC without changes in ACTH at 17 days post-infection (dpi), while an increase of intra-adrenal expression of pro-inflammatory cytokines was observed. In this study, we determined at different dpi the expression of PKA/PKAp (as a measure of ACTH-pathway activation), IL-1 receptor (IL-1R) and Epac2 (as a potential modulator of adrenal steroideogénesis). C57BL/6 mice were infected with Tc or inoculated with saline (Co). Data are showed as mean±SEM (n=3-5/day/group). Assessments were made between 10 to 16 dpi. IL-1β is increased in plasma during infection [IL-1β (ELISA, pg/ml); Co:22.7±9.0, Tc(11dpi):59±34.6*, Tc(16dpi):82.13±27.2*, *p<0.05 vs Co]. The PKA/PKAp ratio was increased at 11 dpi [Western blot, PKA/PKAp: Co=1±01; Tc(10dpi)=1.1±0.1; Tc(11dpi)=2.4±0.7*; Tc(12dpi)=0.8±0.5; Tc(13dpi)=1.0±0.2; Tc(14dpi)=1.1±0.2; Tc(15dpi)=0.9±0.2; Tc(16dpi)=0.8±0.1; *p<0,05 vs Co]. Tc mice displayed an intra-adrenal elevation of Epac2 after 14dpi (p<0,05 vs Co), decreasing after 16dpi. In parallel, the expression levels of IL-1R start to increase from 13dpi (Western blot, p<0,05). Tc infection activates the steroidogenic synthesis which results in remarkably increased GC production. Our results suggest that at the beginning, GC secretion may be increase by the ACTH-mediated response, but in a late phase of infection, GC secretion might be maintained by other mediators including IL-1 and Epac2.

Keywords: Glucocorticoid; Cytokines; Trypanosoma cruzi; HPA axis

(847) **ROLE OF ALTERNATIVE BACKDOOR PATHWAY METABOLITES ON HUMAN ADRENOCORTICAL STEROIDOGENESIS**

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The backdoor pathway for androgen synthesis is initiated when 17OHprogesterone (17OHP) is 5α-reduced and then converted to pdiol, substrate for CYP17A1, leading to androsterone and then DHT without the intermediacy of DHEA, androstenedione and testosterone. LC– and GC-MS/MS analysis of urinary steroids showed this pathway is active in pathological states in which adrenal 17OHP accumulates. However, its relevance to human adrenal physiology is unknown. We have previously described the mRNA expression of the backdoor pathway genes to complete all the steps to DHT in the postnatal human adrenal gland. Furthermore, functional studies in H295R human adrenocarcinoma cells gave first hints that DHT may participate in the regulation of adrenal steroidogenesis through inhibition of CYP17A1 activity (ENDO 2014, SAIC 2015). To provide further evidence for the presence of the backdoor pathway and its role in postnatal human adrenal cortex, human adrenal tissues (HAT) were collected from 3 age groups: Gr1: <3 months, n=4, fetal zone involution; Gr2: 3 months to 6 yr, n=4, pre-adrenarche; and Gr3: >6yr (14-40yr; median= 17y), n=7, post-adrenarche. Immunohistochemistry confirmed protein 5α-reductase 1 (SRD5A1) expression in HAT from the 3 age Gr. HAT from Gr3 were used to prepare primary cultures of human adrenocortical cells (HAC). HAC were treated in the presence or absence of ACTH (10nM) with or without DHT or androsterone for 18-48h. In a dose-dependent manner at concentrations ≥1μM, DHT significantly decreased cortisol and stimulated progesterone basal (-1.7 to -3.8 and 1.8 to 3.4 fold, respectively) and ACTH-induced (-1.5 to -4.7 and 1.5 to 2.2, respectively) production (Log Transformation ANOVA-Dunnett's Test; p<0.05). Comparable results were observed with androsterone incubation of HAC. Altogether, our data suggested that intraadrenal backdoor pathway metabolites might have physiological significance as paracrine/autocrine regulators of adrenocortical function.

Keywords: adrenal androgens, backdoor pathway to DHT, human adrenal steroidogenesis, SRD5A1, DHT

(515) **VITAMIN D REGULATES STEROIDOGENESIS AND INFLUENCES THE EXPRESSION OF SEVERAL COMPONENTS OF THE HISTAMINERGIC SYSTEM IN R2C TUMOR LEYDIG CELLS.**

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Leydig cell tumors (LCT) are endocrine tumors of the testicular interstitium. Current evidence indicates that aromatase (CYP19) overexpression and excessive estrogen (E2) production play a crucial part in their genesis. Although most LCT are benign, malignant LCT respond poorly to chemo/radiotherapy, underscoring the importance of developing novel therapeutic strategies. Bioactive vitamin D (calcitriol; CAL) is a steroid hormone with a major role in human health. CAL binds to nuclear vitamin D receptor (VDR), a ligand-inducible

transcription factor that functions to control target gene expression. Vitamin D deficiency has been linked to various malignancies, including cancer. Previously, we reported that histamine (HA) is an autocrine growth factor in LCT. Also, we detected VDR in human Leydig cell (LC) hyperplasia and LCT. Several components of the HA-ergic system are targets of VDR, and CAL regulates CYP19 in many cell types. Thus, to assess the question whether CAL plays a role in LCT, we studied its ability to modulate the expression of genes involved in maintaining the tumor phenotype. Experiments were performed in R2C LC (R2C), the best known *in vitro* model for Leydigioma. R2C were treated with CAL (10^{-11} M-10^{-8} M). Gene and protein expression were evaluated by RT-qPCR and Western blot, respectively. Cell proliferation was measured by ^{3}H-Thymidine incorporation. CAL (10^{-9} M; 5 h) increased the expression of VDR, histidine decarboxylase (HDC) and HA receptors H1 and H4 genes (p<0.05), but diminished the expression of StAR protein and CYP19 (p<0.05). Also, CAL (10^{-9} M; 24 h) inhibited R2C proliferation (p<0.05). Overall, our results suggest that CAL negatively modulates steroidogenesis and proliferation in R2C, either directly or by favoring the action of HA through H1 and H4, whose selective activation has been reported to inhibit such processes. Although further research is needed, CAL treatment should be considered as a potential therapeutic strategy in LCT.

Keywords: Leydig cell tumor, Vitamin D, steroidogenesis, histaminergic system.

(524) **HISTAMINE H4 RECEPTOR AS A NOVEL THERAPEUTIC TARGET FOR THE TREATMENT OF LEYDIG CELL TUMORS IN PREPUBERTAL BOYS**

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Leydig cell tumors (LCT) are rare endocrine tumors of the testicular interstitium, with recent increased incidence. Symptoms include precocious puberty in children; and erectile dysfunction, infertility and/or gynecomastia, in adults. The evidence so far points to aromatase (CYP19) overexpression and excessive estrogen (E2) and IGF–1 production as responsible for Leydig cell tumorigenesis. While LCT are usually benign, the malignant phenotype in adults responds poorly to chemo/radiotherapy, highlighting the need to identify new targets for treatment. Herein, we studied the potential role of the histamine receptor H4 (HRH4) as a therapeutic target for LCT. Experiments were performed in R2C rat Leydig tumor cells (R2C), the best-known *in vitro* model for Leydigioma. The expression of HRH4, StAR and CYP19 was evaluated by qPCR and Western Blot. P4 and E2 levels were quantified by RIA, and cell proliferation was determined by ^{3}H-Thymidine incorporation. *The angiogenic capacity of R2C and the effect of HRH4 agonist treatment on this capacity* were evaluated *in vitro* and *in vivo*, using human umbilical vein *endothelial cells* and by means of the quail chorioallantoic membrane assay, respectively. Also, HRH4 immunoexpression was studied in 2 human LCT *versus* normal human testis samples (NHTS) belonging to four different age groups: neonatal, n=2; infantile, n=1; juvenile, n=3 and pubertal, n=3. E2 and IGF-1 negatively regulated HRH4 mRNA and protein levels in R2C. In agreement, HRH4 expression was weak in LCT but moderate to strong, and confined to the interstitium, in all the NHTS analyzed. No HRH4 was detected in seminiferous tubules or germ cells. Treatment of R2C with HRH4 agonists inhibited StAR expression (p<0.01), P4 and E2 synthesis (p<0.01), CYP19 expression (p<0.05), and cell proliferation (p<0.001). Finally, selective HRH4 activation negatively affected the angiogenic capacity of R2C (p<0.001). Overall, our results point to HRH4 as a novel therapeutic target in LCT.

Keywords: Leydigioma – Cell proliferation – Angiogenesis – HRH4 – Histamine

(1478) **IMMUNOENDOCRINE INTERACTIONS IN PATIENTS WITH PULMONARY OBSTRUCTIVE DISEASES**

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Resistance to corticosteroids in patients with severe asthma and with chronic obstructive pulmonary disease (COPD) is an important barrier to an effective treatment. Different lines of evidence indicate that glucocorticoids (GCs) resistance is the result of significant changes in the cellular microenvironment that occur over time during disease progression. Several factors have been involved in this process and recent findings indicate that miRNAs may also be playing a role in the development of GC resistance during chronic inflammatory states. In this work we contribute to the characterization of the immunoendocrine interactions observed in patients with both severe asthma and COPD as a preliminary approach to subsequent studies on the role of sRNA in the development of GC resistance in these diseases. Both sputum and blood samples from volunteers (n=20) were employed to compare the events occurring at the lung with what happens in the peripheral compartments. As expected, most asthmatic patients, and some with COPD showed abnormally high IgE blood levels and mild leukocytosis, with elevated eosinophils. Interestingly, in most cases sputum cytology did not show an eosinophilic infiltrate and neither of these findings correlated with the severity/stage of the diseases. Levels of endogenous cortisol were also quantified to confirm that there is no adrenal suppression in these patients despite treatment with inhaled corticosteroids. Also, RNA was purified from both sputum and blood samples and CG receptor transcript and miR-223, let-7 and tRNAh Glu were quantified by RT-qPCR, being able to detect sRNAs in all analyzed samples, including extracellular fractions. In summary, these results provide a first approach for the better understanding of the immunoendocrine alterations associated with pulmonary obstructive diseases and present a useful experimental design for the study of regulatory mechanisms mediated by miRNAs in secretions of patients with asthma and COPD.

Keywords: asthma, COPD, sRNA, glucocorticoids

(764) **HYPOGONADISM IN MEN WITH STABLE CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD). ASSOCIATION BETWEEN TESTOSTERONE AND COPD SEVERITY. CROSS SECTIONAL STUDY**

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The prevalence of hypogonadism in patients with COPD is estimated in 22-69%. The association between testosterone (T) levels and COPD severity is controversial. Aim: To estimate the prevalence of hypogonadism in men with stable COPD and the association between T and COPD severity. Methods: 47 men, 69.1±8.9 years and BMI: 27.8±4.3 kg/m^2 were included. COPD severity was evaluated with BODE index. Laboratory: total T(TT), estradiol (E2), LH, SHBG and prolactin.Free (FT) and bioavailable T (BT) were calculated. ADAM and IIEF-5 score were performed. Hypogonadism was considered when TT<3 ng/ml, FT<6.1 ng/ml or BT<1.2 ng/ml. Results: Laboratory (X±SD): TT 3.3±1.3 ng/ml, FT 5.4±1.8 ng/ml, BT 1.2±0.4 ng/ml, LH 4.2±2.1 mU/ml, SHBG 46.1±18.8 nmol/l, E2 24.9±7.4 ng/ml and prolactin 8.2±3.5 ng/ml. Hypogonadism: TT 48.9%, FT 63.8%, BT 42.6%. ADAM score was positive in 80.9% patients, 38.3% in those with TT>3 ng/ml and 86.7% in those with TT<3 ng/ml. IIEF-5 was performed in 28 patients, 82.1% had erectile dysfunction. A negative correlation between BODE index and TT: -0.28, p=0.05; FT: -0.38, p=0.007 and BT: -0.39, p=0.0065, was found. Conclusions: Men with stable COPD have a high prevalence

of biochemical and clinical hypogonadism. ADAM score has a high sensitivity and a low specificity, T measurement is recommended. T values have a negative correlation with the severity of COPD, lower T are associated with high severity.There is a high prevalence of erectile dysfunction in this population.

Key words: hypogonadism, chronic obstructive pulmonary disease

(1399) **HYPERCOAGULABLE STATE AND RISK OF TROMBOSIS IN THE DOG WITH CUSHING'S DISEASE**

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Hypercoagulable state is a situation of high morbidity and mortality in the dog with Cushing's Disease (EC). The most serious condition is pulmonary thromboembolism which is a cause of death. D-dimer (DD), a degradation product of fibrin, is an indicator of thrombosis. The Von Willebrand Factor (FvW) and the inhibitor of plasminogen activator-1 (PAI-1), are increased in plurimetabolic syndromes. The objective of this study was to evaluate whether the activity of Antithrombin III (ATIII) and DD are useful as indicators of formation or presence of thrombosis and to analyze the concentrations of FvW and PAI-1 in the dog with EC compared to a group of healthy dogs. We studied a total of 26 dogs with EC. To make the comparison 12 of them were taken and then 12 dogs from the bioterior, as a control group. All patients underwent platelet counts, coagulation times (KPTT and prothrombin time (PT)), fibrinogen (F), ATIII expressed as ATIII activity (normal> 80%) and DD (positive> 200, DD +). FvW and PAI-1 were measured by ELISA. Proteinuria in relation to creatinine (PRC, normal value <0.30). Results were expressed as average ± SEM, the comparison of the averages were made by the t-test of unpaired samples. P <0.05 was considered significant. Fisher's Test was also used. The platelet count, KPTT, TP and F were normal. In 9/26 (34.6%) both the ATIII and the DD were altered, with an inverse correlation (r = -0.5). The PRC was elevated in 10/26 (38.5%), associating with the lowest ATIII activity and DD +, PRC values correlated with ATIII (r = -0.75) and with DD (r = 0, 67). The concentrations of FvW and PAI-1 were significantly higher in the group with EC (FvW: 4,2±0,3 vs 2,7±0,3 ng/ml; PAI-1: 836,6±153,2 vs 295,2±24,1 pg/ml). Routine coagulation studies aren't relevant for diagnosing thrombosis. The increase in FvW and PAI-1 shows the presence of hypercoagulability and higher risk of thrombosis. The evaluation of DD and ATIII is recommended for the diagnosis of thrombosis.

Keywords: Hypercoagulable - Cushing's Disease – Thrombosis - Von Willebrand Factor - Inhibitor of plasminogen activator-1

HEMATOLOGY 1

(1355) **COORDINATED REGULATION OF HEPCIDIN AND IRON IMPORTERS THROUGH IRON EXCESS AND ERYTHROPOIETIN SIGNALS IN ENTEROCYTE AND HEPATOCYTES IN MICE MODELS.**

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The erythropoietic activity is the main inhibitor of the hepcidin expression, the key regulator of the iron metabolism that reduces the intestinal absorption of iron and the release from macrophages. **Objective:** To evaluate the response of functional axis "HEPCIDIN-DMT1-ZIP14 using mice models of iron-overload and also an erythropoiesis induced by EPO. **M*aterials and Methods*:** CF1 mice (25±5g) were divided into 4 groups (n=4/group;block design):1)*Iron-overload and* erythropoietin(IO+EPO) Fe-Saccharate ip (days 0,4,8,12;1800mg/kg) and EPO ip(days 17,18,19;20000UI/kg);*2)Iron-overload*(IO);3)Erythropoietin(EPO);4*)control (C).*The Protocol was approved by the Committee on Experimental Animal Use and Care-UNS. Immunohistochemistry: DMT1, ZRTIre like protein14 (ZIP14),prohepcidin,ferritin. **Results:** *Duodenum:* In control and EPO, ZIP14 was found in enterocytes apical membrane, while a slight basolateral expression was seen in iron-overload with and without EPO. DMT1 in enterocyte cytoplasm was moderated in Control, while in Iron-overload it was slight. In EPO and IO+EPO, the apical DMT1 was intense. *Liver:* The prohepcidin expression was slight in EPO, moderated in control and intense in IO and IO+ EPO. Both ZIP14 and ferritin in hepatocyte cytoplasm were weak in C and in EPO, they were intense in iron-overload and IO+EPO. Evident DMT1 expression was detected in the cell membrane and in cytoplasm of hepatocytes in EPO, while a weak expression was observed in C. In IO+EPO and IO, DMT1 expression was slight in hepatocytes cytoplasm. **Conclusions:** Two signals could induce specific response for each group: (i) *iron-signal,* induced prohepcidin synthesis**,** which reduced the duodenal iron uptake through DMT1 and ZIP14 and increased the hepatic iron storage through ZIP14, reducing iron availability; (ii) *EPO-signal,* which affected duodenal iron uptake by DMT1 and, therefore, allowed an iron supply to the bone marrow.

Key words: erythropoiesis, iron-overload, mice models, enterocytes, hepatocytes.

(1393) **PROTEINS ASSOCIATED TO UPTAKE OF IRON IN HUMAN NEUROBLASTOMA CELLS**

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Introduction: Alterations in the neuronal iron uptake are frequently associated to brain diseases. Therefore, the aim was to study the effect of iron excess on divalent metal transporter1 (DMT1) and Zrt-Irt-like Protein14 (ZIP14) expressions in human neuroblastoma cells. Materials and Methods: To evaluate cellular viability a dose-response curve was assayed with low and high iron concentrations (ferric ammonium citrate-FAC) 30μM-80μM and 200μM-600μM, respectively at different times. The iron uptake was assessed in the culture medium in 30μMFAC/72hs and in 600 μM/24hs treatments. Pre-treated cells with NAC 2Mm/12hs plus FAC30μM/72hs for immunocytochemistry: DMT1, ZIP14, prohepcidin, ferritin. Viability was assessed by a nuclear red method. Iron levels: Wiener kit. Results: In treatments with low FAC concentrations (30μM, 60μM and 80μM) it was observed a decrease in cellular viability at 72hs ($p<0,05$). However, in treatments with high FAC concentrations (450μM and 600μM), the decrease in the cellular viability was seen at 24hs($p<0,05$). The iron content in the culture medium decreased by 25% in 600μM-24hs and by 42% with 30μM/72 hs treatments. The ZIP14, prohepcidin and ferritin expressions were higher in cells with FAC 30μM/72hs compared with those found in the control. The DMT1 immunoreactivity was lower in cells with FAC30μM/72hs than that observed in the control. The NAC pre-treatment reversed the change induced by the iron in the ZIP14, the prohepcidin and the ferritin expressions. Conclusions: The increase in the ZIP14 expression could reflect a high iron uptake that produced neuronal death, being DMT1 the transporter without a relevant role. The high prohepcidin expression could suggest a decrease of iron export through deregulation of axis hepcidin-FPN. Thus, the change of the ZIP14 expression in neurons could be one of the mechanisms that produce neuronal death by high iron uptake.

Key Words: Iron, Neuroblastoma Cells, Hepcidin, Divalent Metal Transporter 1, Zrt-Irt-Like Protein14

(440) **ASSOCIATION BETWEEN GENOMIC REARRANGEMENTS AND INSTABILITY IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA**

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Chronic lymphocytic leukemia (CLL) is a heterogeneous disease with a highly variable clinical course. Genomic instability (GI), evidenced by acquired genetic aberrations, has an important role in CLL pathogenesis, as the number of genomic alterations was shown to increase throughout the course from newly diagnosed to progressive and further to relapsed CLL. GI can be assessed by means of chromosome aberrations (CA) and micronucleus (MN) analysis. Cytogenetic and FISH (fluorescence in situ hybridization) studies are important prognostic factors in CLL. We have analyzed GI in 81 untreated CLL patients (41 males; mean age: 65.6 years; range: 42-83 years) and 6 normal controls. Cytogenetic analysis was performed on stimulated peripheral blood lymphocyte cultures. FISH was performed using the CLL panel according to manufacturer's protocol. For each patient, CA was evaluated on 50 metaphases while 250 interphase nuclei were analyzed for MN frequency. An increased number of CAs in CLL patients compared to controls (6.89±5.4% vs 0.25±0.4%, p=0.004), with the higher value in cases with abnormal (8.54±5.8%) vs normal (5.2±4.5%) karyotype (p=0.008), was observed. Considering FISH risk groups, the analysis showed a higher frequency of CA in patients with deletions 11q22/17p13 (8.4±5.6%) associated to poor prognosis than those with no alterations or deletion 13q14 (5.1±3.8%) (p=0.012) related to a better outcome, and +12 (3.6±2.3%) with intermediate risk (p=0.010). MN analysis displayed an increased frequency in CLL patients (3.1±1.7%) compared to controls (0.7±0.3%) (p=0.001) but no significant differences between cytogenetic or FISH groups were observed. Our results show the presence of basal genomic instability in untreated CLL patients as measured by both CA and MN techniques, as well as an association with cytogenetic and FISH risk groups, being to our knowledge the first study taking into account these prognostic factors.

Keywords: chronic lymphocytic leukemia, genomic instability, FISH, cytogenetic analysis.

(1232) **CANONICAL AND NON-CANONICAL STIMULATION OF T-ALL TUMOR CELLS AND MESENCHYMAL STROMAL CELLS DETERMINES TUMORAL NICHES WITH DIFFERENTIAL BEHAVIOR**

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T-cell acute lymphoblastic leukemia (T-ALL) constitutes 20% of diagnosed ALL. As other hematological tumors it presents a close connection with the stromal counterpart. Leukemia cells can adjust the niche affecting the communication between the stroma and HSC in models of T-ALL. Thyroid hormone (TH) modulation may be critical in determining cancer progression. In this context we investigated the acquired functional behavior of mesenchymal stromal cells (MSC) when regulated by T-ALL cells in a TH-modulated microenvironment. We demonstrated that TH stimulation on T-ALL cells via surface receptors (non-canonical TH action) induced a higher chemotactic response and stronger morphogenic rearrangements, while canonical TH stimulation on tumor cells induced less chemotactic response (1.5 fold higher, 148±23 MSC/field, p<0,05) and less morphogenic rearrangements in MSC. Further, direct stimuli exerted by canonical TH on MSC induced higher MMP2 activity (1,55±0,25 vs. 1,24±0,15 MMP2 relative activity, p<0,01) and a high secretory activity via microvesicles (MV) on these cells (0,44 ug/ml/cm2 vs. 0,3 ug/ml/cm2). We proposed based on the outlined data a working model where canonical TH stimulation generates a niche that induces MSC to stay and rearrange the tumor microenvironment, while non-canonical TH stimulation generates a tumor microenvironment with MSC more prone to leave the niche. Thus, signaling triggering a prevailing canonical or non-canonical TH action could be critical in determining a tumoral niche of T-ALL cells interacting with MSC.

(885) **SMC3 HAPLOINSUFFICIENCY RESULTS IN GERMINAL CENTER HYPERPLASIA AND COOPERATES WITH BCL6 TO INDUCE B-CELL LYMPHOMA**

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The cohesin complex is a multiprotein complex composed of four core subunits, being Smc3 a unique subunit that cannot be replaced by any other protein. We recently showed that in the germinal center, B cells undergo a massive reorganization of chromosomal architecture, with the cohesin complex preferentially localizing in those new interactions. In the present work we aimed to identify the role of cohesin during GC B cell development. We used a conditional knockout mouse model for Smc3 with Cre excision controlled by Cg1 or CD19 genes. Complete knockout of Smc3 resulted in abrogation of GC formation, as evidenced by flow cytometry and immunohistochemistry (t test, p<0.001). Curiously, excision of only one allele of Smc3 resulted in hyperplasia of GC (t test, p<0.001). Since the cohesin complex is known to regulate chromosomal folding and promoter-to-enhancer interaction, we performed HiC in sorted GC from wild type or Smc3 haploinsufficient mice. We observed that cohesin haploinsufficiency caused a mild but consistent decrease in long range interactions in the order of 100 Mb. No changes in gene expression or overall chromatin accessibility were observed, nor major changes in enhancer and promoter usage between wild type and Smc3 haploinsufficient GC B-cells. However, Smc3 haploinsufficient GC B-cells had a larger S-phase duration and a higher mutational load. To address If GC hyperplasia translated into differential lymphomagenesis, we crossed Smc3 mice to ImBcl6 mice, which develop lymphoma over time, and we observed that Smc3/Bcl6 mice developed lymphomas faster and more aggressively than any othergroup (Long-rank test, p<0.001). Our results provide evidence for an Smc3 role during GC development and demonstrate that alterations of the cohesin complex can create vulnerabilities leading to malignant transformation in conjunction with known oncogenes.

(1070) **ANALYSIS OF *MYC* REARRANGEMENTS IN PATIENTS WITH MULTIPLE MYELOMA**

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Multiple myeloma (MM) is a malignant B-cell lymphoproliferative disease characterized by the infiltration of clonal plasma cells in the bone marrow. At the genetic level, it is a heterogeneous disorder characterized by multistage accumulation of genetic abnormalities deregulating different pathways. The *MYC* proto-oncogene is a transcription factor that plays a role in cell cycle progression, apoptosis and cellular transformation. Rearrangements of this gene have been thought to be rare events in MM. In this study, we have evaluated *MYC* rearrangements in MM patients by FISH (fluorescence in situ hybridization) analysis. A total of 46 MM patients (26 females; mean age: 59.5 years; range: 46-86 years) were analyzed. Cytogenetic study was performed on unstimulated bone marrow cultures. FISH analysis was performed using TP53, RB1, IGH and MYC probes (Live-Lexel, Argentina) according to manufacturer´s protocol. *MYC* rearrangements were observed in 25 (54.3%) patients: 19 (76%) cases showed split signals, 5 (20%) had *MYC* gains and one case (4%) showed both split and gain. The correlation with recurrent alterations detected by FISH revealed a significant association with the presence of IGH rearrangements (p<0.007). Although no significant differences were found, the analysis taking into account chromo-

some alterations showed a higher frequency of *MYC* rearrangements in cases with normal karyotype (28%) compared to those with abnormal karyotype (9%). The evaluation according to the International Stage System (ISS) found higher frequency of *MYC* alterations in cases with ISS 2 and 3 (45%) than in ISS 1 (37.5%). No association with the other clinical parameters was observed. The high prevalence of *MYC* rearrangements in MM patients of our series indicates the importance to perform this study in order to refine the prognostic evaluation and treatment decisions. To our knowledge, this is the first analysis of these alterations in Argentinean MM patients.

Key word: Multiple myeloma; *MYC* rearrangements; FISH

(595) **DETERMINATION OF ALLELE BURDEN OF JAK$_2$V617F MUTATION IN GENOMIC AND PLASMA CELL FREE DNA IN MIELOPROLIFERATIVE NEOPLASMS**

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Cell free DNA (cfDNA) in plasma has the clinical potential to be a more specific tumour marker for diagnosis and prognosis, as well as for early disease detection. Currently, determination of the JAK $_2$ V617F mutation and measurement of its allele burden (AB) is performed in genomic DNA (gDNA) using both peripheral blood (PB) and bone marrow (BM) samples from patients with chronic myeloproliferative neoplasms (MPNs). With this procedure, not all malignant alleles could be detected. In this work we compared the %AB of JAK$_2$V617F (percentage of alleles JAK$_2$V617F / total alleles JAK$_2$) in gDNA and cfDNA samples using a quantitative rel time PCR methodology. For quantification we used a calibration curve made with a mixture of gDNA from healthy individuals and gDNA derived from a homozygous JAK$_2$V617F-Human-erythroleukemia cell line (ATCC Hel 92.1.7). JAK2 status mutation was analyzed in 10 patients with MPNs, primary and secondary myelofibrosis (MF) [n: 4], Escential thrombocytosis (ET) and Policitema Vera (PV) (n: 6), which were diagnosed according to the 2008 World Health Organization (WHO) classification. gDNA was obtained from peripheral blood using a modified Miller and Dykes technique, while cfDNA was obtained from plasma using the QIAmp DNA Blood Mini kit. We showed a highly significative correlation between the %AB of JAK$_2$V617F in cfDNA and gDNA (Spearman P=0.0028). Also, when we analyzed paired samples, we observed a higher %AB in cfDNA (P=0.001) compared to the gDNA compartment. These data show that plasma is enriched with tumour-specific nucleic acid and could be the sample of choice for testing JAK$_2$ mutations.

Keywords: JAK $_2$V617F mutation, chronic myeloproliferative neoplasms, cell free DNA

(437) **METHYLATION OF TUMOR SUPRESSOR GENES P15, P16, RARβ2 AND SOCS1 PROMOTERS IN LEUKEMIA PATIENTS**

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DNA methylation plays a critical role in the transcription regulation. Its increase, mainly in promoter genes regions, generates a reduction or transcription blockage in patients with different types of cancer. It has been found that some tumor suppressor genes are hypermethylated (inactive). This could be a factor leading to the progress or malignancy of this disease. In this work we propose to study the methylation profile of 4 tumor suppressor genes (CDKN2B (p15INK4b), CDKN2A (p16iNK4a), RARβ2 and SOCS1) in 30 patients with acute myeloid leukemia and chronic myeloid leukemia (16 AML and 14 CML) and in 30 controls samples by the methylation-specific PCR technique, MSP, with previous treatment of DNA with sodium bisulfite, for the conversion of unmethylated Cytosines into Uracil bases. We analyzed 25/30 patient samples and 26/30 controls. Significant correlations in patients methylation status ($p < 0.05$) were found in p16 and SOCS1 gene's promoters by Fisher's test analysis. We found 40% (8 methylated + 2 hemimethylated) and 48% (5 methylated + 7 hemimethylated) in p16 and SOCS1 respectively. No methylation was found in p15 gene promoter neither patient analysis nor in control samples. Finally, RARβ2 shows that 36% of the patients analyzed, contain partial methylation (hemimethylation) in their promoters, which could mean a decrease in their expression. These results suggest that methylation in the promoters of the p15INK4b and SOCS1 genes could be related to the events that generate the onset or progression of the disease. The silencing by hemimethylation in the promoter of the RARβ2 gene would lead to a decrease in its expression, which could generate effects that favor the process of leucemogenesis.

Key words: Methylation, Leukemia, Epigenetics, Supressor genes, MSP

PHARMACOLOGY 3

(717) **EUGENOL AS AN ALTERNATIVE FOR THE MANAGEMENT OF COMMON CARP (*Cyprinus carpio*)**

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Common carp (*Cyprinus carpio)*, has been introduced in different parts of the world. Is adapted to different culture systems and is the specie of greater world production. These systems expose fish to neuroendocrine and physiological changes affecting productive performance and survival; which are reduced with the use of depressants. The objective of the work, carried out in La Helvecia shallow lake (Córdoba), was to evaluate in *C. carpio* the activity of eugenol, an agonist of GABA, at different concentrations. Fish were randomly collected from a batch of 100 housed in a 5 cubic meter cage located within the lake, forming 2 groups of 10 fish (A: 134.0±21.1 g, 23.8±1.8 cm and B: 116.0 ±26.4 g, 23.0±2.3 cm). Three containers of 50 liters each were used to avoid acclimatization stress. Concentrations were 25 and 50 ppm for groups A and B respectively. The work sequence consisted in: a) acclimatization of fish in a container to register basal respiratory rate after adopting normal swimming behavior; b) transfer to the second container with eugenol, recording times of partial balance loss, lateral decubitus, reaching anesthesia, presence of excitation and respiratory rate in anesthesia, c) placed in a third container to control behavior and recovery. Data were analyzed using the non-parametric Mann-Whitney test. Results indicates that eugenol exerts a significant effect on the respiratory activity, decreasing from 106.4±5.7 to 84.4±7.6 and 107.8±12.5 to 68.0 ±12.9 opercular movements per minute ($P < 0.05$) at 25 and 50 ppm respectively. Differences were observed in achieving anesthesia, demanding 5.5 ±1.0 minutes at 25 ppm and 2.4±0.5 minutes at 50 ppm ($P < 0.05$) and total recovery time: 3.7±0.8 and 6.5±1.5 minutes for 25 and 50 ppm respectively ($P < 0.05$). No fish had signs of excitation, no deaths were recorded 96 hours after the study. Preliminary results show eugenol as a good alternative for the management and anesthesia of *C. carpio* under natural conditions.

Key words: *Cyprinus carpio*, eugenol, anaesthesia

(1695) **RELATIONSHIP BETWEEN SEVERITY OF PERIODONTAL DISEASE AND RHEUMATOID ARTHRITIS CLINICAL MANIFESTATIONS**

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Periodontal disease (PD) is a pathological inflammatory condition leading to the destruction of the soft and hard tissues and eventually to tooth loss. PD is initiated by a bacterial infection that spreads immune systemically and has been associated with the raised risk of systemic diseases such bacterial pneumonia, cardiovascular heart disease, stroke and autoimmune diseases. Rheumatoid arthritis (RA) is a progressive and chronic, inflammatory autoimmune disease associated with articular and systemic effects although the etiology of RA remains unknown. RA progression and severity are influenced by genetic, environmental and infection factors. RA clini-

cal presentation can be of erosive and nodular presentation. RA and PD share analogous pathological features. Little is known about the prevalence of both inflammation diseases in Argentina. The **objectives** are to investigate the prevalence of periodontal disease in RA patients in Buenos Aires, Argentina and the relationship between the severity of PD with RA clinical expression (erosive and nodular). **Materials and Methods**: 111 patients with RA were included. Clinical evaluation and radiological studies were performed. Presence and severity of periodontal disease (attachment level, pocket depth and bleeding index) was assessed. Control 101 people from the general population without RA**. Results:** RA patients have a higher prevalence of severe periodontal disease compared to controls (12,2% vs 7,4%, p=0.002). PD is found in both arthritis presentations (nodular and erosive). The erosive presentation of RA is predominant in patients with mild (45%) and moderate PD (51%). The percentage of patients presenting both clinical manifestations increases with the grade of the severity of PD, reaching 42% of patients with severe PD. **Conclusion:** this is the first approach that investigates the relationship between the presence of erosive and nodular RA with the severity of the periodontal disease that showed a positive correlation.

Words: Periodontal disease, Rheumatoid arthritis, erosive, nodular

(1602) **KNOWLEDGE THE STUDENTS WHO ARE CURSING GENERAL PHARMACOLOGY HAVE ON MEDICINAL PLANTS PHARMACOLOGY CATHEDRAL - FCM – UNC**

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The consumption of medicinal plants has increased considerably in recent years. It is important to take precaution in the use of them because it is not advisable to self-medicate even if, in general, they are considered harmless. Objectives: To evaluate students' knowledge of medicinal herbs. Determine if training is needed during the grade. Materials and Methods: Prospective, descriptive, cross - sectional study carried out in August 2017 in students who were in the 3 year course of Medicine - FCM - UNC Cát. Of General Pharmacology. We used a previously validated survey which was random, anonymous, voluntary, semi-structured with general sociodemographic and specific data referring to medicinal plants, the degree of acceptance and use. The sample was 246 out of a total of 647 students. Within the study population 162 (65.9%) were women and 84 (34.1%) were men. The most frequent age range was 20-21 for both women and men. Results: There is no knowledge about medicinal plants 127 students (52%). They did not consume medicinal plants 153 (62.2%). They believe that 185 (75.2%) are effective, although they do not consider them as 181 (73.6%). Regarding knowledge about their indication, they indicated that: from Poleo 190 (77.2), Ambay 223 (90.7%) and Cola de Caballo 192 (78%) were unaware of their indication, Aloe 160 (65%) antiulcerous, Manzanilla 101 (41.1%) sedante. The responses of the herbs that can be used in pregnancy were incorrect in 68.8% of the respondents. They do not know if they have interactions with other drugs 134 (54.5%) and consider training on medicinal herbs 217 (88.2%) necessary. Conclusions: A high percentage of those surveyed stated that they did not know about medicinal herbs. Most believe that they are effective but do not consider them safe. Due to the results obtained and, to the interest shown by the students, the incorporation of these contents in the subject is considered.

(193) **ESTABLISHMENT OF CELLULAR MODELS DERIVED FROM A BILATERAL PATIENT WITH RETINOBLASTOMA**

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Retinoblastoma (RB) is the most common intraocular cancer in children. Previous research groups have built cells lines modeled after RB patients in developed countries, but there has never been cell lines derived from a patient who was enucleated upfront from both eyes(naïve)**.** Therefore, our research focused on developing cell lines that are modeled after a naïve patient which can later be used for translational research.

Intraocular tumor samples taken by the ophthalmologist at the time of enucleation were used to make the cell culture. After the cell culture was established, evaluations were made to assess the origin of the cell using different types of lineage specific markers includingdiasylganglioside GD2,arrestin3 (ARR3) and synaptophysin by immunohistochemistry and the expression of cone-rod homebox (CRX) through RTq-PCR.Double time (DT) was also calculated. Pharmacological characterizations were made by the determination of theconcentration of drugs that inhibit 50% of the cell viability (IC50) using melphalan, carboplatin and topotecan. The study protocol and informed consent were approved by the Institutional Committee Review board (Protocol #N662).

We established two cell models derived from a bilateral patient with RB: one from the left eye tumors and one from the right eye tumors.Both cell models expressed GD2 and synaptophysinillustrating that they derived from a neuroectodermal tumor. Also, both cultures expressed ARR3 and CRX demonstrating that the cultures were indicative of retinal progenitor cells. The culture derived from the right eye was observed to be growing more slowly (DT=4.5 days) than the one derived from the left eye (DT=3.4 days). However, there was no significant difference observed between the IC50 for all three drugs between both eyes (p>0.05).

Altogether, we were able to develop an effective cell models. These models may be useful to understand the progression and tumor development in a bilateral patient and in other preclinical models.

Keywords : Retinoblastoma, cell models, naïve, bilateral.

(82) **EVALUATION OF DRUG-DRUG INTERACTIONS IN CATTLE USING INTESTINAL EXPLANTS**

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The concurrent administration of drugs is currently used in veterinary medicine. In cattle, the coadministration of anthelmintics may be a useful pharmacological tool to delay the parasite resistance. Different transporter proteins such as P-glycoprotein (P-gp) are involved in the excretion process of anthelmintics. Previous studies corroborated *in vivo* pharmacokinetic changes obtained after the coadministration of two macrocyclic lactones. The aim of the current trial was to evaluate the modulation of intestinal transport of macrocyclic lactones in cattle using the intestinal explants model. Bovine ileum samples from Aberdeen Angus/Hereford crossbreed steers were obtained from a slaughterhouse located in Tandil area. Immediately following sacrifice, a segment of caudal ileum (30 cm) was opened by the mesenteric border, rinsed with ice-cold 1.15% KCl and immersed in ice-cold Euro-Collins solution containing abamectin (ABM) alone (0.5 μM) or ABM plus ivermectin (IVM) (1μM). Containers were covered, chilled in ice, and transported to the laboratory within 30–40 min for subsequent procedures. Intestinal explants (19 mm of diameter) were prepared and transferred to 6-well culture plates with 6 ml of Wiliams´ medium E inside a container with a humidified atmosphere of 95% O2:5% CO2 at 37 ºC. Intestinal explants were harvested between 15 and 60 minutes post-incubation and frozen at -20 ºC. ABM concentrations were measured by HPLC with fluorescent detection. The concentrations of ABM in the explants were significantly higher after the coincubation with IVM ($P<0.05$). ABM accumulation in the intestinal explants was 2.3 fold higher at 30 minutes post-incubation with IVM. The ABM accumulation rate during the 60 minutes of incubation was 50 % higher in the presence of IVM. As was previously corroborated *in vivo*, the intestinal explants were a useful model to evaluate the drug-drug interaction between macrocyclic lactones in bovine.

Keywords: intestinal explants, cattle, drug-drug interactions, P-glycoprotein

(747) **FLUOROQUINOLONE DEPLETION IN EDIBLE COMPARTMENTS OF EGGS IN LAYING HENS**

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Fluoroquinolones are antimicrobials approved for use in domestic animals but are not allowed in laying birds because they generate residues in eggs that compromise food safety, although they are usually applied against infectious diseases that defy animal life. This study was performed to establish depletion in the edible compartments of the egg. Laying hens in postural peak were used, divided into two groups, group A (N = 15) and B (N = 10) receiving 7.3 and 1.5 mg / kg of danofloxacin (DFX) and marbofloxacin (MFX) respectively in the drinking water 5for 11 days. At the end of the administration, eggs were collected daily and separated into clear and yolk. In group A the extraction of the analyte was performed with 200 µL of sample, 200 µL of water, 800 µL of a solution of methanol: water: perchloric acid 50: 50: 2 v / v / v and norfloxacin as internal standard and in group B with methanol: water: perchloric acid: phosphoric acid (50: 50: 2 v / v / v) and enrofloxacin as internal standard.The whole was centrifuged 25 minutes at 13500 rpm at 4 ° C. Separation and quantification was performed by HPLC reverse phase isocratic elution with C-18 column, fluorescence detector at λ_{ex} 295 nm and λ_{em} 490 (DFX) and 500 nm (MBX) and mobile phase composed of water, acetonitrile and triethylamine (79: 19:1 v/v/v) at pH 3. Using peak areas of known concentrations, the concentrations of test samples were calculated by simple linear regression. According to the complexity of matrix studied, the procedures applied are simple, fast and sensitive for monitoring programs and control of residues. Levels that declined significantly after the second day were established, and persists until 9 and 15 (DFX) and 8 and 9 days (MBX) in clear and yolk, respectively, Being more relevant in yolk according to the liposolubility of fluoroquinolones and the time required by each egg compartment for its formation, according to the extension of the treatment.

Key words: fluoroquinolones, eggs, disposition

(996) **NEW UHPLC-MS/MS METHOD FOR THE IDENTIFICATION OF BENZNIDAZOLE AND REDUCTION METABOLITES IN BIOLOGICAL FLUIDS**

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Benznidazole (BNZ) is the drug of choice for the treatment of Chagas' disease, but its metabolism and elimination have never been studied in depth. The aim of this study was to develop the methodology required for BNZ metabolism studies, using liquid chromatography linked to mass spectrometry (UHPLC-MS/MS). A new UHPLC-MS/MS method was developed for the rapid identification of BNZ, reduction metabolites like aminobenznidazole (BNZ-H2) and hydroxyaminobenznidazole (BNZ-HOH), and their glucuronidated forms (BNZ-OH-Gluc) and (BNZ-H-Gluc), in biological fluids. Spectrometric parameters were optimized by direct infusion of a BNZ-Water:Acetonitrile solution in an *ABSciex QTRAP 6500* triple quadrupole mass spectrometer. After optimizing the mass spectrometry parameters, the chromatographic conditions were optimized by injecting 3 µL of the BNZ solution through a C18 Shim-Pack XR-ODSII column 75 mm long, 3 mm internal diameter and 2.2 µm particle size, on a Shimadzu Nexera X2 UHPLC, coupled to the spectrometer using electrospray ionization in positive mode. Once BNZ identification was optimized, we proceeded to study urine samples from BNZ-treated Chagas' disease patients. Samples were extracted in acetonitrile and centrifuged, and the supernatants injected in the UHPLC-MS/MS system. BNZ and its reduced derivatives were detected and characterized using enhanced mass scans (EMS), Q1-Multiple Ion, enhanced product ion (EPI) and MS3 modes, whereas glucuronic acids were recognized by the neutral loss of glucuronic acid (+176 m/z) in neutral loss mode (NL), in conjunction with EPI and MS3. The method developed enables the five compounds to be monitored in 3 minutes by the *MRM Scheduled Scan* mode with high reliability, based on their transitions and characteristic chromatographic times. This method could allow rapid identification and exploration of BNZ metabolites in patients, an especially useful tool in the follow-up of pediatric patients treated for Chagas' disease.

Keywords: UHPLC-MS/MS, benznidazole, aminobenznidazole, glucuronides, MRM-*scheduled*.

(555) **HYPERGLYCOSYLATED IFN-ALPHA2B MUTEINS WITH IMPROVED PHARMACOKINETIC PROPERTIES AND HIGHER *IN VIVO* ANTITUMOR ACTIVITY**

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IFN-alpha2b is a pleiotropic cytokine that has been used for the treatment of many viral and tumor diseases. Its pharmaceutical relevance has inspired the development of many new IFN-based drugs. IFN4N constitute a hyperglycosylated IFN-alpha2b that was developed in our laboratory by glycoengineering strategies. This new molecule exhibited reduced *in vitro* antiviral and antiproliferative activity compared to the non-glycosylated IFN. However, IFN4N showed improved pharmacokinetic properties as well as higher *in vivo* antitumor activity. Since the mutation R23N has been identified as the main responsible of affecting IFN4N antiproliferative capacity, here we propone the design, production, purification and characterization of new highly glycosylated IFN-alpha2b muteins with better *in vitro* and *in vivo* biological activity.

Different groups of muteins have been designed and produced in CHO-K1 cells in order to reach the goal. Group A involves IFN variants with the same amount of potential glycosylation sites but higher *in vitro* antiproliferative activity compared to IFN4N. Group B presents muteins with higher glycosylation degree but lower *in vitro* activity (R23N mutation is present). Group C combines the best mutations in new muteins that exhibited improved *in vitro* antiproliferative activity as well as higher glycosylation degree. Two muteins of each group were purified in order to analyze their pharmacokinetic properties in Wistar rats. The result showed that the higher the apparent molecular mass, the slower the plasmatic clearance. *In vivo* experiments, performed in nude mice implanted with prostate cancer derived cells, revealed that the new hyperglycosylated variants were able to reduce the growth rate of the tumors. Particularly, their weight at the end of the treatment was significantly reduced for muteins belonging to group A and C, compared to the control.

Our results show the importance of pharmacokinetics as well as *in vivo* antitumor activity in order to improve the performance of IFN-alpha2b as a biotherapeutic.

(254) **INTESTINAL PERMEATION OF NEW ZIDOVUDINE PRODRUGS DESIGNED TO OPTIMIZE BIOPHARMACEUTICAL PROPERTIES**

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Zidovudine (AZT) is an anti-VIH drug widely used to treat AIDS. Despite its clinical efficacy, AZT presents variable and low bioavailability which in turn result in severe adverse effects. The present work reports the intestinal permeation studies using a *ex vivo* intestinal gut sac technique of four new AZT derivates: AZT-Suc-Fen-O-met (**1**), AZT-Suc-Iso-O-met (**2**), AZT-Glu-Fen-O-met (**3**), AZT-Adi-Fen-O-met (**4**), designed to enhance the bioavailability and protein binding properties of AZT.

In a first stage, a bioanalytical method was developed and validated to quantify AZT and the studied prodrugs prodrugs in TC199, with the corresponding chemical stability being also evaluated. In a next stage, the intestinal permeation of AZT and **1-4** was evaluated in proximal jejunum, at two concentrations (0.45×10^{-5} M and 1×10^{-5} M) in the presence and absence of the Pgp inhibitor Verapamil.

The results showed that the magnitudes of the apparent permability (P_{app}) was **3<2<1<4**. The P_{app} of **4** is not statistically different to that of AZT (p >0.05), while the rest of the derivativves exhibited a less overall permeability compared to AZT AZT (p< 0.05). In particular, compound **1** permeated by passive diffusion and was not subjected to Pgp mediated efflux, while **2** permeated by passive diffusion and was subjected to Pgp efflux. Respect to **3**, this derivative presented a low permeability and a high efflux, while prodrug **4** presented the highest intrinsic permeability among the studied compounds and was also able regenerate AZT by metabolism inside the enterocyte.

In conclusion, in this study we report four new AZT derivates that exhibiting different absorption mechanisms. In particular, the prodrug **4** presented a similar overall permeability to that observed for to AZT without being subject to Pgp efflux. These findings constitutes a significant advantage for the design of new prodrugs able to optimize AZT biopharmaceutical properties.

Keywords: Intestinal permeation, AZT, prodrugs.

(997) **NATURAL TREATMENTS: LACTOFERRIN EXERTS ANTIGIARDICIDAL EFFECTS THROUGH STRESS AND CRITICAL MORPHOLOGICAL DEFECTS**

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Understanding how parasites respond to mechanisms of cellular stress is necessary to identify essential processes on their life. *Giardia lamblia* is a protozoa parasite that is the most common cause of non-viral non-bacterial diarrheal disease world-wide. Despite the effectiveness of metronidazole, a major antigiardial drug, its strong side effects has been matter of concern. Consequently, the search for natural treatments is important for the control of the illness. Glycoprotein bovine lactoferrin (bLF) and its peptide lactoferricin (bLFcin), have exhibited antimicrobial activity in *Giardia*. However, there is little evidence of the underlying mechanisms for their effect. Thus, our goal was to understand how these molecules are internalized and how their microbicide mechanism beyond cell death takes place. To address this point, we performed uptake experiments and different fluorescence and electron microscopy techniques. Our results showed that both molecules are internalized by receptor-mediated endocytosis and are localized in the endo-lysosomal compartments. By transmission and scanning electron microscopy, we observed that both bLF and bLFcin produce morphological changes correlated with the trigger of ER stress. Moreover, trophozoite treatment leads to a partial encystation process probably as a consequence of the general stress induced in the cell. Our findings reinforce the knowledge of a safety use of lactoferrin as treatment for giardiasis.

INFECTOLOGY 2

(1925) **TEGUMENTARY LEISHMANIASIS, KNOWLEDGE AND SOCIAL BEHAVIOR OF THE TARGET POPULATION**

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Leishmaniasis are caused by trypanosomatid protozoans of the genus Leishmania spp, transmitted by sandflies. The different species of Leishmania cause a wide spectrum of manifestations: L Tegumentary (LT) that can be cutaneous, or mucous, and visceral. Leishmaniasis has a high public health impact due to its morbidity and mortality and is associated with environmental factors and anthropogenic modifications.

The INP receives samples from different health centers for the diagnostic confirmation of leishmaniasis, as a National Reference Laboratory.

We did an exploratory study based on interviews among those who attended the INP for diagnosis in 2016. Socio-demographic data, knowledge, preventive practices and perception of the disease were obtained. Results: 14 men and 8 women were interviewed with an average age of 40 years. They came from Paraguay, 5; NEA, 5; NOA, 3; BsAs, 5; Sta Fe 1; Bolivia, 1; Peru, 1; Senegal, 1. They lived 13/22 in GBA and 9/22 performed rural tasks. Low knowledge about LT was detected; 5/19 recognize or have heard speak; 2 knew the form of transmission and recognized the vector 13/22 recognized the "polvorín, mosquita, carachay, zancudo", mainly those of Paraguay and Misiones: People avoided insect bites by covering limbs (6), repellent (13) use of mosquito nets (5) and fume at home and peridomicilio (10). The lesions are mainly found on mucosa (8) and extremities (6). Some patients remember the prime infection in their youth, more than 20 years ago. Most do not consider LT a serious or contagious disease. In the social aspect they do not perceive a differential relationship between family and friends. However some, due to exposed lesions had modified the dress and avoided the concurrence to spaces of social encounter. At the time of the interview 8/22 they were under sick leave. Conclusion: They recognize the vector from their common names, especially those who lived in NEA, Paraguay and did rural work. The results show little knowledge of the disease and the prevention measures seem to have been insufficient. The need appears to provide accessible and easily understood information for a medical consultation, a quick diagnosis and a timely treatment.

Key Words: Leishmaniasis Tegumentaria- Social Knowledge and Behavior

(661) **THE TLR AGONIST PROFILIN PROTEIN FROM *TOXOPLASMA GONDII* GENERATES PROTECTION IN AN EXPERIMENTAL VACCINE**

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Toxoplasmosis is a disease that affects 30% of the world's population. At present, there are no pharmacological treatments that eliminate the parasite or vaccines that confer protection to the host. In this work, we studied the adjuvant and/or immunogenic value of a recombinant form of *T. gondii* Profilin protein (rTgPF), a TLR ligand, in a vaccine strategy using a murine model of chronic toxoplasmosis. BALB/c mice were intradermal immunized 3 times with a 2-week interval with: rGRA7 (recombinant *T. gondii* dense granule 7 protein), rTgPF, rGRA7+rTgPF or rGRA7+ACF.The anti-GRA7 humoral response showed a Th1 profile when rTgPF was used as adjuvant (IgG1/IgG2a ratio: 0,6±0,5), while rGRA7+ACF showed a mixed profile (IgG1/IgG2a ratio: 1,3±0,5). Splenocytes from rGRA7+rTgPF and rGRA7+ACF mice stimulated with rGRA7, showed similar levels of proliferative responses that were significantly higher than the control group (p <0.05), and also significant production of IFN-γ (p <0.05 vs control). In addition, although rTgPF generated a low humoral response, this presented a Th1 profile both in rTgPF and rGRA7+rTgPF groups (IgG1/IgG2a ratio: 0,4±0,1 and 0,4±0,1 respectively). After rTgPF *in vitro* stimulation of splenocytes, significant proliferative responses and also significant production of IFN-γ were detected in rGRA7+rTgPF mice compared to the control group (p<0.01). When vaccinated mice were orally challenged with a non-lethal dose of *T. gondii* cysts, rGRA7+rTgPF mice presented a 62% reduction in the cysts number per brain compared to the control group (p<0,05). This reduction resulted similar to the observed for the rGRA7+ACF group. Together these results demonstrate the dual role of rTgPF: as an adjuvant with capacity to enhance immune responses against another antigen, and also as an immunogen given its ability to induce specific responses. We conclude that Profilin is a valuable candidate to include in a vaccine against toxoplasmosis

Key words: Toxoplasma gondii, Vaccine, Chronic infection, Profilin, GRA7

(848) **TARGETING PROTEIN A DURING STAPHYLOCOCCUS AUREUS OSTEOMYELITIS**

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Staphylococcus aureus is a major causative agent of osteomyelitis in adults and children. The increasing incidence of antimicrobial resistant isolates and the morbidity of this type of infection denote that alternative therapeutic approaches are required. We have recently demonstrated that staphylococcal protein A significantly contributes to increased osteoclast differentiation and activation as well as cortical bone destruction during the course of disease. Therefore, we hypothesize that targeting protein A in addition to antimicrobial treatment could be an adjunctive strategy to control bone damage during the initial course of S. aureus osteomyelitis. The aim of this study was to evaluate the potential of using anti-protein A antibodies to neutralize the deleterious effect of this protein on bone homeostasis using and in vivo experimental model of osteomyelitis. Groups of BALB/c and C57BL/6 mice received anti-protein A antibodies by intraperitoneal route (75 mg/kg) or normal rabbit serum (sham control) one day before the subsequent challenge with S. aureus (2 x 106 UFC) in the left tibia. Two days later the tibias were excised and the bone marrow cells collected and cultured in the presence of M-CSF (50 ng/ml) to obtain osteoclast precursors. Immunization with anti-protein A antibodies significantly reduced the priming of osteoclast precursors in response to S. aureus infection as evidenced by their decreased capacity to differentiate into mature osteoclasts after in vitro stimulation with RANKL (BALB/c: p<0,01; C57BL/6: p<0,001). Moreover, administration of anti-protein A antibodies on day -1, 6 and 9 after S. aureus challenge significantly decreased the levels of IL-1, IL-6 and TNF-α in the bone at day 14 after the onset of experimental osteomyelitis (p<0,05). These results indicate that anti-protein A antibodies could locally reduce the undesirable effects of S. aureus induced osteoclastogenesis during the initial stages of osteomyelitis.

Keywords: Staphylococcus aureus, osteomyelitis, protein A, osteoclastogenesis, immunization.

(874) **TNFR1 SIGNALING DRIVES THE IMMUNOSUPPRESSIVE FUNCTION OF MDSC DURING *Staphylococcus aureus* SEPSIS**

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TNF-α signaling is strongly associated with the inflammatory response that characterizes the initial phase of sepsis. The events that govern the concomitant and subsequent immunosuppression, however, are not completely elucidated. Myeloid derived suppressor cells (MDSC) have been described as an immature population of cells that is able to suppress T cell responses. It has been described that TNF-α participates in MDSC accumulation and activation during chronic inflammation.

The aim of this study was to determine the role of MDSC and TNFR1 signaling in the modulation of T cell function during *S. aureus* sepsis.

We used a murine model of *S. aureus* sepsis of peritoneal origin. At day 8 after inoculation, a significant increase in the percentage of MDSC accumulated in the spleen (p<0,001) and significantly reduced T CD4$^+$ cell responses (p<0,05), measured as the proliferative capacity in response to Con A, were observed in *S. aureus* infected mice compared with control mice. Treatment of septic mice with 5'Fluorouracil, at a dose proven to specifically deplete MDSC, significantly improved the proliferative capacity of CD4+ T cells. Conversely, when *S. aureus* sepsis was induced in *tnfr1*$^{-/-}$ mice, besides a significant increase in the percentage of MDSC in the spleen was observed (p<0,01), the proliferative capacity of T CD4$^+$ cells did not differ from that of control mice suggesting that in the absence of TNFR1 signaling MDSC might lose their suppressive capacity. MDSC are known to exert their suppressive function trough the expression of arginase 1 and iNOS. The levels of *arg*1 and iNOS were decreased in MDSC from *tnfr1*$^{-/-}$ septic mice compared with MDSC from wild type mice. Taken together these results indicate that MDSC contribute to T cell dysfunction during *S. aureus* sepsis and that TNFR1 signaling determines the suppressive capacity of these cells highlighting the role of TNF-α in both, the inflammatory and the immunosuppressive, phases of staphylococcal sepsis.

Keywords: sepsis, immunosuppression, *Staphylococcus aureus,* myeloid derived suppressor cells.

(877) **PHENO AND GENOTYPIC CHARACTERIZATION OF AN *ENTEROCOCCUS FAECIUM* STRAIN AND ITS VIRULENCE IN A MURINE MODEL OF MASTITIS**

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Abstract: Bovine mastitis is a disease that causes significant economic losses to the dairy industry in the world, being streptococci and enterococci the main environmental bacteria involved. The aim of this work was to characterize pheno and genotypically an *Enterococcus faecium* strain collected from a bovine mastitis case. In addition, bacterial counts and histopathological alterations in the mammary tissue were evaluated using a mouse mastitis model. For phenotypic characterization bacteria growth curve, antibiotic susceptibility testing, enterococcal virulence factors and biofilm assays were performed. For genotypic characterization, amplification of virulence associated genes by PCR assays and PFGE assay were performed. The maximum cell growth was determined at 6 h. The strain only showed susceptibility to teicoplanin, presented gamma hemolysin and did not present gelatinase activity. It could be characterized as moderate biofilm producer and yielded *gelE1* and *efa* genes. PFGE analysis showed the presence of 19 bands. Balb/c lactating mice were inoculated as follows: Control: 100 μl of PBS and Group 2: 100 µl of 1x10^{8}CFU/ml of *E. faecium*. Animals were sacrificed 24, 48, 72, 96 and 360 h post-inoculation (p.i.). Bacterial count decreased at 48 hours (p<0.001 with respect to the initial inoculum) and was constant since 72 to 360 h p.i. Group 2 showed an intense infiltration of PMNs since 24 to 96 h p.i and *loss of alveolar* structure *integrity.* The study contributes to the development of experimental intramammary infection model in mice and would facilitate the study of mastitis caused by this pathogen.

Keywords: Enterococcus faecium, mouse mastitis model

(1066) **ROLE OF PROSTAGLANDIN E2 DURING THE HUMAN IMMUNE RESPONSE AGAINST *MYCOBACTERIUM TUBERCULOSIS***

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Tuberculosis (TB) is, alongside HIV, the leading cause of death from an infectious disease. In fact, *Mycobacterium tuberculosis* (*Mtb*) causes nearly 10 million of new cases and 1.5 million deaths per year. Prostaglandin E2 (PGE2), an active lipid compound derived from arachidonic acid, is a key mediator of immunopathology in chronic infections and cancer by regulating different stages of immune responses. Manipulation of PGE2 levels was proposed as an approach for countering the Type I IFN signature of TB patients and to alleviate active TB disease, but very limited information exists about this pathway in patients with active TB and the role of autophagy, a cellular process involved in defense mechanism against *Mtb*. Here we investigated the role of PGE2 on modulation of human immune responses against *Mtb*. First, we analyzed the expression of surface molecules that play an important role on T cell activation. Peripheral blood mononuclear cells from healthy donors (HD) and TB patients were cultivated for 16 h without stimulus to allow monocyte adherence. Cells were then stimulated with *Mtb*-Ag ± PGE2 (2 μg/ml) for 24 h or 5 days. Flow cytometry (FC) analysis revealed that PGE2 significantly reduced SLAM and CD31 expression on CD3$^+$ T lymphocytes and CD80 and MHCII expression on CD14$^+$ monocytes from all groups under study. Interestingly, PGE2

reduced the lymphocytes proliferation from HD and TB patients. Besides, autophagy levels were analyzed by FC and Confocal Microscopy against LC3B-II. We found that PGE2 significantly increased LC3 puncta accumulation on monocytes from HD and TB patients. Finally, we observed an interesting increase in apoptotic blebs formation in cells treated with PGE2. Taken together, PGE2 has immunomodulatory features in both innate and adaptive immune responses during human TB. Our findings might contribute to deepen the mechanisms that operate on *Mtb*-resistance mediated by PGE2 and the potential of PGE2 as a tool to improve anti-TB treatment.

Palabras Clave: PGE2, TUBERCULOSIS, AUTOFAGIA, PACIENTES, RESPUESTA INMUNE

(1380) **COMPARATIVE VIRULENCE ANALYSIS OF COMMUNITY-ASSOCIATED AND HEALTHCARE-ASSOCIATED METHICILLIN-RESISTANT Staphylococcus aureus STRAINS CIRCULATING IN ARGENTINA USING THE Galleria mellonella MODEL.**

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Community-associated MRSA (CA-MRSA) strains are genotypically distinct from healthcare associated MRSA (HA-MRSA), and there is evidence that CA-strains have a more virulent clinical phenotype than HA-MRSA strains causing infection. Nevertheless, in Argentina the behaviour of the circulating strains remains unclear. We aimed to analyse virulence between a representative collection of CA- and HA-MRSA strains recovered throughout Argentina since 1999 in an *in vivo Galleria mellonella* killing model. MRSA were studied by SCC*mec* and spa-typing, PVL, PFGE and MLST. CA-MRSA isolates [I-ST5-IVa t311 PVL^+ (n=2), N-ST30-IVc t019 PVL^+ (n=2) and USA300-ST8-IV t008 PVL^+ (n=2)] and HA-MRSA isolates [A-ST5-I t149 (n=2) and B-ST239-III t037 (n=2)] were analysed. 8 caterpillars were inoculated in triplicate with 5 μl ($6x10^8$ CFU/ml) of each strain and incubated at 37°C and observed every 24 h for 80 h. Log-rank tests were used to compare Kaplan–Meier survival curves for all CA-MRSA vs HA-MRSA strains and for all clonal groups. Statistical analysis showed that CA strains killed more larvae than HA strains ($P<0.001$) and there were no significant differences by CA or HA clones ($P>0.05$). Although, the USA300 clone killed *G. mellonella* larvae more quickly than N or I strains (mean survival time, 47 h for USA300 vs 64 h for N and I strains, P=0.051), there were no significant differences between the CA strains. We also analysed separately the most prevalent CA clones in Argentina (N and I strains), excluding USA300 strains vs. the most prevalent HA clones (A and B strains).This analysis, also showed that CA clones killed more larvae than HA clones ($P<0.018$). These results demonstrate that there are differences regarding virulence in the most prevalent CA- and HA-MRSA strains circulating in Argentina and suggest that the increased virulence phenotype of these clones could be associated with increased killing in *G. mellonella* virulence model.

Keywords: CA-MRSA, HA-MRSA, *Galleria mellonella*

(1560) **EARLY AND RAPID DETECTION OF DENGUE VIRUS SEROTYPES 1–4 BY COLORIMETRIC RT-LAMP AMPLIFICATION ASSAY**

Adrián Vojnov

Dengue viruses (DENV), a member of the Flaviviridae family, are responsible for one of the most important emerging viral diseases. This mosquito-borne disease has a great impact in tropical and subtropical areas of the world in terms of illness, mortality and economic costs, mainly due to the lack of approved vaccine or antiviral drugs. Infections with one of the four serotypes of DENV (DENV-1–4) result in symptoms ranging from an acute, self-limiting febrile illness, dengue fever, to severe dengue haemorrhagic fever or dengue shock syndrome. The aim of this work is to develop an assay for early and rapid detection of DENV infection during the febrile period. An early detection is crucial for proper patient management and prevention of disease spread, especially in the resource-limited rural healthcare settings where dengue is endemic. Here, a specific, sensitive, and robust prototype colorimetric reverse transcriptase loop-mediated isothermal amplification (RT-LAMP) assay was developed for detection and differentiation of DENV1-4 serotypes. These techniques are used as a powerful tool for screening and diagnosis of infectious diseases. This Isothermal method, as an alternative to polymerase chain reaction (PCR), require no thermocycling machine and can mostly be performed with reduced time, high throughput, and accurate and reliable results. The reaction was performed in one step in a single tube by mixing primers, reverse-transcriptase and DNA polymerase together with the tested samples (RNA genome international reference for Dengue serotypes) at 65 □C for 60 min, with the addition visible pH indicator dye prior to amplification. The pH indicator dye change resulting from amplification reactions performed with minimal buffering, by eye without a need for instrumentation. The result showed no cross reactivity to other closely related arbovirus (Zika). Our assay is more sensitive than quantitative reverse transcription-polymerase chain reaction (qRT-PCR).

KEYWORD: Dengue virus, Dengue serotypes 1–4, Diagnostic test, Reverse transcriptase loop-mediated isothermal amplification, Serotype detection.

(1814) **HANTAVIRUS INDUCED CHANGES IN T-CELL SUBPOPULATIONS AND SHOWED UP-REGULATION OF PRO-INFLAMMATORY CYTOKINES RELATED WITH DISEASE SEVERITY**

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Hantaviruses are emerging human pathogens. These zoonotic viruses are responsible of two different clinical presentations, hemorrhagic fever with renal syndrome (HFRS) and hantavirus pulmonary syndrome (HPS). Hantaviruses predominantly infect microvascular endothelial cells causing capillary leakage. The hallmark of hantavirus diseases is the vascular permeability, which leads to pulmonary edema in HPS patients. HPS is endemic in Argentina where it was associated to high case fatality rates.

In order to evaluate the role of the immune response on pathogenesis we performed cytokine profile analysis and initial T-cell phenotypic characterization in HPS patients.

For biomarkers analysis we study 109 HPS patients including 39 dead patients (D) and 69 survivors (S). Control samples were obtained from healthy adult volunteers (HV). Serum cytokine and granzyme levels were analyzed using multiplex magnetic bead-based technology by a Luminex 200 analyzer. Fresh blood samples were collected to analyze surface markers on T cell subpopulations by flow cytometry in 19 patients. Statistical analysis was performed by Student's *t*-test using GraphPad Prism 6 software. We found significant higher levels of IL-2, IL-4, IL-6, IL-8, IL-10, INF-γ, GM-CSF and TNFα in patients compared with HV. Interestingly, we showed an increased of Granzyme A and B in patient´s sera compared with HV. Analysis of T cells subsets in patients showed increase in $CD8^+$ cells, resulting in the inversion of the CD4/CD8 ratio compared to HV. This preliminary phenotypic analysis of T cell subpopulations showed an increase of $CD38^+$ /$CD27^-$ in $CD4^+$ and decrease in CD28, CD27 and CD127 in $CD3^+$/$CD8^+$ cells. In addition to this, we observed high number of classic monocytes ($CD14^+CD16^+$) in patients compared with HV. Our results showed a differential biomarker expression according to disease severity. This, together with the preliminary T cell analysis, would suggest an active role of immune response in pathogenesis.

Key words: Hantavirus, T cells, cytokines

(1917) **HISTOLOGYCAL CHARACTERIZATION OF *Clostridium septicum* TRAUMATIC INFECTION IN MOUSE MODEL**

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Among the main histotoxic clostridia is *Clostridium septicum*, gram positive bacillus, anaerobic and sporulated. It is the causal agent of malignant edema and traumatic gas gangrene in cattle, sheep and goats. Its main virulence factor is the alpha toxin with a necrotizing and cytotoxic activity previously studied in our laboratory that also produces apoptosis. One of the characteristics of this infection is the low number of phagocytic cells at the site of infection. The objective of this work was to evaluate the *in vivo* interaction of *C. septicum* with phagocytic cells in a mouse model. Histological damage was characterized in the muscle infected with pathogen cells and the cell infiltrate was analyzed. A batch of BALB-C mice was inoculated intramuscularly into the hind paw with a DL_{50} of *C. septicum* ATCC 12464 and a 1/10 dilution thereof. After 48 h the mice were euthanized and the legs were surgically removed and embedded in paraffin and stained with hematoxylin-eosin. Inoculation sites in the mice were evaluated by observation of pathological changes in the area The formation of acute or chronic lesions and the infiltration of phagocytic cells at the site of infection were analyzed. The affected animals showed clinical signs at approximately 48 hours after inoculation (depression, muscle swelling, stiffness). The muscular lesion initially presented hyperemic, warm, soft and delimited; and later dark, cold, firm and necrosed. At necropsy serosanguinolent inflammatory fluid was observed in the subcutaneous tissue. In the histopathological examination, necrosis, apoptosis, swelling and vacuolization of muscle fibers were observed. There was edema, hemorrhage, large numbers of bacilli and areas with discrete neutrophil and monocyte infiltration. The ability to eliminate phagocytic cells by both necrosis and apoptosis ways, is an efficient mechanism of evasion of the innate immune system by this pathogen.

EPIDEMIOLOGY AND VECTORS 1

(1476) **A TRANSCRIPTOMIC APPROACH TO UNDERSTAND THE STATE DEPENDENCY AND POST ECDYSIS TIME IN THE ANTENNAE OF A CHAGAS DISEASE VECTOR**

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Abstract: Triatomine bugs transmit Chagas disease to humans in most countries of Latin America. Several authors have clearly established that the behavioral responses of these bugs to host and sexual signals present strong plasticity as a function of the time elapsed since ecdysis. Whether these alterations are based on changes at peripheral or central nervous processes (or both) deserves to be clarified at molecular level to allow better control strategies based on manipulating bug physiology and behavior. A transcriptome of 5th instar larvae antennal samples (seven RNA samples per age point: 0, 2, 4, 6 and 8 days after ecdysis) were sequenced using Illumina HiSeq-2000. The corresponding sequencing results from the 35 libraries were analyzed by means of CLC genomics software. The expression of several chemosensory receptor genes, including odorant, gustatory and ionotropic receptors increased significantly during the first week after ecdysis. Odorant binding and chemosensory proteins presented high antennal expression and some of them increase significantly their transcript abundance after ecdysis. The relevance of these results will be discussed in the context of state dependent behavior.

Keywords: Triatomine, RNAseq, sensory receptors

(1863) **AMERICAN TEGUMENTARY LEISHMANIASIS (ATL) IN YOUNG AGE GROUP IN OUTBREAKS OF THE DISEASE FROM THE PROVINCE OF CORRIENTES**

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Leishmaniasis, a parasitic disease transmitted by the bite of some species of sandflies affects various age groups. American tegumentary leishmaniasis (ATL) is caused by different *Leishmania* species ranging from cutaneous forms to severe mucosal lesions.

In America, the observed magnitude of ATL in children has led to the study of increased risk of exposure of this group due to the possibility of peri- and intradomiciliary transmission.

The objective of this study was to describe cases of ATL in children and adolescents in the province of Corrientes, where is endemic.

The diagnostic methods used to confirm the clinical diagnosis: Montenegro Skin Test (MST) and skin smear after MGG coloration.

Thirty-five children and adolescents were diagnosed with ATL in five leishmaniasis epidemic outbreak in the province of Corrientes from 2010-2015.

From the total number of evaluated patients (34), 65% were school-age children (6-12 y-old) and 35% adolescents, of whom 19 (56%) were boys and 15 (44%) girls,

Most of the cases proceeded from the locality of Bella Vista (38%) and from Corrientes city (38%). The rest of localities were: Paso de la Patria, Empedrado and Itatí.

These patients had a mean clinical evolution of 2.9 months of their lesions. Most patients presented just one lesion (86%), which were located mostly in extremities (45% in legs, 19% in arms,11% cases in the face, 13% were in hands). One girl of eight months of age presented one ulcer in the face.

All patients were positive by MST, and 48.8% were positive by smear.

All cases were treated successfully with Glucantime® IM.

Leishmania braziliensis was identified as the main etiological agent of mucosal leishmaniasis in all epidemic outbreak. Infection of *Lutzomyia longipalpis* by *L. amazonensis* was observed in Corrientes city.

Although study participants came mostly from an endemic area, mucosal compromise was rare. A high frequency of LTA was shown in young age groups and cases clustered in urban neighborhoods of Corrientes.

(1835) **COMPARATIVE STUDY OF ENTEROPARASITOSES AMONG IMMIGRANT AND NON-MIGRANT POPULATIONS LIVING IN PERIURBAN OF GRAN LA PLATA (BUENOS AIRES, ARGENTINA)**

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The mass movement of people creates opportunities for the spread and establishment of infectious diseases, such as enteroparasitoses. For both geographical and historical reasons, Gran La Plata is receiving an increasing number of immigrants. We aimed to compare the prevalence of intestinal parasites in immigrant populations (IP) from Bolivia, Paraguay and Peru, and non-migrant populations (NMP**)** resided in periurban of Gran La Plata. A cross-sectional study was conducted between April 2016-July 2017 in two groups of 127 individuals matched for age (0-59 years old) and sex (47.2% males and 52.8% females). Coproparasitological samples were analyzed by Willis and Ritchie concentration methods, anal swabs and conventional PCR. Furthermore, socio-environmental factors were surveyed through semi-structured questionnaires. The overall prevalence of intestinal parasites was 82.7% in IP and 66.1% in NMP (χ^2=9.1,p<0.05). However, the Sørensen's Coefficient showed

a similar parasite species composition in both groups (90%). In IP, *Blastocystis* sp. was significantly more prevalent than NMP (66.9% vs 40.1%) (χ^2= 18.3,p<0.05). We also identified a higher prevalence of *Giardia lamblia* (23.6% vs 17.3%) and *Enterobius vermicularis* (29.9% vs 25.2%) in IP respect to NMP. *Chilomastix mesnili* only was observed in IP and *Ascaris lumbricoides* only was presented in NMP. The multiple infections was more common in IP (21.3% vs 15.0%). In both, socio-environmental data revealed that the most of population did not have access to public services; most of adults had incomplete primary education and did not have stable employment. In contrast, most of IP were not house owners, raised animals for consumption and had hygienic practices more adequate. Although the prevalence of intestinal parasites in IP was higher than NMP, the species composition was similar. Thus, the socio-environmental conditions in both populations adversely affect the parasite transmission.

Keywords: Immigrants, non-migrants, intestinal parasites, La Plata.

(916) **DEVELOPMENT OF A TAQMAN MULTIPLEX REAL TIME PCR ASSAY FOR THE DETECTION OF TRYPANOSOMA CRUZI IN BLOOD SAMPLES OF WILD AND DOMESTIC ANIMALS**

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Chagas disease affects about 7 million people worldwide and is caused by the protozoan parasite *Trypanosoma cruzi*. In the argentine Chaco, an endemic area of Chagas disease, little is known about *T. cruzi* domestic and wild transmission cycles. Therefore, a field trial in rural areas near Añatuya, Santiago del Estero, for eco-epidemiological purposes, is under way. Due to the large number of samples we estimate to obtain in the trial, our goal was to develop a TaqMan multiplex PCR assay that simultaneously allowed *T. cruzi* detection and DNA integrity control. To achieve this, we choose the interphotoreceptor retinoid-binding protein (IRBP) gene, because it is highly conserved in all mammals and its use as a DNA integrity control in a conventional PCR was previously described. Based on an IRBP sequence alignment of several domestic and wild species, we designed a TaqMan probe to a highly conserved region within the amplified zone. The detection of satellite *T. cruzi* DNA was performed with the primers and probe already developed in our laboratory. Once the parameters for the multiplex PCR were established, the assay was tested in a total of 45 blood samples of wild mammals. So far, all samples analyzed were *T. cruzi* undetectable. Our DNA integrity control worked well for *Conepatus chinga, Molossus sp, Lagostomus maximus, Lycalopex gymnocercus, Rattus rattus, Calomys sp, Galea musteloides* and *Leopardus geoffroyi*. However, it did not work for *Chaetophractus villosus, Tolypeutes matacus, Graomys chacoensis* and *Myotis sp*. DNA integrity was tested by amplification of beta-actin in these cases. A sequence analysis to identify mutations that prevent amplification of the selected IRBP region in these species will be done. The performance of the multiplex PCR in domestic animal samples remains to be studied.

Keywords: Chagas disease, *Trypanosoma cruzi* wild and domestic reservoirs, TaqMan Multiplex PCR

(917) **DISTRIBUTION OF POTENTIAL SCHISTOSOMIASIS HOST SNAILS ALONG THE URUGUAY RIVERS BASINS ARGENTINA, IN THE PROVINCE OF CORRIENTES**

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Schistosomiasis is an important neglected tropical disease caused by parasitic worms of the genus *Schistosoma*, with a significant socioeconomic impact. The only species of *Schistosoma* that occurs in the Americas is *S. mansoni* and the transmission occurs largely in Brazil, affecting million people. Natural transmission of schistosomiasis does not exist in Argentina. The distribution of the *S. mansoni* is determined by the presence or absence of *Biomphalaria* snails, which act as the parasite's intermediate host.

This study aims to access the relative abundance and the natural infection of *Biomphalaria straminea* and *B. tenagophila* by *S. mansoni* from seven Department in the Uruguay River basin.

The survey collections were made from March 2015 to September 2016 from different suitable snail habitats. Live snails were kept at the laboratory in aquaria for to identify species and for the screening of positive snails. Snails that did not produce cercariae, were crushed for the search for *S. mansoni* larval stages.

Freshwater snail specimens were collected in different sampling sites in all Department surveyed.

The following species were identified: *B. tenagophila, B. straminea* and *B. orbigny*.

B. tenagophila was the most frequent species, as it was observed in 17 districts of six Departments: five places of Paso de los Libres, five from Mercedes, four from Monte Caseros, two from Curuzú Cuatiá and one from General San Martín

B. straminea was identified in Santo Tomé and *B. orbigny* in Mercedes.

No specimens of collected snails were found infected with *S. mansoni*.

The World Health Organization (WHO) has emphasized the need to create predictive maps for expected schistosome distributions.

Among the *Biomphalaria* species that occur in Brazil, three are regarded as intermediate hosts of *S. mansoni: B. glabrata, B. tenagophila* and *B. straminea*.

The present study extended the geographical distribution of *B. tenagophila* in the Province of Corrientes.

Keywords: *Biomphalaria,* schistosomiasis mansoni, Provincia Corrientes

(1648) **Epidemiology of American Tegumentary Leishmaniasis in the northern province of Salta during the year 2016**

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Tegumentary leishmaniasis (LT) is endemic in northern Argentina and is caused by parasites of the genus Leishmania sp. Leishmaniasis is considered by the World Health Organization as "neglected disease". In the departments of Oran and San Martin of the province of Salta, the greatest number of annual cases are reported. The Institute for Tropical Disease Research (IIET) of the National University of Salta is the diagnostic center of the region. The objective of this work is to describe the epidemiology of leishmaniasis from all patients with a compatible ulcer who attended the IIET during 2016. A total of 216 patients with ulcers were analyzed. All the smears were searched for amastigotes. The predominant clinical form was cutaneous (94%), while the rest were mucocutaneous (6%). The 57% (123/216) were smear positive. 74% of the cases were male and 26% were female. Regarding the place of origin of the patients, 62% are SRN Oran, 17% H. Irigoyen and 5% of Pichanal, all localities of the Oran department. The rest of the cases (16%) are neighboring departments (San Martin and Rivadavia). The statistics suggest sanitary measures of promotion and prevention of the leishmaniasis to diminish the incidence of this parasitosis.

(1892) **FIRST CASE OF FELINE LEISHMANIOSIS CAUSED BY LEISHMANIA INFANTUM IN ASUNCION, PARAGUAY**

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The aim of this study was to survey Leishmania infection in a cat with inespecific clinical signs. Evaluation by parasitological, serological and molecular methods. A cat from Asuncion, Paraguay, 3 year

old, male, 3.4 kg had apparent Feline Lower Urinary Tract Disease (FLUTD) fever, depression, lethargy, progressive weight loss, dull hair coat, generalized lymphadenopathy, neurological disorders, mydriasis, pale mucous membranes, slow capillary refill time. The cat was negative for feline immunodeficiency (FIV) and feline leukemia virus (FeLV) (Serology test)

The blood count showed thrombocytopenia and hyperproteinemia, liver and kidney function tests showed elevated FAL, ALT and uremia. A fine bone marrow needle was aspirated and myeloid hyperplasia was determined with the presence of Leishmania sp. The immunochromatography (Rapid Test Kit. Korea. Leishmania infantum antibodies) was positive which was confirmed by PCR method by using 13A/13B primers (Rodgers et al 1990) for Leishmania genus identification followed by a PCR with LV-P1/LV-P2 primers (Piarroux et al, 1994) for Complex Leishmania donovani identification. The DNA was purified from bone marrow tissue in order to improve the sensitivity of the test. The PCR conditions were similar as the publications. It has been described subclinical Leishmania infection in cats in the Mediterranean basin countries and also several studies have demonstrated the infectivity of sand fly vectors from a cat. These facts raise the possibility of reservoir host role for this species.

From a clinical point of view, this case reinforces the importance of including leishmaniosis in the differential diagnoses of feline pathology, especially in cats with inespecific clinical signs. Nevertheless, from a public health perspective it is important to clarify whether this animal species is capable of sustaining and spreading L. infantum infection.

(113) **FUNCTIONAL CHARACTERIZATION OF *T. CRUZI* MUCINS IN THE INFECTION OF THE INVERTEBRATE HOST**

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Trypanosoma cruzi, the etiological agent of Chagas diseases is covered in different glycoconjugates which contribute to parasite protection and to the establishment of a persistent infection. TcSMUG L is a group of genes coding for small *T. cruzi* mucins anchored to and secreted from the surface of replicative, insect-dwelling developmental forms (i.e. epimastigotes). The objective of this work was the functional characterization of TcMUGL mucin family and their role in the infection of *Rhodnius prolixus*. We analyzed TcSMUGL expression levels in different *T. cruzi* strains observing not only differences in expression levels but also in glycosylation patterns. In order to characterize epimastigote mucin function we generated *T. cruzi* strains overexpressing TcSMUGL and TcTSSA (tripomastigote stage mucin) in two different genetic backgrounds CL Brener (high TcSMUGL levels) and Y strain (low TcSMUGL levels and is unable to infect *Rhodnius prolixus)*. Transgenic lines were used to perform in vivo infection assays in *Rhodnius prolixus*. TcSMUGL CL overexpressing parasites presented higher infection rates than control lines. Furthermore TcSMUGL lines belonging to the Y strain were able to stablish the infection in the insect host, presenting high infectivity rates. In order to further characterize mucin function we performed *in vitro* adhesion using different sections of *Rhodnius prolixus* nymph's digestive tract. Our results indicate that TcSMUGL would be involved in parasites adhesion to the posterior midgut, as both transgenic TcSMUGL lines presented 4 times more adhesion percentages than control lines. Finally we performed in vitro lysis experiments with digestive contents observing that TcSMUGL overexpression enhances parasites survival towards stomach and intestinal contents. Together, these data indicate that TcSMUG L mucins are key determinants of the infectivity of *T. cruzi* towards the insect population and, hence, on *T. cruzi* epidemiology

Key words: *Trypanosoma cruzi*, TcSMUGL, *Rhodnius prolixus*

(1210) **URBAN INFESTATION BY *TRIATOMA INFESTANS* IN THE ARGENTINE CHACO: EVALUATION OF A SELECTIVE TREATMENT PROTOCOL**

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Triatoma infestans has increasingly been found in urban areas of the Gran Chaco ecoregion where vector-borne transmission of *Trypanosoma cruzi* still occurs. How to cope with urban infestations in a cost-effective way remains unclear. As part of the longitudinal intervention program "Avia Terai sin Chagas", we assessed house infestation with *T. infestans* in the town of Avia Terai, Chaco, Argentina. The town had last been sprayed with pyrethroid insecticides by the National and Chaco Chagas Programs in 2011-2013 (total, 774 houses). All inhabited (n=1454) houses were visited in February 2016 and manual searches using a flushing-out agent were performed in 412 houses, including those where dwellers reported having seen triatomines, harbored chickens, and in one of every three houses that did not fulfill any of these conditions. *T. infestans* was found in 56 houses located in 27 of the 125 urban blocks. All infested and adjacent houses were sprayed by local vector control personnel using beta-cypermethrin (Chemotecnica) at 50 mg/m^2 in domiciles and a double dose in peridomestic sites. Using a similar survey protocol, the prevalence of house infestation decreased significantly from 13.6 to 2.9% (n=859) in March-April 2017 (Fisher's exact test, $p<0.0001$), whereas block infestation before (21.6%) and after spraying (15.2%) were not significantly different (p=0.33). Eleven blocks and only 6 houses were persistently infested over surveys. Most of the 25 infested houses in 2017 had not been examined (40%) or had been negative for triatomines in 2016 (36%). Most pre- (78,6%) and post- (68,2%) spraying foci occurred in peridomestic structures. Post-spraying house infestation is most likely explained by the existence of unreported, untreated foci within the infested blocks. Our ongoing research efforts are directed to shed light on the cost-effectiveness of alternative treatment protocols, and ensure improved effectiveness, sustainability and local community acceptance.

Keywords: Chagas disease, infestation, urban, vector control.

(1631) **CHARACTERIZATION OF TWO NOVEL *NEOSPORA CANINUM* ISOLATES FROM A DAIRY FARM IN URUGUAY REVEALS GREAT GENETIC DIVERSITY AMONG REGIONAL ISOLATES**

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Neospora caninum causes neosporosis, known as the billion dollar disease, due to its estimated impact on the worldwide economy. Bovine neosporosis *is* one of the leading causes of bovine abortion in the world. However, very little is known about the biology of this parasite. The overarching goal of our study is to tailor both diagnostics and prophylactic strategies to the region´s needs by characterizing prevalent strain(s), at the molecular level. To isolate and genotype *N. caninum* in Uruguay, a group of pregnant cows seropositive for *N. caninum* were identified by ELISA, and followed up to term. Six pre-colostrum seropositive asymptomatic calves were euthanized to attempt parasite isolation. Brain samples were processed for identification of infected areas by PCR analysis and histopathology. Infected brain areas were inoculated into IFNγ-KO mice, and two strains of *N caninum* were recovered. Multi-locus microsatellite typing of genomic DNA revealed a unique genetic pattern, distinct from previously reported isolates from the region, and that of European and North American strains. The genetic diversity revealed by these novel isolates suggests that greater genetic diversity exists than previously reported for this parasite, even among isolates from adjacent geographical regions. These findings reflect a situation akin to what

is observed for other prevalent Apicomplexa such as *Toxoplasma gondii*. We are currently exploring the phenotypic implications of this genetic diversity to determine its impact on virulence, vertical transmission and incidence on abortion rates.

CELL SIGNALING 2

(53) ROLE OF WNT/β-CATENIN AND NOTCH PATHWAYS IN RAT CORPUS LUTEUM DEVELOPMENT

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Wnt/β-catenin and Notch are evolutionarily conserved pathways involved in the regulation of ovarian function. Previous results from our laboratory using XAV939 to inhibit the canonical Wnt pathway demonstrated that Wnt is implicated in ovulation and luteal function. We established that this pathway is involved in luteal progesterone production through the regulation of StAR levels. In addition, this pathway regulates the proliferation and survival of the Corpus Luteum (CL) cells and the luteal vascular development. The objectives of this study were: 1) to confirm our previous results by using another Wnt inhibitor that acts through a different mechanism and 2) to study the effect of hCG on Notch member expression. To this purpose prepubertal eCG-treated rats were injected with either Wnt inhibitor (XAV939 or ICG-001) or vehicle solution (DMSO, Control group) into the bursa of both ovaries the day of hCG administration. Ovaries were collected 48 hours later. CLs from one ovary were isolated by microdissection and used to measure mRNA, total protein or nuclear protein levels. The other ovary was fixed for histological analysis. The level of nuclear β-catenin, which is responsible for transcriptional regulation, decreased after XAV939 administration, as measured by Western blot (control: 1.7±0.04; XAV939: 1.4±0.05; [n=4]; P<0.01). ICG-001 administration produced ovarian cysts (control: 0%; ICG-001: 1.22±0.33%) and decreased the percentage of CL (control: 43.5±4.5% [n=6]; ICG-001: 25.9±1.5% [n=5]; P<0.05), consistent with the phenotype observed with XAV939. In addition, preliminary qPCR experiments showed an increase in the expression of Notch4 receptor and Hes1 (Notch pathway effector) without effect on Notch1 and Dll4 in CL compared to immature granulosa cells. The results observed with ICG-001 strengthen those previously observed using XAV939 as a Wnt inhibitor, while the results obtained by qPCR suggest a differential expression of Notch members in ovarian cells.

Keywords: Corpus Luteum, Wnt, Notch, ICG-001

(805) THE CFTR CHLORIDE CHANNEL REGULATES EGFR LIGANDS EXPRESSION

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The *CFTR* (cystic fibrosis transmembrane conductance regulator) gene is mutated in the cystic fibrosis (CF) disease. CFTR is a cAMP-activated chloride channel, member of the superfamily of ABC (ATP Binding Cassette) transporter proteins, which functions not only as a regulated chloride channel but also as a signalling molecule modulating different genes. Previously, we found that several genes had altered expression due to the CFTR failure (p<0.05), such as SRC (a tyrosine-kinase which in turn regulated MUC1), MTND4 (a mitochondrial gene encoding a subunit of the mitochondrial Complex I), and CISD1 (a mitochondrial protein encoded in the nucleus with a yet ill-defined function). Hence, the aim of the present work was to determine if a failure in the CFTR activity (or expression) determines a differential regulation in the EGF receptor and its ligands and which are the signalling pathways involved. We use a cellular model consisting of Caco-2 cells (human colon carcinoma epithelial cells) expressing wt-CFTR that were previously selected and cloned after transfections with short hairpin RNA interference (shRNA) directed against different regions of CFTR (CaCo-2/pRS26) or with its control plasmid (CaCo-2/pRSctrl). The results obtained, by real time PCR, suggested that CFTR modulates significantly (p<0.05) the expression of TGF-alpha, epiregulin and amphiregulin but not the EGFR expression. As we observed an important regulation of epiregulin (EREG) ligand by CFTR, we continue to study the possible signalling pathways involved. We observed that both IL-1β and JNK participates significantly (p<0.05) in EREG expression. In conclusion, CFTR channel activity failure or CFTR inhibition regulates the expression of different EGFR ligands possible involved in phenotype changes present in CF-like cells. This work was supported by ANPCYT (PICT 2012-1278), CONICET (PIP 2015-2017 GI 11220150100227CO and PUE 22920160100129CO), and research fellowships from CONICET.

Keywords: Cystic-Fibrosis; CFTR; EGFR

(1039) THE INFLAMMATORY RESPONSE OF RETINAL PIGMENT EPITHELIUM CELLS EXPOSED TO HIGH GLUCOSE CONCENTRATIONS: THE ROLE OF CLASSICAL PHOSPHOLIPASES D

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Diabetic retinopathy (DR) is one of the main causes of visual dysfunction and blindness in working-age adults. Chronic hyperglycemia, oxidative stress and inflammation are key players in the pathogenesis of DR. The aim of the present work is to study the role of classical phospholipases D (PLD1 and PLD2) in retinal pigment epithelium (RPE) cells exposed to an *in vitro* DR model induced by high glucose (HG) concentrations.

ARPE-19 and D407 human RPE cells were exposed to normal glucose concentration (Control condition or NG, 5.5 mM) or to HG concentrations (16.5 or 33 mM) for 4, 24 or 72 h. To study the role of classical PLDs and the ERK pathway cells were pre-incubated for 1 h with EVJ (VU0359595, 0.15 μM) to inhibit PLD1 activity, with APV (VU0285655-1, 0.5 μM) to inhibit PLD2 or U0126 (10 μM) to inhibit the MEK/ERK pathway, prior to cell exposure to HG. Statistical analysis was performed using ANOVA followed by Bonferroni´s test and p-values ≤ 0.05 were considered statistically significant.

Our results demonstrate that 72 h exposure to HG reduced cell viability and increased caspase-3 cleavage. In addition, HG exposure for 4 h induced early and concatenated events, as PLD, ERK and nuclear factor kappa B (NFκB) activation. NFκB activation induced by HG correlated with the increment in pro-inflammatory interleukins (IL-6 and IL-8) and cyclooxygenase-2 (COX-2) mRNA levels ($p \leq 0.0001$). The effect of pharmacological inhibitors demonstrated that ERK and NFκB activation were dependent on both PLD isoforms. Furthermore, the increment in IL-6 and COX-2 mRNA levels induced by HG was reduced to control levels in cells pre-incubated with both PLD inhibitors. However, the rise in IL-8 mRNA levels was only reduced in cells incubated with the PLD1 inhibitor, EVJ.

In conclusion, our findings demonstrate that PLD1 and PLD2 mediate the inflammatory response of RPE cells exposed to HG, pointing to the potential use of classical PLDs as a therapeutic target for DR treatment.

Keywords: diabetic retinopathy, retinal pigment epithelium, phospholipase D, inflammation

(957) THE MAGNITUDE OF Ca^{2+} INDUCED Ca^{2+} RELEASE TRIGGERED BY ACTIVATION OF TRPV4 IS DEPENDENT ON THE EXPRESSION OF AQP2 IN RENAL CELLS

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There is increasing evidence indicating that aquaporins (AQPs) may influence cell signaling by affecting TRPV4, a non-selective Ca^{2+} channel. In a previous communication we described that under TRPV4 stimulation, AQP2 can modulate SK3 (a KCa subtype)

leading to hyperpolarization of the membrane potential. Surprisingly, TRPV4-mediated Ca^{2+} signals were not apparently affected by Vm hyperpolarization. Since other works reported that TRPV4 activation triggers Ca^{2+} induced Ca^{2+} release (CICR) from intracellular stores, the aim of the present work was to investigate if CICR can mask the influence of hyperpolarization on TRPV4-mediated Ca^{2+} influx in AQP2 expressing cells. Using fluorescent probe techniques, we studied intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in response to activation of TRPV4 (with 4α-PDD, 10 μM) in two renal cell lines expressing (AQP2-$RCCD_1$) or not AQP2 (WT-$RCCD_1$). In addition, we assessed the colocalization between SK3 and AQP2. We found that in both cell lines, TRPV4-mediated $[Ca^{2+}]_i$ signals were unaffected by preincubation with dantrolene (10 μM), an inhibitor of Ca^{2+} release from ryanodine receptors. However, caffeine (5 mM), an inhibitor of Ca^{2+} release from inositol trisphosphate receptors (InsP3R), significantly diminished the response in both cell lines (Area under curve: Wt-Ctrl: 928±77, n=320 vs. Wt-Caf: 478±54, n=107, p<0.001; Aqp2-Ctrl: 971±38, n=255 vs. Aqp2-Caf: 694±77, n=154, p<0.01). Interestingly, the reduction was lower in AQP2 expressing cells (25%) than in WT cells (50%). These results suggest that TRPV4 activation triggers both Ca^{2+} influx and CICR (via InsP3R) in both cell lines. Nevertheless, when the CICR component was abolished, Ca^{2+}influx was greater in AQP2-$RCCD_1$ cells evidencing a higher Ca^{2+} entry probably due to the hyperpolarization observed as an effect of AQP2-SK3 interaction. Additionally, the immunofluorescence studies showed colocalization of AQP2 with SK3 supporting the idea of an interplay between both proteins.

Keywords : Aquaporin, Trpv4, Sk3, Calcium, Renal Cells

(487) **THE CHLORIDE ANION ACTS AS A SECOND MESSENGER OF THE CFTR ACTIVITY TO MODULATES THE EXPRESSION OF SPECIFIC GENES**

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The impairment of the CFTR channel function leads to several alterations in cystic fibrosis (CF), including as differential gene expression. We have previously demonstrated that the expression of several genes can be modulated by the CFTR activity. However, the signaling mechanisms by which this channel induces the CF phenotype remain unclear. Recently, applying differential display to IB3-1 CF cells cultured under different intracellular Cl^- concentrations ($[Cl^-]_i$), we observed and characterized two Cl^--dependent genes: *GLRX5* (glutaredoxin 5) and *RPS27* (also named metallopanstimulin-1 or ribosomal protein S27). From these results, we hypothesized that Cl^- might act as a second messenger for CFTR signaling. Here, to test this idea, we study *RPS27* mRNA expression in T84 cells (which express wt-CFTR), modulating the CFTR activity by using pharmacological inhibitors. First, we observed that incubation of T84 cells with increasing concentrations of the CFTR inhibitors CFTR(inh)-172 or GlyH-101 determined a progressive increase ($p<0.05$) in the $[Cl^-]_i$ (using the Cl^- fluorescent probe SPQ). The $[Cl^-]_i$ rise was concomitant with a dose-dependent down-regulation ($p<0.05$) of *RPS27*, suggesting that CFTR inhibition produce Cl^- accumulation and that *RPS27* expression can be modulated by the CFTR activity. More importantly, Cl^- behaves as a signaling effector for CFTR in the modulation of *RPS27* expression. In addition, the IL-1β receptor antagonist IL1RN or the JNK inhibitor SP600125, both restored the down-regulation of *RPS27* induced by CFTRinh-172, implying a role of autocrine IL-1β and JNK signaling downstream of Cl^- in modulation of *RPS27*. The results suggest the existence of Cl^--dependent genes and that Cl^- anion might act as a second messenger modulating the expression of specific genes. Supported by PIP 2015-2017, PUE 22920160100129CO and PICT 2012-1278 to TASC and PICT-2015-1031 to AGV.

Keywords: Intracellular chloride; second messenger; RPS27; Cl^--dependent gene expression

(1704) **REGULATION OF NUCLEAR FACTOR KAPPAB SIGNALLING BY FK-506 BINDING PROTEIN 52**

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Nuclear Factor KappaB (NF-κB) is key regulator of the transcription of genes involved in cell death, inflammation and invasion. Recently, we have demonstrated that FKBPs (FK506-binding proteins), especially FKBP51 and FKBP52, are able to modulate NF-κB transcriptional activity. The aim of this work is to determine the participation of FKBP52 in different steps of NF-κB signaling and the expression of NF-κB target genes. Thus, we over expressed FKBP52, FKBP52 F130Y (a mutant lacking peptidylprolyl-isomerase activity (PPIase)), or empty vector (control group). Different hallmarks in NF-κB (p65/p50) activation after PMA or TNF-α stimulation were evaluated: total and phosphorylated p65 protein levels and NF-κB inhibitor´s phosphorylation (p-iκB) by Western blot, p65 nuclear traslocation by immunofluorescence, and the expression of NF-κB target genes by real time PCR. After PMA stimulation neither FKBP52 nor FKBP52 mutant transfection showed significant differences in iκB phosphorylation kinetics compared to control, suggesting an iκB independent mechanism. Also, p65 phosphorylation at Ser536 was not altered by overexpression of FKBP52 or FKBP52 F130Y, compared to control. However, basal p-p65 and total p65 were increased after overexpression of FKBP52 vs. control (p<0,001). Furthermore, after TNF-α treatment FKBP52 favored p65 nuclear retention (vs control and FKBP52 F130Y, p<0,05), in accordance with previous results obtained after PMA simulation. The expression of target genes such as MMP-9 and Bax (evaluated by real time PCR) was regulated by FKBP52 in a peptidylprolyl isomerase dependent manner, since overexpression of FKBP52 F130Y resulted in decreased expression of these genes compared to FKBP52 *wild type*. In summary, we propose FKBP52 as a new key regulator of NF-κB target genes expression, evidencing FKBP52´s PPIase enzymatic activity is essential for this regulation.

Financial support: UBA, CONICET , PICT 2015-1603
Keywords: NF-kappaB, FKBP52, peptidylprolyl isomerase activity.

(306) **PTHrP INDUCES TUMORIGENIC BETA-CATENIN PATHWAY INDEPENDETLY OF WNT SIGNALING IN COLON CANCER CELLS.**

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Abstract: There is evidence that PTHrP is implicated in different cancers such as colorectal cancer (CCR). CCR is the second most common cancer in Argentina, according to data from the National Cancer Institute. Although great progress has been made in the diagnosis and therapy of CCR, the survival rate remains low. It is known that β-catenin is a protein which plays a key role in maintaining the growth and proliferation of CCR. In tumor intestinal cells treated with PTHrP, we previously found that Akt and ERK1/2 signaling pathways are involved in the phosphorylation and subsequent nuclear translocation of β-catenin. The aim of this study was to further investigate the molecular mechanisms involved in these processes induced by the hormone employing two cell lines derived from human colorectal cancer, Caco-2 and HCT116. Results by Western blot analysis suggest that PKC, Src and p38 MAPK signaling pathways modulate the phosphorylation of β-catenin induced by PTHrP. Once in the nucleus β-catenin can bind to transcription factors, such as LEF-1 and TCF family members, promoting the expression of genes such as c-Myc and cyclin D. Immunocytochemistry analysis suggest that PTHrP induces LEF-1 protein expression and also promotes the association of β-catenin with this transcription factor. However, the protein-protein interaction between β-catenin and Transcription factor 7-like 2 (TCFL2/TCF4) observed in basal conditions is not modified by PTHrP at the times studied. These results may be correlated with our previous reports where PTHrP also increases protein levels of cyclin D and c-Myc through ERK1/2. Finally, employing iCRT 14, a specific inhibitor of gene transcription induced by β-catenin, we

found that β-catenin participates in tumor cell proliferation induced by PTHrP. Investigation of the underlying mechanisms for the tumorigenesis of CCR will facilitate the diagnosis and therapy of this disease.

Keywords: PTHrP, colorectal cancer, molecular mechanisms, β-catenin.

(952) **LIPOPOLYSACCHARIDE-INDUCED AUTOPHAGY IN RETINAL PIGMENT EPITHELIUM CELLS**

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Lipopolysaccharide (LPS) can reach the retinal pigment epithelium (RPE) in patients with bacterial endophthalmitis. This unusual pathology (consequence of intravitreal injections, trauma, eye surgery, or sepsis) has generally poor prognosis and often leads to vision loss. Our previous studies demonstrated for the first time that classical phospholipases D (PLDs) participates in the LPS-induced inflammatory process in RPE cells. The aim of the present work is to study the autophagy process in RPE cells exposed to LPS.

For this purpose D407 and ARPE-19 human RPE cells were exposed to LPS (25 µg/ml) for 24 and 48 h. Western Blot (WB) and immunofluorescence (IF) assays were performed in order to evaluate LC3II (an autophagosome marker) content and LC3-positive punctate structures, respectively. To study the role of the PLD pathway, cells were pre-incubated for 1 h with selective PLD1 (EVJ or VU0359595, 0.5 µM) or PLD2 (APV or VU0285655-1 0.5 µM) inhibitors prior to LPS addition. Cell viability was measured using the MTT reduction assay. Statistical analysis was performed using ANOVA followed by Bonferroni´s test and *p*-values ≤ 0.05 were considered statistically significant.

Our results demonstrate that in D407 cells LPS reduced cell viability after 48 h treatment ($p \leq 0.001$). LPS also increased LC3II/LC3I ratio and LC3-positive punctate structures after 24 and 48 h exposure ($p \leq 0.05$). An increment in LC3-positive punctate structures was also observed in ARPE-19 cells exposed to LPS. In addition, PLD1 and PLD2 inhibition highly increases LC3II content in LPS-exposed D407 cells with respect to LPS condition. Furthermore, both PLD inhibitors restore cell viability to control condition levels in D407 cells exposed to LPS.

In conclusion, our results show that LPS treatment reduces cell viability and increases the autophagosome marker LC3II in RPE cells. Furthermore, the inhibition of both PLDs restores cell viability, possibly through the modulation of LPS-induced autophagy.

Keywords: retinal pigment epithelium, lipopolysaccharide, autophagy, phospholipase D

(58) **1α,25(OH)$_2$D$_3$-GLYCOSIDES AND SYNTHETIC 1α,25(OH)$_2$D$_3$ ACTIONS IN CELL CYCLE AND MYOGENESIS OF C2C12 SKELETAL MUSCLE CELLS**

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Solanum glaucophyllum leaf's extracts (SGE) enriched with 1α,25(OH)$_2$VitaminD$_3$-glucosides are available for the organism due to the presence of endogenous glycosidases. We have shown that SGE alike 1α,25(OH)$_2$D$_3$ (1,25D) induces myoblast differentiation. In this work, we investigate Akt role in myoblast fusion to form myotubes during differentiation. The fusion index was determine staining C2C2 cells with MitoTracker Red prior fixation, cells were then stained with DAPI, and examined by fluorescence microscopy. We found that the ability of vitamin D compounds, 1,25D or SGE, to induce myotube formation was impaired in presence of Akt inhibitor LY294002. Since Akt inhibitor suppresses myotube formation, we investigate whether Akt participates in the late differentiation marker MHC2b expression founding that its mRNA expression was induced by 1,25D and SGE in an Akt dependent manner. The commitment of myogenic cells in skeletal muscle differentiation requires earlier irreversible interruption of the cell cycle. To evaluate the effect of SGE on the distribution of C2C12 cells into different phases of the cell cycle, we performed flow cytometry assays. The studies showed that SGE, similarly to 1,25D, prompts a peak of S-phase followed by an arrest in the G0/G1-phase, events which were dependent on the MAP kinases ERK1/2, p38 and JNK. Significant differences of the data between control and treated conditions were analyzed by one way-ANOVA followed by Bonferroni test ($p<0.05$) or t-test (*$p<0.05$, **$p<0.01$). Taken together, these results suggest that SGE, as 1,25D, promotes myotube formation through Akt activation and regulates the cell cycle through ERK1/2, p38 and JNK.

Keywords: Cell Cycle, C2C12 cells, differentiation, vitamin D

BIOPHYSICS 2

(792) **A HUMAN TRUNCATED α7 SUBUNIT CO-ASSEMBLES WITH THE FULL-LENGTH α7 TO FORM FUNCTIONAL NICOTINIC RECEPTORS**

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The α7 nicotinic receptor subunit gene, *CHRNA7*, codes for a subunit that forms the homomeric α7 receptor, which is involved in learning and memory. In humans, exons 5-10 of *CHRNA7* were duplicated and fused to the *FAM7A* gene, given rise to the *CHRFAM7A* gene. The product of the resulting chimeric gene, dupα7, is a truncated subunit that lacks part of the ACh binding site. We here combined cell expression, confocal microscopy, western blot, and electrophysiological recordings in HEK cells to understand the functional role of the dupα7 subunit. We found that cells transfected with dupα7 cDNA express the dupα7 protein but show neither surface binding of an α7 specific antagonist nor agonist-elicited currents. To determine if dupα7 assembles with α7 into functional receptors, we used an α7 subunit carrying mutations in determinants of conductance (α7LC) as a reporter of receptor stoichiometry. Co-expression of α7LC with dupα7 or the reverse combination, α7 with dupα7LC, allowed detection of single-channel openings elicited by ACh, indicating that α7 and dupα7 subunits co-assemble into functional heteromeric receptors. The analysis revealed that a minimum of two α7 subunits is required for forming functional receptors and that activation of the heteromeric receptors occurs through the α7/α7 interface. Our results contribute to the understanding of the functional significance of the partial duplication of the α7 gene.

Keywords: nicotinic receptor, Patch-Clamp, ion channel, electrophysiology

(1108) **EPIGALLOCATECHIN-3-GALLATE INCREASES THE AFFINITY FOR NA$^+$ IN THE NA,KATPASE**

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The kinetics of formation and breakdown of the intermediates involved in the transport of Na^+ is one of the less studied aspects of the Na,K-ATPase reaction cycle. According to the Albers-Post model, binding of 3 intracellular Na^+ to the *E*1 state of the enzyme triggers phosphorylation by ATP in the presence of Mg^{2+} and Na^+ becomes occluded in the phosphorylated intermediate *E*1P. Na^+ is released to the extracellular medium after the *E*1P®*E*2P conformational transition. Occlusion of Na^+ has only been reported in inhibited enzyme, in the presence of oligomycin or Cr-ATP, and in partially proteolized enzyme.

The aim of the present work is to develop a procedure for measuring the kinetics of Na^+ occlusion in the Na,K-ATPase during the normal functioning of the reaction cycle. For this, states with occluded Na^+ need to be rapidly stabilized and isolated.

In this work, we propose to use epigallocatechin-3-gallate (EGCg) as a stabilizing agent (Ochiai et al., 2009, Biochem. Pharmacol., 78:1069-1074) and a rapid-filtration procedure to isolate the species

with tightly bound Na^+.

Experiments were carried out at 25 °C in media with imidazole-HCl 25 mM, pH 7.4, using Na,K-ATPase partially purified from pig kidney. To evaluate the effects of EGCg on the affinity for Na^+, enzyme was incubated with eosin Y in a medium containing RbCl and different concentrations of NaCl and EGCg. The $K_{0.5}$ for Na^+ for the increment in eosin fluorescence decreased as [EGCg] increased. Measurements of tightly bound $^{22}Na^+$ to the Na,K-ATPase in the presence of 100 μM EGCg show that this increment in affinity for Na^+ is compatible with the stabilization of a state containing occluded Na^+. Addition of 100 μM EGCg to the washing solution is sufficient to "instantaneously freeze" the reactions of formation and breakdown of Na^+-bound states during the normal functioning of the reaction cycle.

Our results show that EGCg is a good stabilizing agent for characterizing the steps involved in the transport of Na^+.

Keywords: Na,KATPase, cation transport, Na^+ occlusion, epigallocatechin3gallate

(884) **HTC1 AND HTC2, TWO *HALOMONAS TITANICAE* CHEMORECEPTORS THAT BIND AROMATIC COMPOUNDS**

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Halomonas titanicae KHS3 is a strain isolated from Mar del Plata harbor that is able to grow on aromatic hydrocarbons and displays chemotactic behavior toward them.

Genomic sequencing allowed the identification of 25 chemoreceptor genes. Most of the genes code for proteins with a predicted periplasmic ligand-binding domain and a highly conserved cytoplasmic signaling domain. To identify those chemoreceptors involved in sensing of aromatic compounds, we first looked at their genomic context. We found one chemoreceptor, Htc1, next to genes related to degradation of aromatic compounds. The ligand-binding domain of this receptor was expressed in *E. coli* as a His-tagged protein. The purified protein was subjected to thermal shift assays. In this assay the Tm (melting temperature) of the protein is expected to increase in the presence of stabilizing ligands. Salicylate, 4-hydroxybenzoate and benzoate increased the Tm, among more than 300 compounds present in the screening plates. The binding was confirmed by thermophoresis assays. All three compounds bind to the periplasmic domain of Htc1 with Kds between 80 and 600 μM. Besides, the full-length receptor conferred the ability to control the flagellar movement in response to salicylate when expressed in *E coli*. An *Halomonas titanicae* Htc1 mutant, however, showed only subtle alterations in chemotaxis, suggesting that there is functional redundancy between *Ht* chemoreceptors.

Among other ligand binding domains from chemoreceptor genes that were subjected to thermal shift assays we found another one (Htc2) whose Tm was also increased with salicylate, as well as with malate, malonate and succinate. The binding properties were characterized using isothermal calorimetry (ITC). The C3-C4 dicarboxylic acids bound with high affinities (Kds between 30 and 600 μM), whereas salicylate binds poorly (Kd about 2.5 mM). Full-length cloning and expression for functional characterization in *E.coli* cells and mutant construction are under way.

Keywords: Chemotaxis, Chemoreceptors, aromatic compounds

(1258) **PRELIMINARY FUNCTIONAL CHARACTERIZATION OF A CALCIUM-SENSING RECEPTOR- POLYCYSTIN-2 CHANNEL COMPLEX IN THE PLASMA MEMBRANE OF LLC-PK1 CELLS.**

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Polycystin-2 (PC2, TRPP2) is a Ca^{2+}-permeable nonselective cation channel from the Transient Receptor Potential (TRP) superfamily of cation channels. PC2 is encoded by the PKD2 gene, whose mutations are responsible for autosomal dominant polycystic kidney disease (ADPKD). PC2 has been detected in different cellular locations, including the plasma membrane, the endoplasmic reticulum, and the primary cilium. Recent studies from our laboratory determined that a functional PC2 is regulated by changes in external Ca^{2+} concentration that modify the whole cell conductance of wild type LLC-PK1 renal epithelial cells. The stimulatory effect of high external Ca^{2+} on the PC2 currents was mimicked by agonists of the Calcium-Sensing Receptor (CaSR), including spermine, gentamicin, and the calcimimetic R-568. CaSR was also identified by immunocytochemistry, and observed immuno-colocalized with the PC2 channel protein in a manner that depended on external Ca^{2+}. The present study explored the effect of Ca^{2+} and CaSR agonists in the regulation of PC2 single channel currents from isolated plasma membranes from LLC-PK1 cells reconstituted in a BLM reconstitution system. Addition of "external" R-568 (5 μM) activated cation-selective single channel activity by 84 ± 12% ($n = 4$, $p<0.01$) that were inhibited (91.3 ± 1.1% comparing to control condition, $p<0.0001$) by addition of PC2 carboxy-terminus anti PC2 antibody from the "cytosolic" side of the BLM. The data suggest the presence of a functional/(structural?) CaSR-PC2 complex in the plasma membrane of LLC-PK1 renal epithelial cells, in agreement with a regulatory pathway where external Ca^{2+} modulates PC2 channel function. This receptor-channel complex may be part of a regulatory mechanism whose function may help explain the connection between Ca^{2+} signals and PC2 function in the onset of ADPKD.

Keywords: PC2, CaSR, Calcium

(1350) **QUERCETIN-MAGNESIUM (II) COMPLEX INHIBITS THE PLASMA MEMBRANE CALCIUM PUMP**

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Abstract: Flavonoids are commonly found in fruit and vegetables and have been reported to reach micromolar concentration in the human blood plasma. These compounds have antioxidant capacity and are related to cancer chemoprotective properties, by triggering apoptosis of malignant cells through the Ca^{2+}–dependent mitochondrial pathway. Some flavonoids, like quercetin inhibit the activity of sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase and the plasma membrane Ca^{2+}-ATPase (PMCA), however the mechanism of its effect is not yet defined. PMCA is a calmodulin regulated P-type ATPase that maintains the homeostasis of intracellular Ca^{2+} in eukaryotic cells, coupling the transport of Ca^{2+} with ATP hydrolysis. The aim of this work is to characterize the inhibition of PMCA by quercetin. We measured the Ca^{2+}-ATPase activity in purified PMCA from human erythrocytes and the $[Ca^{2+}]_c$ transport in HEK293T cells culture. Results using purified PMCA show that quercetin inhibits PMCA activity. Quercetin absorption spectra with added magnesium Mg^{2+} show the formation of a quercetin-Mg^{2+} complex. Competitive kinetics with Mg^{2+} has shown that the mechanism of inhibition of quercetin is totally dependent on the concentration of Mg^{2+}. Assays with [γ-^{32}P] ATP indicate that inhibition of the ATPase activity by quercetin led to the increase of phosphoenzyme level (EP), implying that quercetin blocks the dephosphorylation of the pump. The EP formed is sensitive to dephosphorylation by ADP, suggesting a stabilization of the E1P intermediate. To characterize the effect of quercetin at the cellular level we studied its transport in HEK293T cell culture by measuring the intrinsic quercetin fluorescence. On the other hand, PMCA activity in HEK293T cells that overexpress PMCA4 is inhibited on a concentration-dependent behavior, in a similar way that in purified PMCA. Altogether, results show that the quercetin- Mg^{2+} complex is the true inhibitor of PMCA both in purified enzyme and in cultured cells.

Keywords: Plasma Membrane Calcium ATPase, quercetin, magnesium, inhibition

(1740) **REGULATION OF THE MOLECULAR FUNCTION**

OF α7 NICOTINIC RECEPTOR BY PHOSPHORYLATION

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α7 is one of the most abundant nicotinic receptors in the nervous system. It is highly expressed in the brain and contributes to cognition, attention, and working memory. The receptor contains an extracellular domain, which carries the agonist binding sites; a transmembrane domain, which forms the ion pore and the gate; and an intracellular domain (ICD), which contains sites for modulation and intracellular signaling. The concept of α7 as a dual metabotropic/ionotropic receptor is attracting increasing attention. Reciprocal crosstalk between phosphorylation-dependent signaling and receptor function has been proposed. However, the regulation of ion channel function by phosphorylation remains unclear. We here explored how tyrosine phosphorylation at ICD affects single-channel function of human α7 by combining site-directed mutagenesis and mammalian cell expression with patch-clamp recordings. We generated two mutant α7 receptors to prevent phosphorylation of key tyrosine residues (α7Y386F and α7Y442F). Wild-type α7 channel activity elicited by ACh consists of brief and isolated openings and less often as few brief openings in quick succession (bursts). We found that the mutations do not affect single-channel amplitude. However, visual inspection of the recordings showed sporadically long-duration bursts that were not detected in the wild-type receptor. To quantify these differences, we analyzed the burst duration histograms. Whereas wild-type α7 histograms are fitted by 2 components, about 40% of the recordings of α7Y386F and 20% of α7Y442F show a third component. This component corresponds to a novel population of long-duration bursts. Our results show that the decrease of phosphorylation increases open-channel duration, probably by reducing fast desensitization, which is the main pathway for terminating an opening event in α7.These findings reveal a novel regulatory mechanism that may be important in receptor function.

Keywords: ion channel, α7 nicotinic receptor, phosphorylation, patch-clamp, mutagenesis.

(757) **STUDY OF THE CALCIUM BINDING SITE IN THE PLASMA MEMBRANE CALCIUM PUMP BY THE PHOTOREACTIVE PROBE AZIDO-RUTHENIUM**

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Abstract: The Plasma Membrane Calcium ATPase (PMCA) is a P-type ATPase that maintains the homeostasis of Ca^{2+} in eukaryotic cells. It couples the transport of Ca^{2+} with the hydrolysis of ATP. The structure of PMCA is still not solved, and only limited information is available of ligand binding sites. The purpose of this work is to identify and characterize the calcium binding site of PMCA. We synthesized azido-ruthenium (AzRu), a photoactivatable reagent designed to obtain structural information, which binds covalently and specifically to Ca^{2+}-binding proteins after irradiation at 290 nm. The experiments were performed with purified PMCA from human erythrocytes. The results show that AzRu irreversibly inhibits PMCA activity. The time course of production of inorganic phosphate was measured before and after the addition of AzRu, in conditions where photolysis of the inhibitor was prevented. It was observed that the rate of inhibition by AzRu decreases by increasing $[Ca^{2+}]$ in the medium, whereas this rate remains unaffected by increasing $[Mg^{2+}]$. These results suggest that the calcium binding site would be involved in the inhibition process. Assays with $[\gamma\text{-}^{32}P]$ ATP indicate that inhibition of the ATPase activity was accompanied by an increase in the phosphorylated intermediate levels, which suggests that AzRu could be blocking the dephosphorylation of the pump. The photolabeling experiments with AzRu were observed by mass spectrometry using calmodulin (CaM) and lysozyme. CaM-AzRu adducts were observed but not with lysozyme. The results confirm the specific interaction of AzRu with calcium dependent proteins. Photolabeling mass spectra of PMCA with AzRu showed the disappearance of signals from certain peptides from the cytoplasmic domains suggesting an interaction with AzRu. Due to the difficulty of studying membrane proteins by mass spectrometry, we are evaluating an optimization of the study of the transmembrane domain where the PMCA-AzRu adduct would be found.

Keywords: Plasma Membrane Calcium ATPase, Azido-Ruthenium reagent, Ca^{2+} binding site, mass spectrometry

(692) **STUDY OF THE MECHANICAL GATING OF THE AQUAPORIN FAPIP2;1 FROM STRAWBERRY**

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Recently, we published the first works that experimentally demonstrate the direct regulation of both plant and animal aquaporins by membrane tension (s) changes. Our recent work with *Bv*TIP1;2 and *Bv*PIP2;1 from red beet show that the first one is mechanosentive while the second one is not. This different behavior could be related to the differential distribution of GxxxG sequences (suggested to be responsible for mechanosensitivity in ion channels) observed by homology modeling. Previously we demonstrated that hAQP1 is a mechanosensitive channel. Both the water permeability (P_f) and the elastic volumetric coefficient (E) are negatively correlated in experiments with hAQP1 and *Bv*TIP1;2 (R^2>0.98), indicating that these aquaporins close with s increments. Phylogenetics analysis indicate that AQP1 and PIPs share a common ancestor and that divergence of the AQP1-PIP and TIPs groups occurred earlier in evolution. Therefore, three hypotheses arise for mechanosensitivity: 1) it would had been present in the ancestor of AQP1-PIPs and TIPs and was lost in PIPs; 2) it appeared in AQP1-PIPs and TIPs by separately; 3) *Bv*PIP2;1 is a mechanosensitive channel but less sensitive than hAQP1 and *Bv*TIP1;2. Now, we are studying the mechanosensitive properties of *Fa*PIP2;1 from strawberry. The homology model of *Fa*PIP2;1 shows differences with *Bv*PIP2;1 and similarities with *Bv*TIP1;2, suggesting that *Fa*PIP2;1 could behave as a mechanosensitive aquaporin. By means of simultaneous V and P measurements in Xenopus oocytes expressing *Fa*PIP2;1 we determine P_f and E under osmotic gradients. Our previous results with osmotic gradients up to 200 $mOmol.Kg_{H2O}^{-1}$ (E=0.5-0.8 KPa) showed that *Bv*PIP2;1 is not regulated by membrane tension changes. Preliminary results with *Fa*PIP2;1 show that this aquaporin does not behave as a mechanosenstive channel, at least up to changes induced with 200 $mOmol.Kg_{H2O}^{-1}$.

Keywords: water permeability; aquaporin; PIP; membrane tension; volumetric elastic modulus

(1757) **AMYLOID β PEPTIDE DECREASES α7 RECEPTOR POTENTIATION**

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Amyloid β peptide (Aβ) is a key player in the development of Alzheimer disease (AD). Aβ is visible as the primary component of senile plaques in the brains of Alzheimer's patients. Cholinergic activity mediated by human α7 nicotinic receptors is decreased in AD, and potentiation of α7 by positive allosteric modulators (PAMs) is emerging as a novel therapeutic strategy for improving memory and cognition. There are reports showing functional interaction of Aβ with α7, but the reported effects are very varied and the underlying mechanisms are not clear. Here we explored the effect of Aβ1-40 and Aβ1-42 on human α7 at the patch-clamp single-channel level. α7 channel activity elicited by 100 μM ACh consists of brief and iso-

lated openings. In the presence of PAMs, open channel lifetime is increased and openings appear grouped in long activation episodes. The type II PAM PNU-120596 (1 μM) prolongs open durations and elicits activation episodes of ~2 s. In the presence of Aβ there is a statistically significant decrease in the mean duration of the potentiated activation episodes, which is 2.6-fold at 100 nM Aβ1-40 ($p<0.001$, n=11) and 2-fold at 100 nM Aβ1-42 ($p<0.05$, n=10). To determine if the effect is specific for PNU-120596, we also tested NS-1738, which is an α7 type I PAM. Again, a 2-fold reduction in the duration of the activation episodes is observed ($p<0.001$). Complementary fluorescence spectroscopic studies using a fluorescent channel blocker, crystal violet, that binds with different affinities to resting and desensitized receptors provide insights into the functional changes. Our results demonstrate that Aβ inhibits potentiation of human α7, probably through an allosteric mechanism which involves slow block or increased desensitization. Deciphering the functional interaction between α7 and Aβ contributes to the understanding of the involvement of α7 in the pathophysiology of Alzheimer disease.

Keywords: Amyloid β peptide, nicotinic receptor, Patch-clamp, ion channel

(547) **ALL YOU NEED IS COFFEE**

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Cholinergic deficit is regarded as an important factor responsible for Alzheimer's disease symptoms. Two molecular targets for the treatment of this disease are acetylcholinesterase (AChE) and nicotinic receptor (nAChR). Caffeine (CAFF) acts as a non-competitive inhibitor of AChE but its mechanism of action on nAChR is still unknown. To this end, we first explored if CAFF influences the nAChR conformational state using the AChR conformational-sensitive probe crystal violet (CrV) and AChR-rich membranes from *T. californica*. CAFF induced changes in the KD value of CrV in a concentration-dependent manner taking the nAChR to a state close to the desensitized one. In the presence of α-bungarotoxin, a specific nAChR competitive antagonist, high concentrations of CAFF increased the KD value of CrV, compatible with a competition for the CrV site in the channel pore. The same effect was seen with galantamine, an AChE inhibitor and partial agonist of nAChR. To understand the molecular mechanism underlying the conformational changes of the nAChR, we expressed adult muscle or neuronal a7 nAChRs in BOSC cells, and performed single channel recordings with different CAFF concentrations in the presence or absence of ACh. At low concentrations (1-300 μM), CAFF activated muscle and a7 nAChRs, and the activation profile was independent of CAFF concentration. On the other hand, at high CAFF concentrations (up to 20 mM), the mean open duration decreased, the relative area of the briefer closed component and the cluster duration increased, and a flickering behavior was observed, these suggesting that CAFF acts as an open channel blocker. Thus, we here demonstrate a dual effect of CAFF on muscle and a7 nAChRs, behaving as a weak agonist at low concentrations and as a negative modulator at high concentrations. Our results bring new information about the mechanism of modulation of pharmacology targets for the design of new therapies for the intervention in neurological diseases.

Key words: Caffeine, nicotinic receptor, crystal violet, single channel recordings.

STRUCTURAL AND FUNCTIONAL BIOCHEMISTRY 2

(224) **CHARACTERIZATION OF THE CHDL DOMAINS OF RapA, AN EXTRACELLULAR LECTIN FROM *Rhizobium leguminosarum* INVOLVED IN BIOFILM MATRIX ASSEMBLY**

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Abstract: In natural environments microbes live in multicellular structures called biofilms, in which cells are embedded in a matrix of self-produced biopolymers. The extracellular matrix determines the immediate conditions of life of biofilm cells, and also provides adhesion to surfaces and mechanical stability. Despite the importance of the matrix in the biofilm mode of life, very little is known about the mechanisms leading to matrix assembly and the extracellular proteins involved in this process. We have recently characterized the RapA lectin secreted by *Rhizobium leguminosarum*, which has a profound impact in the organization of the biofilm matrix. The RapA lectin interacts specifically with the acidic exopolysaccharides (EPS/CPS) produced by *R. leguminosarum* in a calcium-dependent manner. The protein is composed of two CHDL domains that are similar to the extracellular domains of eukaryotic cadherins. Aiming to obtain a tool to study the development of the matrix during biofilm formation, we dissect the protein in its two halves, and study the properties of the individual CHDL domains. The domains were amplified by PCR using specific primers, cloned as His tag fusions and purified from the soluble fraction of *Escherichia coli* BL21(DE3) induced cells. The purified domains were analyzed by CD spectroscopy with the addition of calcium ions, and in a functional test by means of a binding inhibition assay (BIA) with the EPS. Our results show that the lectin activity is confined to the carboxy terminal CHDL domain of RapA, which contains the calcium binding site and is able to bind to the EPS, although with less affinity than the entire RapA lectin.

Keywords: lectin, calcium, biofilm matrix, exopolysaccharide.

(279) **STRUCTURE OF DIFFERENT PHOSPHORYLATED STATES OF PLASMA MEMBRANE CALCIUM PUMP**

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The control of the cytoplasmic calcium homeostasis relies on many transporters like the Plasma Membrane Calcium Pump (PMCA) and the Sarcoplasmic Reticulum Calcium Pump (SERCA) which belong to the P-ATPase family. At difference with SERCA, structure and function of PMCA has not been fully elucidated yet, because obtaining suitable preparations for X-ray crystal diffraction and NMR techniques was unsuccessful.

To elucidate the structural changes produced in PMCA during the reaction cycle we tested a purified preparation of the pump with fluoride complexes of beryllium, aluminum and magnesium, each one stabilizing different analogues of phosphorylated intermediates in P-ATPases. These blockers were previously assayed in SERCA leading to different conformational states. To follow the binding of fluoride complexes by fluorescence we employed eosin, which binds to the N domain of PMCA. Quantum yield of the bound probe decreased in the presence of fluoride complexes indicating more exposure to solvent. The magnitude of this change depended on the presence of calcium and the complex identity.

Following the kinetics of the conformational change, we propose a model to explain how these complexes stabilize phosphorylated states in PMCA. The detectable conformational change associated with an intermediate of the reaction cycle also allows us to develop a method to measure PMCA activity using a fluorescence approach. Finally, we prepared and refined PMCA structural models based on homology with SERCA and Na^+/K^+ ATPase at different states, to understand the changes at the nucleotide-binding domain during the catalytic cycle of PMCA.

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Keywords: fluoride complexes, fluorescence, homology modelling

(727) **HOW DEEP IS YOUR BLUE? A NOVEL COPPER CONTAINING NITRITE REDUCTASE FROM A THERMO-**

PHILIC BACTERIUM ISOLATED FROM THE SOUTH AFRICAN DEEP SURFACE

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Abstract: many microorganisms of the genus *Thermus* have been isolated from hot environments around the world but particularly *Thermus scotoductus* SA-01 was isolated from fissure water collected 3.2 km below the surface in a South African gold mine. Denitrification is a relevant energy pathway in this kind of microorganisms and the presence of both a cytochrome cd_1 (NirS) and a copper containing nitrite reductase (NirK) is exceptional. The genome of *T. scotoductus* SA-01 showed the presence of genes for both enzymes. Here we present the expression, purification and the structural and functional characterization of NirK from *T. scotoductus* SA-01 (*Ts*NirK). *Escherichia coli* BL21(DE3) Gold cells were used to express the optimized Tsc_c17620 gene inserted into pET22b(+). The recombinant *Ts*NirK was purified by several chromatographic steps and the turquoise pure enzyme showed different spectroscopic features from those observed in green/blue NirKs. Gel filtration analysis showed an elution peak corresponding to a homotrimer and metal analysis reported 3 mol copper per mol monomer. We proceeded on protein crystallization screening assays and *Ts*NirK crystals developed in several conditions. Single crystals grown in 0.2 M $CaCl_2$.2 H_2O 0.1 M HEPES sodium pH: 7.5 28% w/v PEG 400 and diffracted at 1.63 **Å** (beamline I04-I, Diamond Synchrotron, UK). The obtained overall structure reveals a homotrimer with 3 copper atoms per each subunit. The catalytic type 2 copper center (T2Cu) is 12.6 **Å** His-Cys bridged to a non-common type 1 copper center ($T1Cu_{N\text{-}ter}$). An extra cupredoxin domain is located at the C termini of each monomer and binds a normal type 1 copper center ($T1Cu_{C\text{-}ter}$) that resembles amicyanin. Thermal stability, kinetic properties and structural features make this enzyme a good candidate for the development of nitrite biosensors.

(851) **BIOPHISICAL AND BIOCHEMICAL CHARACTERIZATION OF PLASMID-MEDIATED COLISTIN RESISTANCE DETERMINAT MCR-1**

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Polimyxin antibiotics, as colistin, are commonly referred as a last-chance to treat multidrug-resistant gram-negative infections. This positively charged peptide can binds to the negatively charged lipid A core, disrupting the bacterial outer membrane. Unfortunately, in 2016 a transferable colistin resistance mechanism was reported, a plasmid-encoded phosphoethanolamine transferase MCR that has rapidly disseminated worldwide. MCR catalyzes the transfer of positively charged phosphoethanolamine onto lipid A, decreasing the net negative charge and preventing colistin binding and antibiotic success. It is a metalloenzyme embedded in the inner membrane, with the catalytic center in a periplasmic soluble domain. Recently, four structures of the soluble catalytic domain have been reported, depicting a zinc metalloprotein. Despite structural equivalence, the stoichiometry on metal content is controversial. This information is pivotal for further mechanistic analysis to gain information for inhibitor design.

Based in previous studies, we have cloned the catalytic periplasmic domain in different expression vectors. According to soluble protein yields, BL21 (DE3) *E. coli* cells expressing the catalytic domain with a His 6x tag and a TEV protease recognition site were selected for further protein production. Bacterial culture condition were tested with different Zn(II) ion concentrations, in order to evaluate the metal content in the purified protein using PAR as a Zn indicator. In all tested conditions, the catalytic domain was able to take up to one Zn(II) ion equivalent.

Metal quelator EDTA was used to obtain apo-proteins derivatives, to further characterize the metal active site using Co(II) as spectroscopic probe. CoCo and ZnCo adduct were produced and the ligand field bands showed a tetrahedral geometry pattern. Secondary and tertiary structural features and relative stability of the apo and metal adducts were also tested by circular dichroism spectroscopy.

(1277) **DESK THERMOSENSING RELIES ON A COILED COIL SWITCH**

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Propagation of a signal through transmembrane segments of a sensor protein is key at the initial stage of many complex signaling processes. The thermosensor histidine kinase DesK from *Bacillus subtilis* senses changes in membrane fluidity initiating an adaptive response. Structural changes in DesK have been implicated in transmembrane signaling, but direct evidence is still lacking. Based on structure-guided mutagenesis, we have proposed a mechanism of DesK-mediated signal sensing and transduction. Specifically, stabilization/destabilization of a 2-helix coiled coil, which connects the transmembrane sensory domain of DesK to its cytosolic catalytic region, is crucial to control its signaling state. Computational modeling and simulations reveal couplings between protein, water and membrane mechanics favoring such conformational changes. We propose that membrane thickening is the main driving force for signal sensing, and that it acts by inducing helix stretching and rotation prompting an asymmetric kinase-competent state. At present, we are dedicating our efforts to crystallize full-length DesK for X-ray structure determination, expecting to shed light on DesK's transmembrane sensing mechanics.

(1327) **ARCHITECTURE OF MECR1: A MEMBRANE-EMBEDDED METALLOPROTEASE DOMAIN POISED TO RECEIVE INFORMATION FROM THE EXTRACELLULAR SENSOR DOMAIN**

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Abstract: **The signal transducer protein MecR1 from methicillin resistant *Staphylococcus aureus* regulates the expression of PBP2a, a protein that is not inhibited by clinical concentrations of most β-lactam antibiotics**. In this study we aimed at identify a structural model that present a better comprehension of the signal transduction mediated by MecR1, which is an attractive target for drug development.

By integrating homology modeling, residue co-evolution analysis, dynamic simulations and docking, we generated a model for the full length MecR1 protein. This model was used to design a series of constructions to further evaluate the topology in-vitro. Fluorescence spectroscopy and Proteinase K susceptibility assays in MecR1. eGFP truncated fusions and TEV peptidase susceptibility assays in MecR1.E205A.TEV insertions were carried out. As a whole, these data allowed us to corroborate the orientation of most transmembrane helixes, it showed that the peptide S63-T102 is extracellular (and not a TM helix) and that Loop 4 in the metalloprotease domain N194 to D213 has low flexibility, which could be due to compaction or membrane interaction. To further evaluate if the effector domain has membrane localization, the cytoplasmic domain (cytMecR1), was expressed in *E. coli*, and we found it was membrane embedded. These results support the existence of a reentering alpha-helix in the metalloprotease domain that tightly anchors this domain to the membrane, in accordance with our computational model.

In conclusion, we presented a model for full length MecR1 in which the metalloprotease domain is embedded in the membrane, defining a hydrophilic chamber. A reentering loop connecting the metal ligands tightly anchors the metalloprotease domain to the membrane (even in the absence of the rest of the TM helixes) and reaches to the outer leaflet of the membrane where it is posed to interact with

the sensor domain loops that show altered mobility upon antibiotic binding.

Keywords: Staphylococcus aureus, b-lactam antibiotics, resistance, MecR1, signal transduction.

(1285) **CHARACTERIZATION OF THE MECHANISM OF ACTIVATION OF THE *vraSRT* SYSTEM OF *Staphylococcus aureus* USING ANTIBIOTIC-DERIVATED PHOTOPROBES**

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Staphylococcus aureus is the leading cause of both nosocomial and community-acquired infections. In *S. aureus* the *vraSRT* three-component system acts as a sentinel that can rapidly sense cell wall peptidoglycan damage and coordinate a response that leads to resistance to β-lactam and glycopeptide antibiotics. VraS and VraR encode a histidin-kinase and a response regulator, respectively. However, the rol of VraT is yet unknown. We still do not understand how VraS is activated in response to cell wall-active antibiotics.

The interaction between VraS (the possible sensor), VraT and different photoprobes derived from ampicillin was studied. The photoaffinity probes were used for covalent labelling of VraS and VraT in *E. coli* spheroplasts, and the interaction was evidenced by an electrophoretic mobility shift in the case of VraS, although no interaction was seen with VraT. We used a *S. aureus* reporter strain to confirm that the ampicillin-derived photoprobes effectively activate the *vraSRT* system. The VraS-photoprobe complexes were purified and analyzed by MALDI-TOF/TOF. In this study we also addressed the topology of VraT in membranes. Using a Proteinase K susceptibility assay we determined that the C-terminal domain of VraT has extra-cytoplasmatic location.

Keywords: Staphylococcus aureus; *vraSRT*; photoaffinity ampicillin-derived probes.

(1381) **FUNCTIONAL EXPRESSION OF HUMAN PARK9-ATP13A2 P5-ATPase IN *Saccharomyces cerevisiae*.**

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The P-type ion pumps are membrane transporters energized by ATP-hydrolysis. They were classified into five subfamilies termed P1-P5; the substrate specificity of P5 subfamily is still unknown. Five genes named *ATP13A1-ATP13A5* that belong to the P5-ATPases are present in humans, while two named Spf1p and Ypk9p were found in the yeast *S. cerevisiae*. By DNA sequence alignment it was shown that P5-ATPases were classified in groups P5A and P5B; the mouse gene Atp13a1 and the yeast gene coding Spfp1 (Yel031w) are members of the first group, while the mouse genes Atp13a2-Atp13a5 and the yeast gene coding Ypk9p (Yor291w) are clustered into the second one. Mutations of the ATP13A2 gene, also known as PARK9, are associated with a form of Parkinson's Disease (Kufor-Rakeb syndrome), a form of Neuronal Ceroid Lipofuscinosis (CNL12) and hereditary spastic paraplegia (SPG78). ATP13A2 is localized in lysosomes and late endosomes (LEs). Dysfunction of this protein diminishes the lysosomal degradation, the autophagic flux and the exosome externalization. In order to advance the biochemical characterization of ATP13A2, *S.c* cells were transformed with the pMP625 vector coding for GFP tagged-ATP13A2 protein and the clone exhibiting the highest GFP-fluorescence intensity selected. Yeasts expressing GFP-ATP13A2 showed a reduced growing rate and an autophagic phenotype characterized by vacuolar multivesicularization. The recombinant fusion protein was purified from microsomal membranes by pseudo-affinity chromatography. Analysis of the eluate by SDS-PAGE and western blot indicated that ATP13A2 was successfully expressed and had the expected molecular size. Preliminary experiments measuring the release of free phosphate from ATP by the Baginsky's assay showed that the purified GFP-ATP13A2 is a functional ATPase.

Keywords: P5-ATPases, Structure and function, Parkinson´s disease

(154) **STRUCTURE-FUNCTION RELATIONSHIP OF A NOVEL LECTIN FROM *Pomacea diffusa* SNAIL EGGS**

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Pomacea freshwater snails lay eggs above water exposing them to environmental stressful conditions and predation. Despite this, *P. canaliculata* and *P. maculata* have been able to establish themselves out of their native range and became important invasive species. The perivitelline fluid (PVF) of their eggs contains antinutritive carotenoproteins and a neurotoxin, which participate in the embryo defence system against predation. By contrast, the PVF of the non-invasive species *P. scalaris* lacks of neurotoxin and its carotenoprotein (PsSc) besides its antinutritive role, displays a potent lectin activity also involved in the egg defence system.

In this work, we studied the PVF of *P. diffusa* a closely related species to *P. scalaris* searching for neurotoxins and isolated and characterized a carotenoprotein named PdPV1. Molecular weight and global shape of PdPV1 were estimated by PAGE, SEC and SAXS. N-terminal and mass spectrometry followed by full cDNA sequencing and bioinformatic analysis were performed on each subunit. PdPV1 resistance to gastrointestinal digestion was evaluated *in vitro* and its lectin activity characterized by hemagglutination assays.

PdPV1 is an anisometric oligomer of 422 KDa resistant to *in vitro* gastrointestinal digestion. It is composed by 6 subunits of ca. 26 KDa showing >90% sequence similarity with PsSc. Though PdPV1 was able to agglutinate different erythrocytes no sequence similarity to any known lectin could be found. Its activity was inhibited by galactose, galactosamine and N-Acetylgalactosamine. No neurotoxins were found in the PVF.

The combination of a carotenoprotein with lectin activity and the absence of neurotoxins as was previously described for *P. scalaris,* suggest an alternative defence system against predation involving lectins. These remarkable defensive differences among *Pomacea* species may explain the invasiveness and the wider geographical distribution of *P. canaliculata* and *P. maculata* compared to *P. scalaris* and *P. diffusa*.

Keywords: lectin, predation, egg defences

(1391) **STRUCTURALLY EXPOSED SITES AND INTERACTORS OF BCY1, THE REGULATORY SUBUNIT OF PROTEIN KINASE A FROM *Saccharomyces cerevisiae*, ANALYZED BY MASS SPECTROMETRY.**

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Protein kinase A is an inactive tetramer formed by a dimer of regulatory subunit (R2) bound to two monomers of catalytic subunit (C). When two molecules of cAMP bind to each R, the affinity between R and C is decreased and active C subunit is released. In mammals, the N-terminus of R subunits (DD) is responsible for the homo-dimerization and for anchoring the AKAPs and thus localizes the signal transduction pathway. In yeast, PKA is also a tetramer; the N-terminus of Bcy1 (R subunit) is responsible for dimerization and for its own localization. No AKAPs have been described yet except for our own preliminary report.

Our study was carried out by proteomic approaches. On one hand, the goal was to obtain information on exposed sites in Bcy1 to reconstruct/model the whole structure, studying three proteolytic bands derived from aged Bcy1 by MALDI TOF-TOF and nano-LC-ESI-Or-

bitrap (QExactive). On the other hand, to investigate the oligomeric of structure Bcy1 *in vivo* and search for *in vivo* interactors by overexpression in yeast of its dimerization/docking domain, 1-50 Bcy1 (DD), tagged with thioredoxin-His in its N-terminus and analysis of the pulled down proteins after Ni-agarose.

The first approach indicates that Bcy1 has clear points of endogenous proteolysis that speak about its folding.

The results of the other approach suggest tetramer formation *in vivo* because of the intact endogenous Bcy1 presence in the pulled down using overexpressed His-Tagged DD domain. Together with Bcy1 a good number of proteins were also pulled down. Some of these proteins were selected for future studies as putative Bcy1 interactors according to rigurous criteria.

In conclusion, we have obtained information regarding the apparently exposed/labile sites of Bcy1 and the potential Bcy1 interactors, and demonstrate the tetramer formation *in vivo*.

Keywords: PKA, Bcy1, mass spectrometry, interactors, proteomics.

ONCOLOGY-ONCOIMMUNOLOGY 5

(336) ABERRANT O-GLYCOSYLATION MODULATES AGGRESSIVENESS IN NEUROBLASTOMA

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The role of glycans in the interaction between cells and microenvironment in adult cancer is widely reported, but little is known about their role in pediatric cancer such as Neuroblastoma (NB). Hence, the study of aberrant glycosylation is a promising field to understand NB transformation. In this work, we characterized the expression of glycans by flow cytometry, particularly truncated and Lewis family in Core 2 O-glycans, and the expression of enzymes involved in their biosynthesis (glycoenzymes) by qRT-PCR in five human NB cell lines and patients' derived primary tumor samples with different MYCN status. We also studied the role of Core 2-O-glycans in cell behaviour by C2GNT1 silencing, a key enzyme in their synthesis, as well as the binding to selectins. Results showed minimal expression of truncated glycans in all cell lines tested and higher expression of Lewis family antigens, associated with the MYCN-amplification status. High expression of C2GNT1 demonstrated Core 2 O-glycans association to MYCN-amplified cells as well ($p<0.001$). The main glycoenzymes responsible for Lewis antigens expression were sialyltransferase ST3Gal4 and fucosyltransferases (FUT) FUT4, 7 and 9. Similar results were obtained in patients' samples, showing overexpression of ST3Gals and FUTs in MYCN-amplified tumors in contrast with MYCN-non amplified ones, which suggest an overlapping role in these functions. Furthermore, MYCN-amplified cells showed higher E- and P- Selectin binding. Silencing of C2GNT1 provoked a decrease in cell attachment, proliferation and migration capacity *in vitro* ($p<0.05$). We also found a downmodulation in the expression of Lewis family glycans and accordingly, a significant decrease in E- and P-selectin binding ($p<0.05$). In this work, we demonstrated the impact of O-glycans in malignant NB cell behaviour, in particular of Lewis family glycans associated with Core-2 branching, the enzymes involved in their biosynthesis and their relation to MYCN amplification.

Keywords: Neuroblastoma, Glycans, Lewis Family, Glycoenzymes, Selectins

(1779) AMINOFLAVONE SENSITIZES BREAST CANCER CELLS TO TAMOXIFEN AND ENHANCES THE EXPRESSION OF TUMOR SUPPRESSOR MIRNA205

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Breast cancer is the most common malignancy in women. The anti-estrogen Tamoxifen (TMX) is often used to treat patients with estrogen receptor positive (ER+) tumors. However, 40% of these tumors acquire resistance to TMX. This drug promotes breast tumor initiating cell (TIC) formation, which could lead to relapse. Aminoflavone (AF), a ligand of the aryl hydrocarbon receptor, exhibits antitumor efficacy in patients with ER+ breast cancer and reduces TIC number in breast cancer. The purpose of our current study is to determine whether AF combined with TMX (co-treatment) increases anticancer efficacy and reduces the tendency for TMX to increase TICs. We studied the cytotoxic effect of TMX, AF and both drugs combined in the LM05-mix (ER+) cell line using the MTS assay. AF induced 6-fold greater cytotoxicity than TMX ($p<0.05$) and co-treatment resulted in greater cytotoxicity than either drug used singly (60-fold higher than TMX and 9-fold higher than AF) ($p<0.05$). Treatment with AF alone or in combination with TMX caused a significant decrease in mammosphere numbers as compared to control (vehicle exposed): 18 fold and 50 fold respectively ($p<0.05$). The number of mammospheres formed after treatment with TMX was 15-fold higher than mammospheres after treatment with AF and 42-fold higher than mammospheres after co-treatment ($p<0.05$). Treatment with AF or co-treatment generated mammospheres of a smaller diameter than those obtained in the control: AF (1.5 fold decrease and 1.6 fold decrease respectively) ($p<0.05$). Finally, we investigated the miRNA profile in control and treated MCF-7 cells using miRNA-sequencing. AF caused a nearly 2-fold increase in miRNA-205 as confirmed by qPCR ($p<0.0001$). miRNA-205 exhibits tumor suppressor actions and tends to be down-regulated in TMX-resistant cells and in tumors of patients who relapse after TMX treatment. In vivo studies are underway to determine whether AF decreases the pro-tumoral effect of TMX.

(1150) DRUG REPOSITIONING FOR CANCER TREATMENT: METRONOMIC CHEMOTHERAPY WITH CYCLOPHOSPHAMIDE (CY) AND LOSARTAN (LOS) FOR THE M-234p MURINE MAMMARY ADENOCARCINOMA

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* contribuyeron igualmente

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Metronomic chemotherapy (MCT) refers to the chronic, equally spaced, delivery of low doses of chemotherapeutic drugs, without extended interruptions. Drug repositioning in oncology refers to the use of drugs formulated for other indications that showed antitumor potential. Cy is an alquilating agent with toxic action on proliferating cells. LOS is an antagonist of angiotensin II receptor, used to treat hypertension. Our aim was to study the combined effect of Cy+Los administered metronomically. Female BALB/c mice were challenged s.c. with M-234p (day 0) and distributed, on day 8, into 4 groups (n=6/group) treated as follows, GI: Control, no further treatment; GII: Cy 25mg/kg/day in the drinking water; GIII: LOS 200mg/kg/day in the drinking water; GIV: Treated as GII + GIII. Mice were weighted and tumor volume measured 3 times/week. When tumors were exponentially growing, mice were euthanized, tumors excised and used for immunohistochemistry (IHC). A significant inhibition of tumor growth was evident in group IV compared to GI, GII and GIII ($P<0.001$). Tumor doubling and survival times were also significantly higher in GIV ($P<0.001$). No weight losses were observed. IHC analysis showed higher N° of $CD4^+$ and CD^+8 lymphocytes and lower N° of $Ki67^+$ cells in GII and GIV, without reaching statistical significance. To evaluate the anti-metastatic effect of the therapy, mice were inoculated i.v. with $5x10^5$ M-234p cells. On day 3, mice were distributed in the same experimental groups. When the first mouse showed signs of metastatic disease, all of them were euthanized, lungs excised and stained to highlight metastasis. The N° of lung metastasis was lower in GII and GIV. In conclusion: MCT with Cy+LOS inhibited

M-234p growth and increased mice survival without general toxic effects; the combined treatment was more effective than the individual drugs; Cy and Cy+LOS diminished lung metastases; these results show the convenience of using Losartan in antitumor treatments.

Key Words: Metronomic chemotherapy, Drug repositioning, Breast cancer, Cyclophosphamide, Losartan

(112) **EFFECT OF RETINOIC ACID (ATRA) TREATMENT ON GROWTH AND MALIGNANT PROGRESSION OF HUMAN MAMMARY TUMOR CELLS OVEREXPRESSING PROTEIN KINASE C ALPHA (PKCα)**

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Abstract: PKC enzymes constitute a family of serine-threonine kinases that control numerous cellular functions, such as growth, apoptosis and malignant transformation, whereas the retinoid system is involved in cell differentiation.

In this work, we studied the effect of retinoid treatment on characteristics associated with tumor progression of MDA-MB231 and T47D cell lines overexpressing PKCα.

PKCα overexpression was unable to alter the proliferative capacity of both cell lines *in vitro*. When we assess the effect of ATRA, T47D-PKCα cells significantly decrease their proliferative capacity, whereas MDA-MB231-PKCα did not respond to retinoid treatment. Cell cycle analysis, by flow cytometry, showed that ATRA induces an arrest in the G1 phase, only in the T47D subline.

Next, we analyzed the functionality of Retinoic Acid Response Elements (RARE) by a gene reporter assay. Both T47D and MDA-MB231 derived sublines responded to ATRA increasing RARE activity. Then, we studied AP1 sites since retinoids effects also involve the trans-repression of this element. Although T47D sublines showed a decrease in AP1 activity when treated with ATRA, no effect was observed in MDA-MB231.

Retinoid receptors expression (analyzed by RT-qPCR) showed that MDA-MB231 cells were unable to increase RARβ and RARγ levels in response to retinoid treatment, as it was observed in T47D cells. As the repression of AP1 sites, induced by ATRA, has been assigned to RARβ we overexpress this receptor in MDA-MB231 cells. Now a clear trans-repression of AP1 sites was observed after ATRA treatment.

Our results suggest that PKCα sensitize T47D cell to retinoid treatment, which correlates our previous observations in murine lines. On the other hand, the lack of response to retinoid treatment of MDA-MB231 could be due to the inability to trans-repress AP1 sites, due the lack of RARβ expression.

Keywords: PKCα, ATRA, RARE sites, AP1 sites.

(786) **THE ANTITUMOR EFFECT OF CIGB-300 PEPTIDE, A PROTEIN KINASE CK2 INHIBITOR, IN MAMMARY CANCER MODELS**

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CK2 is a serine-threonine kinase that has been involved in growth, proliferation and cell apoptosis. CK2 has a constitutive expression and it has more than 300 substrates. In recent years, CK2 became an interesting target for anticancer therapies. Inhibition of CK2 showed antitumor activity in different types of cancer. CIGB-300 is a peptidic inhibitor of CK2, designed to bind to the phospho-acceptor domain of CK2 substrates, impairing the correct phosphorylation by the enzyme. Because breast cancer is one of the main tumor types in which CK2 is overexpressed, we focus on the effect of CK2 inhibition as a modulator of key features of breast cancer cell biology.

Previously, we showed that CIGB-300 reduced the proliferation, spreading capability and ERK phosphorylation of tumor cells in a murine breast cancer model. The aim of the present study was to evaluate the role of inhibition of CK2 by CIGB-300 in murine and human mammary carcinoma cell lines. For this purpose, we evaluated the action of CIGB-300 on viability, apoptosis and clonogenic capacity using three different breast tumor cell lines, MCF7 (positive for estrogen, progesterone and HER2-neu receptors), MDA MB 231 (representative of triple negative tumors) and F3II, a murine mammary carcinoma. As compared the potential of CK2 inhibition in these models we included another chemical inhibitor of the enzyme, CX-4945.

Our results showed that CIGB-300 reduced the viability of MDA MB 231 (IC50=120 μM) and MCF-7 (IC50=140 μM) ($p<0.05$, ANOVA). We observed that in both human and murine cell lines CIGB-300 exerts a pro-apoptotic action (TUNEL assay, $p<0.05$, $\chi 2$). We also found that the inhibition of CK2 by CIGB-300 decreased the clonogenic capacity of F3II, MCF-7 and MDA-MB-231 ($p<0.05$, ANOVA).

These preliminary results support the relevance of CK2 enzyme as molecular target in breast tumor cells.

Keywords: CIGB300, CK2, breast cancer models

(971) **EFFECTS OF METFORMIN AND GLUCOSE RESTRICTION ON THE INVASIVE CAPACITY OF HCC CELLS: MODULATING AMPK ACTIVATION**

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AMPK is an antioncogenic kinase whose dysfunction has been related to hepatocellular carcinoma (HCC) etiology. We have demonstrated that AMPK induces cell-cycle arrest and death in HCC cells undergoing glucose restriction, and that AMPK is inhibited by PKA phosphorylation (S173), which prevents apoptotic death. AMPK decreases migration/invasion in different cell lines, but its effects in HCC cells remain unclear. We have previously shown that metformin and other AMPK activators decrease HCC cells migration. Metformin possesses promising antitumor effects via AMPK and inhibits PKA, as well.

We aim to study AMPK role on migration/invasion of HCC cells treated with metformin and its modulation by PKA.

Invasion assays showed that 1 (M1) and 5 (M5) mM metformin decreased invasion of C3A (M1:52.3±11.8*, M5:16.5±9.3* %) and HuH-7 (M1:67.5±4.9*, M5:24.8±12.1* % vs C) cells. AMPK knockdown prevented this effect. Besides, metformin induced apoptotic death.

When C3A cells were subjected to glucose starvation (GS), decrease of invasion (M1GS:24.8±14.1* % vs M1) and apoptosis percentage (M5GS:71.5±3.4* vs M5:52.4±6.8 %) were enhanced.

P-AMPKα(T172) and PKA phosphorylated substrates in cell lysates were analyzed. Metformin increased AMPK activation in a dose dependent manner, and decreased PKA activity at 5 mM. AMPK activation was significantly potentiated when cells were incubated with M5 combined with GS, whilst PKA activity was almost undetectable.

We performed invasion assays in C3A cells stable expressing WT and S173C forms of AMPK. S173C cells had lower invasion levels in response to AMPK activation (S173C: GS:36.5±3.9*, M1:16.5±1.3*, AICAR:4.0±0.4* % vs WT: GS:62.6±3.7, M1:51.9±7.4, AICAR:23.2±2.8 %).

We demonstrated that effects of metformin are enhanced by glucose restriction, and this is related, at least in part, to a greater activation of AMPK associated to PKA inhibition. We conclude that AMPK(S173) phosphorylation by PKA prevents AMPK anti-invasive effects. *$p<0.05$.

Keywords: hepatocarcinoma, metformin, AMPK, PKA, invasiveness

(464) **FLUOXETINE AND SERTRALINE TREATMENT PREVENTS STRESS-INDUCED ALTERATIONS IN TUMOR CELL CYCLE PROTEINS RELATED TO ENHANCED TUMOR PROGRESSION IN EL4 LYMPHOMA. PARTICIPATION OF THE IMMUNE SYSTEM.**

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Selective serotonin reuptake inhibitors, as fluoxetine (F) and sertraline (S), are frequently used for the treatment of stress-associated disorders. However, there is contradictory evidence about their effect on the immune system and cancer prognosis. We have previously reported that both F and S are able to revert chronic stress enhancement of EL-4 lymphoma growth. In the present work we studied the effect of F or S on chronic stress-induced alterations in tumor cell cycle progression and in the anti-tumor immunity. Female C57BL/6J mice were treated with of F or S and subjected (E) or not (C) to a heterotrophic chronic stress for five weeks. Then, mice were subcutaneously injected with EL4 T lymphoma cells to generate a solid tumor. Tumor mRNA expression of proteins related to regulation and promotion of cell cycle was assessed by qRT-PCR. mRNA levels of cyclins A2, D1 and D3 were increased in tumor from E mice compared to C ($P<0.01$). In addition, cell cycle inhibitors p15, p16, p21 and p27 mRNA levels were decreased in tumors from E animals compared to C ($P<0.01$). Both F and S treatment resulted in C values of mRNA expression of these regulatory proteins. To determine the involvement of alterations in anti-tumor immune response in the stress-induced tumor growth, adoptive transfer experiments were performed. For this purpose, irradiated animals were tail vein injected with lymphoid cells of C and E animals treated or not with F and S and then inoculated with tumor cells. Data indicated that tumor growth was increased in mice injected with lymphocytes from E mice as compared to those transferred with immune cells from C animals. Furthermore, mice injected with immune cells from E animals treated with F and S did not show this effect. These results suggest that antidepressant treatment prevents stress induced tumor growth by avoiding chronic stress effects on the antitumor immunity.

CHRONIC STRESS – ANTIDEPRESSANTS – LYMPHOMA – ANTI-TUMOR IMMUNITY – TUMOR PROGRESSION

(984) **META-TYROSINE INHIBITS THE PROLIFERATION OF ESTABLISHED METASTASES IN A HUMAN MAMMARY ADENOCARCINOMA AND INDUCES APOPTOSIS IN TUMOR CELLS WITHOUT APPARENT TOXIC EFFECTS FOR THE ORGANISM**

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The development of new strategies to limit metastatic growth after tumor removal is one of the greatest clinical challenges today. In previous work we have shown that meta-tyrosine (m-tyr), an isomer of tyrosine absent in normal proteins, is responsible, at least in part, for the phenomenon of Concomitant Tumor Resistance (CR); in which a tumor-bearing host inhibits the growth of secondary tumor implants. In this work we propose a deeper understanding of the molecular and antitumor mechanisms involved in CR as well as the study of the effectivity of its administration by the intraperitoneal (i.p) route. NOD *scid* gamma mice with a 41-day MDA-MB-231 human tumor received a daily i.p injection of 2.5 mg/mouse of m-tyr or saline (control) for 18 days. After the treatment all mice were sacrificed and lung metastases counted. At day 59 the number of metastases (Median [range]) was 10.0 [4-39] and 4.0 [0-14] for the control and m-tyr group respectively ($p<0.02$). We also analyzed signs of toxicity in BALB/c mice by the daily i.p administration of 2.5 mg/mouse of m-tyr for 39 days. Herein a hematological study was performed and the spleen was analyzed by flow cytometry. An increase in CD8+ T ($p<0.01$) and B220+ B cells ($p<0.02$) was observed in m-tyr group. Hematological analysis did not revealed significant differences in the number of granulocytes, monocytes and red blood cells. The number of total splenocytes was also higher in the m-tyr group ($p<0.03$). In addition, we observed apoptosis of murine LMM3 tumor cells cultured during 24 h in the presence of m-tyr ($p<0.05$). These findings suggest that the i.p route is effective in reducing the number of metastases and has no apparent toxic effects at the level of the organs studied. The increase in CD8+T lymphocytes caused by the daily administration of m-tyr could be associated with antitumor cytotoxicity and activation processes. The mechanism of activation of apoptosis caused by m-tyr in tumor cells still remains unknown.

Keywords: m-tyr, metastases, toxicity, human tumor, murine tumor.

(114) **NEW DRUGS FOR CANCER TREATMENT. EFFECT OF NORCANTHARIDIN AND LEVOGLUCOSENONE ON MALIGNANT PROGRESSION OF HUMAN AND MURINE MAMMARY TUMOR CELLS**

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Abstract: In the present work, we start the evaluation of norcantharidin (NCTD) and levoglucosenone (Levo) on *in vitro* processes associated to malignant progression, using human and murine mammary tumor-derived cell lines.

Levo results from pyrolytic treatment of microcrystalline cellulose or cellulose-containing materials, and it has been used as the basis for the synthesis of a wide variety of compounds with different biological activities. Although it was shown that it could alter the cell cycle, its usefulness in oncology remains unexplored till nowadays. NCTD is a less toxic and demethylated form of cantharidin, an active constituent of Mylabris beetle, used in traditional Chinese medicine.

The effect of NCTD was analyzed on the triple negative mammary cell lines 4T1 and Hs578T, while the effect of Levo was analyzed on LM3 and MDA-MB231 cell lines. Both drugs proved to have a strong antiproliferative effect. NCTD exhibited an IC_{50} of 35 and 55 uM for 4T1 and HS578T cells respectively. In the case of Levo, the IC_{50} was 10 µM for the two cell lines analyzed. By flow cytometry we have determined that both drugs induced an increase in the sub-G1 fraction of the cell cycle, compatible with the presence of apoptotic cells. Related to *in vitro* malignant progression, we studied adhesion, migration (by wound healing assays) and secreted matrix metalloproteinases activity (by zymography) in all cell lines mentioned above, treated or not with both drugs. A significant reduction in all parameters associated to tumor progression was observed in a dose-dependent manner.

The important response to the treatments observed *in vitro*, suggests that these drugs could be useful for *in vivo* assays, hoping that in the future they could be suitable for breast cancer treatment.

Keywords: Norcantharidin, levoglucosenone, Breast cancer

(78) **NITRIC OXIDE INHIBITION REDUCE MIGRATION AND INVASIVENESS IN BLADDER TUMORS THAT EXPRESS iNOS BY DOWN-REGULATING VIMENTINA, CARMIL AND INCREASING RSU1**

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Bladder cancer (BC) is classified according to the invasion status in non-muscle invasive (NMI) and muscle invasive (MI) tumors. The progression to the invasive illness leads to radical cystectomy. Identification of novel molecules involved in tumor progression, raise the possibility to implement new therapeutic strategies in the beginning of this pathology.

Previously, we described that nitric oxide (NO) production by the inducible isoform of NO synthase (iNOS) is a bad prognosis marker in patients with BC. Using a murine BC model that express iNOS we demonstrated that iNOS inhibition with L-NAME or the stable silencing of iNOS expression by a sh-RNA (sh-iNOS), reduced tumor growth, invasion and metastases.

By a proteomic study comparing NMI MB49 and MI MB49-I orthotopic bladder tumors we selected to validate and analyze three proteins involved in migration and invasiveness processes such as

Vimentina, Carmil and Ras Suppressor Protein 1 (RSU1). The objective of the present study was to evaluate their modulation by the inhibition of NO using Western Blot (WB), Immunofluorescence (IF), Immunohistochemistry (IHC) and quantitative PCR (qPCR) techniques.

Our results showed that L-NAME reduced the pro-migration and invasiveness proteins Vimentina and Carmil and increased the pro-adhesion protein RSU1 either in MB49 and MB49-I (IHC or IF, WB and qPCR, $p<0.01$). Similar results were observed in MB49-I/sh-iNOS.

Conclusion: NO inhibition down-regulates the expression of key proteins involved in tumor progression and increases a protein related to cell attachment and adhesion. These findings added to previous results reinforce the idea that the NO inhibition could be a good therapeutic strategy for bladder tumors that express iNOS.

Keywords: bladder cancer; nitric oxide; inducible nitric oxide synthase; proteomic analysis.

ONCOLOGY-ONCOIMMUNOLOGY6

(1207) CANCER STEM CELLS IN THE RESISTANCE TO PHOTODYNAMIC THERAPY

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Photodynamic therapy (PDT) is an oncologic treatment. It is based on the administration of a photosensitizer and its activation through visible light. The main problem of all oncological treatments, including PDT, is the occurrence of resistant cells. It is proposed that a small fraction of neoplastic cells, called cancer stem cells (CSCs), are the responsive of tumor regeneration and the resistance to the therapies. In our laboratory, we have obtained human cells of skin cancer (SCC-13) and glioblastoma (T98G), resistant to several cycles of PDT with Me-ALA. The resistant populations present characteristics of CSCs, such as a higher tumorigenic capacity, higher proliferation in 3D cultures, lower photosensitizer accumulation and higher cross-resistance to a chemotherapy drug with respect to parental population. The objective of this research was to identify and compare the expression of marker proteins of CSCs in parental population, parental population after PDT (LD50) and resistant population (flow cytometry). In SCC-13, CD44+ percentage was higher in parental cells after PDT and resistant population, respect to parental cells. In T98G was observed higher expression of CD133 marker in parental cells after PDT and in the resistant cell, comparing with parental population. Sox-2 also increased its expression in T98G (fluorescence mean 22.90 ± 3.96 U.A.) cells after PDT comparing with parental cells (15.15± 6.43 U.A.) and SCC-13 post-PDT (335.5 ±160.5 U.A.) respect to parental cells (199.50 ± 65.70 U.A.). The results encourage us to continue the studies. We will determine the degree of resistance of CSCs isolated with the markers CD133 and CD44. In addition, we will develop a strategy for elimination of these CSCs resistant to PDT through a treatment with nanoparticles conjugated to antibodies anti-CD133 and anti-CD44, in order to improve the effectiveness of PDT.

Keywords: cancer stem cells, photodynamic therapy, glioblastoma, skin cancer

(880) *IN VITRO* SECRETOMIC ANALYSIS OF PROSTATE CANCER CELLS AND BONE PROGENITOR CELLS GROWING IN CO-CULTURE SYSTEMS

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Currently our view of cancer has evolved to include, in addition to the transformed cells that have deregulated homeostatic mechanisms, a wide spectrum of cells of the tumor microenvironment. The dialogue established between the tumor cells and its microenvironment, is an essential determinant of the characteristics of the tumor progression. The dissemination profile of Prostate Cancer (PCa) shows tendency to develop in the bone, where tumoral cells interact with the microenvironment disrupting the bone tissue balance. This work aimed at analyzing, which soluble factors could be potential mediators of the chemical cross-talk between PCa cells and bone progenitor cells. For this purpose, we used co-culture transwell systems of PC3 (human PCa cells) with the pre-osteoclastic Raw264.7 or pre-osteoblastic MC3T3 cell lines (murine cells), where cells shared the culture media (CMs) without physical contact for 24h.

We employed mass spectrometry -nanoLC-MS/MS(Orbitrap)- for the secretome analysis of the CMs. The obtained spectra were analyzed with the Proteome Discover Software and compared with both, human and murine protein databases. Results highlight a differential profile of proteins released to the CM when tumor cells are grown in co-culture compared to controls. These results could potentially explain the altered mRNA expression levels assessed by RTqPCR in PCa cells growing in-culture, displaying significant up-regulation for HO-1 ANXA2, ANXA2R,OPG and PTHrP ($p<0.05$). Of note co-culture secretomes showcase highly bone-associated proteins released by tumoral cells such as periostin, Cartilage oligomeric matrix protein, OPN, BMP and ILGF-II, IL-7, FGF, Mx-1 -involved in prostate carcinogenesis- compared to controls, evidencing a misregulated microenvironment.

We hereby report that these results may offer a new set of potential biomarkers in addition to the existing diagnostic tests that could significantly improve sensitivity and specificity in PCa diagnosis.

Keywords: Prostate Cancer; Bone metastasis; Proteomic; Secretomic

(1041) INTERACTION OF TGFβ2 AND HEREGULIN FOR RAC ACTIVATION AND MIGRATION IN BREAST CANCER CELLS.

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Current therapies for breast cancer often fail attacking the main focus that directs it. Rac1 is a Rho-GTPase that promotes migration of breast cancer cells. We aim to study different signaling pathways that converge in Rac1 for breast cancer progression. Heregulin (HRG) is an ErbB ligand that activates Rac1 in a time-dependent manner. To determine whether a short exposure to HRG is necessary and sufficient for maintaining Rac activation over time, T-47D cells were stimulated for 5, 15 or 30min, washed to remove the stimulus and assayed for Rac activation at 1h. When HRG was supplied for 5min, Rac activation significantly decreased at 1h compared to control ($p< 0.05$). Addition of HRG immediately after washing was sufficient to recover Rac activation. When HRG was removed at 15min, slight changes on Rac activation were observed. However, removal of the conditioned medium after 30min suppressed the maintenance of Rac activation, and a second addition of HRG failed to recover the effect. We conclude that HRG is necessary for initial Rac activation and, subsequently, cells start producing autocrine factors that are necessary for maintenance of HRG-initiated Rac activation. Previous data showed that Rac activation by HRG leads to an increase in TGFβ2 expression in breast cancer cells. In fact, the TGFβ family is involved in cell migration and metastasis. Here, we show that the increase of TGFβ2 mRNA after HRG stimulation is time-dependent: it significantly increases ($p<0.05$) after 30min, with maximum at 2h. Wound healing assays reveled that TGFβ2 itself induce migration of MCF7 cells in a dose-dependent manner, with maximum at 10ng/ml. Combined addition of HRG and TGFβ2 produced the same increase on cell migration as HRG alone. These results suggest that there is a sequential relationship between the Rac1 activation peak and the maximal increase of TGFβ2. We propose that this cytokine is necessary for the maintenance of HRG-triggered phenotype of breast cancer cells.

Breast Cancer, Signaling, Heregulin, Rac, Tgfβ2.

(1043) MIGRATION CAPACITY OF BONE MARROW-MESENCHYMAL STEM CELLS FROM PATIENTS WITH ADVANCED BREAST CANCER: A NOVEL APPROACH OF BONE PRE-METASTATIC NICHE

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Recent reports indicated that self-renewal, proliferation, as well as migration capacity of bone marrow (**BM**) mesenchymal stem cells (**MSC**) are essential properties for bone regeneration processes.

We found that MSC from untreated advanced breast cancer patients (**BCP**, invasive ductal, stage III-B) have lower cloning, proliferation and osteogenic differentiation capacities than healthy donors (**HD**)-MSC. Also, BCP-MSC favoured osteoclastogenesis from BM hematopoietic precursors.

Here, we aimed to evaluate migratory capacity of MSC from BM of advanced BCP and different parameters that regulate it, which is indispensable for the processes of osteogenesis and bone vascularization. For this purpose, we studied the % of MSC that express CD146 and the level of expression/MSC (*Flow Cytometry=FC*), as well as TERT activity (*RT-PCR*), telomere length (*RT-PCR*), and intracytoplasmatic/ mitochondrial ROS production (*FC*) in MSC of BCP and HD. Also, we analyzed the expression of total and phosphorylated (P) β-catenin in these stem cells (*Western-blot and immunocytochemistry*) and the production of pro-MMP-2, active-MMP-2, SDF-1 and CCL-2 levels (*ELISA*) in conditioned media of fibroblast colony forming units (1CFU-F = 1MSC). Finally, we studied their migration capacity (*Transwell assay*).

The results indicated that BCP-MSC vs HD-MSC had: lower % of MSC-CD146(+) [p=0.0446]; decreased CD146 relative fluorescence index (p=0.0023); decreased TERT activity (p=0.0416); increased P-β-catenin expression (p=0.0001); increased P-β-catenin/ total β-catenin ratio (p=0.0410); increased total ROS levels (p=0.0286); decreased SDF-1, pro and active MMP-2 levels (p=0.0334, 0.0010, 0.0030); increased CCL-2 level (p=0.0370) and poor migration capacity (p=0.0376).

Findings suggest that alteration of MSC migration provide more insight to better defining the development of the BM and bone "pre-metastatic niche". Also, data show some possible targets aimed to restore migratory capacity of BCP-MSC.

Key words: breast cancer, bone marrow, mesenchymal stem cells, migration capacity.

(1230) **OCT-4 EXPRESSION WAS INCREASED IN RESISTANT CANCER STEM CELL-LIKE SUBPOPULATION IN COLORECTAL CANCER**

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Radiochemoresistance of tumors has been associated with the presence of a small subpopulation of tumor cells called cancer stem cells (CSCs). These cells had been shown not only linked with high resistance to chemotherapeutic drugs but also are associated with metastatic recurrences. Embryonic markers such as Oct-4, Nanog, Sox-2 are associated with resistance to chemotherapeutic drugs in some cancer. However, little has been reported about the expression of these embryonic markers in colorectal (CRC) CSCs subpopulation. The aim of this work is the study of the mentioned embryonic marker in sensible and oxaliplatin-resistant (OXA) human CRC cancer lines, and in cultures derived of them enriched with CSC-like subpopulation, in order to detect novel markers associated to resistance in these cells. We characterized the expression of Oct-4, Nanog and Sox-2 by immunofluorescence, cell cytometer and real time-PCR in sensible or resistant human CRC cell to oxaliplatin (T-84 and HCT-116) and in cultures enriched by CSC-like subpopulation. The last cultures were obtained by growing tumor cells as colonospheres (CSPHE) in minimum medium in low adherence surfaces. On the other hand, we also measured the reactive oxygen species (ROS) levels as a characteristic associated to CSC-like subpopulation. The expression of Nanog and Sox-2 was not changed in sensible and resistant cell lines grown in monolayer or as CSPH cultures. However, the expression of Oct-4 was positive in 6-12% and 40-50% of sensible cells that were grown in monolayer or as CSPHE respectively. About chemoresistant cells lines, 40-60% and 75-85% of cells were positively for Oct-4 depending if cells were grown as monolayer or as CSPHE respectively. About ROS level, cells that were grown as CSPHE had shown significant lower levels in respect to cells that were grown as monolayer. These results showed that Oct-4 could be a promising marker to identify chemoresistant cells with CSC-like characteristics in CRC.

Keywords: Oct-4, Cancer Stem Cell, Chemoresistance

(452) **RANK RECEPTOR IN SPINDLE-SHAPED STROMAL CELLS, A PROGNOSTIC DETERMINANT OF EARLY BREAST CANCER.**

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In breast carcinomas (BC), most stromal cells are fibroblasts, and 80% of them are tumor-associated fibroblasts (TAF), characterized by increased expression of α-SMA and FSP, among others markers. One source of TAF is bone marrow (BM)-mesenchymal stem cells (MSC) and independent of the source, TAF are spindle-shaped stromal cells with negative CD34 expression. Other markers of BM-MSC such as CD105 and CD146 could be present in TAF.

In previous work we found that SSC, not associated with the vasculature, sampled from early (I/II) breast cancer patients (BCP), express RANKL and RANK at significantly higher levels vs. non-neoplastic breast tissues. Also, RANK expression in these SSC had a significant association with RANKL expression in these SSC and in tumor cells of these BCP. These SSC were CD34(-) and CD105(+) like TAF and MSC.

Recently work showed that RANKL is a critical molecule for BM-MSC clonogenic and differentiation potential, in particular osteogenic. Other authors found that RANKL soluble induced a migratory response in osteoblasts and osteoblast progenitors (MSC and pre-osteoblasts).

The aim of this study is to explore the clinicopathological significance of RANK in these SSC, as prognostic determinants of early BCP.

We conducted immunohistochemical analysis for RANK expression in these SSC of primary tumors from early BCP (invasive ductal), and analyzed their association with clinicopathological characteristics. Here, we demonstrate that the elevated level of RANK in these SSC was significantly associated with a higher risk of metastatic occurrence (0.0119). Moreover, high expression of RANK was associated with shorter disease-free survival and metastasis-free survival (p = 0.007 and 0.014).

This study is the first to demonstrate that high levels of RANK expression in SSC, not associated with the vasculature, could be used to identify early BCP with poor outcomes.

Keywords: mesenchymal stromal cells, RANK, breast cancer, metastatic occurrence.

(718) **RELEVANCE OF iNOS EXPRESSION AND NITRIC**

OXIDE PRODUCTION IN THE MAINTENANCE OF CANCER STEM CELLS IN A MURINE BLADDER CANCER MODEL

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Introduction: Bladder cancer (BC) is the second most common tumor of the male urogenital tract, and an important worldwide cause of death. BC is classified as no muscle invasive (NMI) when it is localized in the urothelium and muscle invasive (MI), when it reaches the detrusor muscle. Increasing evidence has indicated the presence of cancer stem cells (CSCs) in many types of cancers, associated with cancer aggressiveness, chemoresistance and relapse. Nitric oxide (NO) is a free radical produced by enzymes called NOS. The inducible isoform (iNOS) produces high levels of NO in response to inflammatory stimuli. The expression of iNOS in human BC is a poor prognostic factor associated with increased invasion and tumor recurrence. Although there is evidence supporting the role of NO/iNOS in the promotion and progression of bladder cancer, the possibility that there are CSCs dependent on endogenous NO generation has not yet been clearly defined. The objective of this study was to evaluate the role of NO in CSC maintenance, modulating it production, using pharmacological inhibitors or silencing iNOS expression in a murine BC model.

Results: The number of CSCs, determined as spheres forming efficiency (SFE), generated by MB49-I (MI BC cell line) resulted higher than the NMI MB49 line ($p<0.05$), in concordance with the higher expression of stemness related genes, Sox-2, Oct-4 and Nanog (qPCR) ($p<0.001$). iNOS inhibition with L-NAME or 1400 W, diminished SFE in both cell lines ($p<0.05$ vs MB49;$p<0.001$ vs MB49-I), and in MB49-I, significantly inhibited the expression of pluripontency markers ($p<0.05$). Moreover, the reduction of this markers and SFE were more evident when MB49-I-shiNOS was tested ($p<0.0001$ vs MB49-I scr; $p<0.001$ vs MB49-I-scr).

Conclusion: Together, this results show that iNOS plays an important role in regulating the self-renewal ability and stemness of bladder cancer cells, and that it inhibition could be a potential therapeutic target in BC that inhibit CSCs.

Keywords: Bladder cancer, cancer stem cells, nitric oxide, iNOS

(743) **SPECIFIC INHIBITION OF PROSURVIVAL FACTOR BCL-XL DISTINCTLY SENSITIZES PATIENT DERIVED GLIOMA STEM CELL LINES TO CHEMOTHERAPY DRUGS**

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High-grade gliomas are the most prevalent and lethal primary brain tumors. They display a hierarchical arrangement with a population of self-renewing and highly tumorigenic cells called cancer stem cells. These cells are thought to be responsible for tumor recurrence, which make them main candidates for targeted therapies. High levels of anti-apoptotic molecules in cancer cells have been shown to both result in resistance to chemotherapy and promotion of unrestrained growth. Herein, we examined the sensitivity of patient derived glioma stem cell enriched cell lines (GSC-ECLs) to a panel of chemotherapy drugs. Flow cytometric analysis of PI staining revealed that treatment with temozolamide (TMZ) (50-500μM) or lomustine (CCNU) (20-200μM) for 7 days increased cell death in a dose-dependent manner ($p<0,05$) while no significant change in GSC-ECLs viability was observed upon vincristine (VCR) (0.5-5μM) exposure. Also, by RT-qPCR and Western blot we determined that GSC-ECLs express higher levels of anti-apoptotic Bcl-xL and Bcl-w and lower levels of Bcl-2 than untransformed neural progenitors ($p<0,05$). Pro-survival Bcl-2 family members counteract apoptosis by sequestering BH3 domains. Thus, to gain insight into the mechanisms that control GSC-ECLs decisions in response to chemotherapy agents, we attenuated their anti-apoptotic threshold using small molecules that mimic BH3 motifs. ABT-263 preferentially targets Bcl-2, Bcl-xL and Bcl-w while WEHI-539 targets only Bcl-xL. Using these agents and small interference RNA-mediated gene silencing we studied the contribution of Bcl-xL, Bcl-w and Bcl-2 in cell survival. We found that BH3-mimetic treatment or Bcl-xL gene silencing similarly exacerbate cell death triggered by TMZ or CCNU and render cells sensitive to VCR in a cell line-dependent manner. Given the intertumor heterogeneity present in high-grade gliomas, the knowledge of GSC-ECLs distinct dependence on Bcl-xL activity may help the development of tailor-made therapies.

Keywords: glioma stem cells, BH3-mimetic, drug resistance

(1102) **WHOLE EXOME SEQUENCING ANALYSIS OF MELANOMA SUSCEPTIBILITY LOCI, FROM GERMLINE TO PRECURSOR AND TUMOR LESIONS - A CASE REPORT**

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The impact of melanoma susceptibility loci throughout tumor evolution from precursor benign nevi to cutaneous melanoma is unknown. The aim of this work was to analyze susceptibility loci at different stages of progression in a cutaneous melanoma patient. *Methods:* Formalin-fixed paraffin embedded biopsies from primary tumor, its associated nevi and lymph node metastasis were sectioned by laser capture microdissection and DNA was isolated. DNA from PBMC was used as germinal reference. Whole exome sequencing was performed (Agilent SureSelect Human All Exon V5, Illumina HiSeq 4000, 100X effective mean depth per sample). A panel of reported loci associated to melanoma susceptibility was searched in germline and lesions; variants allele frequencies were determined for each sample. *Results:* A total of 56 variants covering 28 genes in 13 chromosomes were found in germline and all lesions. 15/56 variants were homozygous. Variants that required LOH to be functional and found heterozygous in lesions samples were not considered as putative susceptibility loci in this patient. Only tumor suppression variants found to be homozygous, heterozygous with haploinsuficiency or oncogenes detected in all samples might have had a role in tumor development and progression. These variants included genes related to hallmarks of cancer such as proliferation (CDK4), abolition of tumor growth suppression (PTCH1), escape from cell death (CASP8), invasion and metastasis (CTSS), inflammation (CPNE7), genome instability and mutations (ATM), and cell replicative immortality (CLPTM1L). *Conclusion:* combined analyses of melanoma susceptibility loci in germline and lesions at different stages of progression provide insight of trunk variants involved in tumor evolution, with impact in patient´s outcome.

(711) **COMBINED RADIOTHERAPY AND HISTAMINE FOR EFFECTIVE TREATMENT OF BREAST CANCER**

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Previous studies demonstrated that histamine enhanced ionizing radiation (IR)-induced response *in vitro* and *in vivo* in human triple negative breast cancer (TNBC) and melanoma models. The aim of this work was to further explore the effect of the combination treatment of histamine and *gamma* IR on tumor immunity, employing a syngeneic TNBC model, with intact immune system, developed in

Balb/c mice with 4T1 cells. Proliferation, apoptosis and oxidative damage studies were performed. Tumor infiltrating lymphocytes and lymphocyte subpopulations in spleen were evaluated by FACS.

Results indicate that histamine potentiates IR response mainly *in vivo*. Radiation administered as 3 doses of 2 Gy in consecutive days reduced tumor growth and weight. Treatment of 4T1 tumor-bearing mice with histamine (1 mg/kg.day) injected *sc* starting 1 day before irradiation until the end of the experiment, potentiated IR-induced decrease in tumor growth ($P<0.05$) and also reduced splenomegaly. The analysis of the distribution of the immune cell subsets showed decreased CD4+ and CD8+ T together with reduced CD19+ B lymphocytes in both histamine-treated and non-treated irradiated tumors. Conversely, histamine treatment increased the percentages of NK cells (4.6±0.8 vs. 1.7±0.3, $P<0.01$) and myeloid derived stem cells (26.7±3.8 vs. 8.9±1.0, $P<0.05$) in irradiated tumors. Non-significant changes in the percentage of lymphocyte populations were observed in spleen of tumor-bearing animals. We conclude that histamine could improve radiation therapy in part by modulating tumor immunity.

Keywords: histamine, radiotherapy, breast cancer, tumor immunity, NK cells

(1104) **EZH2 AS A TARGET OF PROGESTERONE RECEPTOR-MEDIATED BREAST CANCER GROWTH**

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On the basis of Estrogen Receptor (ER) presence, breast cancer (BC) patients are treated with selective ER modulators (SERMs) such as tamoxifen. However, approximately 40% of women receiving TAM experience tumor relapse. We and others have previously reported Progesterone Receptor (PR) participation in BC growth. We therefore propose that, being blocked ER, PR may take control of tumor growth, progression and endocrine resistance. We have described that the progestin medroxiprogesterone acetate (MPA) induces the interaction between PR and EZH2 to promote transcriptional repression of tumor suppressor GATA3. EZH2, a histone methyltransferase, is often overexpressed in different types of cancers and associated with poor overall survival. The objective of this work was to study the role of EZH2 in MPA-induced BC growth. In vitro studies were carried out in the T47D human BC cell line and in primary cultures of the MPA-dependent C4HD tumor. Firstly, MPA (10 nM) induced EZH2 expression after 18 and 24 h of treatment, seen by increased levels of both mRNA (qPCR, $p<0.05$) and protein (SDS-PAGE/WB). RU486, a PR antagonist, prevented these effects. Interestingly, when EZH2 was knocked-down by a specific siRNA or when EZH2 activity was specifically inhibited by GSK126 (5 uM), MPA-induced cell proliferation was prevented ($p<0.001$). In a pre-clinical study, 12 BALB/c mice carrying C4HD tumors (via sc) were randomly separated in two groups, and injected via ip for 14 days with either vehicle (captisol 20%) or GSK126 (50 mg/kg/day). Blockage of EZH2 activity diminished MPA-induced tumor growth ($p<0.001$). Lastly, EZH2 protein expression was analyzed by IHC in a cohort of 118 ER+/PR+ BC patients treated only with tamoxifen. Patients with higher EZH2 expression showed to have lower DFS probability ($p=0.0099$). These results show a key role of EZH2 in progestin-induced breast cancer and point it out as a possible novel therapeutic target for TAM-resistant breast cancer.

Key words: breast cancer, progesterone receptor, EZH2, GSK126, tamoxifen

GENETICS AND MOLECULAR BIOLOGY 5

(509) **PROTEIN SIGNATURES THAT ALLOW PROPER METAL RECOGNITION AND SIGNAL TRANSDUCTION IN MerR METALLOREGULATORS**

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Transcriptional regulation is the main cellular mechanism bacteria employ to control homeostasis/resistance to essential and harmful metal ions. Our group focuses on metalloregulators of the MerR family, dimeric proteins that detect the ions in the cytoplasm and activate transcription of specific transporters or detoxification enzymes to eliminate the intoxicant. Among them, two groups with similar structure can be clearly distinguished depending on the charge (+1 or +2) of the metal ion they sense. Previously, we discerned the molecular bases that allow GolS, a *Salmonella* specific sensor, to discriminate Au(I) from other metal ions including Cu(I) or Ag(I), or divalent ions. We found that two residues from the metal binding loop (MBL) between the two coordinating cysteines (A113 and P118) are crucial for Au discrimination, while a single serine residue (S77) that is located near the metal coordination environment is essential from excluding +2 charge metals from the binding site. Here, we analyzed how the size and identity of the residues of the MBL determine proper recognition of metal ions and transcriptional activation. We swapped the MBL of GolS or CueR (the paralogous non-selective monovalent metal ions sensor) for the same region of ZntR or MerR, two representatives of the divalent metal ion sensor group, and analyzed the response of the new variants to different monovalent (Au and Cu) or divalent (Hg, Cd, Pb and Zn) metal ions by measuring transcriptional activation of specific reporter genes. Our results suggest that besides the presence and proper location of essential ligands, the size of the metal binding loop is the main determinant of proper metal recognition and signal transduction that allow activation of transcription of target genes.

Keywords: Metals,Transcriptional regulation, Metalloprotein, MerR regulator

(1841) **ANTI- *Helicobacter pylori* ACTIVITIES OF *Origanum vulgare* AND *Larrea divaricata* CAV EXTRACTS IN VITRO.**

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Helicobacter pylori is a gram-negative bacillus and is the cause of peptic ulcer disease and gastric cancer. Due to antibiotic resistance, there is great interest in the search for new therapies. Urease and Flagella are virulent factors that are required for the colonization and survival of *H. pylori* in the human stomach. *Origanum vulgare* (Orégano) and *Larrea divaricata* Cav. (Jarilla) are plants known for their properties in folklore medicine. The aim of this work was to investigate the effects of *O. vulgare* and *L. divaricata* extracts on antimicrobial activity and inhibition of urease and flagella activities in *H. pylori* strain. In this study, the antibacterial activity of both aqueous extract against *H. pylori* strain was assayed according to CLSI guidelines (CLSI, 2007). The extracts were diluted to obtain the final concentrations from 10 to 0,009 mg/ml for each. Serial dilutions of Amx (Sigma-AldrichCo., StLouis, MO) were used as a control in the susceptibility test. Bacterial suspension was adjusted to a scale of 0.5 McFarland standard ($1x10^8$ CFU/ml). Minimal inhibitory concentration (MIC) was measured by determining the lowest amount of extract or antibiotic needed to inhibit the visible growth of the microorganism. The reference strain HP146128 was treated with sub-inhibitory concentrations of Orégano extract (0,5 mg/ml) and Jarilla extract (1 mg/ml) and was incubated for 26h at 37°C in microaerophilic atmosphere. The gene expressions were determined by RT-PCR using 16s RNA as housekeeping gene. The results obtained showed that the MIC of Orégano and Jarilla were 1,25 mg/ml and 2,5 mg/ml respectively. Orégano extract down-regulates the ureA gene and fla gene expressions; while the Jarilla extract showed no decrease in the expression of the same genes to the concentration assayed. The use of Orégano extract can contribute as alternative

and complementary medicines, safe and less costly in the treatment of *H. pylori* infections.

Keywords: Helicobacter pylori, Origanum vulgare, Larrea divaricata CAV, Antimicrobial activity.

(1491) **SYNTHETIC BIOLOGY FOR THE DEVELOPMENT OF A CYANOBACTERIAL HIGH-VALUE-MOLECULES PRODUCTION PLATFORM**

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Cyanobacteria are the only prokaryotes that perform plant-like oxygenic photosynthesis and convert sunlight-derived energy into biomass. They possess several advantages as hosts for biotechnological applications, including simple growth requirements, ease of genetic manipulation and robust high-scale production. At present, many bioindustrial processes rely on the fermentation of heterotrophic bacteria to produce various fine chemicals. Nevertheless, the economic viability of these production schemes is limited by the cost of carbon substrates used in the fermentation processes. Cyanobacteria, endowed with photosynthesis system to fix carbon dioxide into reduced form, are ideal biosynthetic machinery for sustainable production of various chemicals and biofuels. In this context, we aim to develop a cyanobacterial platform for the production of high value molecules of industrial interest. For this purpose, and as a proof of concept, we designed and constructed recombinant cyanobacteria strains capable of producing methyl-branched chain lipids (MBL), which have exceptional physicochemical properties for their application in the bio-lubricants, pharmaceuticals and cosmetics industries.

The construction of different heterologous pathways for the biosynthesis of the substrates and the final products was done using a combinatorial modular design. After the recombinant-cyanobacteria strains were obtained, we tested if the enzymes of each pathway were expressed and finally we carried out different bioconversion assays to test if the desired products were synthesized. These bioconversion assays demonstrated that the cyanobacterial strains successfully produced MBL.

In summary, we have developed a cyanobacterial high-value-molecules production platform with a modular-functional design that allows a versatile, interchangeable and combinatorial construction of the desired cyanobacterial strain.

Key Words: Cyanobacteria- Synthetic Biology- Lipids

(174) **BOVINE MACROPHAGES INTRACELLULAR TRAFFIC OF *Leptospira interrogans***

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Pathogenic leptospires are able to infect humans and many domestic and wild animals. This bacterium can survive and grow in animal tissues by escaping natural defense mechanisms. We have recently obtained the whole genome sequence of a representative strain from a major clon circulating in Argentina that causes bovine reproductive losses The objective of this study was to analyze the cellular traffic between this virulent strain and its attenuated counterpart in bovine macrophages to understand their differential virulence.

We have infected bovine macrophages derived from peripheral blood mononuclear cells (PBMCs) with stained leptospires from both virulent (P1) and attenuated (P19) strains. Infections were analyzed using flow cytometry and confocal microscopy. In order to evaluate the cell entrance we have used cytochalasine D (cytoD) as a phagocytosis inhibitor. The intracellular localization was studied using Lysotracker and immunofluorescence with LAMP-3.

Both techniques showed a higher number of P1 inside the cell compared with P19. Cells pre-treated with cytoD showed 50% less intracellular P1 than untreated cells. No significative difference was observed for the attenuated strain with cytoD. The intracellular colocalization of Lysotracker with P1 was significantly higher than the observed for P19, both in cytoD treated and untreated cells. The same was observed for the colocalization of LAMP-3 with both strains. Particularly, in cytoD treated cells, the colocalization of Lysotracker with P1 was 50% lower than in untreated cells.

The ability of leptospires to invade bovine macrophages was confirmed and the virulence of P1 could be explained by the higher amount of bacteria inside the macrophages in comparison with P19. Our results allowed us to postulate a possible intrinsic mechanism of cellular entrance not dependent of phagocytosis for P1. This could lead to a possible evasion of leptospires from the innate bovine macropaghes mechanisms of bacteria control.

(1544) **PARTIAL PURIFICATION OF ANTIMICROBIAL PEPTIDES ACTIVE AGAINST FOOD BORNE PATHOGENS**

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Bacteriocins are small, ribosomally synthesized antimicrobial peptides that kill bacteria closely related to the producer strain. Hybrid bacteriocins were made by the fusion of genes encoding Enterocin CRL35 (*munA*), Pediocin PA-1 (*pedA*) and Microcin V (*cvaC*) separated by a hinge region. These bacteriocins were constructed in order to obtain peptides with broad antimicrobial spectrum. The aim of this work was to purified different hybrid bacteriocins and microcins available in our laboratory. Microcin E492, Microcin V and Microcin H47 were obtained from supernatants of the producer strains. Hybrid bacteriocins with different hinge regions (Ent35-GGG-Mccv, PedA-GGG-MccV, PedA-GIG-MccV) were purified by disruption of the producing strains and subsequent precipitation of the proteins with 40-50% $(NH_4)_2SO_4$. The cellular extracts were loaded onto a C18 chromatography cartridge, eluted with acetonitrile and then were concentrated. The antimicrobial peptides were found to be active against different food borne pathogens such as *Listeria monocytogenes*, *Escherichia coli* O: 157, *Salmonella enterica* serovar pullorum and gallinarum. Serial double dilutions of the partially purify peptides were performed. Antibiotic activity units (AU/ml) were calculated as the reciprocal of the highest dilution showing growth inhibition halo against *E. coli* MC4100. The antimicrobial peptides have potential biotechnological applications as food biopreservative and in the pharmaceutical industry.

Keywords: bacteriocins, biopreservative, food borne pathogens.

(1488) **RNA-SEQ TRANSCRIPTIONAL PROFILING OF *Herbaspirillum seropedicae* IN RESPONSE TO SUFFICIENT AND HIGH PHOSPHATE CONCENTRATION**

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Herbaspirillum seropedicae is a diazotrophic and endophytic bacterium associated with important agricultural crops, promoting plant growth and increasing productivity. Its agricultural use requires studies on its physiological response to prevailing levels of nutrients such as phosphate (Pi). It has been previously reported in different bacteria that environmental Pi modulates the levels of intracellular polyphosphate (polyP), a molecule that plays important roles in microbial metabolism. The aim of this work was to evaluate the intracellular polyP levels and the differentially expressed genes in *H. seropedicae* grown in sufficient or high Pi conditions. *H. seropedicae* SmR1 was grown in NFbHN-malate medium modified with 5 or 50 mM Pi; culture absorbance at 600nm and polyP levels were measured at different times during 24 h. Results showed that cultures grown in high Pi medium reached higher cell densities than cultures grown in sufficient Pi media and maintained high polyP levels in stationary phase. To analyze if these differences in pol-

yP levels could generate changes in gene expression, mRNA from cell grown in different media was purified and used for RNA-seq profiling. Comparison of RNA profile between sufficient and high Pi conditions revealed that 620 genes were differentially expressed, being 53 and 43% repressed and induced by high Pi, respectively. Most of the induced genes in the high Pi condition were involved in energy production process, two component regulatory systems, amino acid transport and metabolism, chemotaxis and inorganic ion transport and metabolism. The ability to respond to environmental signals which modulates polyP production could be critical in diverse aspects of *H. seropedicae*.

(1753) **ROLE OF DIFFERENT COUPLING CHEW PROTEINS IN THE CHEMOTAXIS-RELATED PATHWAYS OF THE HYDROCARBON-DEGRADING STRAIN *Halomonas titanicae* KHS3**

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In the canonical *E. coli* chemotaxis pathway, chemoreceptors transmit information from the extracellular medium to an associated histidine kinase (CheA), through a coupling protein (CheW). Many environmental bacteria possess more than one chemosensory pathway. The protein components show a high conservation and mechanisms that avoid crosstalk between the different pathways are not clear yet.

Halomonas titanicae KHS3 was isolated from Mar del Plata harbor. It can grow using polyaromatic hydrocarbons as the sole carbon source and displays chemotactic behavior toward these compounds.

Two chemotaxis-related systems were identified in its genomic sequence. Cluster 1 has a canonical organization, similar to the *E. coli che* cluster. On the other hand, cluster 2 shows a different gene organization including a diguanylate cyclase protein and two CheW-like coupling proteins.

The aim of this work was to investigate the properties of the different CheW proteins. The three proteins were modelled using Swiss-model software. Both *Ht* CheW1 and CheW2 showed a structure similar to *E. coli* CheW whereas *Ht* CheW3 displayed an structure that deviated from the conserved one, suggesting a different role.

CheW1 and CheW2 were expressed in *E. coli* cells. CheW1, from cluster 1, complemented chemotaxis function in an *E. coli* strain lacking native CheW in soft agar plates, indicating that it fulfills a similar role in complexes that control flagellar rotation. When expressed at very high levels, it interfered with chemotactic function, consistent with the ability of CheW proteins to interact with chemoreceptor dimers and disrupt important receptor-receptor interactions.

In contrast, CheW2 failed to restore chemotactic function, and did not interfere with *E. coli* chemotaxis, even when overexpressed. This indicates that CheW2 does not interact with *E. coli* chemoreceptors, suggesting that it has specificity determinants for proper interactions with cognate receptors.

(823) **USE OF SUICIDE PROBES TO STUDY CLASS II BACTERIOCINS MECHANISM OF ACTION AGAINST A RECEPTOR-FREE HOST**

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The increasing resistance to common antibiotics is highly worrisome, thus, it has led to the study of alternative antimicrobials as a primary concern. Bacteriocins are a promising solution to this problem and the understanding of their mechanism of action is fundamental for development of new variants with potential use in the pharmaceutical industry. Class II bacteriocins are unmodified membrane-active peptides that act over a narrow spectrum of bacterial targets. They are believed to bind a specific receptor on the membrane that would participate in the formation of a pore, leading to membrane permeabilization and cell death. In order to reveal whether or not the pore structure involves a specific receptor, we designed chimeric peptides fusing the bitopic membrane protein EtpM with different class II bacteriocins: enterocin CRL35 (Ent35), pediocin PA-1 (PedA-1) and microcin V (MccV). We chose *E. coli* as an expression host because this bacterium is naturally insensitive to Ent35 and PedA-1, since their specific receptor Man-PTS is not present on its inner membrane. In addition, an *sdaC* mutant *E. coli* strain was employed as a receptor-free host for MccV, as it does not express SdaC, the specific membrane receptor for this microcin. If the fusion EtpM-bacteriocin kills the expressing host cell, it would mean that the specific receptor could be dispensable for the final step of membrane disruption. As these constructs exert a lethal effect when they are expressed, they are called “suicide probes”. This hybrid proteins EtpM-Bacteriocins were heterologeously expressed in *E. coli* and *E. coli* ΔsdaC respectively, and the results suggest that, indeed, the specific receptor would act simply as docking molecule and it would not participate in pore formation mechanisms. The effect of these suicide probes in some membrane properties is also analyzed.

Keywords: bacteriocin, mechanism, receptor, microcin, antibiotic

(1844) **DISSECTION OF VIRULENCE-ASSOCIATED TRANSCRIPTIONAL NETWORKS IN *Brucella*: SEEKING FOR NEW REGULATORY PROTEINS THROUGH BIOINFORMATIC, BIOCHEMICAL AND MOLECULAR APPROACHES**

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In facultative intracellular bacteria of the genus *Brucella*, significant progress has been made in identifying virulence factors. However, the genetic program orchestrating the *Brucella* intracellular adaptation process is still poorly understood. In this regard, our group recently identified the regulon of VjbR, a transcription factor known to play a major role in the pathogenesis of *Brucella*. Our ChIP-seq and RNA-seq analyses showed that VjbR controls bacterial functions relevant for survival during the initial stages of the intracellular infection, and revealed that VjbR indeed acts as a global regulator exhibiting a large amount of binding sites across the *Brucella* genome. However, further analysis of our data indicated that the VjbR transcriptional network is highly complex. For instance, we found that VjbR failed to bind to many genomic positions containing conserved VjbR-binding motifs, which suggested the possible existence of competitors that prevent binding of VjbR to specific promoters under the assayed conditions. To explore this possibility, we developed a bioinformatic method that allowed us to identify conserved sequences adjacent to VjbR-binding motifs that failed to bind VjbR *in vivo*, which could act as binding sites for competitor transcription factors. EMSA analysis of one of such specific promoters showed that in the absence of competitors, VjbR alone was able to interact *in vitro* with its target DNA sequence. Moreover, in *Brucella* crude extracts we detected two proteins able to bind to the analyzed promoter. Using biochemical and molecular methods we isolated one of these proteins, wich was identified as a transcription factor known to coactivate expression in other direct targets of VjbR. Determination of the second DNA-binding protein, however, will requiere further work. In summary, here we present a pipeline suitable to identify possible competitor and/or coactivator transcription factors in complex transcriptional regulatory networks.

Keywords: transcriptional regulation, functional genomics, LuxR, bacterial pathogen

(849) **CHARACTERIZATION OF SECONDARY METABOLITE BIOSYNTHESIS PATHWAYS IN *S. EUROCIDICUS*.**

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Streptomyces eurocidicus produces at least three secondary metabolites, azomycin, eurocidin and tertiomycin, which its biosynthesis pathways are unknown. Azomycin is a broad-spectrum antibiotic belonging to the group of nitroheterocyclic compounds. Eurocidin

is a pentaene macrolide with antifungal activity. And Tertiomycin is an antibacterial compound from the spiramycin family with activity against Gram-positive bacteria, but the structure is still unknown. With the aim of study the biosynthetic pathwayof these metabolites, we have first validated the production of each of the using *S. eurocidicus* NRRL B-1676 strain. In liquid media culture it was found that eurocidin is produced in the exponential phase while both azomycin and tertiomycin are produced in the stationary phase of growth. Surprisingly, it was found that *S. eurocidicus* has a compound, presumably eurocidin, with a strong fluorescence that stains colonies on solid and liquid media. The identity of each compound was determined by TLC observed by UV at 254/302 nm and by p-anisaldehyde staining, or by high resolution LC-MS. In turn, the activities of each spot observed were determined by inhibition against *Bacillus subtilis* or *Saccharomyces cerevisiae*. Then, we have sequenced the *S. eurocidicus* NRRL B-1676 genome by 100bp paired-end reads using Ilumina technology. Analysis of the genome sequence allowed identifying putative gene cluster for each metabolite using bioinformatic tools. To validate these gene clusters we are constructing knock-out strains by homologous recombination or by UV mutagenesis. Thus, mutant strains for eurocidin gene cluster were obtained and it was found that they lost antifungal activity and are not fluorescent. New mutant strains for the other pathways are being constructed.

Keywords: Streptomyces, Azomycin, Eurocidin, Tertiomycin, Pathways.

GENETICS AND MOLECULAR BIOLOGY 6

(554) A FUNCTIONAL CHARACTERIZATION OF AMP-ACTIVATED PROTEIN KINASE IN *Trypanosoma cruzi*

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INGEBI - CONICET - UBA

The AMP-activated protein kinase (AMPK) is a heterotrimeric enzyme involved in maintaining energy homeostasis in response to different stresses in many organisms. Sequence and structure of its subunits may change between organisms, but they maintain the same function. The α subunit contains a N-terminal kinase catalytic domain, the β subunit acts as a scaffold and intervenes in the localization of the complex and the γ subunits binds AMP. During the transition from the mammal host to the insect vector, *Trypanosoma cruzi* suffers nutritional stress from the absence of glucose in the insect's midgut. The ability to respond to this stress, allows the parasite to differentiate and survive. Recently, it was shown that *Trypanosoma brucei* AMPK is involved in surface protein expression changes in response to nutritional stress and differentiation.

We identified four candidate genes for the AMPK subunits of *T. cruzi* (α1, α2, β and γ). Each of these subunits was capable of reverting the 'glucose dependent' phenotype of *S. cerevisiae* conditional mutants alternatively lacking one subunit of the AMPK ortholog SNF1. Also, we overexpressed the α1, β and γ subunits with a hemagglutinin (HA) Tag in CL Brener epimastigotes and evaluated their localization and possible post-translational modifications. Western blots using anti-PhosphoAMPK antibody showed specific stripes corresponding to the expected MW of the alpha subunits. These stripes are reduced in intensity or completely deleted with Lambda phosphatase treatment. Also, epimastigotes treated with an AMPK specific activator show a shift in the intensity pattern of the stripes. Thus, the phosphorylation pattern of the α subunits can be modified *in vivo*. RT-PCR assays also revealed the endogenous α2 mRNA, but not the α1 mRNA in epimastigotes. Our results show, for the first time, the presence of an AMPK ortholog in *Trypanosoma cruzi*. In the future, we aim to discover its role in the life cycle and stress responses of this parasite.

Keywords: AMPK, stress response, AMP, signaling, transduction pathway

(608) IN VIVO EVALUATION OF THE BIOLOGICAL FUNCTION OF AN AMP-ACTIVATED PROTEIN KINASE ALPHA SUBUNIT IN *TRYPANOSOMA CRUZI*

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The AMP-activated protein kinase (AMPK) is a heterotrimeric evolutionarily conserved enzyme that typically functions as a metabolic sensor and generates changes in energy homeostasis, through transcriptional and metabolic reprograming. In parasitic protozoa, such as *Trypanosoma cruzi*, energy sensing is crucial since they must go through sudden environmental changes during the passage through its different stages. AMPK can also influence other cellular processes such as mitochondrial activity, autophagy, endoplasmic reticulum stress, and apoptosis. This protein kinase is composed of the catalytic subunit α and two regulatory subunits β and γ.

Using conserved domains and secondary structure analysis, we have been able to identify four candidate genes corresponding to two a subunits, named TcAMPK±1 and TcAMPK±2, and ² and ³ subunits, named TcAMPK² and TcAMPK³ respectively. In the present work we have focused on the characterization of TcAMPK±1; for this goal we subcloned into the pRIBOTEX vector the putative orthologous gene of *T. cruzi* (TcAMPKα1), to which we added an HA tag to perform further experiments.

To study its expression, we first evaluated its transcription through RT-PCR and the expression of the HA-fussed protein was confirmed in the soluble fraction by subcellular fractionation and subsequent western blot. After confirming its correct transcription and translation we demonstrated its localization in granules distributed in the cytoplasm by indirect immunofluorescence using an anti-HA antibody.

In the present time, we are assessing epimastigote cultures growth of wild type and TcAMPKalfa overexpressing parasites, with or without Compound C (Dorsomorphin) which is a potent and specific inhibitor of the AMPK activity. These studies will allow us to identify the biological function of this protein, which may be essential to maintain the energy homeostasis of the cell and to complete their life cycle.

Keywords: Chagas disease, metabolic sensor, energy homeostasis, AMPK.

(1410) STUDY OF SMALL GTPASE RAB11 IN *Leishmania mexicana.*

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Leishmaniasis is a human disease produced by species of the genus *Leishmania*, that infect 1 million individuals per year. As *Trypanosoma cruzi* and *Trypanosoma brucei*, *Leishmania* parasites express surface GPI-anchored virulence factors with key roles in infection.

In *T. cruzi*, the small GTPase Rab11 and the contractile vacuole complex (CVC) are key elements in the transport pathway of these proteins to the surface, whereas in *T. brucei* Rab11 mediates endosomal recycling, as in higher eukaryotes. The function of Rab11 in *Leishmania* has not been studied yet. To investigate this, we overexpressed (OE) a *GFP-LmRab11* fusion protein gene in *Leishmania mexicana*, cloned in the pNUS-nGFP vector. Expression was verified by western blot or flow cytometry and the overexpressed populations showed no growth differences with wild type and GFP^{OE} control parasites.

We found that the GFP-Rab11 signal is localized within a small intracellular region, as in *T. cruzi*, compatible with the location of the CVC. We observed morphological changes in Rab11^{OE} parasites with respect to controls. Scanning electron microscopy revealed that Rab11^{OE} parasites have less extracellular vesicles than the wild type.

To explore the effects of interfering with the Rab11 pathway, we transfected *L. mexicana* with mCherry-Rab11 "dominant-negative" (DN): an enzymatically inactive version of the GTPase by the S21N substitution. Despite numerous attempts, it was not possible to obtain Rab11DN^{OE} populations. However, simultaneous transfection with GFP-Rab11 was successful. As expected, we observed that the active GTPase localizes in the CVC region, while the Rab11DN signal is distributed throughout the cytoplasm. These results show that expression of Rab11DN is deleterious, as it was only possible by co-expression in Rab11^{OE} background.

This effect is not observed in *T. cruzi* and suggests that Rab11DN interferes with vital processes in *Leishmania* biology.

Keywords: Rab11, Leishmania mexicana, Contractile Vacuole Complex.

(465) **STUDYING THE ROLE OF NUCLEOSIDE DIPHOSPHATE KINASE 1 FROM *Trypanosoma cruzi* IN DNA INVOLVING MECHANISMS**

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Nucleoside diphosphate kinases are multifunctional enzymes that participate in many cellular processes besides their housekeeping function of maintaining intracellular pools of di and tri phosphate nucleotides. TcNDPK1 is an isoform of the enzyme present in Trypanosoma cruzi, the parasite that causes Chagas Disease. Among the multiple possible functions, several evidences associate TcNDPK1 with DNA processing, for example, the in vitro ability of binding nucleic acids and its nuclease activity. In the present work we evaluate the role of TcNDPK1 in DNA involving processes such as DNA repair using heterologous gene expression systems and over expression in epimastigote cells. We found that bacterias expressing the enzyme decreased five times the spontaneous mutation rate respect to controls, measured by counting rifampicin resistant colonies. In addition, yeasts lacking the endogenous gene YNK1 (YNK1-) but expressing TcNDPK1, grew more efficiently, tolerated high hydrogen peroxide concentrations (IC_{50} of 21.2mM and 6.3mM respectively) and were less sensible to UV irradiation *than control*. All these results support a possible role of TcNDPK1 in DNA repair mechanisms; however parasites *over expressing the enzyme had an opposite phenotype. We observed a lower growth rate with higher mortality, reaching parasite densities two to three folds lower than control in the stationary phase.* In addition, these parasites had augmented expression levels of activated PARP, an enzyme involved in DNA repair machinery, and twofold increased content of ATP. Such observations are in accordance with the generation of DNA damage, what was observed by gel electrophoresis of the genomic DNA. The non-protective behavior in parasites could be caused possibly by the over expression and deregulation of TcNDPK1. Altogether, these results exhibit the complexity of TcNDPK1 operation and strongly suggest its participation in DNA involving processes, giving rise to novel functions.

Keywords: *Trypanosoma cruzi*, nucleoside diphosphate kinase, DNA repair machinery, mutation

(324) **DEFINING THE FUNCTION OF AN HYPHOTETICAL CALCIUM BINDING PROTEIN IDENTIFIED IN THE PATHOGEN *Trypanosoma cruzi***

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Trypanosoma cruzi is the causative of Chagas disease, a neglected illness affecting ~6 million people in Latin America. Calcium and proteins that binds Ca+2 are important for parasite differentiation and infection into mammalian cells. Several channels and proteins that bind Ca+2 are expressed in *T. cruzi*, but most of them remain uncharacterized. We selected a new, hypothetical Ca+2 binding protein (*Tc*CAL1) from *T. cruzi* proteome to study its role in the parasite physiology. *Tc*CAL1 is a 103 amino acid protein containing two EF-hand motifs and it is expressed in all life cycle forms of *T. cruzi*. To define its function, a yeast two-hybrid assay, using a cDNA library from *T. cruzi* has been employed to analyze the association of *Tc*CAL1 with other proteins. We identified two proteins that interact with *Tc*CAL1. One was a protein containing an Armadillo-like domain. The Arm domains are present in several proteins and some of them depends their function on Ca+2 levels or small Ca+2 binding proteins (like S-100 proteins). The other was a hypothetical protein with homology to a prefoldin-like subunit. These proteins form multimeric complex that collaborate with chaperons. In certain complexes, the presence of Ca+2 is essential for an adequate protein folding. As a result, we hypothesize that *Tc*CAL1 can modulate the function of these proteins in different processes. Current work concerns the validation of *Tc*CAL1 associations in co-immunoprecipitation assays from *T. cruzi* extracts. Future work involves studies of *Tc*CAL1 deregulation and their effect on differentiation and infection, in order to establish its role in these processes.

Keywords: Ca+2 binding protein, *Trypanosoma cruzi*

(393) **CHARACTERIZATION OF *Trypanosoma cruzi* ALPHA-TUBULIN ACETYLTRANSFERASE**

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Among the numerous isoforms of α-tubulin found in the different microtubular structures of *Trypanosoma cruzi*, acetylated α-tubulin is one of the most abundant. In other organisms, one of the enzymes responsible for this modification is the α-tubulin acetyltransferase, or αTAT.

We have identified a coding sequence for the putative αTAT in the genome of *T. cruzi*, established lines that overexpress the protein of interest in an inducible manner, using the pTcINDEX-GW vector and characterized the mutant parasites phenotypically. As a first approach, we evaluated the overexpression of the αTAT in the mutants by western blot analysis (using anti-α-tubulin and anti-acetylated α-tubulin as primary antibodies), showing an increase in the levels of tubulin acetylation when αTAT is overexpressed. Besides, other experiments indicated that the protein localizes mainly in the cytoskeleton and flagellum of *T. cruzi*. Subsequently, we studied the growth of the mutant epimastigotes and a remarkable reduction was observed as a result of an increased expression of αTAT. Furthermore, we studied the morphology of the mutants by phase contrast microscopy and immunofluorescence analysis. The overexpression of αTAT led to an abnormal morphology in the parasites; aberrant nuclei and parasites containing more than one flagellum were observed. In addition, a higher concentration of the protein was detected mainly in the perinuclear region. Finally, we evaluated the phenotypic effect generated by orizalyn, a microtubule depolymerizing drug. Wild-type parasites lose their normal morphology after the treatment in a dose-dependent manner but differences in drug resistance were observed in the overexpressing parasites upon the induction.

These findings suggest that the mechanisms of tubulin modification –particularly acetylation- could influence the functional role of the microtubules, both in cell division and differentiation during the parasite's life-cycle.

Keywords: *Trypanosoma cruzi*, alpha-tubulin, acetylation, cytoskeleton

(1455) **CHARACTERIZATION OF HISTONE DEACETYLASE ENZYMES OF CESTODE PARASITES AS POTENTIAL DRUG TARGETS OF NEGLECTED DISEASES**

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Cestode parasites cause neglected diseases such as hydatidosis. These parasites have complex life cycles undergoing metamorphic events that comprise cell proliferation, differentiation and death. This suggests the involvement of a complex system of gene expression control that has been associated with changes in chromatin struc-

ture in trematode parasites. Histone deacetylase enzymes (HDAC) remove acetyl groups from histones and other cellular effectors, thus directly influencing the chromatin structure and thereby regulating gene transcription and other cellular processes. HDAC have been validated as drug targets for the treatment of cancer and other diseases including parasitic infections. However, knowledge of HDAC in cestode parasites is lacking. Previously, we have shown the presence and transcription of HDAC genes in several species of cestode parasites. In this work, we aimed to study the effect of the pan-HDAC inhibitor Trichostatin A (TSA) in *Mesocestoides corti*, a cestode laboratory model. We found a decrease in the viability, measured by AlamarBlue assay and motility index determination, and observed phenotypic alterations in *M. corti* larvae upon incubation with TSA. To assess the molecular target of TSA, we evaluated changes in the total amount of acetylated histone H4 by western blot using anti-acetylated histone H4 antibody. We observed a band corresponding to acetylated H4 histone in parasites treated with TSA but not in control parasites, suggesting that TSA strongly inhibits H4 histone deacetylation. This effect was not observed in parasites treated with praziquantel and albendazole suggesting a specific effect of TSA. These findings suggest that HDAC could have an essential role in cestode development and survival. This work provides a first step into the study of epigenetic mechanisms in cestode parasites and explores new alternatives to treat the diseases they cause.

Keywords: HDAC, neglected diseases, drug targets, cestode parasites.

(1055) **VIRTUAL SCREENING OF FLAVONOIDS AS POSSIBLE INHIBITORS OF *TRYPANOSOMA CRUZI* ARGININE KINASE.**

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Arginine kinase from *Trypanosoma cruzi* (*Tc*AK) is an enzyme involved in the energetic homeostasis and response to stressors in the parasite. The activity of enzyme, among some of its homologues from diverse phyla, is inhibited by some flavonoid compounds. In addition to it, one of these inhibitors, the resveratrol, affected the replication and survival of cultured epimastigotes as well as amastigotes/trypomastigotes infecting mammal cell cultures. In order to test other molecules that can throw light on the mode of interaction between the enzyme and the flavonoids, we arranged a simple pipeline that narrows the focus of the testing efforts to a handful of compounds.

For this, we downloaded the (now unavailable) database of medically active plant substances (MAPS), specifically the subset of flavonoid molecules, that comprised 326 compounds, and modeled the molecular interactions between these flavonoids and a homology model of *Tc*AK obtained from the iTASSER online server. These models were carried on two different molecular docking softwares, the open source AutoDock Vina, and the private licensed OpenEye.

Results from each software were ordered according to its docking score, and then both lists were compared to take only the molecules that were present between the best 100 scores of both programs, from which only 5 where shared among the first 20, 7 among the first 50 and 29 among e first 100. From these molecules, only 7 are purchasable, and three of them (delphinidine, malvidine and petunidine) were also present as possibly active compounds in the previous virtual screening that pointed out resveratrol as an inhibitor of the *Tc*AK activity, making them of high interest for future *in-vitro* tests.

Keywords: virtual screening, flavonoids, Arginine kinase, molecular docking

(1068) **DETECTION OF SPECIFIC IGE ANTIBODIES AGAINST *Trypanosoma cruzi* ARGININE KINASE.**

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The Arginine Kinase from *Trypanosoma cruzi* (*Tc*AK) is thought to be the product of gene transfer from an invertebrate host to a *Trypanosoma* ancestor. The function of this enzyme in the parasite is still unknown, but since it was found in extra-cellular *T. cruzi* vesicles, and it shares a very conserved sequence with invertebrate AKs, which are well known allergens in a very wide range of species, we suspect that *Tc*AK might have some immune modulating action by making immune response incline towards a Th2 response that is ineffective against the parasite, instead of a Th1 response that could control the infection.

To start testing this hypothesis we decided to measure the presence of *Tc*AK specific IgE antibodies in the serum of individuals infected and non-infected with the parasite. Samples were taken from individuals in Venezuela (49 Chagas positive and 46 Chagas negative) and in Argentina (20 positive and 10 negative), in order to take into account the regional differences in parasite strains and in host immune response. Detection was carried on by ELISA against recombinant *Tc*AK, using secondary anti-human IgE coupled with alkaline phosphatase and pNPP dephosphorilation. Results for each regional sera origin were obtained and treated separately, using for both groups a ROC curve and Mann-Withney test.

With both regional origins of the sera we found statistical significant differences in the *Tc*AK recognition between the non-infected and infected sera, with an Area Under the ROC curve >0,75 and a $p < 0,005$ for both the ROC analysis and the Mann-Withney test. These results points out that infection with the parasite trigers the production of IgE antibodies against *Tc*AK, but wether this priming of the response towards IgE secretion is relevant for the infection is still to be determined.

Keywords: IgE, Arginine kinase, Trypanosoma cruzi, ELISA.

IMMUNOLOGY (ADAPTATIVE IMMUNITY) 2

(311) ***Chlamydia muridarum*** **INDUCE THE EXPRESSION OF CO-INHIBITORY MOLECULES ON IMMUNE CELLS**

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Chlamydia trachomatis is the most commonly reported agent of sexually transmitted bacterial infections worldwide. This pathogen frequently leads to long-term persistent subclinical infections, both in male and female. This is in part due to certain mechanisms, not yet fully elucidated, that suppress the immune response to the pathogen. Recently, the role of co-inhibitory pathways in some chronic infections has been studied, and reports suggested that the overexpression of co-inhibitory molecules would be related to pathogen persistence. In the present work we aimed to evaluate the expression profile of co-inhibitory molecules using an *"in vitro"* model with *Chlamydia muridarum* (Cm) stimulation. For that purpose, splenocytes from C57BL/6 and NOD mice were in vitro stimulated for 24-48 h with inactivated Chlamydia Elementary Bodies at different bacteria/cell ratios. After that, PD-L1 and PD1 expression was determined by flow citometry in different cell subpopulations. A significant and dose dependent increase in the percentage of $PD\text{-}L1^+CD20^+$ lymphocytes was found after Cm stimulation when compared with non-stimulated cells ($P < 0.01$). Not only major percentages but also higher mean fluorescence intensity for PD-L1 were observed in B lymphocytes. Cm stimulation also induced higher percentages of $CD4^+PD\text{-}L1^+$ and $CD8^+PD\text{-}L1^+$ T lymphocytes, although in minor proportions when compared with B lymphocytes. Non-significant changes were observed in $CD11b^+PD\text{-}L1+$ and $CD11c^+PD\text{-}L1+$ cell populations. The expression of PD1 showed a similar pattern to

those observed for PD-L1. Moreover, although B and T lymphocytes from C57BL/6 and NOD mice showed PD-L1 overexpression, the NOD strain showed a more pronounced response after Cm stimulation. Our results demonstrate that Chlamydia is able to induce the expression of co-inhibitory molecules mainly in B lymphocytes, possibly attenuating the immune response and favoring chronic and persistence infections in genital tract.

Keywords: Infection ,Co-inhibitory pathways, Chlamydia muridarum , PD-L1

(548) **REGULATORY T CELLS DYSFUNCTION IN INDIVIDUALS WITH TRISOMY 21**

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Trisomy 21 (T21) is the most common genetic disorder in human population, occurring in approximately 1 in 700 live births. Individuals with T21 have a unique disease spectrum and a hyperactivated Type I interferon signaling, which could be a result of increased gene dosage of the four IFN receptor subunits encoded on chr21. T21 causes widespread alterations in gene expression across the genome, including, most prominently, consistent activation of the interferon transcriptional response. Based on this and the fact that individuals with T21 are predisposed to develop autoimmune diseases, we decided to dissect Treg functionality in individuals with T21, focusing on the effect that the type I IFN-IFNAR axis could have on its biology. We characterized the circulating Treg cells and T cells subsets in individuals with or without T21 using multicolor Flow Cytometry. Individuals with T21 (n=9) have a significantly alteration in the CD4/CD8 ratio ($p<0,001$), and higher numbers of CD8+ T cells compared with controls (n=14, $p<0,05$). Also, these individuals present more CD8+ T cells that produce effector proteins (IFN-γ $p<0,05$ and GzmB $p<0,05$). When we focused on Treg cells, individuals with T21 have a higher number of CD4+Foxp3+ T cells in peripheral blood ($p<0,05$) but the expression of more than 20 phenotypic markers analyzed were similar to controls. Interestingly, individuals with T21 have an increased frequency of IFNAR1+ Treg cells ($p<0,05$). When an *in vitro* suppression assay was performed, a reduced inhibitory potential of Treg was clearly observed in individuals with T21, whereas the proliferative capacity of the responder T cells was the same than their controls.

We hypothesize that the inappropriate Type I IFN activation could contribute with the alteration in the suppressive function of Treg in individuals with T21, and this could explain the increased risk of leukemia and autoimmune disorders, as well as many developmental abnormalities also observed in interferonopathies.

Keywords: Trisomy 21, Regulatory T cells, Type I Interferon Signaling, Suppressive Function

(643) **EVALUATION OF THE EFFECTS OF SKIN ULTRAVIOLET LIGHT EXPOSURE ON PNEUMOCOCCAL VACCINATION EFFECTIVENESS**

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Cutaneous exposure to UV radiation (UVr) promotes well-known detrimental effects on health, as skin cancer development and specific immunosuppression. However, the impact of this immunosuppression on human vaccination has been poorly studied.

Previously, we have reported that a single high UV dose (shUVd - 400 mJ/cm^2) promotes skin inflammation and decreases CHS reaction. In contrast, repetitive low UV doses (rlUVd - 4 consecutive days, 20 mJ/cm^2) do not induce inflammation and increase CHS.

The aim of the present work was to study the effect of cutaneous exposure to shUVd and rlUVd on the effectiveness of a non-conjugated pneumococcal vaccine and on B-1 cells expansion.

C57BL/6 mice were intramuscularly immunized with Pneumovax23 (MSD), 24h after irradiation. Non-irradiated vaccinated and non-vaccinated mice were used as controls. Specific antibodies (IgM and IgG) were measured by ELISA at different time-points post-vaccination.

Specific IgM and IgG levels were significantly increased in all vaccinated groups ($p<0.01$), but only IgG levels were significantly decreased in shUVd-exposed mice compared with rlUVd ones ($p<0.05$). No significant differences were observed on the B-1 cells percentage (CD45$^+$, CD3$^-$, CD19$^+$, CD43$^+$) in peritoneal cavity 4 and 24 h after immunization (non-irradiated mice).

Therefore, we decided to apply the vaccine intraperitoneally in mocked-irradiated mice. We observed a non-significant increase of the percentage of B-1 cells 4h after immunization and a significant decrease 24h after ($p<0.01$). Specific IgM and IgG levels were significantly increased in all IP vaccinated groups ($p<0.01$), but there were no significant differences between them.

These results show that UVr is able to modulate immune responses to pneumococcal vaccine, promoting a detrimental effect on the humoral response of shUVd exposed-mice. Further studies should be done to elucidate the effects of UVr in the modulation of B-1 cell population.

Keywords: UV, immunosuppression, vaccine, B-1 cells

(821) **DENDRITIC CELLS PLAY A CENTRAL ROLE IN THE PATHOGENESIS OF *Yersinia enterocolitica*-INDUCED REACTIVE ARTHRITIS IN TNFRp55 DEFICIENT MICE**

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Dendritic cells (DC) orchestrate immune responses presenting antigens to T lymphocytes and driving their activation and differentiation to effector T cells. In previous studies, we demonstrated that TNFRp55-deficient (*TNFRp55*$^{-/-}$) mice develop reactive arthritis (ReA) after orogastric infection with *Yersinia enterocolitica* (Ye). The purpose of the present work was to study the contribution of DC in the pathogenesis of Ye-induced ReA in this mouse model. The DC were purified after their *in vivo* expansion by injection of Fms-like tyrosine kinase 3-Ligand (Flt3L)-transfected BL16 melanoma. Isolated WT and *TNFRp55*$^{-/-}$ DC were stimulated with lipopolysaccharide (LPS), and IL-12/23p40 levels were assessed in culture supernatants by ELISA. Moreover, these cells were *in vitro* infected with Ye, and then co-cultured with purified CD4$^+$ T cells obtained from spleen of WT and *TNFRp55*$^{-/-}$ mice 5 days post-infection with Ye. After 5 days, T cell differentiation to Th1 and Th17 profiles were analyzed by flow cytometry, and IFN-γ and IL-17 levels were measured in culture supernatants. We found that *TNFRp55*$^{-/-}$ DC secreted higher levels of IL-12/23p40 compared with WT DCs (*p<0.05*). In co-culture of *TNFRp55*$^{-/-}$ DC with WT CD4$^+$ T cells, we found a significant higher frequency of IFN-γ$^+$CD4$^+$T cells and IL-17$^+$CD4$^+$ T cells (*p<0.05* compared with WT DCs). Accordingly, higher levels of IFN-γ and IL-17 were detected in these culture supernatants (*p<0.001* compared with WT DC). These findings indicate that DC play a critical role in the pathogenesis of Ye-induced ReA in *TNFRp55*$^{-/-}$ mice by overproduction of IL-12/23p40, and consequently driving Th1 and Th17 programs. The results suggest the existence of a TNFRp55-mediated anti-inflammatory circuit in DC. Therefore, these cells may be considered as a novel target in the treatment of ReA.

Keywords: dendritic cells, reactive arthritis, TNFRp55, *Yersinia enterocolitica*

(1004) **DEVELOPMENT AND VALIDATION OF A RECOMBINANT PROTEIN BASED COMPETITIVE-INHIBITION**

ELISA FOR DETECTION OF NEOSPOROSIS IN CATTLE

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Infection with *Neospora caninum*, an apicomplexan protozoan parasite, causes significant economic losses in cattle worldwide due to abortions. The reference method for serodiagnosis of neosporosis is the indirect fluorescent antibody test (IFAT), in spite of its complexity and subjectivity. The aim of this study was to develop and validate a competitive-inhibition ELISA (cELISA) based on major surface antigen protein (SAG1) of *N. caninum* and the monoclonal antibody (mAb) P1C2D8F8, for detection of antibodies to *N. caninum* in cattle. A truncated variant of NcSAG1 was expressed as a recombinant protein (rSAG1) in *Escherichia coli.* The mAb was generated by immunizing mice with sonicated *N. caninum* tachyzoites. $cELISA_{rSAG1}$ was standardized under different physicochemical conditions. Sera (n=1085) from 264 dairy cows, with known status of neosporosis infection were used as gold standard. Infected and uninfected population were defined as those with at least three consecutive positive (n=385) or negative (n=700) results by IFAT and $cELISA_{tach}$ (in-house test based on lysed tachyzoites), over four years monitoring. The prevalence of neosporosis in 262 beef cows from 12 herds of Santa Fe province was evaluated using the $cELISA_{rSAG1}$, and the results compared with IFAT, $cELISA_{tach}$ and commercial $cELISA_{VMRD}$. The efficiency of the $cELISA_{rSAG1}$ and agreement (*kappa*) with other tests was evaluated by Receiver Operating Characteristic analysis. The rSAG1protein had high yield, solubility and stability. $cELISA_{rSAG1}$ showed 97.1% sensitivity and 97.6% specificity with a cutoff point of 28% of inhibition. The prevalence of neosporosis in beef cattle was 36%. The agreement between $cELISA_{rSAG1}$ and IFAT or $cELISA_{tach}$ or $cELISA_{VMRD}$ was 92% *(k*= 0,820); 95% (*k*= 0.880); and 96% (*k*= 0.905*)* respectively. The $cELISA_{rSAG1}$ is simple, fast, and could be used for large-scale epidemiological studies or disease-monitoring programs of multiple animal species.

(1096) **EFFECT OF CIRCADIAN VARIATIONS IN MUCOSAL IMMUNE RESPONSE TO FOOD ANTIGEN**

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Many immune parameters oscillate rhythmically with the time of day and the circadian clock has emerged as an important gatekeeper that enhances fitness of the organism. In the present work we have characterized the influence of biological rhythms on the immune function of the intestinal mucosa and its impact on the tolerance towards oral antigens. Using multivariate statistic tests (PCA , ADL, MANOVA), we evaluated 17 parameters (cell subsets, homing receptors, activation markers and cytokines) in mesenteric lymph nodes (MLN) of B6 WT mice exposed to light-dark 12:12 conditions at four times (ZT5, 11, 17 and 23). PCA and ADL tests confirmed that over a 24-hr period, three different immune configurations can be described in MLN: at the light period (ZT 5); at the dark period (ZT17) and at the transition periods (ZT11 and 23). The distribution in these groups was dependent on the integrity of the molecular clock, as mice deficient in the clock gene *Per2* (Per2KO), showed significant differences with WT animals ($p<0.05$) as well as a reduction on the oscillatory pattern. We evaluated if immune configurations observed at ZT5 and ZT17 could be both competent to induce tolerance toward a fed antigen (ovalbumin-OVA). Using two models of tolerance, we found that the intake of the antigen during the late dark phase (ZT23) induced more tolerance evaluated as lower proliferation rates. Moreover, OVA specific T cells ($CD4^+$ $OT\text{-}II^+$) with increased expression of Foxp3 transcription factor ($p<0.05$) and selective localization in the lamina propria was elicited by the daily administration of OVA at the end of the dark phase. Our results demonstrate that in a period of 24 h, the feeding during the dark period and the development of tolerogenic responses seem synchronized in the MLN promoting the tolerance development that is essential for intestinal homeostasis.

Keywords: Mucosal immunology – Biological Clock - Tolerance -

(1289) **DISSECTING THE ROLE OF GALECTIN-3, IFN-γ AND MICROBIOTA IN GERMINAL CENTER B CELL FATE DECISIONS.**

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Germinal Centers (GC) are unique structures where high affinity antibodies and memory B cells are generated. With the aim of ensure fast selection of rapidly evolving clones in GCs, follicular dendritic cells, GC B cells and T cells engage in multiple interactions. So far, only co-stimulatory or inhibitory membrane immune receptor/ligand pairs and cytokines have been investigated and are known to be involved in this process. However, there is a pressing need to understand other factors that regulate GCs in order to optimize and control humoral responses following vaccination, antibody-mediated autoimmune diseases, and GC B cell-derived malignancies. Here, we report a novel effector function by which Galectin-3 (Gal-3), a β-galactoside binding protein, is critically involved in the development of GCs. Gal-3 KO mice exhibit high frequency of spontaneous GC (sGC) B cells and T follicular helper (Tfh) cells that correlated with hypergammaglobulinemia, high amounts of IFN-γ in serum and lupus-like autoimmune manifestations. We found that the main source of IFN-γ in Gal-3 KO mice were CD4+ T cells (including Tfh), since there are no significant differences between IFN-γ-producing CD8 T, NK, NKT and DCs cells between groups. IFN-γ blockade in Gal-3 KO mice prevents the autoimmune condition, demonstrating that IFN-γ overproduction sustain lupus-like disease. We were also wondering whether specific microbiota in Gal-3 mice could influence the induction of sGCs. Microbiota modulation by antibiotic treatment could not prevent sGCs induction in Gal-3 KO mice and, interestingly, these mice did not modify the generation of GCs in Peyer's patches after treatment, unlike the WT mice which showed a deep reduction of them. Finally, using chimeric mice with B cell-specific deficiency of Gal-3, we reveal that intrinsic Gal-3 signaling in B cells control sGC formation. Together, our data provide the first evidence that Gal-3 acts directly on B cells regulating GCs responses via IFN-γ.

Keywords: Germinal Centers; Galectin-3; Autoimmunity; IFN-γ; B cell Immunology.

(1598) **EFFECT OF *Trypanosoma cruzi* CYTOSOLIC TRIPAREDOXIN PEROXIDASE ON PERIPHERAL BLOOD MONONUCLEAR CELLS FROM PATIENTS WITH CHRONIC CHAGAS DISEASE**

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Trypanosoma cruzi, the etiological agent of Chagas disease (ChD), has a highly efficient detoxification system to deal with the oxidative burst imposed by its host. One of the antioxidant enzymes involved is the cytosolic tryparedoxin peroxidase (TcCPx) which catalyzes the reduction of hydrogen peroxide, small-chain organic hydroperoxides and $ONOO^-$. This enzyme is present in all parasite stages, and its over-expression renders parasites more resistant to oxidative defenses of macrophages, indicating an important role in

parasite survival. However, TcCPx not only constitutes a relevant virulence factor in ChD, but also promotes inflammatory reactions in *T. cruzi*-infected mice. The aim of this study was to analyze the specific humoral and cellular immune response triggered by TcCPx in chronic asymptomatic (n=7) and cardiac patients (n=6), and non-infected individuals (n=5). Results showed that levels of IgG antibodies against TcCPx were low (titer<1/400) in sera from individuals across all groups. However, after five days of *in vitro* stimulation, TcCPx induced proliferation of peripheral blood mononuclear cells (PBMC) from asymptomatic patients (p<0.01). In addition, only interferon-γ, but not GM-CSF or IL-10, were detected in the supernatant of PBMC from asymptomatic patients (p=0.04) under the same culture conditions. No response of either type was observed in cardiac patients and non-infected individuals. NetMHCpan and NetMHCIIpan were used to predict potential binding peptides within TcCPx for HLA-class I and II, respectively. The algorithm highlighted four regions of 16-23 aminoacids long containing peptides with high binding probability to most prevalent alleles of α and β chains of HLA-II in central and Northern Argentine population, while potential HLA-I epitopes were mostly found in N-terminal region of the protein. We expect that our research can contribute to understand the role of TcCPx on the immune response of chronic Chagas disease patients.

Keywords: Cytosolic tryparedoxin peroxidase, *Trypanosoma cruzi*, chronic Chagas disease, epitope prediction.

(1810) **DESMOGLEIN-4 DEFICIENCY INCREASES RESIDENT CD3+ T CELL SUBSET IN A RAT PSORIASIS MODEL**

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It is known that desmogleins are involved in cell adhesion mechanisms and are crucial in keeping structural integrity of different tissues, including skin. They also play important roles in differentiation, cell activation and migration. Keratinocytes (KC) produce several inflammatory factors that modulate leukocytes. Psoriasis is a chronic inflammatory skin disease, characterized by KC hyperproliferation, vasculature growth, and leukocyte infiltration into the dermis and epidermis. Imiquimod (IMQ) is an immunomodulator used in mice to induce lesions closely resembling human psoriasis. The aim of our work was to assess the impact of desmoglein-4 deficiency in the amount of skin leukocyte infiltration in an IMQ induced psoriasis model in rats. To this end, OFA hr/hr rats, which are mutant for the desmoglein-4 gene and Sprague-Dawley (SD) wild type rats were used. IMQ was administered to both strains in shaved skin for four days to generate psoriasis-like lesions. Skin biopsies from treated and untreated OFA and SD rats were weighed and minced to obtain cell suspensions that were stained with monoclonal antibodies against CD45 (panleukocytary lineage) and CD3 (T cell lineage) conjugated with fluorochromes and analyzed by flow cytometry. Interestingly, we found that IMQ treatment to both groups increased CD45+ CD3+ cells in OFA skin compared to controls, but this difference was much greater and significant in OFA rats (SD 0.79±0.24 vs OFA 4.12±0.75, p<0.05) that correlated with increased inflammatory area and histopathologic changes in OFA rats. These results suggest that desmoglein-4 absence contributes to psoriasis progress, promoting expansion of leukocyte population in skin. Although further research is needed, these results could have a potential impact on the design of clinical therapies for psoriasis progression.

Keywords: Desmoglein-4, psoriasis, imiquimod, immune response

(1894) **DENDRITIC CELLS ARE RESPONSIBLE FOR THE FAILURE IN CTLs GENERATION IN LSP1-DEFICIENT MICE**

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Leukocyte-specific protein 1 (LSP1) is an important regulator of actin cytoskeleton remodeling, modulating leukocytes motility, due to its F-actin binding domain. We have previously shown that *Lsp1*$^{-/-}$ mice have an impaired CTL response after antigen exposure. Moreover, *Lsp1*$^{-/-}$ dendritic cells (DCs) fail to induce a strong CTL response in vivo, migrate to lymphoid tissues, present antigens and produce IL-12 when transferred into WT mice.

In order to deepen the mechanisms operating this diminished CTL generation in *Lsp1*$^{-/-}$ mice, we first analyzed the ability of their CD8$^+$ T cells to proliferate and become activated. After *in vitro* stimulation with αCD3/CD28, *Lsp1*$^{-/-}$ CD8$^+$ T cells proliferated and up-regulated CD25 as strongly as WT CD8$^+$ T cells. Granzyme B production and IFNγ release were slightly lower (p<0.05) in *Lsp1*$^{-/-}$ vs WT CD8$^+$ T cells, with no difference in IFNγ production.

Then, we evaluated *in vitro* activation of CD8$^+$ T cells purified from OT I (*Lsp1*$^{+/+}$) mice after culture with BMDCs (differentiated from bone marrow precursors with Flt3-L), previously pulsed with latex beads coated with ovalbumin, plus CpG-ODN. Cell proliferation, CD25 up-regulation and IFNγ secretion was significantly lower in T cells cultured with *Lsp1*$^{-/-}$ BMDCs than with WT BMDCs (p<0.01). Finally, we compared the ability of Flt3L-BMDCs from *Lsp1*$^{-/-}$ and *Lsp1*$^{+/+}$ mice to translocate antigen from endosomes to cytosol by performing the βLactamase export assay. *Lsp1*$^{-/-}$ BMDCs showed a slower escape of βLactamase to the cytosol, indicating a slower Ag translocation than in WT BMDCs.

In conclusion, these results reveal that the diminished generation of CTLs we observed in *Lsp1*$^{-/-}$ mice is due to a deficiency in the ability of DCs to cross-present Ag rather than a failure in their CD8$^+$ T cells. This deficiency in *Lsp1*$^{-/-}$DCs is related to an alteration in the escape of the antigen from endosomes to the cytosol, which led to fewer antigens associated to MHC-I to be presented to CD8$^+$T cells.

Keywords: LSP1; Dendritic Cells; Cross-presentation; CTLs.

IMMUNOLOGY (IMMUNOTHERAPY) 1

(182) **THE TSf-ISPA FORMULATION CONFERS PROTECTIVE CAPACITY AGAINST *Trypanosoma cruzi* INFECTION AFFECTING CELLULAR POPULATIONS WITH REGULATORY/SUPPRESSOR PHENOTYPE**

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Introduction: Previously, we have described that a fragment of the trans-sialidase protein (TSf) formulated with an adjuvant developed by our group (ISPA) conferred protective capacity against *Trypanosoma cruzi* (*T. cruzi*) infection.

Objective: to advance in the characterization of the regulatory immune response generated after TSf-ISPA immunization as well as after immunization and *T. cruzi* infection.

Material and methods: BALB/c mice were immunized 3 times every 15 days with 10ug TSf plus 5ug ISPA (TSf-ISPA group) (PBS was used as a control). Fifteen days after the last immunization, mice were challenged with 1000 trypomastigotes (Tulahuen). 7 days after immunization and 14 and 21 days post infection (p.i), flow cytometry was carried out to measure the percentage and number of spleen CD4+ Foxp3+ (Treg) cells and CD11b+GR-1+ myeloid-derived suppressor cells (MDSC).

Results: After immunization, the TSf-ISPA group showed similar levels of Treg cells and a slight but significant increase in the percentage and absolute number of MDSC cells in the spleen. (mean% CD11b+GR1+ ±SD): TSf-ISPA: 5.3±1.7; PBS: 3.2±0.5, p<0.05, n=4 per group.

At 21 days p.i. an increase in the number of Treg cells in the spleen of the immunized and infected mice (TSf-ISPA Tc+) was registered

as compared to the PBS infected group (PBS Tc+). In addition, a decrease in the percentage and absolute number of MDSC cells in the TSf-ISPA Tc+ group was observed as compared to PBS Tc+. Example: (mean% CD11b+GR-1+ ±SD) TSf-ISPA Tc+: 9.5±1.3; PBS Tc+: 17.1±2.1, p<0.05, n=4 per group). In addition, the results showed that the TSf-ISPA formulation affected significantly (p<0,05) the granulocitic-MDSC subtype (CD11b+Ly6G+LY6C+/-) and not influenced significantly (p>0,05) the monocitic-MDSC subtype (CD-11b+Ly6C+Ly6G-).

Conclusion: these results show that the protection conferred by TSf-ISPA immunization correlates with alterations in cellular populations with regulatory/suppressor phenotype.

Keywords: *T. cruzi*, vaccine, trans-sialidasa, CD4+Foxp3+ Treg cells, Myeloid-derived suppressor cells

(195) **EVALUATION OF PEPTIDE FORMULATIONS AND ADJUVANT DOSES REQUIRED TO ELICIT A SPECIFIC CD8+ T CELL RESPONSE AGAINST AN ENDOGENOUS PROSTATE CANCER OVER-EXPRESSED ANTIGEN**

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Active immunization has been proposed as a promising strategy for treatment of advanced Prostate Cancer. However, few tumor associated antigens have demonstrated to elicit an efficient immune response and moreover, our knowledge about how to perform such immunizations remains incipient. Previously, we postulated Galectin-1 as a novel immunogen in PCa and immunizations with a Gal-1 derived peptide induced specific cytotoxicity. This work evaluates different conditions to ameliorate the immunization protocol. First, we decided to comparatively study the use of short peptides (Gal-1 MHC class I and PADRE MHC Class II) *vs* a long peptide (both peptides joined by a cleavable link). Second, we evaluated the adjuvancy doses. Both peptide formulations combined with conventionally used doses of adjuvants (900mM PolyU + 25uM CpG) do not induced specific cytotoxicity while 1/32 lower adjuvant doses induced 15% for the long peptide *vs* 2% for the short ones (p <0.05, t-test) at day 7 post-priming. This prompted us to comparatively characterize at the cellular level these different immunization protocols. Immunization with long peptide and lower doses of adjuvant demonstrated a moderate total lymph node cell burst in contrast to the great expansion seen with conventional doses of adjuvant. Additionally, lower doses of adjuvant decreased Tregs and more importantly, induced a 2.8 fold increase in the absolute numbers of tetramer specific CD8+ T cells. These parameters could explain the higher response obtained. To gain better insights about parameters required for an optimal boost, we demonstrated that low levels of adjuvant are required in re-stimulations but, in this phase, long peptides do not increase the specific cytotoxicity while the short ones do, suggesting that the use of long peptides is only crucial at priming. In summary, our results challenge current ideas about the parameters required to perform a better immunization against an endogenous, tumor-overexpressed antigen.

Key words: cancer immunotherapy, novel therapeutic targets, specific CD8+ T cells

(256) **ASSESSMENT OF BENZNIDAZOLE AND CCR4 ANTAGONIST COMBINATION FOR *Trypanosoma cruzi* CHRONIC INFECTION TREATMENT**

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Benznidazole (Bz) is the main drug used to treat *T. cruzi* infection. Besides its parasiticide effect it is able to stimulate T cell specific effector responses. Taking in mind that this immunological modulation has shown to be important for parasite eradication, in this work we hypothesized that the efficacy of Bz treatment could be improved by inhibiting T regulatory cells, using a CCR4 antagonist receptor (CCR4a). BALB/c mice were infected with 10^6 trypomastigotes of Sylvio *T. cruzi* strain. Treatment was administered at 120 days post infection (dpi) and groups were assigned as follow: CCR4a+Bz (treated with subcutaneous CCR4a and oral administration of Bz 100 mg/kg); CCR4a alone; Bz alone (100 mg/kg); PBS (untreated infected group) and NI (non-infected group). At 188 and 273 dpi, parasitemia was evaluated by qualitative PCR. Also, specific IgG1 and IgG2a levels were measured by indirect ELISA. At 273 dpi, electrocardiograms (ECG) were carried out and heart parasite load was determined by PCR real time. In all infected groups, the presence of the parasite was found before and after different treatments. Heart parasite load at day 273 was not significantly different in all infected groups. Levels of specific antibodies were meaningfully increased in all groups after treatment (p <0.05), being IgG2a levels higher than IgG1 levels in all cases (p <0.05). IgG2a levels decreased in CCR4a+Bz in relation to the PBS group (p <0.05). NI mice did not present electrical disturbances in ECG. In the same way, CCR4a+Bz group demonstrated a trend to a lower frecuency of arrhythmias comparing to PBS (p = 0.059). Otherwise, groups with CCR4a and Bz alone did not decrease these alterations. Our results suggest a possible synergy of both drugs although it would be necessary to enlarge the groups to corroborate these findings.

Keywords: Benznidazole, *T. cruzi* infection, CCR4 antagonist receptor.

(791) **ALLOGENEIC AND SYNGENEIC CELLS AS ANTIGEN SOURCE AND THEIR EFFICIENCY IN DC-BASED ANTI-MELANOMA VACCINATION**

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A major obstacle to obtaining relevant results in cancer vaccination has been the identification of immunogenic antigens. In particular, dendritic cell (DC)-based cancer immunotherapy can be achieved by loading DCs with syngeneic or allogeneic cells, but the consequences brought upon anti-tumor protection are not clear. When using syngeneic tumor cells, tumor self-antigens, including cancer testis antigens (CTA), other tumor-associated antigens (TAA) and neoantigens generated through mutations during tumor progression, are provided. On the other hand, allogeneic tumor cells could only supply shared CTA and other TAA. To assess the advantages of each system, we have analyzed in a murine model the effect on anti-melanoma protection of loading DCs with irradiated syngeneic B16-F1 melanoma, allogeneic Cloudman melanoma, or allogeneic 4T1 mammary carcinoma cells (DC-ApoNec vaccines). The cells were fully characterized by whole Exome Sequencing and RNAseq. Using Spinning Disk Confocal Microscopy we observed that irradiated tumor cell components were efficiently internalized by DCs, and transported to MHC-class II-positive tubulovesicular compartments. Loading DCs with allogeneic cells induced significantly higher DC maturation than syngeneic cells, measured by Flow Cytometry as CD86 and I-A^b surface expression. DC-ApoNec vaccines were administered to C57Bl6 mice, followed by B16-F1 tumoral challenge. Allogeneic melanoma cells induced effective anti-melanoma protection (p<0.05), but syngeneic melanoma cells established a more potent (p<0.001) and long-lasting protection. Interestingly, when allogeneic mammary carcinoma cells were used to load DCs, short-

term (p<0.001) and long-lasting anti-melanoma protection were also obtained. By RNAseq, we determined that B16-F1 neoantigens were not present in allogeneic cells, while shared CTA and other TAA and were identified in syngeneic and allogeneic cells, suggesting they play an important role on efficient anti-tumor protection.

Keywords: anti-melanoma vaccination, dendritic cells, allogeneic cells, syngeneic cells.

(891) **CO-ADMINISTRATION OF *Toxoplasma gondii* SERINE-PROTEASE INHIBITOR-1 WITH ALLERGEN INDUCES REGULATORY CELLS IN ASTHMATIC MICE**

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UNSAM, ECyT, CESyMA

By using a therapeutic experimental asthma model we previously showed that treatment with *T. gondii* serine protease inhibitor-1 (TgPI-1) significantly reduced airway hyper-responsiveness and inflammation. Moreover, co-administration of TgPI-1 with the allergen showed an enhanced improvement compared with the administration of TgPI-1 alone. Besides, we observed that allergen-specific Th1 and Th2 responses were diminished. Based on these results, in the present study we sought to explore whether regulatory cells may account for the effect of TgPI-1. For this purpose we first evaluated if TgPI-1 treatment modulates allergen-specific T cell proliferation. BALB/c mice ip. sensitized with ovalbumin (OVA)/Alum and aerosol challenged, were intranasally treated with TgPI-1 (PI) or TgPI-1+OVA (OPI). Controls included non-sensitized mice (N), and asthmatic mice treated with PBS (O) or with OVA (OO). One week after the treatment, mice were re-challenged. Only mice treated with OPI showed a significant reduction in thoracic lymph node cells OVA-specific proliferation (OPI vs OO; p<0.001). This treatment also induced a significant increase in $CD4^+Foxp3^+$ Treg cells compared to *naïve* mice (p<0.05). These results were not accompanied by an increase in TGF-β or IL-10 production. A significant expansion of Tregs (p<0.05) and a diminished allergen specific proliferation (p<0.01) in OPI group compared to OO and N groups was also detected at systemic level. Similar to lymph nodes, PI treatment didn`t result in a higher frequency of $CD4^+Foxp3^+$ cells nor a reduced OVA-specific splenocyte proliferation. These results might explain the better outcome of co-administration of TgPI-1 with allergen and suggest that $CD4^+Foxp3^+$ Treg cells might be involved. Functional studies will be undertaken to confirm this hypothesis. Overall, TgPI-1 emerges as a promising tolerogenic adjuvant to be included in therapeutic vaccine formulations.

Key words: Asthma, Serine-protease inhibitor, Immunotherapy

(959) ***BRUCELLA ABORTUS* U-OMP19 IMPROVES THE IMMUNE RESPONSE AND PROTECTIVE CAPACITY OF ENTEROTOXIGENIC *ESCHERICHIA COLI* (ETEC) ANTIGENS**

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Enterotoxigenic *E. coli* (ETEC) is the most common cause of bacterial diarrhea both among children in developing countries and in travelers to these regions. Both naturally acquired infection and oral-mucosal vaccination against ETEC enterotoxins, colonization factors or adhesins can induce protective immunity. EtpA, an extracellular ETEC adhesin, is a novel antigen (Ag) with immunogenic and protective capacity found recently.

We propose to use a bacterial protease inhibitor, U-Omp19 from *Brucella abortus*, as platform to deliver antigens in oral formulations against infectious diseases since this protein can protect antigens (Ags) from digestion and it can also trigger and direct the type of immune responses. Thus in this work our aim was to investigate the effect of U-Omp19 co-delivery on EtpA immunogenicity and protective efficacy. To this end inbread BALB/c and outbread CD1 mice were orally immunized with i) saline ii) EtpA iii) EtpA+Omp19 iv) EtpA+dmLT or v) EtpA+dmLT+Omp19. Different doses of EtpA were studied alone or plus adjuvants. Fecal and serum anti-EtpA and anti-LT antibodies (Abs) were evaluated by ELISA. Cellular immune responses or the protective capacity of formulations against oral challenge with ETEC H10407 were also evaluated.

Results obtained indicated that U-Omp19 co-delivery increased mucosal anti-EtpA or anti-LT Abs (IgA in fecal extracts) and induced EtpA-specific IgA and IgG producing memory B cells in bone marrows. U-Omp19 improved Ag-specific IFN-γ and IL-17 response in spleens (P<0.05) and mesenteric lymph nodes (P<0.01). Moreover, U-Omp19, when co-delivered with EtpA or with lower doses of dmLT and EtpA induced significant protection (P<0.05) against oral challenge with ETEC H10407, while the Ags alone did not.

All together our results indicate that U-Omp19 can help to reduce Ag dose and could be a good candidate to be included in new vaccine formulations against ETEC.

Keywords: U-Omp19; ETEC; vaccine; adjuvant; EtpA

(1212) **A LIQUID CRYSTAL NANOSTRUCTURE, USED AS VACCINE PLATFORM, MODIFIES BIODISTRIBUTION OF VACCINE COMPONENTS**

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In the last years much effort in vaccinology has focused on the new formulation strategies for subunit vaccines. We formulated OVA (antigen) and CpG-ODN (TLR-9 agonist) with a nanostructure formed by self-assembly of 6-O-ascorbyl palmitate (Coa-ASC16). *We have previously shown that* this nanovaccine (OVA/CpG-ODN/Coa-ASC16) elicited an adaptive immune response superior to those induced by an aqueous formulation (OVA/CpG-ODN). However, we still do not know exactly the mechanisms of action of Coa-ASC16. Hence, the aim of this work was to test the impact of this nanoformulation on biodistribution of vaccine components and early immune response. Methods: mice were s.c. immunized with OVA/CpG-ODN or OVA/CpG-ODN/Coa-ASC16. OVA and CpG-ODN were labeled with near-infrared fluorescent dye, and both signals were measured with an Odyssey® CLx at several time points post immunization (pi). Cytokines/chemokines were evaluated in plasma by a multiplex assay at 1.5h pi. Results are indicated as OVA/CpG-ODN vs OVA/CpG-ODN/Coa-ASC16. Liver: OVA signal was 1.2×10^7 vs 0.6×10^7 (p<0.01), 4.3×10^7 vs 1.0×10^7 (p<0.001) and 2.0×10^7 vs 0.8×10^7 (p<0.01) at 20 min, 2 and 4h pi. No signal was observed in spleen and kidney in any of groups. Injection site: OVA signal was 1.6×10^6 vs 6.9×10^6 (p<0.001) and 0.05×10^6 vs 2.45×10^6 (p<0.05) at 0.3 and 5 days pi; for CpG-ODN there was no significant differences between both groups at any time. Lymph node: OVA signal was 1.8×10^5 vs 0.3×10^5 (p<0.01), 0.5×10^5 vs 4.5×10^5 (p<0.001) and 0.1×10^5 vs 2.8×10^5 (p<0.01) at 20 min, 2 and 24h pi; in contrast CpG-ODN signal was similar between two groups at 20 min and 2h pi. In addition, soluble vaccine elicited higher amounts of systemic TNF-α and MCP-1 than nanovaccine immunization (p<0.05). Conclusions: Coa-ASC16 retains antigen at the injection site but not CpG-ODN, and promotes co-delivery of both molecules to lymph node without concomitant induction of systemic inflammation.

Keywords: vaccine, adjuvant, nanostructure, CpG-ODN

(1848) **EFFECTS OF EDIBLE MUSHROOMS EXTRACTS ON PLATELET ACTIVATION AND AGGREGATION**

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Mushrooms have been used as food and medicine in different cultures. Beside their high nutritional value, mushrooms are rich in many bioactive compounds of high medicinal values such as small organic molecules, polysaccharides and proteins. These bioactive components have become popular sources of natural antitumor, antiviral, antimicrobial and immunomodulatory agents. In particular, immunomodulators have gained much interest because of the increasing growth of the immunotherapy sector. The immunomodulators are substances which can stimulate, suppress or modulate any of the components of the immune system, including platelets. Although they are known mainly for maintaining vascular integrity, platelets are potent modulators and effectors of inflammatory and immune processes.

Taking into account mushroom properties and the role of the platelets in the immune response, the aim of this work was to study the effect of different extracts on platelet aggregation and activation. In order to achieve this goal, protein enriched extracts of *Agaricus bisporus* (Champignon and Portobello), *Ganoderma lucidum* (Reishi) and *Lentinula edodes* (Shiitake) were prepared, and platelets were exposed to them for one hour. After that, an aggregation assay using thrombin as an agonist was performed. In addition, secretion of different cytokines was analyzed by ELISA.

The results showed that Champignon, Reishi and Shiitake extracts modified platelet aggregation kinetics. On the other hand, Portobello extract was able to reduce platelet aggregation. Regarding cytokines secretion, none of the extracts increased IL-6 expression, but *Agaricus bisporus* extracts (Champignon and Portobello) were able to induce INFγ secretion.

In conclusion, these findings suggest that extracts of the edible mushrooms studied have compounds that could be used as modulators of platelet activation. Thus, the extracts obtained are a promising source of immunomodulatory compounds.

Keywords: edible mushrooms, immunomodulators, platelet activation

ANIMAL BIOLOGY 3

(1624) **EFFECTS OF *Lactobacillus johnsonii* AJ5 METABOLITES ON *Apis mellifera* NUTRITION, IMMUNITY AND THEIR TOXICITY AGAINST *Varroa destructor***

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Apis mellifera L. is an essential pollinator affected by several stressors linked with colony losses and the disturbance of their ecological function. Among this, *Varroa destructor,* an ectoparasitic mite affects seriously the colonies. The relationship between stressors and bee symbionts is being studied to enhance bee health. The goal of this study was to evaluate the effect of cell-free supernatants (CFS) produced by *Lactobacillus johnsonii* AJ5*,* a bee bacterial symbiont, on *A. mellifera* nutrition, immunity and their toxicity against *V. destructor.* Bees in cages were fed *ad libitum* with a solution of 40% v/v (CFS/sugar syrup) for 6 days. Two controls were performed: one with sugar syrup (50% w/v) and the other with a solution of MRS broth and syrup (6.25 % v/v). Assays were performed by triplicate. Soluble protein was assessed in samples of 20 abdomens per treatment. Samples of each replicate were used to analyze by quantitative real-time PCR (qRT-PCR) the expression of immunity genes: Vitellogenin (Vg), Insulin-like peptides 2 (Ilp2), Domeless (Dom), Hymenoptesin (Hym) and Propheniloxidase (Ppo), using beta actin as housekeeping gene. Toxicity against *V. destructor* was assayed in glass Petri dishes (n=5) feeding 3 bees *ad libitum* as mentioned above. After three days, six female mites were incorporated per dish, and their mortality was registered at 24, 48, and 72 h. Results were analyzed by ANOVA and means were compared with the Tukey test. Soluble protein in bees fed with the CFS was significant different respect to controls ($p<0.05$). Toxicity against *V. destructor* was observed for CFS with a 56.6% of effectiveness with differences respect to controls ($p<0.05$). Non statistical differences were detected in mRNA expression levels of Vg, Ilp2, Dom, Hym and Ppo between treatments. Further studies are needed to consider *Lactobacillus johnsonii* AJ5 CFS as a natural alternative for the enhancement of bee health.

Keywords: *Apis mellifera*, *Varroa destructor*, *Lactobacillus johnsonii* AJ5, nutrition and immunity

(1189) **THE INDUCTION BY BEAUVERIA BASSIANA OF A SPECIAL INNATE IMMUNE RESPONSE IN THE INSECTS CERATITIS CAPITATA AND TENEBRIO MOLITOR**

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Insects trigger a multifaceted innate immune response to fight microbial infections. We previously demonstrated that a response to microorganisms (bacteria and yeast) infection is the induction of N-β-alanyldopamine (NBAD) synthesis in the insects epidermis. Here we show for the first time the ability of entomopathogenic filamentous fungi to induce that special innate immune response (SIIR) through the induction of NBAD- synthase. We tested the pathogenicity of two novel strains of *Beauveria bassiana* selected for their infectivity in dipterans (strain 436) and coleopterans (strain 075) in the Medfly *C. capitata* and in the mealworm *T. molitor*. We here demonstrated the induction of the NBAD- synthase in cell-free extracts of both insects.

We show that *B.bassiana* 436 is pathogenic for *C.capitata* when topically administered with a suspension of 1.10^6 conidia/mL. When infected, survival in the medfly was 19 ± 1.73 days compared to controls 32 days (Test Mantel-Cox p=0.00284 (S). No significant differences were observed between sexes. Surprisingly, the strain 075, entomopathogenic for coleopteran, also proved to be infective for diptera using a puncture method ($p<0,05$).

Larval malformations in *C. capitata* were observed in sublethal infections with the strain 436 in *C. capitata*, whereas strain 075 induced in some cases unusual cuticle tanning in *T. molitor* adults. Although the insects reinforced the cellular and humoral main immunological system by expression of the SIIR they were unable to neutralize the fungal infection.

Keywords: RIINS- Survival- Beauveria bassiana- Insects

(1737) **GONADOTROPIN INHIBITORY HORMONE (GNIH) A LINK BETWEEN FEEDING AND REPRODUCTION IN THE CICLID FISH *Cichlasoma dimerus*.**

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GnIH downregulates reproductive function in vertebrates. Moreover, there are reports that propose that GnIH acts as an orexigenic peptide in birds and mammals. Previously, we characterized *Cichlasoma dimerus'* GnRH variants (sGnRH, sbGnRH, cGnRH), the orexigenic peptide Neuropeptide Y (NPY) and GnIH peptide, and demonstrated that GnIH inhibited gonadotropin but stimulated growth hormone release in adults of this specie. The general ob-

jective was to start studying GnIH as a link between feeding and reproduction in *C. dimerus*. We evaluate possible morphological relationships between neurons and fibers of GnIH, GnRH variants and NPY, and we analyze variations in the expression of those peptides in animals after fasting. By double label confocal immunofluorescences, we found that neurons of the *nucleus olfacto retinalis* (NOR) co-expressed GnIH and sGnRH, and that some fibers co-expressed both peptides while others were immunoreactive (-ir) to each one at hypothalamic level. There were no contacts between sbGnRH or cGnRH fibers with GnIH neurons in the NOR. GnIH-ir neurons of the hypothalamus did not co-express any GnRH variant. GnIH-ir fibers were observed in close proximity to NPY-ir fibers at hypothalamic level and few contacts between cGnRH-ir and GnIH-ir fibers were observed in midbrain. On the other hand, animals were isolated in aquaria and daily fed at a fix time. After an adaptation period, they were randomly assigned to feeding or fasting groups. Four weeks later, variations in mRNA levels of those peptides were determined by real time PCR in telencephalon, hypothalamus and medium-posterior brain. Fasting conditions induced a significant decrease in GnIH mRNA levels ($p=0.0375$) in the hypothalamus, a clear tendency of decreasing in NPY expression but no changes for GnRH variants in telencephalon and medium-posterior brain. Taking into account our results, we proposed that GnIH could act as a link between feeding status and reproduction in *C. dimerus*.

Keywords: GnIH, GnRH, NPY, Fasting, Fish.

(895) **IMPACT OF DIETARY PHYTOCHEMICALS AND ACARICIDE EXPOSITION ON LONGEVITY AND STIMULATION OF DETOXIFICATION MECHANISMS IN *Apis mellifera***

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Recent studies have reported how pesticides impact on bees, affecting their behavior and survival. The main pollutants in bee colonies are due to chemical control of *Varroa destructor*, an ectoparasite mite of *Apis mellifera*. It has been shown that the nutritional quality of *A. mellifera* diet appears to play an essential role in honey bee health, particularly in the presence of pesticides. In this work, we examined the effect of phytochemicals (p-coumaric acid, indole-3-acetic acid and gibberellic acid) in lifespan of honey bees. Besides, we evaluated if administration of phytochemicals in the diet increases the tolerance of bees to a synthetic acaricide, by stimulation of detoxification mechanisms. We conducted a series of longevity assays with nurse bees fed with sugar syrup in the presence or absence of p-coumaric, indole-3-acetic acid and gibberellic acid. Then, bees were exposed to DL_{50} of fluvalinate topically. We further explored if administration of indole-3-acetic acid in the diet can impact the expression of mRNA of genes involved in detoxification pathways. Indole-3-acetic acid enhanced tolerance of fluvalinate (log-rank: 3.823; $p \leq 0.05$); p-coumaric acid had a similar effect, although of reduced magnitude (log-rank: 2,433; $p \geq 0.05$) and gibberellic acid did not produce a significant result. mRNA expression of cytochrome P450 6AS4 and carboxylesterase genes was upregulated in bees fed with indole-3-acetic acid diet. Although, in bees with indole-3-acetic acid diet exposed to fluvalinate, the expression of these genes was lower than in not exposed bees. The expression of cytochrome P450 9Q3 was downregulated in bees fed with indole-3-acetic acid diet. Our results underscore the importance of phytochemicals naturally present in nectar and pollen, and their influence in honey bee longevity and pesticide stress.

Apis mellifera; *Varroa destructor*; phytochemicals; detoxification; nutrition.

(1236) **PROLACTIN INDUCING FACTORS ARE UP-REGULATED IN THE HYPOTHALAMUS OF THE SOUTH AMERICAN PLAINS VIZCACHA FROM MID-PREGNANCY**

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Abstract: Considering that prolactin (PRL) promotes luteal steroidogenesis in rodents and that the South American plains vizcacha (*Lagostomus maximus*) shows ovulation with corpora lutea (CL) formation at mid-gestation, with increased hypophyseal PRL and CL PRL receptor (PRLR) expression, we investigated the effect of PRL over the expression of hypothalamic factors related with its own production throughout the reproductive cycle of *L. maximus*. Firstly, adult non-pregnant (both non-ovulating and ovulating) and pregnant (early-, mid-, and term-gestation) vizcachas (n=5 per group) were used to study hypothalamic PRLR and tyrosin hydroxylase (TH) immunoexpression and their colocalization by immunofluorescence with confocal microscopy. In addition, we developed a hyperprolactinemia model by injecting non-pregnant females with two doses of 10 mg/Kg sulpiride during 7 days (SULP, n=5) to study the influence of PRL over the expression of its modulators. Animals injected with vehicle were used as controls (CTL, n=5). ANOVA followed by Bonferroni or *t*-test were used to determine significant differences among groups ($p<0.05$). PRLR and TH expressions were studied in arcuate nucleus (ARC). Significant increments in immunoreactive PRLR and TH areas, and in the number of neurons with expression of both markers, were detected in mid- and term-pregnancy related to the other groups. Regarding to the hyperprolactinemia model, the efficiency of sulpiride was confirmed by a significant increase ($p<0.05$) in the hypophyseal PRL immunoreactivity in SULP vs CTL. In the hypothalamus, ARC Immunoreactive PRLR area and, PRLR and TH colocalization were significantly increased in SULP vs CTL. These results suggest a hypothalamic regulation of PRL expression in the vizcacha from mid-pregnancy which supports its involvement in CL formation and maintenance of ovulation from mid- and up to the end of pregnancy of *L. maximus*. Fundación Científica Felipe Fiorellino, PIP110/14, PICT1281/2014.
Keywords: prolactin, hypothalamus, vizcacha

(1824) **ROL OF THE GONADOTROPIN INHIBITORY HORMONE (GNIH) IN THE DEVELOPMENT OF THE CICHLID FISH *Cichlasom adimerus* RELATED TO FEEDING AND GONADOGENESIS**

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GnIH is a dodecapeptide that belongs to the RFamide family. Its acts on the pituitary to inhibit gonadotropin (GTH) release in birds and mammals, but this function in other vertebrates is unclear. Previously, in our lab, we showed that GnIH inhibited GTH and stimulated growth hormone (GH) release in adult of *C. dimerus*. We also studied the ontogeny and we found that GnIH is expressed in neurons of the *nucleus olfacto retinalis* (NOR) and the *nucleus posterioris periventricularis* (NPP). GnIH immureactive (-ir) fibers were widely distributed in the brain and other cephalic regions, even in the pituitary. Our first goal was to study if the expression of GnIH presents variations related to changes in the feeding and gonadal development during ontogeny. Our second goal was to evaluate a possible co-expression of GnIH and GnRH variants, and also if there were contacts between GnIH fibers and GTH and GH cells. Methodology: larvae were obtained from 4 independent spawnings. Samples were taken from hatching to 85 days post-hatching (dph) and processed for immunohistochemistry/double label immunofluorescence or real time PCR. Neurons of both nuclei were quantified (repeated measures AN0VA) and measured. We observed that the number of neurons in the NOR increased by 17 and 85 dph ($p<0.01$), and in the NPP by 14 and 85 dph ($p<0.01$). No changes were observed in the size of NOR (14±3μm) or NPP (8±2μm) cells. mRNA of GnIH was detected on day 1, with an increase in its levels

between 12-20 dph. Analyzing the co-expression of GnIH and GnRH variants, we observed that NOR neurons co-expressed GnIH and salmonGnRH. We found contacts only between GnIH-ir fibers and GH-ir cells at pituitary level. Considering that gonadogenesis begins and the yolk-sac is completely consumed by 12 dph, these results indicate that GnIH could act in the regulation of both processes. The present work comprises starting point for future experiments about the role of GnIH in the development of teleost fish.

Keywords: GnIH, Ontogeny, GnRH, GH, Teleost.

(1359) **ARACHIDONIC ACID DERIVED ENDOCANNABINOIDS AS MEDIATORS OF CHOLESTEROL TRAFFICKING IN *CAENORHABDITIS ELEGANS***

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Proper control of cholesterol transport is crucial for physiological homeostasis in most eukaryotic cells, although the underlying mechanisms of this process remain unclear. We describe a novel role for endocannabinoid signaling molecules in cholesterol trafficking mechanisms. Loss of polyunsaturated fatty acids (PUFAs) greatly enhances the dauer constitutive phenotype of DAF-7/TGF-β *Caenorhabditis elegans* mutants, which are already intrinsically deficient in intracellular sterol trafficking. Further biochemical and functional analysis established that this exacerbated phenotype is provoked by suppression of the synthesis of the PUFA-derived endocannabinoids 2-arachidonoyl glycerol and anandamide. These endocannabinoids not only rescue the arrest brought on by PUFAs deficiency, but also abolish the larval arrest caused either by impaired cholesterol trafficking (derived from either defective synthesis of glucosylceramides or from deficiencies in Niemann-Pick C1 (NPC1)-like proteins) or by cholesterol starvation of wild type worms for two generation. Thus, endocannabinoids play a pivotal role in C. elegans development acting as regulators of nematode cholesterol transport processes. These findings reveal a previously unsuspected mechanism for the physiological activities of endocannabinoids, uncovering new opportunities for therapeutic intervention in cholesterol disorders.

(1862) **MEIOSIS IN *COLUMBIA LIVIA* (BIRDS: COLUMBIFORMES)**

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Some authors associate active caspase 3 with events of cell proliferation and differentiation. With the objective of establishing the role of this protein during the events of the ontogeny in *Columba livia*, detection of active caspase 3 was carried out in histological preparations of C. livia. corresponding to stages (E) 40-43 and neonates by inmunohistochemical (IHQ). Were tested using an active anti-caspase 3 antibodies 1:600 and developed according to the indirect “L-streptoavidin biotin” protocol. Expression of said protein was examined at quantitative level via Western Blot (WB). By IHQ said molecule was expressed in the left ovary from the E41. In contrast, oogonias dispersed in the right gonad, do not express active caspase 3, indicating that these, in the right gonad, do not enter into meiosis. Previous determinations by TUNEL and by IHQ for bax were positive, indicating in these cells apoptosis by another common effector caspase different from 3 or by another mechanism of programmed cell death. The expression of caspase 3 active in oogonia in division allows inferring that this molecule would be involved during the gonadal ontogeny in the processes of cell division. This inference is confirmed by the previously presented results of the expression of GnRHR in ogonias from E41, at which point the cells are in cell division. By WB, an increase in the expression of caspase 3 was detected from E41, indicating the onset of meiosis at this time. Taken together with the results of IHQ, these data indicate that active caspase 3 is expressed in the embryonic ovary from E41 and its expression is gradually increased, concomitantly to the increase of germ line cells entering meiosis. Therefore, active caspase 3 is not a key regulator of apoptosis in *C. livia* gonads since positive immunodetection is associated with germline cells in differentiation.

Keywords: cell proliferation, ovogenesis, columbidae

(216) **THE CNS ACTS AS A TRANSDUCER OF STRESS-INDUCED MASCULINIZATION THROUGH CORTICOTROPIN-RELEASING HORMONE B**

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Exposure to environmental stressors during early development has important implications for the rescheduling of many cellular and molecular mechanisms. In many fish, environmental stressors like high temperatures (HT) increase cortisol levels that in turn induce gonadal masculinization of genotypic females, overriding genetic factors related to gonadal development. Here, we show for the first time that the CNS is acting as the transducer of environmental stressors-induced gonadal masculinization. The mRNA of Corticotropin-releasing hormone b (*crhb*) and its receptors (*crhr1* and *crhr2*) were elevated at HT during the critical developmental period when the gonadal primordium was sexually labile. Mutations of both *crh* receptors by CRISPR/Cas9 in genetic female medaka embryos prevented them to masculinize when exposed to HT. However, some XX individuals exhibited an intersex gonad (testis-ova) with the loss of *crhr1* function. The prevention of masculinization in the *cas9*+sgRNA-*crhr1* and -*crhr2* injected larvae was likely due to the lack of Acth release from the pituitary gland, which in turn did not induce cortisol production, the downstream effector of the hypothalamic-pituitary interrenal (HPI) axis. Finally, we could rescue HT-induced female-to-male sex reversal in *cas9*+sgRNA-*crhr2* injected embryos by the addition of cortisol. Taken together, these results revealed the importance of the central nervous system (CNS) as the transducer of stress-induced masculinization in genetic females.

Key words: Environmental stress, CRH, Masculinization, CRISPR/Cas9, Medaka

(1886) **CRISPR/CAS9 GENOME EDITING IN *Drosophila melanogaster***

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Drosophila melanogaster, has been used as a model organism for developmental studies for more than 40 years. Investigation in *Drosophila* developed a set of sophisticated techniques for the study of gene function. Genome editing technologies take advantage of RNA-guided nucleases, which can generate directed mutagenesis in selected targets in the genome. In this study, we show the ability of the CRISPR/Cas9 technique to generate mutant alleles that enable the study of spermatogenesis and the role of small ORFs (sORF) in development. We designed and produced five sgRNA expressing plasmids to target *me31B* and two to target sORF-7; and initiated preliminary studies to validate the genome editing in both loci. The proposed objectives are to generate mutations within *me31B* and sORF-7 loci through the injection of sgRNAs expression vectors in blastoderm embryos and to screen for mutant alleles via the use

of PCR techniques. We generated a deletion in the *me31B* locus through the injection of a combination of two plasmids in *vasa-cas9* and *nos-cas9* embryos. In *vasa-cas9* embryos analyzed 24hs post-injection we found fragments indicative of the amplification of a deletion, however, the embryos did not fulfill their life cycle. In contrast, after injecting *nos-cas9* embryos we obtained adult viable flies in which progeny we revealed the fragment indicative of the deletion via PCR. The CRISPR-Cas9 technology proved to be useful to point out the critical role of the sORF-7 in the development of *Drosophila*. We obtained high levels of embryonic lethality with percentages of up to 90% when injecting the plasmids in *act-cas9* embryos. These results indicate that the sORF-7 is transcribed, at least, from the zygote's DNA and is required for the proper development of the egg. The biological function of sORF-7 remains unknown and opens the possibility to assess the importance of other sORFs using this method.

NEUROSCIENCE 5

(618) **5-HT2A RECEPTOR IN MPFC CONTROLS CONTEXT-GUIDED RECONSOLIDATION OF LONG- TERM OBJECT MEMORY IN PERIRHINAL CORTEX**

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The object recognition memory retrieval is a complex process that required the interaction of multiples structures. It has been proposed that mPFC interacts with the hippocampus during contextual-guided versions of the spontaneous object recognition paradigm. Using a pharmacological disconnection experiment, we have shown that mPFC 5-HT2aR modulation and hippocampus (HIP) interacts in an ipsilateral way during the resolution of an object-in-context recognition memory task. Since the information regarding the identity of the object could be stored in other structures such as the perirhinal cortex (PRH) then, the mPFC-HIP interaction could control the reactivation/reconsolidation in the PRH. To test this idea, we infused a 5-Ht2aR antagonist (MDL) in mPFC before the reactivation phase and immediately after a protein synthesis inhibitor (EME) in the PRH or dorsal dHIP. We also evaluate the interaction between the ventral hippocampus (vHIP) and the mPFC using a disconnection approach. We infused MDL in mPFC and muscimol in the vHIP before the retrieval and EME in the PRH after the reactivation session. We found that blocking 5-HT2aR signaling in the mPFC affects the reconsolidation in the PRH but not in the dHIP. In the disconnection experiment, only contralateral infusions made memories for both objects susceptible to the action of EME. Our results suggest that the interaction between mPFC 5-HT2a modulation and HIP activity controls the reconsolidation of object memory traces in PRH during the retrieval phase.

Keywords: Serotonin – Memory – Retrieval – Prefrontal Cortex - Hippocampus

(1721) **ENDOGENOUS ANTIOXIDANTS AND OXIDATIVE STRESS ON COGNITIVE DIFFICIENCIES**

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Introduction: Oxidative stress is identified as a fundamental component in the onset and maintenance of vascular pathologies. In neurocognitive disorders (TNC) the oxidative mechanisms are altered. Nitric oxide (NO) plays a key role in these processes, whereas superoxide dismutase (SOD) is part of the endogenous antioxidant system. Objective: To study in patient the relationship between NO and SOD values and the cognitive state. Material and Methods: Include all subjects who agree to participate and signed informed consent. Demographic data and cardiovascular risk factors were assessed. Patients with pathologies with increased oxidative stress were excluded. The Montreal cognitive test (MoCA) was performed to establish cognitive status. Nitric oxide (NO) (**µ**M) and superoxide dismutase (SOD) (U / ml) were quantified by spectrophotometry. The relationship between variables was analyzed by linear regression analysis, level of significance p <0.05. Results: 96 patients were evaluated, 18 were excluded. Female gender was defined. Age: 68.28 ± 11.90. Years of schooling: 08,13 ± 04,04. MoCA: 22.36 ± 6.30. Hypertension, sedentary lifestyle and dyslipidemia were the most prevalent cardiovascular factors. From the analysis of the linear regression between the values of NO plastic observed an increase of 0.27 points in the MoCA test for each 3.24 uM of NO; CI = LI (95%) 8.63-S (95%) 32.85 (p = 0.0011). While there is a decrease (0.16 points) in MoCA with increasing SOD; IC = LI (95%) 179.46 - LS (95%) 293.27 (p <0.0001). Conclusions: The excessive generation of free radicals leads to oxidative, nitrous, or both, damaging proteins, lipids and DNA. The brain is more susceptible to oxidative damage because of its high metabolic rate, high polyunsaturated lipid content, and lower levels of endogenous antioxidant activity and protection mechanisms. An increasing number of studies indicate that oxidative stress correlates with the pathogenesis of AD, and vascular dementia

(189) **EFFECT OF GEOMETRICALLY DIFFERENT ENVIRONMENTS AS NOVELTY ATTRACTORS ON THE LATERALIZED EXPLORATORY RESPONSE IN MATURING RATS**

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It is known that the brain is functionally lateralized to control several biological responses in man and animals. However, its intrinsic neural mechanisms are not known. Previously in our laboratory, results were shown that in the Double Hole-Board Labyrinth (LDHB), maturing normal rats show a left-biased exploration induced by novelty. Since the LDHB is composed of two solid rectangular compartments disposed in 90º to each other (inverted "T" form), question was raised if the rat's brain lateralized behavioural response should be constant in any geometrically shaped environment. The answer to this question should enlighten the possible plasticity in the lateralized responses of the animal. Maturing normal rats at 30 days of age were used. They were tested in the LDHB (n=20) as a complex geometrical environment (SRT); in a rectangular cage (n=18), as a rectangular environment (SR), and a cubic cage (n=18), as a square environment (SQ). Exploration activity was video recorded and measured by digital automatic counter. Results are expressed as Counts/min. Results shown that in the SQ, right-biased exploration was found (74.5±9, left side Vs 118±12, right side, p<0.01), with a percentage of 77.8 of right explorers. In the rectangular environment, no left or right preference was found (47.5±10, left side Vs 84.5±9, right side, n.s.); and in the LDHB, left-biased exploration was found (75.5±9 left side Vs 37.5±3 right side, p<0.01), with a percentage of 70% of left explorers. In conclusion, results show that the spontaneous natural biased exploratory response showed by animals is depending on the geometry of the novel environment, suggesting that in the rat's brain the lateralized neural circuits are modulated by the geometric aspect of the environment.

(1808) **NEUROPEPTIDE W30 IMPROVES HIPPOCAMPAL-DEPENDENT MEMORY AND SYNAPTIC TRANSMISSION BY PROMOTING NOS1 AND CREB EXPRESSION**

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Homeostatic regulation of feeding and energy balance involves cognitive, emotional, sensorial and autonomic events, as well as the activity of a highly integrated and redundant neurohumoral system. Neuropeptide W (NPW) is part of this complex net. There are two mature forms derived from a common precursor peptide, pro-NPW, by proteolytic processing: NPW30 (the 30 amino acids form) and NPW23 (consisting of 23 amino acids). The peptide and its receptors are present in the rat hippocampus, amygdala, hypothalamus, and pituitary. We have previously found that hippocampal administration of NPW30 increased latency time in the step-down test. Objective: to assess the effect of hippocampal NPW30 acute administration on neuroplasticity and expression of several genes related to the molecular cascade of memory in rat hippocampus. Male adult rats were infused into the hippocampus with artificial cerebrospinal fluid (Control group) or NPW30 (0.3 and 3 nmol/µl) and 24h later, the hippocampus was dissected to perform electrophysiological procedure (n=5/each group) or RT-PCR (n=10/each group). Statistics: Student's t-test or ANOVA followed by Bonferroni. The highest dose of NPW30 reduced the threshold to generate long term potentiation (LTP) (NPW30, 3 nmol/µl: 31.67±5.43 Hz vs. Control: 90.00±17.32 Hz; $P<0.05$) and increased the relative expression of cyclic AMP response element binding protein (CREB) 1b ($F(27,2)=8.07$; $P<0.05$) and neuronal nitric oxide synthase (NOS1) ($F(23,2)=9.31$; $P<0.05$). It has been demonstrated that NOS1 activity is relevant in early stages of memory formation, and CREB is a transcription factor that plays a key role in the hippocampal plasticity, especially in LTP induction and maintenance and long term memory establishment. In conclusion, these findings suggest that NOS1 and CREB participate in the mechanism by which NPW30 can modulate neuroplasticity and behavioral expression of memory.

Keywords: Neuropeptide W30, NOS1, CREB, LTP, memory.

(558) **POSITIVE EMOTIONAL INDUCTION INTERFERES WITH THE RECONSOLIDATION OF NEGATIVE AUTOBIOGRAPHICAL MEMORIES.**

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After reactivation, a previously consolidated memory can enter into a labile state followed by a re-stabilization process defined as reconsolidation. The aims of this study were to explore whether an existing negative autobiographical memory can be modified by using a non-invasive interference (audiovisual positive preparation) after reactivation and to determine if this effect could be dependent on the reconsolidation process.

Participants 'memories were reactivated on day 1 (retrieval session) by means of the Autobiographical Memory Test (AMT) by the presentation of the negative adjective "angry" and they were instructed to remember one specific event from their own past and to write down the event in 4 min. Ten min later half of the subjects were shown the positive audiovisual inductor (POSITIVE INDUCTOR, PI group) and the other half was shown an equivalent neutral audiovisual inductor (NEUTRAL INDUCTOR NI group). The memories were tested 7 and 30 days later by means of the presentation of the title of the event and they had again 4 min to write down the event. The results shown that only in women the presentation of a positive inductor reduced significantly the negative emotional content 7 and 30 days later (post hoc, $p< 0.05$).

In sum, we found that a positive emotional experience after a negative autobiographical memory reactivation may lead to a change in the emotional information of the original trace and that such effect can be mediated by the reconsolidation process. These results suggest that a positive audiovisual induction may play a potential role in psychotherapeutic techniques for the modification of dysfunctional autobiographical memories.

(1700) **ROLE OF THE SEROTONERGIC RECEPTOR 2a (5-HT2a) IN COGNITIVE FLEXIBILITY**

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Living organisms inhabit environments that are being permanently modified, so adapting their behavior to such changes could determine their survival. The concept of "cognitive flexibility" refers to this ability and serotonin (5-HT) has been identified as an important player in decision making. So, it would be reasonable to assign the serotonergic system a major role in processes of cognitive flexibility. Pharmacological experiments support this statement. However, it is still questionable which are the receptors that mediate these processes.

One of the most important post-synaptic receptors of the serotonergic system is the type 2A (5-HT2aR). This receptor is highly expressed in the limbic system as well as frontal regions of the cortex and has been associated with various psychiatric disorders. The lack of specific antagonists makes complex the identification of its function. Then, we assessed the role of the 5-HT2aR using protocols of extinction and reversal learning as measures of cognitive flexibility in genetically modified mice. Our preliminary results indicate that WT mice extinguish a fear conditioned memory faster than 5-HT2a$^{-/-}$ mice (two-way ANOVA, $p<0.01$). This would indicate that 5HT2aR is necessary for cognitive flexibility.

Since cognitive rigidity is a common behavior symptom of many psychiatric disorders, it has clinical relevance to identify its underlying neurobiological substrate to generate new and specific pharmacological tools.

Palabras clave: cognition, behavior, serotonin.

(242) **SENSITIZATION-DEPENDENT NICOTINE PLACE PREFERENCE IN THE ADULT ZEBRAFISH**

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IFIBIO.

Tobacco smoking is a risk factor for subsequent cocaine abuse, but the neural substrates underlying this risk remain unknown. Locomotor sensitization is a robust and readily assayed effect of psychostimulants, while conditioning place preference (CPP) is a more naturalistic model of addictive behavior. To understand the biological basis of nicotine induce reward, we analyzed first sensitization to nicotine and/or cocaine in zebrafish by exposing animals five days (20 min-day) to either drug followed by five days of abstinence before a challenge dose. We tested next if sensitized zebrafish can induce nicotine place preference using a biased design. Locomotor sensitization was increased by 103% with nicotine and 166% with cocaine. In addition, both nicotine and cocaine previously sensitized animals showed positive nicotine CPP, using a nicotine dose that cannot generate CPP by it self. Zebrafish sensitization and CPP was corroborated by behavioral analysis of several indicators of drug exposure and preference, such as locomotor activity, time spent in the upper-zone, time spent in the drug-paired side, number of entries to the drug-paired side, and distance traveled. The behavioral results obtained were also accompanied with chances in the expression of some molecular markers, measured by qPCR, such as nicotine receptors subunits, DAT and Pitx3, which are associated with drug reward. These results from zebrafish strongly suggest that repeated exposure to nicotine and/or cocaine induce sensitization by increasing the vulnerability and facilitating the rewarding properties of nicotine, although the dose of nicotine is not sufficient to induce seeking behavior *per se*. The behavioral characterization of these responses in zebrafish may be the first important step towards establishing this species as a tool for the analysis of molecular and neurobiological mechanisms underlying human drug seeking behavior.

Keywords: Sensitization, nicotine, cocaine, zebrafish, CPP.

(671) **UNDERSTANDING MEMORY LOSS: DEVELOPMENT OF A RETRIEVAL-INDUCED FORGETTING PARADIGM IN RODENTS**

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Miranda, Noelia Weisstaub, Pedro Bekinschtein
FAVALORO - CONICET - INECO

In the last two decades there´s been a growing human literature on a phenomenon called retrieval-induced forgetting (RIF). RIF has pointed to inhibitory control processes that resolve retrieval competition as a cause of adaptive forgetting. Using spontaneous recognition memory in rats, we have developed a rodent paradigm for RIF. We were able to show that forgetting of an item associated with a particular context happens under conditions that cause competition between memory traces for two items that share a particular retrieval cue. Under these conditions, forgetting is long lasting and independent of the selected retrieval cue. We used local pharmacological inactivation to show that this kind of forgetting requires the activity of the medial prefrontal cortex (mPFC). With pharmacological inactivation, we showed that the Ventral Tegmental Area (VTA) is necessary for the forgetting to occur and that the infusion of a D1/5 agonist in the mPFC is sufficient to rescue the expression of the RIF phenomenon impelled by the inactivation of the VTA. These results are consistent with the idea that the RIF occurs via a top-down inhibitory control mechanism exerted by the mPFC on structures linked by hypothesized memory traces. With the latter results, we bring new evidence supporting the role of dopamine in the resolution of interference via mPFC inhibitory control.

Keywords: mPFC – Memory - Forguetting

(1932) **SURVIVAL AND DIFFERENTIATION EFFECTS OF PIGMENT EPITHELIUM DERIVED FACTOR (PEDF) ON RETINA NEURONS**

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PEDF promotes survival of photoreceptor cells and in an R28 retinal progenitor cell line by binding to PEDF membrane receptors. However, the intracellular molecular effects of PEDF in retina neurons are still unknown. Here we investigated these effects using pure neuronal cultures of photoreceptors and amacrine neurons, prepared from newborn rat retina and grown in a chemically defined medium. Cultures were supplemented at day 2 with either recombinant human PEDF (10 nM); PEDF plus P1 (a PEDF receptor blocking peptide, 100nM); or vehicle (control). At day 5, cells were fixed and cell death and apoptosis were evaluated by DAPI staining, Propidium Iodide and TUNEL assays, respectively. Mitochondrial activity was assessed with Mitotracker and opsin expression and axonal outgrowth were determined by immunocytochemical methods.

PEDF decreased the percentages of Propidium Iodide and TUNEL-positive photoreceptors by 55% and 65%, respectively, with respect to controls without PEDF, and significantly prevented the loss of mitochondrial membrane potential. Pre-incubation with the blocking P1 peptide abolished PEDF effects. PEDF had no effect on opsin expression but promoted its apical localization in photoreceptors as occurs in mature retinas, in contrast to its diffuse distribution on the cell body in controls. PEDF also stimulated axon outgrowth in amacrine neurons, by 2-fold, in neurons treated with PEDF, compared to controls. These effects were blocked by the blocking P1 peptide pre-treatment.

In summary, this work shows that PEDF acts as a survival factor for photoreceptors, preserving their mitochondrial functionality, and induces the differentiation of amacrine and photoreceptor neurons during their development in vitro.

Keywords: PEDF, apoptosis prevention; retina photoreceptors; amacrine neurons

(1935) **SUPERIOR CERVICAL GANGLIECTOMY INDUCES DRY AGE-RELATED MACULAR DEGENERATION IN MICE: A NEW EXPERIMENTAL MODEL**

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Dry age-related macular degeneration, a prevalent cause of blindness, is a progressive and degenerative disease, characterized by alterations in Bruch´s membrane, retinal pigment epithelium, and photoreceptors exclusively localized in the macula. Despite there are experimental murine models, the vast majority take too long to develop retinal alterations, which in general are ubiquitous, many result from non-eye specific genetic manipulations, and most do not always reproduce the hallmarks of human age-related macular degeneration. Choroid vessels receive sympathetic innervation from the superior cervical ganglion, which together with the parasympathetic system, regulate the blood flow. Choroid blood flow changes have been involved in age-related macular degeneration development and progression. At present no experimental models take this factor into account. The aim of this work was to analyze the effect of superior cervical gangliectomy on the choroid, Bruch´s membrane, retinal pigment epithelium, and retina. Adult male C57BL/6J mice were submitted to unilateral superior cervical gangliectomy and a contralateral sham procedure. Although superior cervical gangliectomy induced ubiquitous choroid and choriocapillaris changes ($p<0.01$), it induced Bruch´s membrane thickening, retinal pigment epithelium melanin content and retinoid isomerohydrolase loss, drusen-like deposit occurrence, and retinal pigment epithelium and photoreceptors atrophy, exclusively localized in the temporal side ($p<0.01$). Moreover, superior cervical gangliectomy provoked a localized increase in retinal pigment epithelium and photoreceptors apoptosis ($p<0.01$), and photoreceptors electroretinographic function decline ($p<0.01$). Therefore, superior cervical gangliectomy recapitulated the main features of human dry age-related macular degeneration, and could become a new experimental model of dry age-related macular degeneration, and a useful platform for developing new therapies.

Keywords: age-related macular degeneration, superior cervical ganglion, choroid, retinal pigment epithelium, experimental model.

NEUROSCIENCE 6

(1530) **OLIGODENDROCYTES MATURATION AND METABOLIC PROFILE UPON IRON DEFICIENCY**

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Iron deficiency (ID) represents one of the most prevalent nutritional deficits, affecting almost two billion people worldwide. Gestational iron deprivation as an experimental model is a useful tool to describe specific oligodendrocyte (OL) requirements for progression to a mature and myelinating state. Previous work demonstrated that ID-OL appear to exhibit intrinsic alterations in terms of proliferation, migration and maturation.

To better understand OL maturation, we explore the hypothesis that ID constrains OL maturation by impairing metabolic pathways. *In vivo* and *in vitro* studies were conducted to evaluate: a) OL lineage composition along ontogenetic myelination; b) OL lineage cell number regulation; c) Glial cell metabolic profiles. We used an eGFP::CNPase transgenic mouse which renders the whole OL-lineage-committed cells as green-fluorescent (CNPase-positive cells). Pregnant mice were fed an ID diet (4mg/g/kg) as from gestational day 5. Postnatal development of myelination was evaluated 15 and 30 days after birth (PND15 and 30). At PND15, ID-OL lineage exhibited an increase in CNPase-positive cells but this redundancy failed to attain complexity and maturity ($p\leq 0.01$). At PND30, the number of CNPase-positive cells decreased, through an increase in cell death ($p\leq 0.01$).

To further describe ID effects, the correlation was explored between maturational stage and ID metabolic signature. Energy me-

tabolism was assessed in OL and astrocyte (AST) primary cultures from newly born ID WT pups. Measurements of glycolysis and mitochondrial respiration showed that ID glial cell (OL and AST) maximum rate of respiration was lower than control ones ($p \leq 0.001$). In addition, control AST exhibited a higher basal glycolitic capacity than ID AST ($p \leq 0.001$). These findings further prove that the regulation of cell metabolism may impact cell fate decisions and maturational status.

Keywords: Iron deficiency, maturational status, metabolic pathway

(1172) **GLUN2A KNOCKDOWN UP-REGULATES SYNAPTIC PROTEIN EXPRESSION IN MATURE PRIMARY NEURONAL CULTURES**

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It is well recognized that the NMDA receptors (NMDAR) are involved in synaptic plasticity, learning processes and memory, but their specific regulation is little known. NMDAR are composed by two GluN1 obligatory subunits and two regulatory subunits: GluN2 (A-D) or GluN3 (A-B). GluN2A and GluN2B are the most expressed regulatory subunits in hippocampus and other memory related brain structures, where GluN2A and GluN2B transcription and translation are tightly regulated. This results in GluN2B expression in immature synapses, and GluN2A in mature and stable synapses. In order to understand the role of GluN2A during memory acquisition and plasticity induction we built two AAV-eGFP vectors: one of them codifying a shRNA anti GluN2A (AAV-sh2A), and the other carrying a shRNA scramble as control (AAV-shSc). We infected mature primary neuronal cultures with AAV-sh2A or AAV-shSc and analyzed not only the specificity of GluN2A knockdown but also the effects of this decrease on other synaptic protein expression: Synapsin-1 and PSD95. As was expected, we observed by qPCR, a decrease in GluN2A mRNA only in primary cultures infected with AAVsh2A, without changes in GluN1 or GluN2B expression. Interestingly in those cultures, GluN2A decreased expression was accompanied by a significant diminution on GluN1 protein level. Furthermore, GluN2B levels were similar to control cultures infected with the AAV-shSc. On the other hand, we found an increased expression of Synapsin-1 and PSD95 in GluN2A knockdown cultures. These results, suggest that although GluN2A decrease does not change NMDAR subunit expression at transcriptional level, it could activate some postranscriptional regulatory mechanisms that change GluN1 protein levels. Moreover, that change seems to induce a rise in the synapsis without NMDARs increase.

Keywords: NMDAR, neuronal cultures, PSD95.

(1532) **SPATIO-TEMPORAL CHANGES OF APOPTOSIS IN CEREBELLAR PURKINJE CELLS AFTER PRENATAL BLOCKADE OF ANGIOTENSIN II AT2 RECEPTOR**

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Angiotensin II (Ang II) exerts its physiological effects through binding to two receptor subtypes: AT_1 and AT_2. These are differentially blocked by Losartan or PD123319, respectively. Ang II receptor expression is highly modulated during development. AT_2 receptors are highly expressed in neonatal tissues suggesting a role of these receptors in growth and organogenesis. In cerebellum, AT2 receptors are located only in the Purkinje cells (PC) membrane; these cells are the primary organizer in the postnatal cerebellar corticogenesis. The aim of the present study was to evaluate apoptosis of PC in neonatal cerebellum of animals treated with AT_2 receptor antagonist. Treatment was performed in Wistar rats during the last week of pregnancy with vehicle (control) or PD123319 (AT_2 antagonist, 1.0 mg/kg/day) and offspring were analyzed at different ages: P3, P5, P8 and P15. Apoptosis was evaluated at the different folia of the cerebellar cortex at the level of the vermis using acridine orange staining and fluorescence microscopy. The control pups showed increased apoptosis in PC in P3–P5 respect to P8-P15 ($p<0,001$). Likewise, apoptosis occurs in these animals, following a spatio-temporal pattern similar to that which they follow in their maturation and migration process. Animals born from mothers treated with PD123319 showed a delay in the apoptosis process. In P3 and P5 treated animals had a significantly decreased in the apoptotic process PC ($p<0,001$). In P8, treated animals showed a significant increase respect to control ($p <0.001$). A detailed spatial analysis shows that the significant difference occurs only in posterior folia: X, IX,VIII ($p <0.05$) and anterior folia: III, II, I ($p <0.01$). In P15 there were no significant changes. These results suggest that there is a developmental stage-specific mechanism of apoptosis in cerebellum and that renin-angiotensin system could be involve in growth and organogenesis process of cerebellum.

(1770) **STUDY OF THE IMPACT OF THE PRO-INFLAMMATORY RESPONSE ON THE DIFFERENTIATION OF HUMAN DOPAMINERGIC PRECURSORS**

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Parkinson´s Disease (PD) is a neurodegenerative disorder characterized by the loss of dopaminergic (DA) neurons of the substantia nigra pars compacta. Transplantation of dopamine-producing cells into the striatum has already shown its effectiveness in animal models and clinical trials. Here, we aimed to study the host primary response related to the graft of human DA precursors (DA14) *in vivo* and the impact of pro-inflammatory factors on the viability and differentiation process of DA14 cells *in vitro*. DA differentiation protocol from human neural stem cells was standardized and characterized by immunofluorescence for the following markers: beta III Tubulin (neuronal cells), GFAP (astroglial cells), Foxa2 (DA precursors) and Tyrosine hydroxylase (TH) (DA neurons). We analysed cell population at two stages: DA14 (Foxa2: 71,5%; TH: 5,2%) and final stage, DA28 (beta III Tubulin: 59,4%; TH: 17,4%; GFAP: 3,9%). For *in vivo* assay, DA14 were transplanted into the striatum of adult immunosuppressed rats and host-primary response was analysed by immunofluorescence. Preliminary results showed host-MHCII and GFAP positive cells within the graft after 7 days of surgery. In order to study the effect of microglial activation on DA14 maturation *in vitro*, BV2 microglial cells were activated with bacterial lipopolysaccharides (LPS). DA14 cells were then exposed to conditioned media (CM) from basal and activated BV2 cells for 4 days and TH positive cells were determined at DA28 stage. TH cell counting revealed that exposure of DA14 to CM from activated microglia decreased the number of DA neurons at final stage ($P<0.05$). These findings suggest that, *in vivo*, DA14 cells grafting induced host primary response and *in vitro*, activation of microglia could have a negative impact on survival and/or maturation of DA precursors.

Keywords: Parkinson's disease; inflammation; dopaminergic precursors

(1877) **STUDY OF THE INVOLVEMENT OF CB1 RECEPTOR IN ANXIETY LIKE-BEHAVIOUR RELATED TO ACETIC ACID-INDUCED VISCERAL PAIN IN ADOLESCENT MALE AND FEMALE MICE LACKING CB_1 RECEPTORS**

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CB_1 cannabinoid receptors are widely distributed in the central nervous system where they mediate most of the cannabinoid induced responses. Here we evaluated the interaction between the CB_1 cannabinoid receptors and the endogenous opioid system by assaying anxiety like-behaviour related to acetic acid-induced visceral pain in adolescent mice of both sexes.

The elevated plus maze and acetic acid-induced writhing tests were used in order to evaluate the effect of morphine (MOR) on the anxiety like-behaviour associated to visceral pain in mice lacking CB_1 receptors, respectively. CB_1 knockout (CB_1 KO) and their wild-type (WT) littermates mice were pre-treated with MOR (3 mg/kg or 9 mg/kg i.p.) or saline (SAL) injection 20 minutes before acetic acid (1.5%, 10 ml/kg i.p.) or SAL administration, and immediately after, the total number of writhes or anxiety-like responses were registered for a period of 20 min.

We observed significant differences in the *effect of* MOR *on the number of writhes, expressed as percentage of writhing* inhibition. MOR (3 mg/kg) decreased and MOR (9 mg/kg) completely blocked the *number of writhes* compared to their control groups (mice only treated with acetic acid) ($p<0.001$) in both sexes and genotypes. On the other hand, an increase in the anxiety-like behaviour (expressed as the percentage of time and entries in the open arms) was observed in mice only treated with acetic acid ($p<0.01$), in both sexes and genotypes. In addition, MOR 9 mg/kg was able to attenuate the anxiogenic-like effect (expressed as the percentage of entries in the open arms) in CB_1 KO, but not in WT male mice ($p<0.05$). On the contrary, this attenuation was not observed in CB_1 KO female mice ($p<0.05$).

In conclusion, the lack of CB_1 receptor could modulate the anxiety-like behaviour associated to acetic acid-induced visceral pain in male mice pre-treated with MOR but not in females.

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Key words: morphine, CB_1 receptor, visceral pain, anxiety

(485) **TGF-β AND ITS INTERACTION WITH THE NOTCH SIGNALING PATHWAY ON OLIGODENDROGLIAL DIFFERENTIATION OF ADULT NEURAL PROGENITOR CELLS**

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Neural progenitor cells (NPCs) from the subventricular zone (SVZ) are the source of new neurons, astrocytes and oligodendrocytes in the adult brain. Both Notch and TGF-β signaling pathways have been shown to act synergistically in co-cultures of mesenchymal stromal cells and NPCs, inducing NPC differentiation into astrocytes and oligodendrocytes. Taking into account the possible participation of NPC in the response mechanisms upon demyelination, the aim of the present work is to study the changes induced by TGF-β and the underlying interplay between TGF-β and Notch signalling on cell populations arising from adult brain SVZ cultures.

Wistar rat adult brain SVZ neurospheres were plated in coated coverslips and cultured for 48 h and then treated with TGF-β for different times. Results obtained 6 days after plating show a significant increase ($p<0.05$, two way ANOVA) in the percentage of glial cell population: PDGFRα+ and GFAP+ cells, concomitantly with an increase in the expression of Notch ligand, Jagged1. Furthermore, Ki67 expression and BrdU incorporation assays rendered a significant increase in total cell proliferation after 24 h culture and a significant increase ($p<0.05$ Student´s t-test) in the proportion of PDGFRα+/Ki67. In addition, 8 day cultures exhibited not only a significant increase ($p<0.05$ two way ANOVA) in the proportion of MBP+ cells but also a higher degree of cell differentiation, as evidenced by the presence of more ramified processes suggesting an effect of TGF-β on differentiation towards a mature oligodendrocyte. TGF-β signaling inhibition resulted in a decrease ($p<0.05$ Student´s t-test) in Jagged1 expression and OPC population; moreover, Notch signaling inhibition showed a decrease in OPC proliferation.

Our results demonstrate the participation of Notch signaling in TGF-β effects on glial cell fate decisions of adult brain SVZ NPCs, as well as on OPC proliferation and maturation.

Keywords: TGF-β, Neural progenitor cell, Jagged1 ligand, Oligodendrocytes

(1548) **THE BMP PATHWAY IS RECRUITED BY DIFFERENT CIRCADIAN CLUSTERS TO COMMUNICATE TIME OF DAY INFORMATION IN ADULT *Drosophila***

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Circadian behavior is controlled by an endogenous clock. In *Drosophila,* this clock resides in about 150 neurons; among them, the sLNvs are particularly relevant to set the free-running period.

To improve our understanding about the communication among different circadian clusters we carried out a miss-expression screen in the sLNvs. This approach uncovered a long period phenotype mutant that affected the levels of a positive regulator of the BMP pathway, a highly conserved retrograde signaling pathway that influences synaptic connectivity through transcriptional control.

Taking advantage of different genetic tools we observed that the activation of the signaling cascade (through expression of a transcription factor or constitutive activated versions of receptors) triggers a long period phenotype, while cluster-specific downregulation of specific ligands triggered loss of rhythmicity under constant conditions compared to controls (statistical analysis included Kruskal Wallis test). To characterize further the steps leading to pathway activation, we examined pMad staining through immunohistochemistry on wild type brains. Interestingly, despite pMad is found exclusively in the nucleus in non-circadian neurons, pMAD is in the cytoplasm in the small and large LNvs suggesting a cluster specific-regulation. In that regard, we explored the possibility that overexpression of the DPP and GBB ligands in specific circadian neurons could increase the amount of pMAD in the nucleus of LNvs neurons. We found that DPP overexpression generates an increase in pMAD nuclear levels exclusively in the LNvs while GBB has no effect (Statistical analysis included a One-way ANOVA).

These results show that not only the BMP pathway is active in LNvs neurons, but also they differentially respond to specific ligands. This finding opens the provocative possibility that the BMP pathway is recruited by different circadian clusters to communicate time information and for fine tuning clock properties.

(30) **PROGESTERONE REGULATES INFLAMMASOME EXPRESSION AFTER SPINAL CORD INJURY: IMPLICATIONS FOR NEUROPATHIC PAIN**

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Abstract: Neuropathic pain, a frequent complication after spinal cord injury (SCI), is refractory to available treatment. Spinal glial cell activation and production of pro-inflammatory mediators, like IL-1β and IL-18, have a critical role in the development of this chronic pain. Neuroinflammatory processes triggered after SCI involve the activation of multiprotein complexes called inflammasomes, containing three main components: NLRP3, ASC and pro-caspase-1. Caspase-1 processes the precursors of IL-1β and IL-18 to its mature forms. We have shown that progesterone (PG), a neuroactive steroid, prevents pain and exerts anti-inflammatory actions. To analize whether these effects involve the modulation of NLRP3 inflammasome, we studied temporal changes in the expression of its components in the acute phase after SCI. Male rats were subjected to spinal hemisection at T13 level and received daily subcutaneous injections of PG (Hx+PG; 16 mg/kg, n=16) or vehicle (HX, n=16). Uninjured rats were used as control (C). Epicenter spinal cord segments were obtained 1 and 3 days post-injury and the mRNA levels of NLPR3, ASC, IL-1β, IL-18 and P2X7 purinergic receptor, involved in inflammasome activation, were determined using RT-PCR. Injured animals showed a significant raise in NLRP3 ($p<0.05$ vs C) and IL-1β ($p<0.001$ vs C) mRNA levels 1 day after SCI, as well as a clear increase in P2X7 ($p<0.01$ vs C), ASC ($p<0.001$ vs C), NLPR3 ($p<0.001$ vs C) and IL-18 ($p<0.01$ vs C) transcripts 3 days after injury. At this time point, animals treated with PG showed significantly lower levels of NLPR3 ($p<0.01$ vs C; $p<0.05$ vs HX), while P2X7, ASC and IL-18 expression levels remained similar to control values ($p>0.05$ vs C; $p<0.05$ vs HX in all cases). These results suggest that PG, by modulating spinal mechanisms associated with NLRP3 inflammasome activation, could stand as a promising therapeutic alternative for SCI-induced neuropathic pain (PIP CONICET 266,

Fundación Barón, Fundación Williams).

Keywords: Progesterone, inflammasome, chronic pain

(62) **HYPOTHALAMIC- PITUITARY-ADRENAL ACTIVITY AND MEMORY IN THE ELDERLY: A NON INVASIVE APPROACH.**

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Abstract: Daily stress in aging has been linked to detrimental effects on memory. However, associated impairment of the HPA axis regulation remains unclear. The aim of this study was to evaluate the relationship between memory and the cortisol feed-back regulation of the HPA axis in aging women. Seventeen women aged 71.5±7 yo without dementia complaining of poor memory were studied. They obtained whole saliva samples to evaluate the circadian rhythm of salivary cortisol (SAF) by the ratio 8.00 and 23.00 ($SAF_8/SAF_{23} \geq 2$). The HPA axis suppressibility was assessed by the overnight 0.5 mg dexamethasone suppression test (DST). SAF and salivary dexamethasone (Dsa) after DST were determined at 8.00 (SAF_{8dex}, Dsa_8) and at 16.00 (SAF_{16dex}, Dsa_{16}). Quality of life (QL) was investigated by the self administered questionnaire SF36. Episodic, logical, working and semantic memories, visuospatial perception and attention were also assessed. The protocol was approved by the Ethics Committee of the IDIM A. Lanari and all subjects gave their written informed consent. SAF (nmol/L) and Dsa (pmol/L) were determined by RIA and ELISA respectively. Statistics were performed by SPSS ($p<0.05$ was considered significant). Memory impairment (MI) was found in 7 patients who had lower QL (Physical Summary Component: 41±7.5) than 10 patients with normal memory (50.2±7.9) p: 0.008. Fifteen women had normal SAF variation, Dsa and DST suppression ($Dsa_8 \geq 450$, $Dsa_{16} \geq 140$, SAF_{8dex} and SAF_{16dex}: ≤2.0). Two MI patients showed normal SAF variation and adequate Dsa (Dsa_8:768±198, Dsa_{16}:263 ±109), but did not suppress with DST (SAF_{8dex}:2.7±0.2, SAF_{16dex}: 3.5±0.7). Conclusions: Poor QL was associated with MI although the latter is not always related to HPA disregulation. Hippocampal images might contribute to establish an anatomical link between MI and abnormal feed-back regulationm.

CARDIOVASCULAR AND RESPIRATORY SYSTEMS 3

(1203) **EFFECTS OF ISOESPINTANOL IN MYOCARDIAL ISCHEMIA-REPERFUSION: INVOLVED MECHANISMS**

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The aim of this study was to determine the effects of isoespintanol (2-isopropyl-3,6-dimethoxy-5-methylphenol, ISO), isolated compound from leaves of *Oxandra cf xylopioides*, on ischemia-reperfusion injury. Isolated rat hearts perfused by Langendorff system were assigned to the following groups: 1. Non-ischemic control: 110 min of perfusion; 2.- Ischemic control (IC): 30 min of global ischemia (GI) and 60 min of reperfusion (R); 3.- ISO: 30 µg/ml ISO was administered during the first 10 min of R. To examine the mechanisms involved other hearts were treated with L-NAME (NOS inhibitor) or wortmannin (Akt inhibitor) or chelerythrine (PKC inhibitor) 10 min before ischemia. Infarct size was determined by TTC staining. Systolic and diastolic function was assessed by left ventricular developed pressure (LVDP) and the left ventricular end diastolic pressure (LVEDP), respectively. Coronary resistance (CR) was calculated as perfusion pressure and coronary flow ratio. The expression of phosphorylated form of endothelial NOS, Akt and PKCε was also measured. ISO significantly decreased the infarct size detected in IC group (11 ± 2 % vs. 31.0 ± 1.5%, $p< 0.05$) and improved post-ischemic recovery of myocardial function (LVPD: 63 ± 7 % vs. 17 ± 3%; LVEDP: 25 ± 5 vs. 52 ± 7 mmHg, $p< 0.05$). In IC, the level of P-eNOS, P-Akt and P-PKCε decreased approximately 45 % and after ISO treatment increased it (approximately 35 %). The increase of CR observed in IC group was significantly attenuated by ISO treatment (0.8 ± 0.2 vs. 3.3 ± 1 mmHg/ml x min^{-1}).The administration of all the inhibitors abolished the effects detected in ISO treated hearts.

The data show that acute treatment with ISO only at the onset of reperfusion decreased the infarct size and attenuated the post-ischemic myocardial contractility dysfunction and coronary resistance increase through Akt, PKCε and eNOS-dependent pathways.

(1427) **ETHOXYZOLAMIDE PROTECTS THE MYOCARDIUM AGAINST ISCHEMIA-REPERFUSION INJURY**

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Carbonic anhydrase (CA) is responsible for catalyzing the reversible hydration reaction of carbon dioxide to form proton and HCO_3^-. The activation of CO_3H-dependent transporters during ischemia-reperfusion contributes to the Ca^{2+} overload and subsequent myocyte damage. Our objective was to determine the effects of CA inhibitor ethoxyzolamide on myocardial alterations produced by ischemia-reperfusion. Isolated rat hearts were submitted after 20 min of stabilization to the following protocols: 1.Non ischemic control (NIC): 90 min of perfusion; 2.Ischemic control (IC): 30 min of global ischemia (GI) and 60 min of reperfusion (R); 3.ETX: ethoxyzolamide 100 µM was administered during 10 min before ischemia and the initial 10 min of R. To examine the participation of p38MAPK, the protocols were repeated in presence of p38MAPK inhibitor SB202190 (10 µM) administered 10 min before ischemia. Infarct size (IS) was measured by TTC staining technique. Left ventricular developed pressure (LVDP), $+dP/dt_{max}$, left ventricular end diastolic pressure (LVEDP) and $-dP/dt_{max}$ served to assess myocardial function. The p38MAPK expression was measured. ETX decreased the IS (12.0 ± 0.4 % vs. 32 ± 2 % in IC, $p < 0.05$) and improved postischemic recovery of myocardial function. At the end of R, LVDP was 62 ± 3 % vs. 15 ± 4 %; $+dP/dt_{max}$: 62 ± 4 % vs. 19 ± 5 %; LVEDP: 36 ± 5 vs. 52 ± 5 mmHg; $-dP/dt_{max}$: 60 ± 5 % vs. 17 ± 5 %, $p < 0.05$). The p38MAPK level increased after ETX treatment (153 ± 9 % vs. 74 ± 8 %, $p < 0.05$). SB attenuated the effects detected by ETX.

The present data demonstrate that CA inhibitor ETX attenuates the post-ischemic myocardial dysfunction and decreases the cell death through p38MAPK-dependent pathways and suggest that an attenuation of Ca^{2+} overload could be the responsible mechanism.

(1526) **FIRST AND SECOND WINDOW OF PROTECTION OF PRE-ISCHEMIC VAGAL STIMULATION ON MYOCARDIAL INFARCTION IN MICE**

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We have previously proven that vagal stimulation (VS) decreases infarct size (IS) when applied before ischemia, thus mimicking classic preconditioning. Our objective was to study the chronology and mechanisms of the protective effect of VS, and to assess whether the protection is comprised of a first and second window. Mice were randomly assigned to different protocols (n=5-7 per group). The control group had 30 min of regional myocardial ischemia and 2 h of reperfusion (I/R). The other groups consisted of 10 min of pre-ischemic VS (pVS) and different times of recovery between VS and I/R protocol: 5 min, 3-6-12-24-48-72-96 h. The 5 min, 3 h and 72 h groups were redone to administer a muscarinic receptor inhibitor (atropine). pVS-5min and pVS-72h were given a mitochondrial K+ ATP channel blocker (5-HD) and a nitric oxide synthase inhibitor (L-NAME). All animals were catheterized to measure ventricular function. Hearts were dyed with Evans Blue and incubated in TTC to measure IS.

pVS-5min had smaller IS in comparison to I/R (44±3% and 57±2%, respectively; p<0.05). pVS-3hs and pVS-6hs had a deeper IS reduction (34±3% and 34±3%, respectively; p<0.05 vs pVS-5min). Cardioprotection was lost at 12, 24 and 48 h post-VS (56±4%, 53±2%, 56±2%, respectively; p=NS vs I/R). Later at 72 h post-VS, cardioprotection reappeared (42±4%; p<0.05 vs pVS-48h) but was lost at 96 h post-VS (56±3%; p=NS vs I/R). The IS-reducing effect of pVS-5min, 3 and 72 h was abolished by atropine (56±2%, 56±3% and 56±3%; p=NS vs I/R), 5-HD in pVS-5min and 72 h (55±2% and 62±5%, p=NS vs I/R) and L-NAME in pVS-5min and pVS-72h (56±3% and 57±2%, p=NS vs I/R). In conclusion, VS has two protection windows similar to classic preconditioning. The first window lasts 6 h and the second window appears at 72 h and is lost at 96 h. The mechanisms underlying the cardioprotective effect include muscarinic receptors, mitochondrial K+ ATP channels and nitric oxide synthase.

Key Words: Ischemia/Reperfusion, Myocardial Infarction, Vagal Stimulation, Preconditioning, Mitochondria

(741) **RELATIONSHIP BETWEEN CARDIOPROTECTIVE EFFECTS OF ROSUVASTATIN (R) AND INACTIVATION OF GSK3B IN RAT HEARTS SUBJECTED TO ISCHEMIA-REPERFUSION.**

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Previously in our laboratory we could establish that the administration of R in hearts subjected to ischemia-reperfusion (I-RP) generated direct cardioprotective effects. These results were accompanied by increased activation of Akt, attenuation of the opening of the Mitochondrial Permeability Transition Pore (MPTP) and preservation of mitochondrial structure. The goal of the present work was to determine the relationship between these events, phosphorylation of GSK3β and the metabolic environment in Langendorff perfused rat hearts subject to I-RP. Wistar female rats (250-300g body weight) fed ad libitum were used. R (3uM) and wortmannin (W 100nM), Akt inhibitor, were added 10 and 15 min before global I respectively. Hearts were subjected to 25 min of I and 60 min of RP. Tissue samples were taken at the end of stabilization and ischemia to determinate lactic acid and glycogen content. The activation profile of GSK3β was studied by western blot taking into account GSK3β-P/GSK3β-T.ANOVA (n=6/group)

Lactic acid content were meaningfully lower in those hearts treated with R (μmoles/g dw): C (control): 146,28±6,60**; R: 56,68±6.77; W: 65,31±5,22; R+W: 61,35±4,10 (**p<0,01 against all groups), although paradoxically glycogen content was similar between hearts treated with R and group C μg/100 mg dw) C: 148,76±32,86*; R: 120,73±25,49*; W: 288,39±21,77; R+W: 324,64±34,75) (*p<0,05 vs W y R+W). Available glucose could be entering the pentose pathway, generating a metabolic environment more beneficial to those hearts subject to IS-RP. Likewise GSK3β presented major phosphorylation in the R group (p<0,05 vs C, W,R+W).

Our results suggest that the cardioprotection exerted by rosuvastatin could be mediated, at least in part, by the phosphorylation of GSK3β, which together with the appropriate metabolic environment could attenuate the opening of the MPTP, promoting alternatives that allow myocyte to face I-RP injury.

Key words: Rosuvastatin, ischemia, cardioprotection, MPTP, GSK3β.

(915) **RELEVANCE OF ANATOMICAL SITE TO ACTIVATE REMOTE ISCHEMIC PRECONDITIONING.**

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Introduction: Remote ischemic preconditioning (rIPC) reduces myocardial infarct size in different animal species, however, in humans the results are contradictory, probably because it has not been considered the anatomical site of stimulation as a variable that could influence the results.

Objective: The aim was evaluate the effects of rIPC on infarct size, considering the optimal anatomical site (arm or thigh) to achieve the myocardial protection. A second objective was to describe some of the pathways involved in the rIPC protection.

Methods: Isolated rat hearts were subjected to 30 min of global ischemia followed by 60 min of reperfusion (I/R). In additional groups, a rIPC protocol of 3 cycles of I/R were performed. This protocol was applied to the right and left femoral artery and in the right and left axillary arteries. We studied the involvement of a vagal neural pathway by performing a bilateral cervical vagotomy prior to the rIPC protocol. The humoral pathway was evaluated by administering DPCPX (adenosine A_1 receptor blocker). Infarct size was measured using triphenyltetrazolium chloride staining.

Results: rIPC performed in the left and right femoral artery significantly decreased infarct size (35,6±1,2 and 30,08±5,2 %, p< 0,05 vs I/R) and this effect was abolished by bilateral vagotomy. In the same way, rIPC performed in the left axillary artery significantly decreased infarct size (27±4,4 %, P< 0,05 vs I/R). This effect was not abolished by bilateral vagotomy. As we mentioned, in order to evaluate the humoral pathway, we administered DPCPX, which completely abolished the beneficial effect of rIPC. Interestingly, rIPC performed in the right axillary artery has not effects on I/R injury.

Conclusions: rIPc performed on hind limb reduces infarct size by a parasympathetic vagal pathway. However, in the left arm, rIPC induced protection by activation of adenosine A_1 receptors; suggesting the participation of an humoral pathway.

Keywords: Ischemic Preconditioning - Myocardial Infarction - Myocardial Protection

(756) **ROLE OF AMP-ACTIVATED PROTEIN KINASE (AMPK) IN MYOCARDIAL ISCHEMIA-REPERFUSION (Is-Rs): DIFFERENTIAL EFFECTS DEPENDING ON THE ENERGETIC SUBSTRATES AVAILABLE.**

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Abstract: AMPK functions as a cellular fuel gauge, being activated to reestablish energy homeostasis at a cell-autonomous level. The role of AMPK in myocardial Is-Rs remains uncertain.

In this study, we explored the effects of the pharmacological inhibition of AMPK by Compound C (CC; 10 μM) in isolated rat left atria subjected to 75 min Is-75 min Rs. Since levels of fatty acids are elevated in most clinical situations of Is-Rs, the influence of palmitate was also studied. For this aim, atria were incubated in Krebs–Ringer containing 10 mM glucose (G) or G and 1,2 mM palmitate (P), 95% O_2–5% CO_2, pH 7,4. For Is, the incubation medium contained 10 mM 2-deoxy-D-glucose, 95% N_2 – 5% CO_2, pH 6,8. ANOVA, n=8.

Results showed a significant increase in AMPK endogenous activation during Is, remaining activated during Rs. CC prevented it. During Is, peak contracture developed earlier in the atria stabilized with P. In this condition, CC increased maximum contracture. These results were consistent with changes in tissue ATP content. Lactate accumulation was reduced by P and even more by CC in both metabolic groups. At the end of Rs contractile reserve (CR) attenuation was more pronounced by P. CC showed dual effect, it increased CR attenuation in the presence of G, but reversed the harmful effect of P. These results were accompanied by similar changes in tissue ATP content, but the rate of mitochondrial ATP synthesis showed no significant differences between groups. PDH activity was reduced in the presence of P. CC reduced PDH activity in the atria incubated with G, but partially reverted P effect. CC also reduced autophagosome like structures and LC3-II/LC3-I ratio, an established indicator of autophagy, in both metabolic conditions. Cellular viability was not affected by P or CC.

The results support that intrinsic activation of AMPK has cardioprotective effects in the reperfused atria when G is the only available energy substrate, but results deleterious when P is also available.

Keywords: AMPK, ischemia, reperfusion, atria, palmitate.

(853) **ROLE OF AUTOPHAGY IN MYOCARDIAL ISCHEMIA-REPERFUSION (Is-Rs): INFLUENCE OF AVAILABLE ENERGY SUBSTRATES**

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Abstract: Autophagy is a prominent feature of myocardial Is-Rs. However, the functional significance is still unclear and controversial.

In this study, we explored effects of autophagy in isolated rat left atria subjected to 75 min Is-75 min Rs, when atria were incubated in the presence of 10 mM glucose (G) or G and 1,2 mM palmitate (P), as observed in most clinical situations of acute myocardial infarction, which are less well established. For this aim, the effects of the autophagy inhibitor 3-methyladenine (3-MA, 5 mM) were assessed. ANOVA, n=8.

Results showed a significant enhance in the LC3-II/LC3-I ratio, an established indicator of autophagy, during Rs, which was achieved more slowly in the presence of P. These results were accompanied by a concomitant decrease of p62, a specific substrate degraded through the autophagy-lysosomal pathway, in both groups. 3-MA prevented LC3 ratio increase and produced p62 accumulation in both metabolic conditions. Electron micrographs showed autophagosome like structures during Rs, which were absent in the presence of 3-MA. Autophagy inhibition was accompanied by a deterioration of mitochondrial morphology and function, although these results were not affected by P. ATP content recovery during Rs was reduced by P and 3-MA in both metabolic conditions, being this result consistent with attenuation in contractile reserve at the end of Rs. In the presence of 3-MA, either in the atria incubated with G or P, spontaneous tachyarrhythmias (270-300 contractions per min) were observed throughout Rs, which were attenuated by the inner membrane anion channel blocker PK11195, suggesting an involvement of mitochondria in the 3-MA-induced arrhythmias. These results were not affected by P.

Present results suggest that autophagy is induced during reperfusion developing cardioprotective effects. P present at high concentrations as an exogenous energy substrate, would not have significant effects on this process in the ischemic-reperfused myocardium.

Keywords: Autophagy, ischemia, reperfusion, myocardium, palmitate

(612) **ROLE OF mKATP AND EFFECTS OF ORAL ADMINISTRATION OF CARVEDILOL TO HYPERTHYROID RATS WITH STUNNING DUE TO ISCHEMIA-REPERFUSION**

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Previously, we showed that hyperthyroidism (HpT) got worse the stunning induced by severe ischemia-reperfusion (I/R) in rat hearts with the opening of mPTP (SAFE 2014). Morever, we found that the perfusion with 0.03 μM carvedilol (Carv) before I/R protected euthyroid (EuT) but not HpT hearts from stunning (SAFE 2015). The aims of this work were to evaluate the role of mKATP and the effects of oral administration of Carv in HpT and EuT hearts exposed to stunning without infarct. HpT was induced by daily SC injection of 20 µg.kg^{-1} T3 during 15 days. Isolated hearts were perfused inside a calorimeter at 37°C to measure left ventricular pressure (LVP, in mmHg) and total heat rate (Ht, in mW.g^{-1}) during exposition to severe I/R (30 minI/45min R). In order to evaluate the role of mKATP, HpT and EuT hearts were pretreated with 30 μM diazoxide (Dzx, mKATP opener). In HpT, Dzx increased the postischemic contractile recovery (PICR, 90.7±10.5 vs 24.7±3.8% in non treated: C-HpT, n=6-5, p<0.05), the total muscle economy (Eco=P/Ht, 5.2 ± 0.5 vs 1.6 ± 0.2 mmHg.g.mW^{-1} in C-HpT) and prevented the diastolic contracture. In EuT, Dzx produced the same effects than in HpT (n=5-6). Carv was given in drinking water (20 mg/kg/day) to HpT and EuT rats during 1 weak before the I/R experiment. In HpT, Carv improved the PICR up to 68.3 ± 10.8 % of initial pressure (Pi), at the end of R (p< 0.05 vs 23.5 ± 3.4 % of Pi in C-HpT, n=4-5) and Eco up to 4.3 ± 0.2 mmHg.g.mW^{-1} (p<0.05 vs 1.6 ±0.2 in C-HpT) while prevented the diastolic contracture. In EuT, Carv increased PICR (55.4 ±17.2 vs 11.6 ± 4.7 % of Pi in non treated: C-EuT, n=4-5; p <0.05), without changing Eco nor diastolic tone. Results suggest that: a) The mKATP opening prevents the stunning in HpT and EuT hearts; b) Oral administration of Carv prevents dysfunction due to severe I/R more in HpT than in EuT. *Grant: UNLP X-795*.

Keywords: heart, hyperthyroidism, carvedilol, ischemia-reperfusion, stunning

(1067) **THE CARDIOPROTECTION CONFERRED BY THIOREDOXIN-1 IN ISCHEMIC POSTCONDITIONING IS ABOLISHED IN MIDDLE-AGED MICE.**

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Thioredoxin-1 (Trx1) maintains the cellular redox status and decreases the infarct size in ischemia/reperfusion injury (I/R). However, it is not fully understood the role of Trx1 in ischemic postconditioning (PostC) in young and aged mice. The aim was to study if Trx1 is involved in the PostC cardioprotection mechanism and if the age can modify this. Wild type mice hearts (Wt), transgenic mice hearts overexpressing Trx1, and a dominant negative (DN-Trx1) mutant (C32S/C35S) of Trx1 were used, mice were divided in young group (4 month) and middle-aged group (12 month). The mice hearts were subjected to 30 min of I and 120 of R (Langendorff technique) (I/R group). In the PostC group, after I, a protocol of 6 cycles of R/I was performed. The assessment of the infarct size was performed using TTC. Also, it was measure hidrogen peroxide (H_2O_2) production, protein nitration, Trx1 activity and expression and pAkt and pGSK3β expression (western blot). Data are expressed as mean±SEM and p<0.05 was considered statistically significant. n= 6 each group.

Previously, we showed that Trx1 is involved in cardioprotection conferred by PostC in young mice. Then we performed the same groups but in middle-aged mice and the cardioprotection was abolished in groups with PostC (Wt-PostC: 53.13±4.58% vs Wt-I/R: 51.75±2.64%; DN-Trx1-PostC: 51.17±5.04% vs DN-Trx1-I/R: 56.57±4.24% and Trx1-PostC: 51.50±3.94% vs Trx1-I/R: 52.88±3.07%). This infarct size behavior was accompanied by a significant lack of survival proteins phosphorylation (Akt and GSK3β) and significant changes in Trx1 expression (in Wt group). Trx1 activity was significantly disminished, H_2O_2 production and protein nitration were significantly increased in all middle-aged groups. Our results showed that Trx1 plays a key role in the PostC protection mechanism in young mice but in middle-aged mice this cardioprotective mechanism was abolished.

Key words: Ischemic postconditioning, myocardial infarction, oxidative stress, thioredoxin-1, aging.

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(1100) **THE ROLE OF THIOREDOXIN 1 IN THE ISCHEMIC POSTCONDITIONING ON MICE FED HIGH FAT DIET**

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Thioredoxin-1 (Trx1) has cardioprotective effects against ischemia/reperfusion (I/R) injury, and participates in mechanisms mediating ischemic postconditioning (PostC). It remains unclear whether the

PostC protective effects are exerted during initial stages of atherosclerotic disease by altering *Trx1* expression. The objective of this study was to generate a murine high-fat diet (HFD) fed model that developed phenotypes consistent with early-stage atherosclerosis, and then evaluate increased oxidative stress, altered mitochondrial bioenergetics and *Trx1* expression in the HFD model. Ultimately, the model was used to determine whether HFD exposure abolished the cardioprotection conferred by PostC. C57/BL6 mice were fed with control diet (CD) or HFD for 12 weeks. Mice isolated hearts was subjected to 30 min of ischemia and 120 min of reperfusion (I/R group). For PostC subjects, after ischemia, six cycles were performed of reperfusion/ischemia (10 sec respectively, per cycle) at the onset of reperfusion. Data are expressed as mean±SEM and $p<0.05$ was considered statistically significant. n= 6 each group. In CD group, the Post had reduced infarct size (CD-I/R: 52.14±2.8 vs. CD-PostC: 36.58±1.8, $P<0.05$). This cardioprotection was abolished in HFD-exposed subjects. HFD increased production of hydrogen peroxide and *Trx1* expression. State 3 mitochondrial oxygen consumption in basal conditions was 24% decreased in HFD-exposed subjects. PostC restored state 3 values in controls more completely than in HFD-exposed subjects. Redox states and mitochondrial bioenergetics were altered in HFD-exposed subjects, leading to increased *Trx1* levels. These changes were accompanied by failed cardioprotection in HFD-subjects that is normally conferred by PostC. In conclusion, this study demonstrated the novel finding that alterations in redox state were enough to abolished cardioprotective mechanisms, such as those induced by PostC.

Keywords**:** High fat diet, thioredoxin, ischemic postconditioning, oxidative stress

REPRODUCTION AND FERTILITY 3

(1654) **EFFECTS OF INTRAGESTATIONAL GHRELIN MISBALANCES ON MATERNAL IMMUNE PARAMETERS AND FERTILITY.**

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Maternal and fetal ghrelin (Ghr) concentrations normally increase during pregnancy suggesting that this hormone exerts important roles on early gestational events. Moreover, some available evidence indicates that Ghr has immunomodulatory properties.

Herein, we aimed to evaluate the effects of Ghr and its pharmacological blockade (antagonist treatment) on implantation events using an already validated mouse model of Ghr misbalance during pregnancy.

Albino Swiss (N:NIH) mice dams were s.c. injected with Ghr (4 nmol/animal/day), an antagonist [Ant: (D-Lys3)GHRP-6; 6 nmol/animal/day] or vehicle (C: saline solution) from day 3 to 8 of gestation. Dams were euthanized at day 8, uteri exposed and analyzed macro and microscopically. The percentages of normal or atrophied fetuses and the number of implantation or resorbtion sites were recorded. In addition, the expression levels of VEGF, MMP9, GM-CSF, IL-10, IL-17 and IL-6 in uterine tissue was analyzed by qPCR. Plasma progesterone (P_4) concentrations were quantified by ELISA. Results was analyzed by ANOVA.

The blockade of Ghrelin (Ant) increased the number of resorbtion sites (C=0.5±0.5, Ghr=1.2±0.4, Ant=3.7±0.5; $p<0.05$) and the percentage of atrophied fetuses (C=4.4±4.0, Ghr=8.9±3.6, Ant=30.2±4.0; $p<0.05$). However, no significant differences were observed in the uterine expression levels of VEGF, MMP9, GM-CSF, IL-10, IL-17 or IL-6 among groups (VEGF=2.72±0.50, MMP9=7.57±0.51, GM CSF=8.73±0.53, IL-10=8.14±0.63; IL-17=8.38±0.85 and IL-6=4.58±0.71; n=14, $p>0.05$). Moreover, plasma P_4 concentrations did not show any significant differences among experimental groups under study ($p>0.05$).

Up to date, our results suggest that Ghr misbalance affects fertility by impairing embryo development not related to immune cytokine alterations at the maternal interface or by altering systemic P_4 levels. Additional experiments are being carried out in order to unveil the putative underlying mechanisms of these effects.

Keywords: ghrelin; implantation; inflammation; angiogenesis; fertility.

(980) **PROINFLAMMATORY RESPONSE OF HUMAN ENDOMETRIAL EPITHELIAL CELLS TO *Brucella abortus* INFECTION**

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Brucella induces abortion in humans and animals. Therefore, the interaction between uterine cells and *Brucella* is important for understanding the pathogenesis of this disease. In this work, we evaluate the inflammatory response (chemokine and metalloproteinases) and the expression of hormonal receptors and various microRNA related to infection or inflammation processes in the human endometrial epithelial cell line Ishikawa, after infection with *B. abortus* 2308 or stimulation with conditioned medium (CM) from infected human phagocytes or trophoblasts. The Ishikawa cells, the monocytic cell line THP-1, the trophoblastic cell line Swan-71 and monocytes and neutrophils isolated from peripheral blood were infected with *B. abortus* for 2 h. After treatment with gentamicin, supernatants were collected at 24 and/or 48 h. Here we show that *B. abortus* invades and replicates in Ishikawa cells without inducing an increase in interleukin 8 (IL-8) or monocyte chemoattractant protein 1 (MCP-1) secretion. However, the stimulation of Ishikawa cells for 48 h with CM from *B. abortus*-infected THP-1 cells, primary monocytes or Swan-71 cells induces a significant increase of IL-8 and MCP-1 as compared to stimulation with CM from non-infected cells ($p<0.01$). The stimulation of Ishikawa cells with CM from infected neutrophils or trophoblasts increases the activity of metalloproteinase 9 (MMP-9) ($p<0.05$) but does not modify the expression of progesterone, estrogen or glucocorticoid receptors, neither the expression of the microRNAs tested (miR-16, miR-21, miR-23a, miR-34a, miR-125b, miR-126, miR-155, miR-200a, miR-222, miR-223, let-7a and let-7c). These results suggest that human endometrial epithelial cell may provide a local inflammatory environment during *B. abortus* infection through interactions with infected phagocytes and trophoblasts, potentially contributing to the pregnancy complications of brucellosis.

Keywords: *Brucella abortus*, abortion, endometrial epithelial cell, inflammation.

(1815) **RESVERATROL AND EPIGALLOCATECHIN GALLATE: IN VITRO STUDY OF TWO NATURAL COMPOUNDS FOR ENDOMETRIOSIS TREATMENT AND THEIR IMPACT ON ESTROGEN ACTION**

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It is known from our previous studies that resveratrol (RES) and epigallocatechin gallate (EGCG), two natural polyphenols with well-known anticarcinogenic properties, have a strong antiproliferative and proapoptotic activity both in-vivo and in-vitro in experimental endometriosis. Moreover, we recently showed the participation of PI3K/AKT pathway in endometriosis, both in patients and experimental models. The aim to this work was to study the in-vitro effect of these compounds on aromatase and estrogen receptor α expression and to evaluate the impact of RES on endometrial cells proliferation induced by E_2. Human endometrial epithelial (ECC-1) and stromal (T-HESC) cell lines were cultured according to standard protocols and were stimulated with different doses of RES (50

and 100μM) or EGCG (20 and 40μM) for 24h. Cell proliferation was assessed using the WST-1 reduction kit, in presence or absence of E_2 and/or the estrogen receptor antagonist ICI 182,780. Levels of aromatase and estrogen receptor α expression were assessed by Western Blot. Both doses of RES inhibited aromatase expression ($p<0.01$) and 100μM RES reduced total estrogen receptor α levels ($p<0.05$) in both cell lines. In addition we found that both RES doses were able to inhibit the ECC-1 proliferation, even that induced by E_2 ($p<0.05$). Preliminary results on ECC-1 show that RES could be using more than one pathway, including that triggered by estrogen receptor. EGCG reduced the aromatase expression in T-HESC ($p<0.01$) as well as in ECC-1 ($p<0.05$) cell line. Besides, EGCG diminished total estrogen receptor α levels ($p<0.05$) in both cell lines. These results, taken together with our previously published ones, encourage us to investigate these compounds as novel strategies to treat endometriosis. More studies need to be undertaken to finally understand if RES and EGCG exert their inhibitory action through estrogen pathway inhibition which may represent an accurate therapeutic for an estrogen-dependent disease.

Keywords: Endometriosis, resveratrol, EGCG, aromatase, estrogen.

(1604) **STUDY OF THE IMMUNE RESPONSE ASSOCIATED WITH GENITAL INFECTIONS AND ITS RELATIONSHIP WITH INFERTILITY**

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Chlamydia trachomatis (Ct), Mycoplasma hominis (Mh) and *Ureaplasma urealyticum (Uu)* are usually associated with infertility. Our aim was to study immunological factors induced by genital infections as a potential cause of infertility. Antisperm antibodies (ASA) were analyzed by ELISA and cytokines and master transcription factor expression were quantified by RT-qPCR using the ΔΔCt comparative method. Sperm parameters were analyzed by WHO recommendations. Vaginal washing were collected from 59 females, Infertile With Infection (IWI-f), Infertile Without Infection (IWoI-f), Fertile Without Infection (FWoI-f) and semen from 27 Infertile male With (IWI-m) and Without infection (IWoI-m). Categorical variables were analyzed by Fisher's Exact test, $P<0.05$ was considered statistically significant. We found a significant association between the type of infection and the patient's sex, for IWI-f with *Mh* and IWI-m with *Uu* infection. No significant difference in sperm parameters between IWoI-m and IWI-m were found. ASA analysis showed no correlation with infertility or infection. Cytokine expression exhibited a significant upregulation in IWoI-f compared to FWoI-f, for IL-6, IL-10, IL-17A, RORγt, FOXp3 and Tbet (Relative Expression [RE]: 5.9, 4.7, 4.4, 12.2, 4.9, 8.7; respectively). In IWI-f with *Mh*, IL-10 was significantly increased (RE: 3.2), while with *Uu,* INFγ, RORγt, FOXp3 were increased, versus FWoI-f (RE: 7.8, 10.7, 12.2, respectively). In IWI-f, the *Mh* and *Ct* were associated with a decrease for IL-17A, RORγt and Tbet, which were significant for *Mh* (RE: 0.25, 0.23, 0.13, respectively) but not for *Ct,* versus IWoI-f. In IWI-m with *Uu* IL-8 was significantly augmented (RE: 8.5) versus IWoI-m. Infertility is associated with an upregulation of several proinflammatory cytokines, in the absence of infection. Alterations in cytokine expression were observed in infertile patients with *Mh* and *Uu* infection. Cytokine disturbance may be associated with infertility.

Keywords: infertility, antisperm antibodies, cytokines, master transcription factors, genital infection

(1524) **THE HYALURONAN SYNTHESIS *INHIBITOR* 4-METHYLUMBELLIFERONE (4MU) EFFECT ON ENDOMETRIOSIS (EDT)**

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It is known from previous studies that 4MU has antiproliferative and antiangiogenic properties. We evaluated the effect of 4MU in EDT models. Stromal T-HESC and epithelial ECC-1 endometrial cell lines, were treated with different doses of 4MU (ranging 0-4mM) and cell proliferation (CP) by the WST-1 assay was evaluated. Mice were surgically induced with EDT and treated orally with 4MU during 28 days. Six groups were included in the in vivo experiment, two different time points for initiating the treatment (2 and 15 days) and two different doses of 4MU (80 and 200mg/kg) with the appropriate vehicle control groups. The number and size of developed lesions were evaluated, as well as vascularization, CP, infiltrating lymphocytes and collagen depot within the endometriotic lesions. CP of T-HESC was significantly inhibited with 2 and 4mM 4MU ($p<0.05$); preliminar results on ECC-1 show that all concentrations of 4MU tested inhibit CP. In vivo, there was a significant reduction in the number of developed lesions in the 200mg/kg group starting treatment at day 2 ($p<0.05$ vs. day 15) and the size of these lesions was significantly reduced too ($p<0.05$ vs. control). Vascularization, evaluated by von Willebrand Factor immunohistochemistry (IHC), did not differ after treatment. CP of epithelial or stromal endometriotic cells was unaltered as seen after PCNA IHC was performed. Lesion-infiltrating lymphocytes were homogenous along all treatment groups as well as collagen depot; demonstrated by hematoxilin eosin and Masson´s trichrome staining, respectively. In vivo, 4MU did not demonstrate to be an efficacious treatment option, although further studies are being held to elucidate what mechanisms have been altered that are reflected in an overall lesser lesion size and number for the 200mg/kg dose at day 2, while in vitro results are promising. Many more studies need to be undertaken to finally understand if 4MU can be thought of as an alternative treatment option in EDT.

Key words: endometriosis, cell proliferation, angiogenesis, hyaluronan inhibition

(704) **ANALYSIS OF *Ureaplasma urealyticum*, *Mycoplasma hominis*, AND *Chlamydia trachomatis* INFECTION OF THE MALE GENITOURINARY TRACT AND SEMEN QUALITY PARAMETERS**

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Male factors account for up to 50% of infertility cases, and infection in the genitourinary tract may play a contributing role in up to 15% of male infertility. In fact, several microorganisms have been proposed to play a pathogenic role in both genital infections and male infertility. Leukocytospermia is a well-known indicator of male urogenital infection or inflammation that can be deleterious to sperm.

This study aimed to analyze leukocytospermia and standard semen quality parameters from infected and non-infected young adult men. A total of 930 semen specimens were collected by masturbation from men attending to the Andrology clinic in a period of seven years. Analysis of *Ureaplasma urealyticum, Mycoplasma hominis,* and *Chlamydia trachomatis* infection were performed by molecular or culture methods. Semen analysis was assessed according to the World Health Organization guidelines. Non-parametric tests were used for statistical analysis.

Analysis of standard of semen quality parameters between non-infected and infected men showed that the presence of *Ureaplasma urealyticum* infection was associated with significantly increased semen leukocyte counts ($p<0.05$), low sperm concentration ($p<0.0001$) and abnormal sperm morphology ($p<0.0001$). On the other hand, the presence of *Mycoplasma hominis* or *Chlamydia trachomatis* in-

fection did not associate with leukocytospermia (p>0.05). However, the presence of the latter both infections were associated with significantly decreased sperm viability (p<0.008) and normal morphology (p<0.004) levels.

Our results revealed that although the presence of any of the infections analyzed herein seemed to negatively influence semen quality, *Ureaplasma urealyticum* infection increased seminal leukocyte concentrations and decreased sperm quality parameters. In conclusion, these data indicate once more that male genital tract infections should be routinely screened in the clinic when assessing potential causes of infertility.

Keywords: male infertility, semen quality, infection, leukocytospermia, inflammation.

(701) **CERAMIDE-1-PHOSPHATE (C1P): SHIELDING OVARIAN FERTILITY FROM CHEMOTHERAPY-INDUCED DAMAGE IN A MICE MODEL OF PREMATURE OVARIAN FAILURE (POF)**

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Cancer-directed therapies cause accelerated loss of ovarian reserve leading to POF. As sphingolipid C1P can regulate angiogenesis and apoptosis, we propose it can modulate ovarian function affected by cyclophosphamide (Cy).

8-week-old mice (C57BL/6 x Balb/c F1) were given either an i.p. injection of Cy (75 mg/kg) or saline solution only (control). Control and Cy mice underwent sham surgery (intrabursal injection of saline solution), while Cy+C1P mice received intrabursa 5 *µ*l C1P (1 mM). After 2 weeks, mice were euthanized for ovary and uterus collection for histology, IHC and western blot studies. ANOVA followed by Tukey test were performed when needed.

We had previously observed that Cy decreases primordial, primary and preantral follicles, while increasing atretic follicles compared to control mice, while in Cy+C1P mice, ovaries recover control numbers of all follicular types. We have now found evidence that Cy increases protein expression of pro-apoptotic BAX (p<0.01) and decreases anti-apoptotic BCLX-L compared to control ovaries (p<0.01), while these protein levels in Cy+C1P mice are no different from control. The apoptotic index (TUNEL-positive follicles/total follicles) in preantral and early antral stages was higher in Cy mice compared to control (p<0.001 and p<0.01), while C1P protected ovaries from Cy-induced apoptosis.

Cy also caused thickening and hyalinization of vascular walls, especially in cortical stromal vessels, which was less evident in Cy+C1P ovaries, with fewer endothelial alterations. Cy-treated ovaries showed discontinuous distribution of α-SMA-positive cells (peri-endothelial marker) around blood vessels. C1P partially prevented this, increasing α-SMA-positive vessel coverage. By H&E, Cy-treated mice showed endometrial alterations in both epithelial and stromal compartments compared to control, while C1P protected endometrial quality.

Thus, C1P administration prior to Cy might be a promising fertility preservation strategy in cancer patients.

Keywords: oncofertility, premature ovarian failure, ceramide-1-phosphate, ovarian reserve

(667) **DIETARY OLIVE OIL IMPROVES SEXUAL GLANDS HISTOLOGICAL ALTERATIONS AND TESTICULAR CHOLESTEROL METABOLISM IN HYPERCHOLESTEROLEMIC RABBITS**

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The relationship between hypercholesterolemia and reduced male fertility has been reported previously. Hypercholesterolemic rabbits (HCR) were associated with deleterious changes in semen and sperm cells: seminal volume was decreased and sperm membrane was overloaded with cholesterol. Olive Oil (OO) supplementation (7% v/p) improved semen parameters affected by high fat diet. A decrease in seminal volume could be explained by alteration in accessory glands coupled to male tract, as prostate and seminal vesicle. The increase in membrane cholesterol could be due to changes in intracellular metabolism of this lipid. New Zealand White rabbits were fed commercial rabbit pellet (normocholesterolemic rabbits: NCR), plus 14% bovine grease (HCR) or 7% bovine grease plus OO (7%) (½HCR + ½OO). Prostate and seminal vesicle were processed for light microscopy and morphometry. In HCR, prostate epithelium height and proprostate villi length significant decreased (p<0.05) compared to NCR. Only proprostate villi lenght was recovered in ½HCR + ½OO. Mucose high in seminal vesicle decreased (p<0.05) in HCR but did not significantly improve with OO addition. Therefore, cholesterol intake affects mainly proprostate villi, prostate epithelium and seminal vesicle mucosa. Within molecular regulation, SREBP (Sterol- Regulatory-Binding-Protein) proteins are essential for biogenesis and membrane homeostasis. At 3 months of diet, SREBP2 mRNA expression did not show significant changes in testis, between NCR and HCR. In contrast, protein expression showed a significant increase in HCR testis. SREBP target molecules followed the same pattern. ½HCR + ½OO showed a recovery in the expression of the mentioned proteins. Taken together, the deleterious impact on reproductive tissues found in HCR could be related to alterations in sex accessory glands as well as changes in metabolic status of testis. OO was able to improve the majority of altered parameters in dietary acquired hypercholesterolemia.

Keywords: hypercholesterolemia, fertility, cholesterol, olive oil

(49) **COCAINE ALTERS THE MOUSE TESTICULAR EPIGENOME WITH DIRECT IMPACT ON HISTONE ACETYLATION AND DNA METHYLATION MARKERS**

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Abstract: Cocaine intake is associated with testicular toxicity and significant reproductive function impairment. Recent evidences suggest that cocaine administration in animal models can trigger non-genetic inheritance of addiction traits from father to offspring, affecting development and behavior. This paternal transmission was proposed to be mediated by the epigenetic reprogramming of testicular germ cells. In the present study, we measured epigenetic and functional markers in testis of adult mice treated with cocaine (Coc 10 mg/kg) compared to vehicle (Veh), in an intermittent binge protocol (3 i.p. injections, 1 h apart, one day on/off for 13 days). We have previously shown that this Coc administration protocol induced testicular germ cell loss together with increased ROS species (González et al., PLoS One. 2015 doi: 10.1371/journal.pone.0142713). Importantly, toxicity occurred in parallel with testicular dopaminergic system dysregulation: Coc induced increased tyrosine hydroxilase expression and dopamine receptors *Drd1* and *Drd2* downregulation. In the present study, we found that chronic Coc intake in mice disrupts testicular epigenetic homeostasis, increasing global histone 3 acetylation and decreasing HDAC1/2 deacetilases mRNA and protein expression (p<0.05). We also found decreased *Hdac3* and *Hdac8* mRNA expression (p<0.05). HDAC1/2 and histone 3 acetylated proteins were localized in Leydig cells and mainly in meiotic germ cells within the seminiferous tubules. We observed altered mRNA expression of DNA methylation markers like increased *Dnmt3a* and *Tet2*, and decreased *Tet1/3* after Coc treatment (p<0,05). Our results further support that cocaine abuse can induce epigenetic changes in different cell types of the testis. Understanding that testicular cells are susceptible to exogenous environmental changes and identifying the type of epigenetic marks that could be altered is critical to detect underlying and transgenerational mechanisms of risk of diseases.

Keywords: cocaine, testis, epigenetic, histone 3, HDAC

(763) **THE IMPACT OF AGE, OBESITY ANDLIFESTYLE UPON ROUTINE SEMEN ANALYSIS PARAMETERS AND SPERM KINEMATICS**

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Over the past decades, paternal age has increased, and attention has been drawn to aging effects on male reproductive potential. This study aims to report the effect of age upon parameters from routine semen analysis and Computer Assisted Sperm Analysis (CASA) kinematics.In addition, the contribution of obesity, alcohol consumption and cigarette smoking to age-related abnormalities was evaluated.

Semen samples were obtained from men for semen evaluation as part of routine andrology workup (n=12,183) and CASA (n=5,363) at the Laboratorio de Andrología y Reproducción (LAR), Córdoba. Statistical analyses were done with GraphPad-InStat software.

This analysis revealed a negative correlation (P<0.05) between age and routine semen parameters (volume, concentration, motility, vitality, morphology, round cells) and CASA variables (VSL, VCL, VAP, BCF, ALH, MAD). Using 40 yr as a cut-off value confirmed a significant decrease in these parametersin older (≥40 yr) compared to younger (<40 yr; n=2,961 each) men, and an increase in their abnormality rates.

Taking into account the negative impact of clinical and lifestyle conditions upon semen quality, aging was re-evaluated in semen from men unexposed to these conditions. As a result, volume, count, motility, vitality, VCL and ALH were still decreased (P<0.05) in older men. Moreover, obesity (BMI ≥30) proved to negatively add to the detrimental effect of age (lowered volume, concentration, count, motility, morphology, peroxidase-positive cells, VSL, VCL, VAP and ALH; P<0.05). Contrarily, alcohol consumption and cigarette smoking barely worsened aging effects (increased vitality and concentration/count abnormalities, respectively; P<0.05).

Thus, a deleterious effect of age was found upon routine semen parameters and CASA kinematics in a large cohort of samples assessed under same laboratory standards. Furthermore, obesity negatively contributed to the age-related reduced semen quality.

Keywords: Andrology, CASA, Aging, Obesity, Lifestyles

ENDOCRINOLOGY 3

(1292) **CONTRIBUTION OF DIFFERENT SIGNALING PATHWAYS ON THE DEVELOPMENT OF ESTROGEN-INDUCED PITUITARY TUMORS: THEIR ROLE IN SENESCENCE PROCESS**

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Recently we have demonstrated signs of cellular senescence as a growth control mechanism during the progression of experimental pituitary tumors. Also, it is known that Ras induces negative feedback response on PI3K/Akt and ERK1/2 pathways, while JNK and p38-stress signalling activation mediate key molecular events, which overall trigger the cellular senescence program. Based on these evidences, we aimed to evaluate the contribution these signalling pathways on pituitary cellular senescence during the development of estrogen-induced tumors.

Pituitary tumors were induced in Wistar adult male rats which were implanted with silastic capsules containing estradiol benzoate (30mg) for 10, 20, 40 and 60 days (E10-60). Control group was implanted with empty capsules. Subsequently, phosphorylated (p) and total ERK1/2; AKT; JNK and p38 protein levels were determined by Western blot. Also, pERK1/2, pAKT and senescent associated -b-Galactosidase (SA-b-Gal) double staining was performed in cryosections from normal and tumoral pituitaries. Statistical analysis: ANOVA-Fischer test (p<0.05).

An early pERK activation was detected at E10, followed by the suppression of this pathway, while pAKT expression was inhibited along tumoral progression. Concomitantly, pJNK and p38 progressively increased expression throughout the pituitary tumoral development. By analyzing the joint progression of senescence and proliferation biomarkers, we discriminated differential immunostained areas in which pERK or pAKT positive cells were predominant and adjacent zones were a higher proportion of cells exhibiting SA-b-gal stain.

These results suggest the existence of different regulatory mechanisms that attenuate the mitogenic signals during pituitary tumour development. Concomitantly, molecular events associated with cell senescence are trigged. This study delineates the existence of an intricate signalling network during pituitary tumour development that may be involved in the control of tumoral growth.

(531) **EFFECTS OF *IN VITRO* AND *IN VIVO* BEVACIZUMAB TREATMENT ON PROLIFERATIVE AND ANGIOGENIC PROCESSES IN PROLACTINOMAS.**

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Abstract: Prolactinomas are the most frequent type of pituitary adenomas and often acquire resistance to conventional therapies becoming more aggressive and locally invasive. Since angiogenesis plays a key role in tumor development we aimed to study the effect of the VEGF-blocking monoclonal antibody, Bevacizumab, on the prolactinoma cell line MMQ both *in vitro* (25, 100 and 250 μg/ml) and *in vivo* (i.p. twice/week, 3 weeks, 25 mg/kg) in Nude mice injected subcutaneously with MMQ cells. Cell viability was evaluated by MTT assay, mRNA and protein levels were measured by RT-qPCR and WB respectively. We determined a trend of reduction of MMQ cell viability with Bevacizumab treatment (p=0,087, N=4), instead, it had no effect on cell cycle progression or apoptosis rate, both evaluated by flow cytometry (ns, N=4). However, protumoral signaling pathways as ERK (p=0,017, N=3) and AKT (p=0,0002 N=3) were activated compared to control and the expression of the oncogene *C-myc* was increased (p=0,025, N=3). Bevacizumab also increased prolactin protein content (p=0,037, N=3). Regarding angiogenesis, 100 μg/ml Bevacizumab decreased secreted VEGF levels (p=0,054, N=5, ELISA) and showed a trend of reduction of the migratory capacity of HMEC endothelial cells evaluated by wound healing assay. However, increased *Vegf* and *Fgf2* mRNA synthesis was found (p=0,040 and p=0,043, N=3) suggesting that blockade of VEGF action promotes an upregulation of proangiogenic factor transcription. *In vivo*, Bevacizumab decreased tumor growth when injected early in tumor development but increased it when administered later, which correlated with *C-myc* expression. However, mRNA levels of *Prolactin*, PCNA and BETA-CATENIN protein levels were increased independently of initial tumor size. Our results suggest that in the no metastatic pituitary tumors, such as prolactinomas, inhibition of VEGF is beneficial in early stages, but compensatory mechanisms may develop a late refractory response.

Keywords: Prolactinoma, Angiogenesis, Proliferation, Vegf

(1071) **EVIDENCES OF OXIDATIVE STRESS DURING THE DEVELOPMENT OF EXPERIMENTAL PITUITARY TUMOR: CELL DAMAGE RESPONSE AND ITS POSSIBLE CONTRIBUTION TO CELLULAR SENESCENCE**

Ezequiel Grondona, Maria Eugenia Sabatino, Liliana Sosa, Bethania Mongi Bragato, Lucía Carreño, Juan Pablo Petiti, Silvina Gutiérrez , Alicia Torres, Alexandra Latini, Ana L. De Paul

The control of reactive oxygen species (ROS) levels is crucial to guarantee cell survival, since their excessive production is able to promote damages in different cell structures. We have previously demonstrated signs of cellular senescence as mechanism of pro-

liferative arrest and alterations in mitochondrial metabolism and dynamics in experimental pituitary tumors. Since mitochondrial dysfunction has been associated with senescence and this organelle is the main source of ROS, we aimed to analyze the activation of cellular damage response mechanisms to counteract the lesions promoted by oxidative stress.

To promote pituitary tumors, estradiol benzoate was implanted subcutaneously in slow releasing silastic brand capsules (10mg) in adult male Wistar rats for 10, 20, 40 and 60 days. Control group: animals were treated with empty capsules. Subsequently, ROS production was determined by flow cytometry, carbonylated proteins by spectrophotometry; γ-H2AX; 8OHdG, total and phosphorylated Nrf2 and HO-1 was assessed by immunohistochemistry and western blot; OGG1 by PCR; and glutathione levels by colorimetry assays. Statistical analysis: ANOVA-Fischer ($p < 0.05$).

From early stages of tumoral development, a progressive increase in ROS levels was detected accompanied by increases in carbonylated proteins and protein expression of DNA damage markers: γ-H2AX and 8OHdG, exposing signs of oxidative stress. Also, significant increases in OGG1, Nrf2, p-Nrf2 and HO-1 oxidative stress response markers were also observed, in addition to fluctuations in glutathione levels, revealing early antioxidant activation in response to oxidative damage.

Our data demonstrate that oxidative stress response activation triggered during pituitary tumoral development was accompanied by detoxifying mechanisms and DNA damage repair, thus favouring the redox balance recovery and cell survival. These results support the emergence cellular senescence as an intrinsic barrier for the pituitary tumoral growth.

Keywords: Oxidative Stress, Pituitary, mitochondria, ROS , DNA damage

(1093) **EZH2 OVEREXPRESION AND ITS EPIGENETIC MARK H3K27me3 IN PITUITARY TUMOR: ROLE ON CELL CYCLE REGULATION**

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Enhancer of zeste homolog 2 (EZH2) is a core epigenetic regulator, which represses genes involved in tumorigenesis and plays a crucial role in cell cycle regulation. This protein catalyzes the repressive mark H3K27me3 and is associated with proliferation and worse outcome in several tumor entities. Considering the few epigenetics studies in pituitary tumors the aim of this study is to elucidate the effect of EZH2 and H327me3 on cell cycle regulation in order to understand epigenetic´s tumor growth. For this propose we used experimental pituitary tumors induced by estradiol benzoate (30mg capsule) in adult *Fisher* male rats. Experiments were performed with the adenohyphophysis and primary pituitary cell culture, from control and induced-tumoral glands. Protein analysis of H3K27me3 and EZH2 was made through Western Blot and the mRNA levels of cell cycle regulators, *cdkn1a, cdk4, ccdn1 and tp53* was quantified by qRT-PCR. Statistical analysis: t-test ($p<0.05$).

The epigenetic mark H3K27me3 as well as EZH2 were increased in pituitary tumor glands compared to control; being similar in primary culture. In pituitary tumor glands *Cdkn1a* and *tp53* mRNA levels displayed a significant reduction respect to control, but in primary culture this diminution was observed only for *cdkn1a.* The C*dk4* and *ccdn1* mRNA levels did not reveled significant variation in the experimental models used.

The present results suggest the presence of the repressive mark H3K27me3 at *cdkn1* and *tp53* promoters, inferring a regulation of these gens by EZH2.

Bearing in mind that target gens of EZH2 in pituitary tumors have yet to be described, further analysis are needed to better understand the cell cycle regulation, being EZH2 a potentially useful diagnostic marker and pharmacotherapeutic target for this tumor.

Keywords: pituitary tumor, epigenetics, EZH2, H3K27me3

(1611) **FGF2-INDUCED ADENOHIPOFISARY CELL PROLIFERATION IS REGULATED BY INHIBITORY GPCR.**

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The metabolic activity of pituitary gland is regulated by numerous factors being the basic fibroblast growth factor (FGF2), one of the main regulator that increase of pituitary cell proliferation. The growth factors effects may be regulated by activation of inhibitory G protein-coupled receptors (GPCRs) at different signaling levels. The aim of this work was to evaluate whether the FGF2 proliferative activity in adenohypophysis cells is regulated by inhibitory GPCRs activated by the somatostatin analogue, octreotide (OCT). Anterior pituitary cell cultures from female rats were treated with FGF2 (10 ng/mL) or OCT (100mM) alone or co-incubated, in serum free condition. The cell cycle was analyzed using propidium iodide by flow cytometry at 24 and 48h. The mRNA levels of cell cycle regulators (tp53, *cdkn1a, cdk4* and *ccdn1)* were determined by qPCR at 3 or 6h and the total and phosphorylated (p) ERK1/2, JNK, p38 and Akt protein expression by western blot at 15, 30, 60 or 90 min. Statistics: ANOVA-Bonferrony. The S/G2M phases were increased by FGF2 whereas OCT decreased these phases. The FGF2/OCT co-incubation significantly increased the G1-phase arrest after 24 and 48 h, effect that was associated with an increase of *cdkna1* and decrease of *ccdn1* mRNA levels at 3 and 6h, while the *tp53* and *cdk4* did not show any significant variation. In addition, even though pP38 and pAkt protein expression levels did not presented changes, a remarkable decrease of pERK1/2 expression was observed in all times analyzed, and a significant increase of pJNK expression at 60 and 90min was detected after combined treatments These findings show that FGF2/OCT treatment inhibited proliferation induced by FGF2 in pituitary cell culture regulating the pERK1/2 and pJNK protein expression and the c*dkn1a* and *ccdn1* cell cycle regulated genes. This regulatory effect may participate in the homeostasis of pituitary cell populations.

Keywords: pituitary cell, , FGF2, inhibitory GPCR, cell cycle, ERK1/2

(284) **MECHANISMS OF THE FUNCTIONAL INTERACTION OF RETINOIC ACID AND BMP-4 IN CORTICOTROPHINOMAS**

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Cushing's disease is an endocrine disorder due to a pituitary corticotrophinoma. We demonstrated in dogs the remission after administration of Retinoic Acid (RA). Bone Morphogenetic Protein 4 (BMP-4) also stops the tumoral progression. Our aim is to establish whether there is a common thread between the signaling pathways of RA and BMP-4. Using the AtT-20 mouse tumor corticotroph cell line, we first confirmed the expression pattern of different type of receptors for RA (RXRα, β and γ ; RARβ and γ). By coimmunoprecipitation we observed the interaction of RXRα, RXRγ and RARβ with Smad1 and Smad4 proteins, key components in the BMP-4 signaling, under RA or BMP-4 stimuli for 24h. The transfection with an expression vector for COUP-TFI produced a reduction in the conformation of these complexes. COUP-TFI blocked from 40-80% to 10% the inhibitory action of RA (100 nM) and the potentiation of the inhibition by BMP-4 (100 ng/ml) on the POMC-Luc promoter. We observed that with a POMC-Luc containing both responding elements, RARE and BMPRE, the inhibitory effect of the co-treatment is max-

imum, while with only RARE, RA inhibited and a potentiation of this inhibitory effect was still exerted by the co-treatment with BMP-4. A point mutation at the BMPRE shows that although the promoter does not respond to BMP-4 it still has the co-potentiation action with RA, indicating that BMP-4 does not need the BMPRE site (the main responding element to BMP-4) to potentiate the inhibitory action of RA, that occurs at the RARE site. RA and BMP-4 form signaling complexes that interact at the transcriptional level, resulting in a potential target to treat this disorder.

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Keywords: Retinoic acid, Cushing's disease, BMP-4, Corticotroph, Pituitary.

(1005) **MODULATION OF ANTI-MITOGENIC SIGNALING IN PITUITARY TUMOR CELLS AS NEW THERAPEUTIC STRATEGIES**

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Clinical treatments for pituitary tumors are focused on stimulating receptors that inhibit proliferation and hormone secretion, such as those for TGFβ1 and somatostatin (SST). However, almost 40% of the patients who receive treatment with SST analogue, Octreotide (OCT), do not respond as expected and the mechanisms underlying this resistance remains still unknown.

Our objectives were to evaluate TGFβ1 and SST receptors expression and to elucidate a possible crosstalk between both pathways in pituitary adenoma cells. We determined the expression of TβRI, TβRII, SSTR2 and SSTR5 by IHC and WB in normal pituitaries (n=7) and in different types of human pituitary adenoma samples: non-functioning (n=9), GH (n=6) and ACTH-secreting tumors (n=2). GH3 rat pituitary tumor cells were treated for 24 h with OCT (10 and 100 nM) and/or TGFβ1 (4 mg/ml). SSTRs and TβRs mRNA was analysed by qPCR, hormone secretion (PRL) by RIA and cell proliferation by ICC for Ki67. Statistics: ANOVA-Fisher, T-Test.

Human pituitary tumor samples exhibited a decreased in SSTRs and TβRs protein expression although variable between different types of adenomas compared to normal pituitaries. In addition, we demonstrated that the co-incubation of OCT with TGFβ1 induced an increase of SSTRs and TβRI mRNA expression, effect that was associated with a decrease of proliferation marker Ki67 and PRL secretion compared to treatments alone ($p < 0,05$).

These findings suggest that pituitary tumor resistance to treatments could be explained, at least in part, by the decreased in anti-mitogenic receptors expression. Furthermore, the synergic effect of OCT and TGFβ1 on cell proliferation and hormone secretion indicate a possible interaction between both anti-mitogenic signals in GH3 cells. Such results highlight the importance of comprehending the connection among different anti-mitogenic signalling pathways and how they can be modulated to obtain a better cell response on the context of tumor resistance to treatments.

Keywords: pituitary adenomas, SSTR, proliferation, hormone secretion, therapeutic targets.

(1032) **PRL-3 EXPRESSION IN HUMAN PITUITARY ADENOMAS AND PROLACTINOMA EXPERIMENTAL MODEL: A POSSIBLE ROLE IN THE TUMORAL DEVELOPMENT**

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PRL-3 (phosphatase of regenerating liver 3) is a subclass of protein tyrosine phosphatases involved in proliferation, migration, invasion and growth tumor, and have been proposed as potential biomarkers of tumor progression. Our aim was to investigate whether PRL-3 is expressed in pituitary human tumor lesions evaluating its presence and correlating with some clinical-pathological parameters. Besides with the objective to analyze a possible PRL-3 participation in pituitary tumorigenesis, this phosphatase and p21 were evaluated in a lactotroph tumoral experimental model. We analyzed PRL-3 in 46 human pituitary adenomas and Fisher male rats treated with estradiol benzoate (EB) during 10, 20 and 30 days (n: 9) were used. Normal human (n: 2) and rats (n: 3) pituitaries were use as control group (CG). Immunohistochemical technique was applied at photonic and electronic level and a semiquantitave study by western blot was used. Statistic analyzes: x^2 for the variables: size, function and ki 67 in human adenomas. Anova-Fisher and correlate analyze in the experimental model. From the total human adenomas, PRL-3 was expressed in 22 out of 46 with immunolocalization predominantly in cytoplasmic level. Interestingly at ultraestructural level some tumors exhibited phosphatase immunolocalization at plasma membrane. There was a positive relation with tumor size (macroadenomas, x^2 $p<0.0001$). Comparing to normal pituitary gland, PRL-3 showed a significant increase in secreting as well as non-secreting tumors. PRL-3 in experimental prolactinoma showed a significant increase at 20 d EB (hyperplasic/adenomatous state), demonstrating a lineal correlation with p21 (r=0,57). At 30 d EB (adenomatous state) PRL-3 expression decreased without reaching the levels observed in the CG. Our investigation is the first to show PRL-3 expression in human adenomas predominantly in macroadenomas and its involving at the early phases of the pituitary adenomatous development.

Keywords: Pituitary adenoma, Inmunohistochemistry, PRL-3, p21, western blot.

(1021) **TRASTUZUMAB INHIBITS PITUITARY TUMOR CELL GROWTH MODULATING THE TGFB/SMAD2/3 PATHWAY**

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In pituitary adenomas, early recurrences and resistance to conventional pharmacotherapies are common, but the mechanisms involved are still not understood. The high expression of epidermal growth factor receptor 2 (HER2)/ extracellular signal-regulated kinase (ERK1/2) signal observed in human pituitary adenomas, together with the low levels of the antimitogenic transforming growth factor beta receptors (TbRs), encouraged us to evaluate the effect of the specific HER2 inhibition with trastuzumab on experimental pituitary tumor cell growth and its effect on the antiproliferative response to TGFb1. Trastuzumab decrease ($p\leq0.05$) the pituitary tumor growth as well as the expression of ERK1/2 and the cell cycle regulators cyclin D1 and CDK4. The HER2/ERK1/2 pathway is an attractive therapeutic target, but its intricate relations with other signaling modulators still need to be unraveled. Thus, we investigated possible cross-talk with TGFb signaling, which has not yet been studied in pituitary tumors. In tumoral GH3 cells, co-incubation with trastuzumab and TGFb1 significantly decreased ($p\leq0.05$) cell proliferation, and effect accompanied by reduction in ERK1/2 phosphorylation and an increase of SMAD2/3 activation. In addition, through immunoprecipitation assays, an increase in TGFbR1-SMAD2/3 association was observed when cells were co-incubated with the inhibitor of the HER2/ERK1/2 pathway and TGFb1. These findings indicate that blocking HER2 by trastuzumab inhibited pituitary tumor growth and modulated HER2/ERK1/2 signaling and consequently the anti-mitogenic TGFb1/TbRs/SMADs cascade. The imbalance between HER2 and TGFBRs expression observed in human adenomas and the response to trastuzumab on experimental tumor growth, may make the HER2/ERK1/2 pathway an attractive target for future pituitary adenoma therapy.

Keywords: Pituitary adenoma, HER2, ERK1/2, TbRs, Smad2/3.

(1782) **IS THE STEM CELL POPULATION CONTRIBUTING TO THE DEVELOPMENT OF THE EXPERIMENTAL**

PITUITARY ADENOMA?

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Our previous results confirmed the presence of stem cell (SC) in adult pituitary gland and provided early evidences of cells expressing cancer stem cells (CSC) markers in experimental pituitary adenomas. The aim of this study was to evaluate possible changes in the expression of CS: GFRa2, Oct-4, Sox2, Nestin, Progenitor (P): Sox9, and CSC: CD133 and CD44 markers, during experimental pituitary tumor development, and later characterization of the SC in culture. The experimental model was performed in female Fischer rats, treated with 30mg of estradiol benzoate during 5 (5D), 10 (10D), 20 (20D) and 30 (30D) days. Control group (C) included rats in diestrous. SC/P and CSC markers were detected by immunofluorescence (IF) and Western Blot (WB). *In vitro* studies were done using C, 5D and 30D pituitary cell suspensions. The spheres were characterized by IF of SC/P and CSC markers and further estrogen stimulation the proliferation of C, 5D and 30D spheres was quantified. SC/P and CSC markers expression was detected in C, 5D and 30D. Oct4, Sox2, GFRa2, Nestin and CD133 showed variations in their distribution within the gland during treatment. We observed positive cells for these markers in the marginal zone (MZ), and low immunostaining in adenohypophysis (AH) of C and 5D. In contrast, their expression was remarkable in AH of 30D, indicating a reorganization of SC from the MZ to AH. The expression levels of GFRa2, Nestin, CD133 and CD44 increased significantly in 5D compared to C and other periods of treatment. Sox2 didn´t show variations on its expression levels and Sox9 (P), decrease in 5D. This allows to propose the participation of CS at early stage of tumor development. The pituispheres in culture were positive for SC/P and CSC markers. A significant increase of proliferation was shown in spheres obtained from 5D and 30D vs. C ($p<0.05$), suggesting that tumoral SC would have a different behavior due to their tumoral imprinting and participate in the neoplastic growth.

Key Words: Pituitary adenoma, Stem cells, Cancer Stem cells

(591) **TRASTUZUMAB REGULATES SRC/FAK/PAXILLIN KINASES CONTROLLING ErbB-2 HUMAN BREAST CANCER CELL MOTILITY.**

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Breast cancer is the most common cancer in women worldwide. The majority of deaths attributed to breast cancer are a result from metastasis. Breast carcinoma is classified into molecular subtypes according to the presence and/or absence of estrogen receptors (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2). HER2+ breast cancers represent 20-25% of all breast cancer diagnoses. Currently, new anti-HER2 strategies that are used in clinical studies have been developed. One of these strategies is a monoclonal antibody that binds to the extracellular domain of HER2 known as Trastuzumab (Tz). In addition, dimerization of HER2/HER3 receptors regulates diverse cellular functions, including cellular motility. Heregulin (HRG), a HER3 ligand plays a role in the development and progression of breast cancer. However, HRG effects on cell motility have not been fully explored in breast cancer cells. Therefore, the aim of our work is to identify new molecular mechanisms regulated by HRG/Tz on the morphology and motility of BT-474 (HER2+) cells, a Tz-sensitive human breast cancer cell line. We observed in HER2+ cells that, the treatment with HRG (1nM) induced firstly, a significantly phosphorylation/activation and dimerization of HER2/HER3 receptor ($p<0.05$). Secondly, after HER2/HER3 dimerization, HRG caused an increased phosphorylation of c-Src, FAK and Paxillin, ($p<0.05$) and the consequent translocation of p-FAK/p-Paxillin to membrane sites where focal adhesion complexes are assembled. This recruitment of c-Src/FAK/Paxillin is required for cell adhesion, migration and invasion in breast cancer cells, but all of these processes were notably reduced ($p<0.05$) by the use of Tz (10 μg/ml). In conclusion, our findings identify a mechanism whereas HRG and TZ control breast cancer cells motility. This information helps to understand therapeutic approaches for the prevention and regulation of breast cancer in patients with overexpression of HER2.

Keywords: Heregulin, Trastuzumab, HER3, cell motility

NEPHROLOGY 2

(1533) **ROLE OF NHE1 AND AQP2 IN BASAL AND ACIDOSIS-ASSOCIATED APOPTOSIS IN A MODEL OF RENAL CORTICAL COLLECTING TUBULE PRINCIPAL CELLS: MPKCCD$_{c14}$.**

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The association between growth mechanisms and intracellular pH (pHi) elicits the possibility that a chronic pH alteration modifies cell growth. Control of pHi is regulated in part by the isoform 1 of Na/H exchanger (NHE1), an exchanger that, depending on the cell type, is involved in apoptotic or proliferative processes. We previously showed in renal cells that the expression of aquaporin 2 water channel (AQP2) modifies growth. The aim of this study was to investigate whether exposure of renal cells to acid medium altered apoptosis and, if so, its association with NHE1 and AQP2. To investigate this, we used a kidney principal cell line whose AQP2 expression can be induced by arginine vasopressin (AVP): mpkCCD$_{c14}$. Cell number counting, apoptosis and immunofluorescence studies were performed. Our studies show that after being cultured for 11 days in the presence of AVP mpkCCD$_{c14}$ cells increase their expression of both AQP2 and NHE1. AVP stimulated cells have a higher basal apoptosis than control ones (% apoptosis control 7.75±1.37 vs AVP 13.33±1.85, $p<0.05$ n=7) which decreases by inhibition of NHE1 with its specific inhibitor HOE-694 1μM (% Apoptosis AVP+HOE= 6.33±1.8%, $p<0.05$ vs without HOE n=6). This inhibition of apoptosis is parallel with a 10% increase in AVP stimulated cell number. After exposing 24hs to acid medium (ΔpH = 0.4) only cells that do not express AQP2 significantly increase their apoptosis (% Apoptosis Control+Acid= 21±1.5%, $p<0.01$ n=6) in parallel with a 10% decrease of cell number. The increase of apoptosis in control cell exposed to acid is even greater by inhibiting NHE1 (% Apoptosis Control + Acid+HOE = 27±1.5%, $p<0.05$ vs Acidosis without HOE n=6). This latter apoptosis increase is parallel with 30% decrease in cell number. In summary AQP2 expression protects renal cells of acidosis-induced apoptosis. In turn apoptotic process in these cells is dependent on NHE1.

Keywords: KIDNEY, ACIDOSIS, AQP2, NHE1

(1618) **EFFECT OF VITAMIN K2 SUPPLEMENTATION ON VASCULAR CALCIFICATION IN PATIENTS IN HEMODIALYSIS: A RANDOMIZED CONTROLLED TRIAL**

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Vascular calcification (VC) is the leading cause of death in patients with chronic renal failure (CRF) on hemodialysis (HD). The vitamin K-dependent Gla protein matrix is one of the most potent inhibitors of VC and in renal patients it is inactive. This risk of mortality is due in part to the development of the calcification of the middle layer. Objective: To evaluate the effect of vitamin K2 on vascular calcification in patients with CRF in HD of the Center of Dialysis Salta. Materials and Methods: prospective, randomized, double-blind study. Inclusion criteria: men and women ≥ 18 years, HD therapy ≥ 6 months, without oral anticoagulants consumption and signed informed consent. The study subjects (n = 58) will constitute a control group: n = 29 (1000 µl of saline solution) or treated group: n = 29 (1000 µl of Vitamin K2). Vitamin K2 will be administered 3 times a week using the same vascular access, at the end of each dialysis session. Blood samples will be drawn for biochemical determinations: Ca, P, PTHi and KPTT. VC will be evaluated before and after 3 months of treatment by carotid artery intima-mediated echocardiography (IME). We performed Shapiro-Wilks test to analyze normality, F-test to compare variances, Student's t test with Welch modification for normal data with unequal variances and Mann-Whitney test. The statistical package StatGraphics and GraphPad Prism 5 were used. Results: Baseline analysis in patients treated and not treated with Vit K2: Ca 9.2 ± 0.2 vs 9.6 ± 0.5 (p = 0.4); P 5.2 ± 0.2 vs 4.3 ± 0.2 (p = 0.006); PTH 441 ± 104 vs 318 ± 62.8 (p = 0.6). Treated and untreated group: Right IME: 0.63 ± 0.07 vs 1.45 ± 2.1 (p = 0.06). Left IME: 0.73 ± 0.09 vs 0.80 ± 0.1 (p = 0.4). Conclusion: It is expected that vascular calcification does not progress in the treated group when compared with the control group.

Key words: patients on hemodialysis, vitamin K2, vascular calcification.

(517) **OXIDATIVE STRESS BIOMARKERS IN LUPUS PATIENTS WITH NEPHROPATHY**

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Renal compromise in patients with systemic lupus erythematosus is an important cause of morbimortality. Experimental evidence suggests the involvement of oxygen reactive species as primary mediators in the pathogenesis of renal damage produced by ischemic, toxic and immunological processes. Our aim was to characterize the redox status in patients with active lupus nephropathy (ALN) and to determine whether any of the oxidative stress (OS) markers studied correlate with the urinary protein/urinary creatinine index (P/Cr), a traditional marker of renal activity. Twenty-two patients with ALN and 25 patients with inactive lupus nephropathy (ILN) were studied. As OS biomarkers we assessed: a) catalase activity (CAT; UI/g Hb) by a kinetic method; b) superoxide dismutase activity (SOD; U/gHb) by a commercial enzymatic technique; and c) lipid peroxidation by the thiobarbituric acid reactive substances method [TBARS; pmol malondialdehyde/g creatinine (pmol MDA/g Cr)]. P/Cr index (g/g) was determined by a colorimetric/kinetic method. The results obtained, expressed as median (range), in patients with ALN and ILN were, respectively, CAT: 33 (6-81) *vs* 35 (6-97); SOD: 971 (258-2280) *vs* 1026 (31-2101); TBARS: 47 (0-172) *vs* 24 (0-157) y P/Cr: 0.83 (0.37-6.6) *vs* 0 (0-0.2). Statistically significant differences between both groups were found for TBARS (p=0.025) and for P/Cr (p=0.001). None of the biomarkers studied significantly correlated with P/Cr. We conclude that, in the sample analyzed, there is a disbalance in the redox status that would be involved in lipid peroxidation of the basal membrane, thus altering its integrity and also affecting tubular function in patients with ALN. Consequently, TBARS could be used as an additional marker of activity in renal disease together with parameters commonly used in clinical laboratory practice.

Keywords: oxdative stress – lupus nephropathy – lipid peroxidation – urinary protein

(760) **EFFECTS OF ESTRADIOL ON CELL PROLIFERATION AND CYCLINS D1 AND B1 EXPRESSION IN PRIMARY CULTURES OF HUMAN RENAL EPITHELIAL CELLS**

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The proximal renal tubule epithelium expresses receptors for estradiol, however, little is known about its functions in the kidney. A sexual dimorphism was found in compensatory renal growth, due to the female sex hormones, but the hormonal role in this process is not clarified. The mechanism of cell proliferation is part of the epithelial regeneration process in renal tubules. The objective of the work was to study the effects of estradiol on cell proliferation and the expression of intracellular signals involved in cell cycle, in primary and three-dimensional (3D) cultures of human cortical renal tubular epithelial cells (HRTEC), developed from nephrectomies performed in pediatric patients at the Hospital Nacional Prof. A. Posadas. HRTEC proliferation rate was evaluated by incorporation of bromodeoxyuridine (BrdU), mRNA expression for cyclin D1 (CYD1) and B1 (CYB1) was assessed by RT-qPCR and tubulogenesis was performed in 3D-HRTEC cultures. HRTEC Incubation with 17β-estradiol (17BE 10 nM, 24 h) stimulated the incorporation of BrdU by 40 ± 4,7 % ($p<0,001$, n=8) with respect to control cells without hormonal treatment. HRTEC pre-treatment with ICI182780, a classical estrogen receptors (ER) antagonist, or G-15, a GPER-1 (protein-coupled estrogen receptor-1) antagonist, totally inhibited the effect of 17BE on cell proliferation, without having effect per se. In primary cultures of HRTEC, 17BE significantly ($p <0.01$) stimulated CYD1 (3.6 ± 0.12 fold) and CYB1 (8.3 ± 1.70 fold) mRNA levels with respect to control cells. In 3D-HRTEC cultures, same treatment with 17BE stimulated ($p<0,05$) cell proliferation per tubular structure, which corroborates the stimulation in tubulogenesis, and also significantly ($p<0,01$) stimulated CYD1 and CYB1 genes expression with respect to control 3D-HRTEC. In conclusion, 17BE regulates HRTEC proliferation by stimulating cyclins involved in cell cycle, evidencing a possible role in the regeneration process of human renal tubular epithelium.

Keywords: estrogen, cell proliferation, cyclins, human kidney primary cultures

(1730) **TLR4 REGULATION IN CIRCULATING MONOCYTES FROM PATIENTS WITH TYPICAL HEMOLYTIC UREMIC SYNDROME (HUS+). ROLE OF Rab7b**

Andrea Fernanda Gil Lorenzo

The inflammatory response of host endothelial cells to Shiga toxin and/or lipopolysaccharides (LPS) of *E coli* is included in typical HUS. LPS stimulation of TLR4 activates signal transduction pathways leading to proinflammatory cytokine secretion. The TLR4-LPS complex is rapidly internalized and TLR4-induced inflammatory signaling is stopped by targeting the complex for degradation. Rab7b, a small GTPase expressed in monocytes, regulates the later stages of the endocytic pathway.

We studied the Rab7b participation on the TLR4 endocytic pathway and its effect on monocyte intracellular cytokine production along the acute course of HUS.

The studies were performed in monocytes from HUS patients by flow cytometry and immunofuorescence confocal microscopy.

Surface TLR4 expression determined by flow cytometry in CD14(+) monocytes from 16 HUS patients significantly increased by

days 1 and 4 compared to 10 healthy children monocytes. We show increased proinflammatory intracellular cytokines, tumor necrosis factor alpha (TNF-α), and interleukin 6 (IL-6) in HUS monocytes at days 1 and 4 vs controls. On the contrary, monocytes display decreased surface TLR4 expression and significant reduction of intracellular TNF-αand IL6 levels released in a time-dependent manner after a disease follow up of 6 to 10 days. Furthermore, immunofluorescence confocal microscopy proved colocalization of increased intracellular TLR4/Rab7b determined by Pearson's coefficient in monocytes from HUS patients on day 1. The highest colocalization of both proteins in monocytes was shown by day 4, then decreased TLR4/Rab7b colocalization was shown 10 days after HUS onset.

The colocalization of TLR4 and Rab7b allows us to suggest that Rab7b participates in the control of the TLR4 endocytic pathway in HUS patient monocytes. A consequential fall in cytokine production throughout the early follow up of HUS is demonstrated.

Keywords: TLR4, LPS, Rab7b, monocytes

(1666) **URINE KALLIKREIN ACTIVITY (UKa) IN AUTOSOMAL DOMINANT POLICYSTIC KIDNEY DISEASE (ADPKD)**

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The kallikrein kinin system (KKS) is involved in blood pressure regulation and has been proven that UKa is a marker of its renal activity. ADPKD progression is characterized by a progressive renal cyst enlargement that led to chronic renal failure with a wide range of severity. Hypertension and microalbuminuria are early features in ADPKD. These parameters has been found in association with a diminished KKS activity in some renal diseases, but no relevant data on ADPKD is available.

To evaluate KKS in ADPKD, we conducted a transversal study of 17 patients (35 ± 1 years, 7 men) and 5 age-matched healthy controls to measure UKa (U/day), urine osmolality (mOsm/Kg H_2O), osmolal excretion (mOsm/day), diuresis (l/day), blood pressure (mmHg), microalbuminuria (in ug/mg), MDRD-estimated glomerular filtration rate (ml/min/1.73 m2), total kidney volume (TKV in ml) and plasma copeptin as a surrogate marker of vasopressin (ng/ml).

Patients showed diminished UKa (2.83 ± 0.73 vs. 12.27 ± 2.31, $p < 0.0001$) and urine osmolality (221 ± 22 vs. 436 ± 81, $p < 0.01$), increased diuresis (2.39 ± 0.25 vs. 1.54 ± 0.24, $p < 0.03$) and trend to a lower osmolal excretion (472.0 ± 47.6 vs. 583.6 ± 67.2, p = 0.07) than healthy controls. No changes in UKa according to blood pressure levels, sex nor antihypertensive treatment were found. UKa levels correlates positively with TKV and negatively with osmolal excretion (R = 0.73 and R = 0.704, $p < 0.002$; respectively). No association with the other variables were found.

ADPKD patients showed impaired KKS activity that could be attributable to the damage in renal architecture caused by cyst burden. Additionally, UKa could be related to impaired urinary concentration capacity, another early manifestation of the disease. Taken together, these data propose the utility of UKa as a marker of ADPKD progression in an extended and longitudinal study.

Keywords: urine kallikrein activity, ADPKD, total kidney volume, urine osmolality.

(1122) **PROXIMAL TUBULE IN EARLY STAGE OF DIABETIC NEPHROPATHY**

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Kidneys play a key role in glucose homeostasis. During hyperglycemia a feature of diabetes mellitus, renal glucose handling is altered which in turn leads to an alteration in water transport. Aquaporin 1 (AQP1) is a water channel strongly expressed in proximal tubules. Although 2/3 of filtered water is reabsorbed in the proximal tubule AQP1 expression in diabetic kidneys and its possible role in the alteration of water transport are unknown. Thus we decided to study if AQP1 expression in diabetic kidneys.

Diabetes was induced in Sprague-Dawley rats after intraperitoneal injection of 65 mg/kg Streptozotocin (STZ). Studies were carried out at 15 days and 5 months post injection.

15 days post STZ injection rats presented normal glomerular filtration rate (creatinine clearance 2.3 ml/min ± 0.16 vs 1.9 ml/min ± 0.15 n=6) and no proteinuria. Histological observation revealed no morphological alterations. We considered 15 days post STZ injection representative of early diabetes where there hyperglycemia without renal damage is found. In these rats AQP1 expression significantly increased in the diabetic group (100 vs 198 ± 28, n=3 p<0.05). Functional studies at 5 months after STZ injection revealed that although diabetic rats showed no alteration in glomerular filtration rate (2.09 ± 0.17 ml/min vs 1.95 ± 0.31, n=6) they presented proteinuria (5.04 ± 0.91 vs 18.30 ± 5.12 p<0.05 n=8) and histological alterations. Kidneys were also enlarged. Here, AQP1 expression was significantly decreased (100 vs 49 ± 4.5 p<0,001)

AQP1 expression increases when no renal damage or functional alterations are present. On the other hand in early times of development of diabetic nephropathy, when kidney damage is evidenced by histological alterations and the presence of proteinuria but glomerular filtration rate is still conserved, AQP1 expression is decreased. Further work is needed to establish the relationship between AQP1 and renal glucose handling.

Key words: Kidney. Diabetes. AQP1. Glucose Handling

(1373) CHRONIC KIDNEY DISEASE INDUCED BY LITHIUM IN AN ANIMAL MODEL

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Lithium (Li) is considered as a first-line drug for the maintenance treatment of bipolar disorder. Interest in the risk of chronic kidney disease and Li-induced renal failure is renewed due to findings suggesting that both complications would be more frequent than previously estimated. The aim of this work was to evaluate renal damage due to chronic exposure to Li. Wistar male rats (n=39) were fed standard diet (Control -C-) or a containing 60 mmol Li/kg diet (Experimental -E-) ad libitum and sacrificed after 1 or 3 months of treatment (1 or 3). Clearance of creatinine (CrCl), lithemia, urin protein excretion (UPE) and fractional excretion of sodium (FENa) were measured. Kidney samples were processed for: 1-histology and morphometric analysis (PAS and H&E); 2-oxidative stress (TBARs and carbonyls) evaluation; 3-immunohistochemistry (Aquaporin-2; AQP2). FENa and UPE were within normal values in all groups. Lithemia (mmol/L) reached therapeutic values in both E groups (E1=0.57±0.18 and E3=0.68±0.12). CrCl was markedly reduced after 3 months of Li administration (C3=2.75±0.47 vs E3=1.55±0.75; t=4.18, p=0.001). Kidney from both E groups showed dilatation of the cortical collecting tubules (CCT), identified with AQP2 positive staining. Some CCT had hypertrophy with enlarged, binucleated cells, protruding into lumen and other CCT had atrophy with flattened cells. Glomerular area -μm^2- was smaller in E rats (C1=11333±2067 vs E1=9757±2178; t=1.58, p=0.135 and C3=13103±1494 vs E3=11053±929; t=3.76, p=0.001). A significant increase in TBARs -nmol/mg-(C1=0.36±0.10 vs E1=0.51±0.11; t=-3.02, p=0.008 and C3=0.36±0.08 vs E3=0.58±0.15; t=-4.02, p=0.001) and carbonyls -nmol/mg-(C1=0.23±0.07 vs E1=0.33±0.06; t=-3.27, p=0.004 and C3=0.24±0.07 vs E3=0.36±0.10; t=-3.24, p=0.005) was observed in both E groups. Exposure to Li from 1 to 3 months induced renal

damage resulting in histological and functional alterations, changes in glomerular area and increase of oxidative stress and damage.

Keywords: Lithium, Chronic Kidney Disease, Histopathology, Renal Function, Oxidative Stress

(1312) **ENDOTHELIN AND RENAL POSTNATAL DEVELOPMENT: MOLECULAR MECHANISMS INVOLVED**

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Disturbances in proliferative and apoptotic processes during development manifest as development abnormalities. Diverse studies have implicated apoptosis as a determinant of nephron number, being mainly implicated the intrinsic or mitochondrial pathway of apoptosis. The aim of this work was to evaluate in the renal cortex of seven days old Sprague-Dawley rats (both male and female) treated with bosentan (dual endothelin receptor antagonist) from the first day of life: 1) cellular proliferation by immunohistochemistry using a primary antibody anti PCNA (Proliferating cell nuclear antigen); 2) cellular apoptosis by TUNEL (Terminal deoxynucleotidyl transferase dUTP nick end labeling) assay and 3) TBARS (thiobarbituric acid reactive substances) to estimate oxidative stress. Four experimental groups were defined as follows: control male (CM); control female (CF); bosentan male (BM) and bosentan female (BF). Two factor ANOVA was used for the statistical analysis.

The number of PCNA positive nuclei present in the immature structures that give rise to glomeruli did not change significantly in bosentan-treated vs non-treated animals in the cortical [CM= 426±11; BM= 406±10; CF= 589±123; BF= 520±165] nor in the juxtamedullary area (JA) [CM= 650±21; BM= 615±69; CF= 856±17; BF= 639±116]. However, the number of TUNEL positive nuclei was significantly higher in bosentan-treated animals, mainly in the JA [CM= 14±4; BM= 79±27; CF= 8±1; BF= 42±17 ($p<0.01$)], in accordance with our previous results showing a decreased glomerular number in that zone (PLOS ONE 11(2): e0148866. doi:10.1371/journal; 2016). There was a tendency to increase TBARS in BM vs CM [0.885±0.171 vs 0.674±0.073 (nmol MDA/mg. prot)].

Endothelin inhibition during the postnatal period produces an imbalance between renal proliferative and apoptotic processes, being exacerbated apoptosis with a decreased proliferation/apoptosis ratio and this may be a consequence of an increased oxidative stress.

Key words: Endothelin, endothelin receptor antagonist, bosentan, renal postnatal development.

(1094) **A MASS SPECTROMETRY-BASED UNTARGETED METABOLOMICS APPROACH FOR STUDYING CLEAR CELL RENAL CELL CARCINOMA USING AN *IN VITRO* MODEL**

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Renal Cell Carcinoma (RCC) is among the 10 most common cancers in both men and women worldwide, with clear cell RCC (ccRCC) being the most common (75%) histological subtype. At molecular level, 90% of all ccRCC patients show mutations in Von Hippel Lindau (VHL) gen which is involved in Hypoxia Inducible Factor 1α expression. Current research has shown that several metabolic alterations are associated with RCC, during tumor progression and metastasis. Mammalian cell metabolomics has emerged as a promising tool for studying cellular biochemistry and investigate altered metabolic networks that contribute to cell proliferation, growth and survival in RCC.

In this study, we have optimized a protocol for harvesting, extraction, lyophilization and reconstitution of metabolites present in conditioned media derived from human ccRCC cell lines 786-O ($VHL^{-/-}$) and Caki-1 ($VHL^{+/+}$), and the non-tumor human cell line HEK-293 (n=22); and we have profiled the exometabolome using a discovery-based metabolomics approach by means of ultraperformance liquid chromatography coupled to quadrupole-time-of-flight mass spectrometry (UPLC-QTOF-MS). Metabolic features (Rt, *m/z* pairs) were analyzed using a cross-validated orthogonal projection to latent structures-discriminant analysis model, using a genetic algorithm variable selection method. A panel of 12 discriminant features with tentative chemical identification allowed differentiating the three cell lines with 100% specificity, sensitivity and accuracy. In addition, 5 of these compounds were present in 10 human serum samples from ccRCC patients and healthy controls. Discriminant metabolic features suggest alterations of the glutathione and phenylalanine metabolism, tryptophan degradation and the pentose phosphate pathway. Current work involves validation of the tentative identification of the compounds with chemical standards.

Keywords: untargeted metabolomics, conditioned media, statistical multivariate analysis, mass spectrometry

(1747) **GENDER DIFFERENCES IN RENAL RESPONSE TO HIGH SODIUM INTAKE IN ADULT NORMOTENSIVE WISTAR RATS**

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There is a gender difference in the regulation of renal function and blood pressure in humans and also in several experimental animal models. We studied the renal response to high sodium intake in male and female Wistar rats.

The experiments were performed in intact male (IM), female (IF) and Gonadectomized (GX) Wistar rats at 150 days of life. Half of them were Orchidectomized (ORX) or ovariectomized (OVX) at 60 days of life and supplemented or not with testosterone propionate (ORXTo), 17β estradiol 3-benzoate (OVXE2) or medroxyprogesterone acetate (OVXP4) at 138 days of life , using cholesterol (CHO) as vehicle. During the last 5 days of the study, half of rats received normal sodium (NS, NaCl 0.24%) or high sodium diet (HS, NaCl 1% in drinking water). Glomerular filtration rate (GFR), renal plasma flow (RPF), sodium excretion (mmol/d/g kw) and mean arterial pressure (MAP, mmHg) were measured. To analyze the response of renal function upon HS, mRNA (Atpa1a) expression by RT-PCR and dephosphorylated Na+,K+-ATPase (dNKA), dopamine-D1-receptor (D1R), cytochromeP450A (CYP4A), Na+,K+,2Cl- (NKCC2) and Na+/Cl- (NCC) cotransporters were determined in renal tissue by western blot.

Under HS, both IM and IF do not change MAP and IF excrete more sodium and showed lower medulla dNKA, Atpa1a, cortical NCC and NKCC2 than IM. OVX excrete less sodium and ORX excrete more sodium than their intact ones and both increase MAP (all $p<0.05$).

The increase of MAP in OVX is a consequence of a defective D1R-CYP4A pathway, which results in more active dNKA and to a higher expression of NKCC2 and NCC, whereas it could be due to changes in GFR and RPF in ORX. E2, but not P4 or To prevented the increase in MAP.

There is a sexual dimorphism in renal function and blood pressure. Gonadectomized rats behave as a model of salt sensitivity. Findings provide a better understanding of gender differences in renal function.

Keywords: renal Na+,K+-ATPase; gonadectomy; dopamine; sex hormones; salt-sensitive hypertension.

PHARMACOLOGY 4

(1725) **ALBENDAZOLE METABOLITES IN SERUM AND URINE: A FIRST STEP IN DEVELOPING AN INDICATOR OF TREATMENT COMPLIANCE IN MASS DRUG ADMINISTRATION PROGRAMS**

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Soil Transmitted Helminth (STH) infections directly impact nutritional status, educational development, individual productivity, and physical and mental development in human populations. Currently, these infections are controlled through mass drug administration (MDA) programs using albendazole (ABZ) or mebendazole. However, not all programs have demonstrated expected impact on prevalence or intensity of infections. These failures may be related to poor programmatic coverage, suboptimal adherence or the exposure of parasites to sub-therapeutic drug concentrations due to poor drug dissolution, insufficient gastrointestinal absorption and/or systemic availability of the active ingredient.

The aims of the present work were to characterize the serum disposition kinetics and pattern of urinary excretion of ABZ and its main metabolites, ABZ sulphoxide (ABZSO) and ABZ sulphone ($ABZSO_2$) in human volunteers, and to determine the optimal time point where ABZ and/or its metabolites can be measured in urine as an indirect assessment of an individual's adherence to treatment. Venous blood and urine samples were collected from eight (8) volunteers between 2 and 72 h for HPLC analysis of ABZ/metabolites, following administration of a single postprandial oral dose of ABZ (400 mg). The ABZSO metabolite was the main analyte recovered either in serum and urine samples from ABZ-treated human. ABZSO serum concentrations reached its peak concentration (Cmax= 1.20 **±** 0.44 µg/mL) at 4.75 h post-treatment. In urine, ABZSO Cmax value was 3.24 ± 1.51 **µg/mL,** reached at 6.50 h post ABZ administration. The urinary AUC value resulted higher (2.3 fold) compared to that measured in serum. The overall, PK-based information reported here demonstrates that the measurement of ABZSO concentrations both, in serum and urine, could be useful to confirm compliance to ABZ treatment and an objective measurement of program coverage.

(1766) **AZILSARTAN AND ITS Zn(II) COMPLEX. SYNTHESIS, ANTICANCER MECHANISMS OF ACTION AND INTERACTION WITH BOVINE SERUM ALBUMIN**

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Azilsartan is the eighth approved member of angiotensin II receptor blockers for hypertension treatment. Considering that some drugs have additional beneficial effects on health when administered, we studied its effects and mechanisms of action on a human lung cancer cell line A549. We have also modified the structure of the drug by complexation with Zn(II) cation and determined the anticancer effect. The crystal structure of the new binuclear Zn(II) complex, for short $[Zn_2(azil)_2(H_2O)_4].2H_2O$ (ZnAzil), was determined by X-ray diffraction methods. A549 and MRC-5 cell lines were used for the anticancer determinations. The results of the MTT assay showed that the complex behaved as good antiproliferative agent (IC_{50} = 155 μM ± 12, p<0.05) without exerting cytotoxic effects on MRC-5 cells. The complex generated reactive oxygen species (ROS) in the cells. This effect was accompanied by glutathione (GSH) depletion and a decrease in the GSH / GSSG ratio. The characteristic apoptotic morphology could be observed by the double staining acridine orange/ethidium bromide and the percentage of apoptotic A549 cells treated with the Zn complex resulted higher (46%, p<0.05) than those of control cells and cells treated with the biometal (10%, p<0.05) and azilsartan (3%, p<0.05). The evaluation was complemented by Western Blot of pro-apoptotic proteins BAX, antiapoptotic BCL-XL and Caspase-9 determinations. The complex showed increase in the Caspase-9 expression and the calculated BAX/BCL-XL ratio (p < 0.05). Additionally, we have evaluated the interactions of the compounds with bovine serum albumin (BSA). Measurements indicated that the complex and the ligand exhibited a strong binding to BSA. All these results suggested that the Zn complex induces apoptosis through ROS-dependent mitochondria pathway in lung carcinoma cells and be effectively transported in plasma. This compound behaved as a potential candidate for alternative cancer treatment.

Keywords: azilsartan, Zn complex, anticancer, BSA binding

(1120) **CHRONIC INTAKE OF *ERUCA VESICARIA* LEAVES EXTRACTS ALTERATES METABOLIC PROFILE AND INTESTINAL EFFLUX TRANSPORTERS ACTIVITY IN RATS**

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Increasing evidence implicates dietary factors in the progression of diseases, including cancer, diabetes and obesity. Diet also regulates ABC transporter expression and function impacting on drug elimination.

The goal of the present study was to evaluate the activity of intestinal ABC transporters and metabolic profile after chronic intake of *Eruca vesicaria* leaves (arugula commercial variety).

Adult male and female Sprague Dawley rats (20 weeks, n = 21) received an oral dose of *E. vesicaria* leaves juice representing human average intake (2g/kg), a methanol extract or saline solution three times a week during 4 weeks. Serosal to mucosal transport of substrates by MRP2, P-gp and BCRP was measured in everted sacs of proximal jejunum, distal jejunum and ileum, respectively. Glycaemia, triglycerides and cholesterol were determined.

Juice intake decreased MRP2-mediated efflux of doxorubicin in males (p<0,05) and females (p<0,001) and increased BCRP-dependent passage of nitrofurantoin selectively in male rats (p<0,01). In turn, methanol extract intake decreased MRP2 activity only in males (p<0,05); while in BCRP a dual behavior was found, increasing its activity in males (p <0.05) and decreasing it markedly in females (p <0.001). In turn, no changes in P-gp activity were observed under any of the treatments. Moreover, methanol extract increased glycaemia in males (p<0,05) and females (p<0,01) as well as triglycerides (p<0,05) in males. No metabolic changes were found after chronic intake of juice.

Present findings indicate that methanol or aqueous extract may enhance and/or suppress xenobiotics or drugs bioavailability and caution should be paid to the possible influence on metabolism. Despite the extracts natural origin, an overall evaluation of the balance between beneficial vs. adverse effects for each secondary active metabolite should be performed.

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Keywords: ABC efflux pumps, pharmacokinetics.

(152) **DERIVATIONS FROM PHARMACOKINETIC ANALYSIS OF A CLARITHROMYCIN EXTENDED RELEASE FORMULATION**

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A study on bioavailability of a new clarithromycin extended release formulation (provided by Química Montpellier SA) let some interesting pharmacokinetic data. A more detailed analysis of elimination kinetics showed 2 groups with important differences. The aim of this paper is to describe these findings. 24 both gender healthy volunteers (33% women, age 20-58 years) participated in this typical cross-design bioequivalence study. Each volunteer randomly received clarithromycin 1 g from different oral formulations in 2 separate rounds by 7 days and 11 blood - 5 urine samples were taken per round to determine the antibiotic profile. It were defined Cmax, tmax, AUCt, AUCinf, elimination half-life, mass of antibiotic recovered in urine during that period, and calculated total Vd(F), total Cl(F), renal and non-renal Cl. As both formulations were equivalent, all elimination data were analyzed by t-test. Population covariates were analyzed by chi-square test. Assuming elimination half-life 10 h as cutoff value, 65% of the volunteers (fast eliminators) had lower values; X = 6.94 ± 1.71 h, and the remaining 35% (slow eliminators) had significantly higher values; X = 16.57 ± 5.58 h (p <0.001). Total Cl(F), renal and non-renal Cl per group in L/h, expressed as X ± SD, were respectively: 27.34 ± 8.10 vs. 19.87 ± 4.82 (p <0.001); 7.51 ± 3.25 vs. 7.85 ± 3.25 (ns), and 19.83 ± 8.50 vs. 12.02 ± 5.05 (p <0.001). The aforementioned data were not correlated with age, gender, or body mass index. The results indicate that such differences in the elimination of clarithromycin could be due to non-renal Cl, and other covariates do not influence this behavior. These differences are not clearly reported previously. Subsequent studies should be performed to confirm or rule out these findings.

Keywords: clarithromycin, pharmacokinetics, non-renal clearance

(1228) **DETERMINATION OF THE PHARMACOKINETICS OF CEPHALEXIN 20% ADMINISTERED SUBCUTANEOUSLY IN LACTATING GOATS**

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The rational use of antimicrobial agents in goats productive systems may contribute to decrease economical lost. Cephalexin is a first generation cephalosporin, active against gram positive microorganism and gram negative bacillus. The sustained release cephalexin formulation (20 %) is used in veterinary medicine, prolonging the duration of the antibacterial activity (long-acting formulations). The aim of this study was to describe the pharmacokinetics of cephalexin 20% after subcutaneous administration in lactating goats. Six healthy lactating goats (weight average 35.52±4.50 kg) received a single dose of 20 mg/kg of a sustained release cephalexin (Cephalexin 20%, Laboratorio Ruminal, Buenos Aires, Argentina) by the subcutaneous route (s.c.) under a skin fold on the lateral of the thorax. Heparinized blood samples (2.5 ml) were collected via jugular venepuncture in preestablished times. Plasma samples were frozen at - 20° C until analysis. Plasma concentrations were determined by the microbiological method, using *Kocuria rizophila* ATCC 9341 as pattern microorganism. Standard curve was validated in plasma for linearity (r2: 0.988), accuracy (13.72%) and precision (3.72%) between 50 and 0.39 *µ*g/ml. The results were analyzed using Graph Pad Prism, Excel and Topfit. The quantification and detection limits of the method were 0.78 *µ*g/ml and 0.39 *µ*g/ml, respectively. Pharmacokinetic parameters were: Cmax: 20.40± 3.24 *µ*g/ml; Tmax: 1.20 ± 0.54 h; t½: 1.29± 0.48 h and TMRinf: 2.44± 0.82 h. The dosage and route of administration are the recommended by the manufacturer for cattle. Cephalexin is a time dependent antibiotic and the pharmacokinetic profile in goats for the sustained released formulation showed that the administration every 12 hours by the s.c. route would be appropiated for the treatment of micro-organisms with MIC £ 1 microgram/ml.

Key words: pharmacokinetics, Cephalexin, goats, lactation

(715) **EFFECT OF FLUNIXIN ON INTRAMUSCULAR ENROFLOXACIN KINETICS IN CALVES**

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Enrofloxacin (EFX) belongs to fluoroquinolone family, targeting gram-negative and some gram-positive bacteria as well as *Mycoplasma* spp. Flunixin (FL) is a nonsteroidal anti-inflammatory drug (NSAID) inhibiting cycloxygenase enzymes in the arachidonic acid cascade, blocking the formation of inflammatory mediators. NSAIDs are frequently coadministered to enhance the rate of recovery in combination with antimicrobial drugs in calves suffering from endotoximia, pneumonia and other viral and bacterial respiratory diseases. However, it has been reported that the distribution and elimination of antimicrobials are altered when they are coadministered with NSAIDs.This work was performed to characterize EFX disposition and establish the influence of the coadministration of FL on kinetic parameters. Lactating calves (N= 6) received 5 mg of intramuscular EFX, a week later 5 mg / kg of EFX and 2.2 mg of flunixin meglumine intramuscularly. At different times for 24 hours blood samples were obtained, centrifuged and stored at -20 °C. The preparative test consisted of extraction of the analyte with 200 µL of plasma, 200 µL of water, 750 µL of methanol: water: acid: phosphoric acid (500: 500: 10: 1 v / v / v / v) and 50 µL of 25% trichloroacetic acid. The whole was stirred 30" in vortex and centrifuged 30 minutes at 13500 rpm at 4 °C. Separation and quantification were performed by reverse phase isocratic elution with C-18 column and reading in fluorescence detector at λex 295 nm y λem 490, mobile phase composed of water, acetonitrile and triethylamine (79: 20: 1 v / v / v) at pH 3. Plasma concentration data were analyzed by PK Solution 2.0 software to calculate robust kinetic parameters. Results indicates rapid absorption and distribution of EFX and moderate permanence in the organism. Statistical non-parametric Mann-Whitney test was used. It can be verified that the coadminstration of FL did not significantly modify (P <0.05), the kinetic parameters of marbofloxacin.

Key words: flunixin, marbofloxacin, pharmacokinetics, calves

(251) **HUMAN PLASMA PROTEIN BINDING OF NOVEL ZIDOVUDINE PRODRUGS: TARGETING SITE 2 OF HUMAN SERUM ALBUMIN**

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The binding of anti HIV drugs to plasma proteins is key to their antiviral efficacy. Human serum albumin (HSA) constitutes the main plasma protein that binds and transport drugs, with several binding sites been reported. In this work, we explore the binding of the zidovudine (AZT) and AZT prodrugs obtained by combination with organic diacids (succinic (Suc), glutaric (Glu) and adipic (Adi) acid) and a methylated phenylalanine moiety. The binding of AZT-Suc-Ph (**1**), AZT-Glu-Ph (**2**) and AZT-Adi-Ph (**3**) to plasma proteins was studied experimental and molecular modeling techniques.

The binding of AZT and **1-3** was measured by incubating solutions of the prodrugs (9*µ*M, 37°C) with human plasma (HP), isolating the free fraction by ultrafiltration. The binding site to HSA was determined using salicylic acid (SA) and diazepam (DZP) as site 1 and 2 markers, respectively. The prodrugs exhibited a significantly increased binding to HP (**1**: 52.1±5.3%, **2**: 59.7±4.3% and **3**: 72.5± 4.3%) compared to AZT (13.8±1.4%), with their bound fraction being slightly displaced by DZP, indicating a high affinity for site 2 in HSA. Molecular docking and free energy decomposition analyses were applied to study the interaction of DZP and **1**-**3**. While DZP maintained the interaction pattern observed in the crystallographic structure, with a binding driven mainly by hydrophobic interactions, prodrugs **1**-**3** positioned the aromatic ring within a highly buried hydrophobic subpocket of site 2, establishing a higher number of interactions. The calculated interaction energies were: -12.26 kcal/mol (DZP), -13.22 kcal/mol (**1**), -14.13 kcal/mol (**2**) and -19.26 kcal/mol (**3**), evidencing that the prodrugs exhibited a more stable interaction with HSA than DZP. A correlation between the bound fractions and the interactions energies was found. In conclusion, the novel prodrugs exhibited a higher affinity for HSA than AZT and a change in

their binding site, which may in turn result in different pharmakinetic properties.

Keywords: Zidovudine, prodrugs, HSA, binding, molecular modeling.

(749) **INTRAMUSCULAR PHARMACOKINETICS OF MARBOFLOXACIN 10% IN WEANING CALVES**

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Marbofloxacin (MBX) is a fluoroquinolone applied only in domestic animals. It exhibits favorable kinetic properties and excellent activity against gram-negative microorganisms and mycoplasmas. This study was to determine kinetic parameters after the single application of MBX (Marbocyl 10%, Vetoquinol, Spain) and to evaluate its therapeutic usefulness. Twelve healthy calves of 29 ± 5 days of age, 46.4 ± 10.5 kg of body weight, clinically healthy, received a single 6 mg / kg dose of marbofloxacin intramuscularly. At different times up to 24 hours post-application blood samples were taken in heparinized tubes, centrifuged and stored until analyzed by HPLC. The preparative test consisted in the extraction of the analyte using 200 μL of sample, 200 μL of water, 800 μL of methanol and enrofloxacin as an internal standard. The assembly was stirred for 30" in vortex and then centrifuged 25 minutes at 13.500 rpm at 4 ° C. Separation and quantification was performed by isocratic elution in reverse phase using C-18 column and fluorescence detector reading set at 295 nm excitation and 490 nm emission, mobile phase composed of water, acetonitrile and triethylamine (79: 19: 1 v / v / v) at pH 3. Plasma concentration data were analyzed by PK Solution 2.0 software to calculate robust kinetic parameters. Intramuscular administration determines prompt absorption, Cmax of 5.0 ± 1.5 µg / ml obtained at 1.7 ± 1.01 hours. MBX exhibits moderate tissue distribution, extensive permanence in the organism according to the parameters of Vd_{area} and TMR. The results obtained agree with the kinetic profile demonstrated by MBX in domestic animals and suggests its application in infectious diseases caused by germs with MIC ≤ 0.13 µg / ml, according to predictors of efficacy used for fluoroquinolones, ABC / CMI and Cmax / CMI.

Key words: pharmacokinetics, marbofloxacin, calves

(892) **IDENTIFICATION OF BENZNIDAZOLE METABOLITES IN URINE FROM PATIENTS WITH CHAGAS DISEASE**

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Chagas disease is a major public health problem in Latin America. Benznidazole is an effective treatment but has several side effects.

Information about benznidazole metabolism it is scarce although there is limited evidence that conjugation with glucuronic acid by glucuronosyltransferase (i.e. phase II reaction) is involved, but it is not clear if benznidazole is directly conjugated or an intermediate phase I metabolic step takes place.

The objective of this study was to characterize benznidazole metabolites in human 24 hours urine samples from patients with Chagas disease receiving benznidazole treatment.

Samples from 3 adult female patients in the chronic stage of Chagas disease, treated with benznidazole 7 mg/kg/day for 30 days were obtained near the end of treatment. Urine from 3 healthy female adult were used as controls.

Samples were analyzed using ultra-high performance liquid chromatography coupled to a triple quadrupole mass spectrometer (UHPLC-ESI-QqQ) in Q_1-Full Mass Scan, Neutral Loss (NL) and Multiple Reaction Monitoring (MRM) mode.

Initially samples were studied using Q_1 -Full Mass Scan mode and NL mode to identify mass loss of 176 units corresponding to glucuronic acid loss after fragmentation. Once relevant peaks were identified by comparison to control urine MRM mode was developed using their transitions to confirm results.

Benznidazole glucuronides were not observed, but two different benznidazole metabolites possibly from phase I reactions were observed: aminobenznidazole (m/z 231) and hydroxyaminobenznidazole (m/z 247). Both metabolites were found in free form and conjugated with glucuronic acid (m/z 407 and m/z 423).

Identification of these metabolites opens the door for future studies to clarify the metabolism of benznidazole in patients and its potential consequences including drug interactions.

Keywords: benznidazole, metabolites, mass spectrometry.

(587) **R-SHINY APPLICATIONS FOR PK-PD: CLARITHROMYCIN PK AS AN EXAMPLE**

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Population PK-PD models play an important rol in drug research and development. The nonlinear mixed effects model is the most used. R is an open-source programming and data analysis language very useful to predict data behavior, a task that invariably requires simulation. R has several advantages over commercial software such as SAS. However, R shows pitfalls when presenting results, such as the static nature of the output. Thus, interactive elements, as Shiny, have recently been added to R libraries in order to improve the user interface.

This work shows a population PK simulation for clarithromycin in an extended release formulation using R and Shiny. For the kelim and total Cl (F) elimination data from 24 volunteers -0.084 ± 0.041 h^{-1} and 25.0 ± 8.4 L/h provided by a bioavailability study; R simulated using 1000 volunteers a population value of -0.073 and 22.66 respectively.

Using the appropriate model, R-Shiny can simulate and display the interesting changes in covariates or in model parameters quickly, through friendly and intuitive widgets. To construct the Shiny interface, medium-level programming skills are required, but simulating a given situation with Shiny (if it had properly programmed ad hoc) is accessible to all biomedical or clinical researcher.

Keywords: Population pharmacokinetics, R-Shiny program language, nonlinear mixed effects model

INFECTOLOGY 3

(1078) **SPERMATOZOA CAPTURE AND EFFICIENTLY TRANSMIT ZIKA VIRUS TO EPITHELIAL AND DENDRITIC CELLS.**

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INBIRS

Zika virus (ZIKV) infection was associated to new born microcephaly and to Guillain-Barre syndrome in adults. Surprisingly, it has been reported sexual transmission of ZIKV, changing our view of arbovirus-host interaction. Infectious ZIKV persist in semen after symptoms onset and at higher viral loads than those found in plasma or urine. Knowing that semen is not merely a carrier for sexually transmitted diseases, we developed experiments to evaluate the role of spermatozoa (sp) in ZIKV transmission.

Sp were obtained from healthy donors by swim up purification. Dendritic cells (DC) were obtained from monocytes (GM-CSF/IL-4 5 days); phenotype was analyzed by flow cytometry and cytokines production by ELISA. ZIKV capture and infection was evaluated by qPCR.

Sp (10, 5, 1, 0.5 $x10^6$) were incubated with ZIKV ($5x10^5$ ufp) 90 min, then cells were washed twice to remove free virus. We observed that sp captured ZIKV (n=3 $p<0.05$) and trypsin pretreated sp (15 min 37°C) showed no ZIKV attached (n=3 $p<0.05$), suggesting a protein mediated interaction. To evaluate if sp was able to transmit ZIKV, sp charged with ZIKV were cultured with epithelial cells monolayers or DC ($2x10^5$/200ul) for 72hs. We observed that sp+ZIKV were able to transmit the virus to cells (n=3 $p<0.001$). Even more, when the same quantity of ZIKV was offer to cells without sp, we found the levels of infection were lower (n=3 $p<0.005$), so sp facilitate the infection.

Lastly we analyzed the phenotype and functionality exerted by ZIKV to DC. DC ($2x10^5$/200ul) were infected with ZIKV ($5x10^5$ or $5x10^4$ ufp), 72hs later we found: an increase of CD86, CD83, CD80 and CD40 expression (n=2 $p<0.05$), an enhanced production of IL-12 and IL-1β (n=2 $p<0.05$), and decreased IL-10 (n=2 $p<0.05$). We also observed a higher ability to induce IFN-γ and TNF-α production by alloreactive lymphocytes (n=2 $p<0.05$).

Our observations support the notion that sp facilitate and might affect the early course of sexual transmission of ZIKV infection.

Key Words: Zika Virus, Dendritic Cells, Spermatozoa, Sexual Transmission

(1097) **ROLE OF *BORDETELLA PERTUSSIS* ADENYLATE CYCLASE TOXIN IN OUTER MEMBRANE VESICLES-MEDIATED IMMUNOMODULATION OF MACROPHAGES RESPONSE**

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Gram-negative bacteria produce and excrete outer membrane vesicles (OMVs) that play a role in the infection process by carrying and transmitting virulence factors into host cells, eventually modulating host defense response. *Bordetella pertussis* (Bp), the etiological agent of whopping cough, is a Gram-negative bacterium that can survive and persist inside human macrophages (MΦ). We previously showed that adenylate cyclase toxin (CyaA) is one of the factors that are involved in modulation of inflammatory host response and intracellular bacterial survival. We recently observed that OMVs released from Bp (Bp-OMVs) induce a decrease in MΦ bacterial uptake. However, once inside the cell, Bp persistence is higher in MΦ treated with Bp-OMVs, suggesting that these vesicles might mediate bacterial host defense manipulation. In the present study we evaluated the potential role of CyaA in this immunomodulatory effect of Bp-OMVs and the related increase of intracellular bacterial persistence. To this end THP-1 MΦ were treated with OMVs isolated from a Bp CyaA deficient mutant (BpΔCyaA-OMVs) or wild type strain (Bp-OMVs) and mRNA was recovered 4 h post-treatment. RT-PCR assays showed that inflammatory cytokine expression, such as IL-8 and TNF-α, was significantly higher in BpΔCyaA-OMVs treated MΦ ($p<0.05$). Accordingly, confocal microscopy studies showed that both bacterial uptake and bacterial colocalization with late endosomal/lysosomal marker LAMP-1 were significantly higher in MΦ incubated with OMVs-BpΔCyaA. In agreement with these results, we observed that the presence of CyaA in the OMVs determined a higher intracellular bacterial persistence over the time of infection. Altogether these results suggest that CyaA plays a key role in Bp host cell response modulation by OMVs and its eventual promotion of intracellular persistence.

Keywords: CyaA, OMVs, macrophages, intracellular survival, *Bordetella Pertussis.*

(1115) **HFQ IS REQUIRED FOR *Bordetella pertussis* ADAPTATION TO THE IRON STARVED HOST ENVIRONMENT**

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Bacterial regulation of gene expression based on small noncoding RNA (sRNA) is usually involved in the response to environmental stress, such as iron starvation. Most of Gram-negative bacteria sRNA-mediated regulation requires the cofactor RNA-binding protein Hfq. It was shown that *Bordetella pertussis* (Bp) is iron starved inside its human host in which is able to survive and eventually persist inside macrophages (MΦ). As strategy to study the infective phenotype, we previously characterized Bp iron-starved proteome and showed that this environmental condition induces a remarkable shift in protein composition. Importantly, we observed that this phenotypic adaptation influences bacterial interaction with immune and epithelial cells facilitating the infectious process. In the present study, we examined the requirement for Hfq in the adaptation of Bp to iron starvation in order to evaluate its relevance in bacterial pathogenesis. By mean of shotgun proteomics we compare the iron-starved proteome of a Bp Hfq deficient mutant (BpΔHfq) with the iron-starved proteome of the wild type strain and found that 375 (out of 1325) proteins required Hfq to respond to low iron availability. Among them, we identified proteins involved in LPS and peptidoglycan biosynthesis, metabolism and transport, suggesting that Hfq plays a role in the regulation of many different pathways during Bp adaptation to iron starvation. Importantly, we observed that under this environmental condition Hfq plays a role in the regulation of the expression of several proteins critical during infection, such as iron acquisition systems, proteins involved in oxidative stress resistance and virulence factors. Accordingly, intracellular survival of BpΔHfq inside THP-1 MΦ was significantly lower than wild type bacteria ($p<0.05$), suggesting that this protein not only is important in the regulation of Bp factors involved in host colonization but also is relevant in intracellular persistence inside host cells.

Keywords: B. pertussis, Hfq, proteome, iron starvation, intracellular survival

(1141) **NOVEL GUANIDINE COMPOUND AGAINST MULTIDRUG-RESISTANT CYSTIC FIBROSIS-ASSOCIATED BACTERIAL SPECIES**

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Chronic pulmonary infection is a hallmark of lung disease in cystic fibrosis (CF). Infections dominated by non-fermentative Gram-negative bacilli are particularly difficult to treat and highlight an urgent need for develop new class of agents to treat these infections. In this work, a small library comprising 13 novel thiourea and guanidine derivatives with low molecular weight were synthetized and studied as antimicrobial agents. The identity of the products was confirmed by FTIR, ^{1}H-NMR, ^{13}C-NMR spectra and elemental analysis. All obtained compounds were tested *in vitro* for their minimum inhibitory concentration (MIC) and their minimum bactericidal concentration (MBC) against two standard Gram negative non-fermentative bacilli species, *Pseudomamonas aeruginosa* PAO1 and *Burkholderia cenocepacia* J2315, and a panel of drug-resistant non-fermentative Gram-negative bacilli recovered from patients with CF (n=37). One novel compound, a guanidine derivative bearing adamantane-1-carbonyl and 2-bromo-4,6-difluouro-phenyl substituents (H-BDF), showed a MIC value less than 2 μg/ml against the standard strains and superior activity than the standard drugs tobramycin, ceftazimide and meropenem ($p<0.05$). Moreover, the 68.4% of clinical isolates had MICs values lower than 4 μg/ml with compound **H-BDF** whereas only 36.8%, 26.3% and 0% of isolates had MICs values lower than 4 μg/ml with meropenem, ceftazimide and tobramycin, respectively. The role that different substituents exert in the antimicrobial activity was determined, highlighting the importance of the halo-phenyl group in the guanidine moiety. Moreover, the new compound H-BDF displays low levels of cytotoxicity against THP-I cells with a selective index (SI) > 8, pointing to its potential for further development as a novel antibacterial drug.

Keywords: Antibiotics, guanidines, thioureas, resistance, cystic fibrosis

(1204) **PRESENCE OF CATHELICIDIN LL37 AND IMMUNOENDOCRINE-RELATED COMPOUNDS IN THP1-DERIVED MACROPHAGES INFECTED WITH *MYCOBACTERIUM TUBERCULOSIS* EXPOSED TO CORTISOL AND/OR DHEA**

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Macrophages (Mps) were found to be influenced in their anti-mycobacterial response since treatment with dehydroepiandrosterone (DHEA) of Mps infected with *Mycobacterium tuberculosis* (Mtb) led to a reduction in intracellular bacterial load and increased autophagy. On the other hand, the Mp effector response in the tuberculosis infection is highly related to the production of antimicrobial peptides such as cathelicidin LL37, whose production was found increased during autophagy, as well.

Given this background, THP-1-derived Mps were infected with Mtb strain H37Rv (MOI 5:1) during 3 h in presence or absence of cortisol (Gc, 1 and 0.1μM) and/or DHEA (1; 0.1 and 0.01μM). Upon washing, cells were cultured for 24 h in the same media with or without hormones and the expression of LL37, glucocorticoid receptors -alpha and beta- (GRα and GRβ), enzymes regulating cortisol availability (11-beta-hydroxysteroid dehydrogenase types 1 -11βHSD1- and 2), the pro-apoptotic protein caspase 3, the inflammasome-related protein 3 (NLRP3) were analyzed by qRT-PCR, and IL1β levels by ELISA.

NLRP3 and caspase 3 showed an increase in all infected cultures ($p<0.05$). –In the same sense, Mtb increased IL-1β production ($p<0.01$) and LL37 expression ($p<0.05$) respect the uninfected cultures. Treated cells with Mtb plus DHEA displayed an increase in IL1β levels ($p<0.01$) and GRα expression ($p<0.05$) compared with those only infected. Irrespective of DHEA presence, Mtb-infected cultures exposed to Gc (1μM) revealed a decreased expression of LL37 and 11βHSD1 respect the Gc-untreated counterparts ($p<0.05$). Unlike this, infected cultures subjected to combined treatment with Gc (at a lower concentration) plus DHEA showed and augmented IL-1β production ($p<0.01$) and a slightly increased LL37 expression, as compared to single Mtb-infected cells. The beneficial effect of DHEA on Mp function, which seems to be also related to LL37 production, continues to be present at low Gc doses.

(1272) **PLASMA EXPRESSION LEVEL OF miRNA IS POSITIVELY-ASSOCIATED WITH CHAGAS CARDIOMYOPATHY IN PATIENTS WITH CHAGAS DISEASE**

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The biomarker value of circulating microRNAs (miRNAs) present in the bloodstream has been extensively studied since the discovery of these small non-coding RNA molecules that regulate gene expression. MicroRNA dysregulation has been linked to cancer development, cardiovascular and neurological diseases, lipid metabolism, and impaired immunity. Our study was designed to evaluate miRNA expression levels in individuals chronically infected with *Trypanosoma cruzi* showing different degrees of cardiac damage by Next Generation Sequencing Technologies (small-RNA-seq). Chronic chagasic patients with different degrees of cardiac involvement were classified as: Indeterminate (IND, n=5); Moderate (M, n=5) and Severe (S, n=5). Parallel sex and age-matched healthy volunteers (Co, n=5). miRNAs were extracted from human plasma using standard protocols and sequenced by Illumina MiSeq system. Finally, sequencing results were processed and analyzed by open software and databases such as Bowtie, R, samtools, ensembl. org and mirbase.org. These analyses revealed high abundance of miRNA expression in chromosome 7 in S vs Co (fold change), in agreement with previous results indicating a positive correlation between this chromosome and heart diseases. miRNASeq also showed differential expression of mature miRNAs in IND, M and S compared to Co. On the other hand, most of the smallRNA found among samples correspond to y-RNA, which has been involved in chromosomal DNA replication and cellular responses to stress, making of them new potential diagnostic biomarkers. miRNAs differentially expressed associated to heart failure (S) correspond to miRNA let-7f-5p, miRNA 125a-5p and miRNA 142-3p. Our results show an association between the presence of specific miRNA and the severity of chronic chagasic cardiomyopathy. New studies are needed for their use of follow-up and prognosis markers of the evolution of this disease.

Keywords: Chagas disease; miRNAs; Next Generation Sequencing Technologies

(1317) **NOVEL IRON REGULATED ANTIGEN PROTECTS AGAINST BOTH CAUSATIVE AGENTS OF WHOOPING COUGH**

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Whooping cough is a highly contagious infectious disease caused by Bordetella pertussis (Bp) and Bordetella parapertussis (Bpp).According to previous studies current acellular pertussis vaccine induces not only low protection against Bp. but also no protection against Bpp. New protective antigens are needed to improve the efficacy of this vaccine against both species. Through the study of proteins induced under physiological conditions, such as iron starvation-our group has identified and cloned two novel antigens protective against B. pertussis. Interestingly, the sequence of these antigens are highly conserved among both previously mentioned Bordetella species, and even more than the sequence of pertactin and FhaB, antigens included in acellular vaccines. In the present work, we studied one of this antigens (Bp1605, AfuA) as a protective immunogen against Bpp. Using western blot and ELISA tests we confirmed that Bordetella parapertussis expresses a homolog of AfuA and that, as found for B. pertussis, it is an iron regulated, surface exposed protein, expressed during infection. This study also showed that antibodies induced by immunization with the recombinant AfuA cloned from Bp (rAfuA) were able to opsonize B. parapertussis and promote bacterial uptake by neutrophils. The inefficacy of current acellular vaccines against Bpp has been attributed to the effect of the O-antigen, absent in Bp. The O-antigenhas been described as a shield that protects Bpp from antibody recognition on the bacterial surface. We here found that the O-antigen has no influence on the recognition of the antibodies raised against AfuA. Accordingly, we found that rAfuA confers protection against B. parapertussis infection in a mouse intranasal challenge model. Taken together, these results point at AfuA as an excellent vaccine candidate for a new generation of acellular vaccines protective against both whooping cough causative agents.

Keywords: Whooping cough, Bordetella parapertussis, Vaccine.

(1385) **ROLE OF PPARγ IN THE GLUCOCORTICOID-MEDIATED EFFECTS ON A MACROPHAGIC CELL LINE STIMULATED WITH *Mycobacterium tuberculosis***

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Tuberculosis (TB) is a major health problem characterized by an immune-endocrine imbalance: high plasma levels of cortisol and pro- and anti-inflammatory mediators (IFN-γ, IL-6, TGF-β and IL-10), with low levels of Dehydroepiandrosterone (DHEA). The etiologic agent, *Mycobacterium tuberculosis* (Mtb) is transmitted by air and captured by lung macrophages (MØ). MØ activation along with an efficient cellular immune response is required for Mtb elimination, which at the same time can mediate tissue damage. Glucocorticoids (GCs) are critical elements to counterbalance the immune-inflammatory reaction, with peroxisome proliferator-activated receptors

(PPAR) being also implicated in this regard. The main forms of these receptors are: PPARα, PPARβδ and PPARγ. TB patients showed an increased expression of the PPARγ transcript in their peripheral blood mononuclear cells, which were positively associated with disease severity of the disease and cortisol plasma levels. Given this background, we now investigated whether PPARγ contributes to the inhibitory effect of GC on inflammatory MØs response (adherent human THP-1 cells) to Mtb (strain H37Rv killed by γ radiation -Mtbi-). Stimulated MØs (n=6-8 cultures) showed increased expression of mRNA PPARγ and IL-1 levels respect the unstimulated counterparts ($p<0.05$ and $p<0.001$ respectively). Cultures exposed to Mtbi+cortisol (10^{-6}M) showed decreased levels of IL-1 compared to those only exposed to Mtbi ($p<0.001$). In addition, cells with Mtbi+cortisol plus a PPARγ specific agonist [15-deoxy-delta(12,14)-prostaglandin J2, 2μM] showed lower levels of IL-1 than those without the agonist ($p<0.01$), along with an increased expression of PPARγ transcripts ($p<0.05$). The relation between PPAR*γ* transcripts and cortisol plasma levels in TB patients together with the *in vitro* results, suggest a contribution of this receptor in the anti-inflammatory effect of GC.

(1394) **PENDRIN EXPRESSION IS AFFECTED IN EPITHELIAL AND MACROPHAGES CELLS BY *B. pertussis* INFECTION.**

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Pendrin is a transmembrane anion exchanger (encode by *SLC26A4* gene) whose expression was first described in epithelial cells and later suggested in other cells of the immune system. In the airway, pendrin mediates the uptake of Cl^- from the airway apical surface and the export of both $HCO3^-$ and thiocyanate into the airway surface liquid. The bicarbonate exported by pendrin produces an optimal pH environment for inflammatory mediators activity and favors the secretion of proteins involved in antimicrobial mechanisms in the lung. A transcriptomic study recently demonstrated the up-regulation of pendrin in mouse lung during B. pertussis (Bp) infection in a pertussis toxin-dependent (PTx) manner. Immunohistochemistry of Bp infected mice revealed pendrin expression in the lungs epithelia and possibly in macrophages. In order to gain a deeper insight into pendrin influence in Bp host infection we here examined the expression of pendrin in human bronchial epithelial cell (HBE) and human monocyte cell line (THP-1) during the Bp infection. qRT-PCR results showed that *SLC26A4* expression in HBE cells is up-regulated soon after Bpwt infection. Ptx was found involved in host cell response since the infection with a PTx deficient mutant (BpΔPtx) induced significantly lower pendrin up-regulation. On the other hand, THP1 Bp intracellular infection assays also showed a significant up-regulation of *SLC26A4* early after Bpwt infection. Again, a lower up-regulation was observed in BpΔPtx infection. As the intracellular infection progressed, however, *SLC26A4* level was found down-regulated in macrophages infected with either Bp strain as compared with uninfected cells showing that this pathogen is able to modulate this response in a PTx independent manner. Altogether these results suggest that pendrin might contribute to host defense response during Bp infection but also show that Bp is able to manipulate its expression in macrophage during the development of intracellular infections.

Keywords: Pendrin, Bordetella pertussis, epithelial cells, THP-1 cells

(1397) **ANTIBODIES AGAINST FIMBRIAL SUBUNITS INCLUDED IN ACELLULAR VACCINES DO NOT HAVE BIOLOGICAL ACTIVITY AGAINST *Bordetella parapertussis***

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Resurgence of whopping cough is partially due to an increase in the incidence of *B. parapertussis* (Bpp) infections whose start concurs with the replacement of whole cell vaccines by acellular vaccines (AV). AV are formulated with *B. pertussis* (Bp) antigens some of which are homologous to those produced by Bpp. Previous studies, however, showed that most antibodies induced by AV fail to protect against Bpp due to the presence of the O antigen (AgO) on the Bpp surface that impairs antibody access to shared antigens. However, controversial results have been reported regarding the role of AV induced anti-fimbria antibodies against Bpp. Fimbriae are proteins exposed on the surface of Bpp and due to its length it is very unlikely shielded by the AgO. Antibodies against these antigens have shown to be protective against Bp but little is known about the cross protection induced by Bp fimbrial subunits 2 and 3 (Fim2/3) against Bpp. In this study we examined the potential of Fim2/3 as vaccine antigens against Bpp infections. To this end, the biological activity of antibodies induced by a vaccine formulated by Bp Fim2/3 was evaluated against Bpp. Whole cell ELISA showed that anti-Fim2/3 antibodies poorly recognize Bpp. Functional assays showed that these antibodies do not agglutinate Bpp and fail to inhibit Bpp attachment to epithelial cells. The AgO was found not involved in the absence of antibody recognition, agglutination activity or inhibition of Bpp attachment to epithelial cells, as determined by the use of an AgO mutant. Proteomic studies showed that Bpp do not express Fim2/3 and bioinformatics analysis suggested that the lack of biological activity of anti-Fim2/3 antibodies against Bpp might be due to a low degree of homology with Bpp fimbriae. Together with previous results this study demonstrates that none of the components of current acellular vaccines protect against Bpp stressing the need for a new generation of preventive strategies to control whooping cough.

Keywords: Bordetella parapertussis, acellular vaccine, fimbriae, fimbrial subunits, protection.

TOXICOLOGY 1

(102) **BISPHENOL S (BPS) INDUCES STRUCTURAL AND MOLECULAR ALTERATIONS IN UTERUS AND OVARIES IN A RAT MODEL OF PCOS**

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Bisphenol A or (BPA) is the most common component of polycarbonates and it has toxic effects in the reproductive system. In order to reduce BPA exposure, the use of alternative compounds such as *Bisphenol S (BPS)* has been proposed. Unfortunately, BPS also displayed estrogenic, antiestrogenic, androgenic and antiandrogenic actions. Therefore, there is a growing concern about the biological effects of BPS in terms of toxicity, genotoxicity, endocrine disruption and neurobiological levels**.**

Objectives: To study the effect of BPS in the uterus and its role in ovarian cysts development in a known rat model of PCOS.

Materials and Methods: Immature Wistar rats (21-22 days old) were injected with vehicle, dehydroepiandrosterone or DHEA (60 mg/kg), DHEA+BPS and BPS alone (1 ug/kg) for 20 days. Tissues were stained with hematoxylin-eosin (H&E) and immunohistochemistry. Statistical differences were evaluated by the Student T-Test.

Results: BPS induces alterations in uterus such as an increase in tissue elasticity and vacuolization of endometrial cells. The administration of BPS in DHEA treated rats, increased significantly the percentage of vacuolization of the endometrial layer (DHEA/BPS 68.77%±17.87 compared to DHEA: 40.29%±19.06, $p<0.001$, n=10 and 9). At the molecular level, we found changes in the expression pattern of the protein Ezrin which is has been involved in cancer progression and metastasis. DHEA-BPS administration increased the citoplasmatic accumulation of EZRIN in the epithelium of endometrial glands compared with DHEA treated animals (97.88%±18.32 vs. 41.38%±5.62, $p<0.001$, n=10 and 10). In ovaries, BPS administration altered the number of structures such as cysts, precysts, and type III follicles induced by DHEA alone, $p<0.001$).

Conclusion: In summary, our work would show that low doses of BPS induce morphological and molecular changes in the uterus and ovaries of rats bearing a PCOS condition.

Keywords: PCOS, BISPHENOL S, UTERUS, OVARIES, EZRIN

(176) **CHANGES IN THE OOCYTE INTEGRITY AND BONE MARROW INDUCED BY 3-METHYLCHOLANTHRENE AND PREVENTED BY ALPHA-NAPHTHOFLAVONE**

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3-Methylcholanthrene (3MC), a polyaromatic hydrocarbon, is an environmental pollutant that causes reproductive toxicity and genotoxicity. Previous studies showed that 3MC alters the ovarian function by affecting the follicle integrity in rodents, and causes DNA damage in peripheral blood, bone marrow and oocytes. Now, we studied the effect of daily exposure to 3MC on the oocyte integrity, induction of chromosome aberrations in bone marrow and the ability of alpha-naphthoflavone (**αNF**) to prevent this action. To this end, immature rats were daily injected with a combination of 3MC (0.1 and 1 mg/kg) and αNF (80 mg/kg) for 20 days. Oocyte integrity by morphological parameters, expressed in **µ**m; oocyte viability by fluorescence dyes; and chromosome aberrations (CA) in bone marrow cells were evaluated. All data are expressed as mean±SEM and all statistical analysis were performed by two-way ANOVA with Bonferroni post-test. Levene's test and a modified Shapiro-Wilk test were used to assess homogeneity of variances and normal distribution, respectively. Compared with controls (C), both doses of 3MC increased the area (C 12.42±0.18; 3MC 14.51±0.64; P<0.001), perimeter (C 77.20±0.89; 3MC 96±2; P<0.001): and perivitelline space of oocytes (C 1.61±0.06; 3MC 2.29±0.09; P<0.001) and decreased both the thickness of the zona pellucida (C 1.69±0.06; 3MC 1.13±0.04; P<0.001) and the number of viable oocytes (C 86%±2; 3MC 60%±3; P<0.001). Likewise, 3MC-treated rats, at both doses, exhibited a higher number of CA (C 7%±2; 3MC 19%**±4; P<0.001)**, especially in the number of metaphases with dicentric chromosomes (C 2.40%±0.52; 3MC 8%±2; P<0.001) and fragments of chromosomes (C 0.97%±0.45; 3MC 3.59%±0.38; P<0.001). All these changes were prevented by daily treatment with αNF. In conclusion: i) daily exposure of 3MC alters the oocyte integrity; ii) low doses of 3MC are enough to induce chromosome aberrations in bone marrow; and iii) αNF prevents both the systemic and oocyte toxic effect of 3MC.

Key words: 3-methylcholanthrene, alpha-naphthoflavone, oocyte, bone marrow.

(1297) **TI(I) AND TI(III) PREVENT PC12ADH CELL PROLIFERATION**

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Thallium (Tl) induces apoptosis in PC12 cells. However, in a PC12 cell adherent variant (PC12adh), this effect was not observed, although a potential cytotoxic effect of Tl on this cell line could not be discarded.

MTT reduction, evaluated in PC12adh cells incubated in the presence of 10-100 μM Tl(I) or Tl(III) (24-72 h), was significantly decreased after 24 h of cell exposure. Tl did not cause cell necrosis, as evaluated from LDH release or nuclear staining with acridine orange and ethidium bromide. To evaluate if Tl could alter PC12adh cell proliferation, the number of cells was estimated by Trypan blue staining. In control samples, the number of cells increased linearly with time. In contrast, in 100 μM Tl-treated samples the amounts of cells remained constant after 48 h of exposure, and were markedly lower than those of the controls (two-way ANOVA). By using specific inhibitors, we observed that Tl did not affect ERK-, mTOR-, PLC-, PKC-, and p53-dependent proliferative pathways. In contrast, inhibition of PI3K enhanced Tl-dependent prevention of cell proliferation, suggesting that this pathway could partially counteract the effects of Tl. Cell cycle analysis by flow cytometry indicated that both Tl(I) and Tl(III) caused partial arrest of the cycle in G2/M phase, without affecting S phase. Accordingly, the amounts of cells in G0/G1 phase decreased with the time of exposure to Tl. Cell volume (forward scatter) and internal complexity (side scatter) also increased with time, in accordance with the appearance of intracellular vacuoles. Tl did not cause cell senescence, indicated by the lack of activation of senescence-associated β-galactosidase. Together, present results suggest that both Tl(I) and Tl(III) prevent PC12adh cell proliferation, and the signaling pathways affected by these cations remain to be elucidated.

Keywords: thallium, cell proliferation, cytotoxicity, cell cycle

(1627) **EFFECT OF CADMIUM BIOTOXICITY AND SOY PROTEIN DURING RAT GESTATION**

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Introduction: the placenta is the main known tissue to protect the fetus from environmental toxins, such as cadmium (Cd). This divalent ion is a cytotoxic endocrine disruptor and produces an imbalance in the transport of nutrients through the placenta, thus causing a decrease in fetal birth weight or premature births. Several studies show that at very low doses, Cd can induce fetal growth restriction, thus increasing the risk of neonatal morbidity and mortality or generating in adult life diseases such as diabetes, obesity or coronary heart disease.

Soy protein is becoming increasingly important in the human diet. Santell et al. (1997) demonstrated that isoflavones (genistein) could cause hypertrophy in the rat endometrium and alter reproductive function in numerous species. Objective: to evaluate the possible protective role of the consumption of soy protein compared to the mechanisms by which Cd exerts its toxicity. Materials and Methods: 4 lots of female Wistar rats were used: 2 lots received casein (Cas) and 2 lots soybean (Soy) as protein source. Within each group, 1 lot received regular water (control-Co) and the other, 15 ppm of Cd in the drinking water during pregnancy period (20G). Results: We determined TBARS, catalase (CAT) and glutation peroxidase (GPx) activity, and nitrite concentration. Placenta tissue total RNA was extracted and RT-PCR was performed using the following primers: MT I; MT II; Nrf-2; NOX-2, SOD-2 and CAT. The concentration of Cd increased in both intoxicated groups ($p < 0.001$). In Soy-Cd group GPx activity; the levels of MDA, nitrite and expression of Nrf-2; SOD and MT I increased ($p < 0.05$, $p < 0.001$, $p < 0.05$, $p < 0.05$, $p < 0.01$ and $p < 0.001$). While CAT activity; expression of mRNA NOX-2 and MT II decreased ($p < 0.01$, $p < 0.01$ and $p < 0.001$).

Conclusion: these results would indicate the presence of an oxidative and nitrosative environment, in placenta at 20G, which would suggest possible alterations in the embryonic development.

(638) **HEXACHLOROBENZENE EXPOSURE INDUCES CELL MIGRATION, INVASION AND ANGIOGENIC FACTORS EXPRESSION IN HUMAN ENDOMETRIAL STROMAL CELLS**

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Endometriosisis is an invasive gynecological disorder of reproductive women characterized by the growth of endometrial glands and stroma outside the uterus. The disease is associated with chronic pelvic pain, dysmenorrhea, and infertility. Exposure to en-

docrine-disrupting environmental pollutants could play a role in the disease etiology. Hexachlorobenzene (HCB) is an organochlorine pesticide found in maternal milk and in lipid foods, that induces toxic reproductive effects in laboratory animals. Degradation of extracellular matrix by metalloproteinases (MMPs) is a basic step in migration and invasiveness in endometriosis. Recently, we demonstrated that HCB enhances cyclooxygenase (COX-2) expression, MMP2 and 9 activation and prostaglandin E2 secretion in human endometrial stromal cells. Pathological angiogenesis is the hallmark of endometriosis. The pro-angiogenic factor, such as vascular endothelial growth factor (VEGF) in the peritoneum of endometriosis patients supports the growth of the ectopic implants. The present study examined the effect of HCB in human endometrial stromal cell line T-HESC on cell migration (scratch motility assay), cell invasion (transwell Matrigel invasion assay), and expression of angiogenic factor VEGF (WB). Cells were exposed to HCB (0.005, 0.05, 0.5 and 5 μM) or vehicle for 24, 48 or 72h. Results showed that the pesticide increased cell migration (47%, $p<0.05$; 81%, 77% and 96%, $p<0.001$) in a dose response manner and through of aryl hydrocarbon receptor (AhR), COX-2, c-Src and estrogen receptor. Moreover HCB (0.5 μM) induced the invasiveness potential of T-HESC (200% $p<0.05$). VEGF secretion was induced by HCB (0.005-0.05 μM) (130-150%, $p<0.05$), while VEGF intracellular content was decreased at HCB (0.05-0.5 μM) (40-60%, $p<0.05$). Our results indicate that HCB exposure could contribute to maintenance and development of endometriosis promoting cell migration, invasion, and angiogenic factors secretion in human endometrial cells T-HESC.

Keywords: Endometriosis, Hexachlorobenzene, VEGF, Invasion, Migration

(209) **HISTOARCHITECTURAL MODIFICATIONS ON POSTPUBERTAL MALE RAT MAMMARY GLAND ARE INDUCED BY PERINATAL EXPOSURE TO A GLYPHOSATE BASED HERBICIDE**

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Glyphosate exposure during critical periods of development induced adverse effects on the reproductive system of male rats, suggesting an endocrine disruption action. In addition, we have shown that the male rat mammary gland is susceptible to endocrine disruption. Here, we evaluated whether perinatal exposure to a glyphosate based herbicide (GBH) alters the mammary gland morphology and endocrine response in postpubertal male animals. Pregnant rats were exposed orally through the diet to vehicle (saline solution), 350 or 3.5 mg/kg/day of GBH from gestational day 9 until weaning. On postnatal day 60, the male offspring were sacrificed and mammary gland and blood samples were collected. Testosterone (T) and estradiol (E) serum levels were measured and, total area, perimeter, longitudinal growth and mammary density were analyzed in mammary gland whole-mounts (WMs). Relative epithelial area, estrogen (ESR1) and androgen receptor (AR) protein expression and proliferation index were also evaluated in histological sections. T and E serum levels were similar between groups; however, the exposure to GBH350 increased the longitudinal growth and reduced the mammary density, without modifying the total area or perimeter of the mammary gland. In accordance with these results, the proliferation index, relative epithelial area and ESR1 expression were also reduced in GBH350-exposed animals, whereas no difference was detected in AR expression. On the other hand, GBH3.5-exposed animals presented similar WM and histological parameters to controls rats. Also, in this last group, ESR1 expression was decreased and AR levels were increased, without altering the proliferation index. Our results demonstrate that perinatal exposure to GBH alters the morphology of the male rat mammary gland long after exposure ended and suggest that the effects may be due to an altered ESR1 expression.

Keywords: Male, Mammary Gland, glyphosate based herbicide

NEONATAL EXPOSURE TO A GLYPHOSATE-BASED HERBICIDE ALTERS UTERINE ADENOGENESIS IN EWE LAMB.

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Abstract: Glyphosate based herbicides (GBH) are one of the most extensively used pesticides in agriculture all over the world. Recently, we showed that a brief exposure to a GBH during the first week of life alters uterine development and induces epithelial hyperplasia in prepubertal rats, and causes post-implantation embryo loss at adulthood. This study investigates the effects of a brief postnatal exposure to a low dose of GBH on the differentiation of the prepubertal ewe lamb uterus. Ewe lambs (Frizone breed) were sc injected from postnatal day 1 (PND1) to PND14 with saline solution (vehicle) or 2 mg/Kg/day of a GBH (reference dose from EPA, USA). On PND45 ewe lambs were hysterectomized and uterine samples were paraffin-embedded or stored at -80°C until mRNA extraction. Cell proliferation was assessed in all uterine compartments by quantifying the expression of Ki-67 protein through immunohistochemistry (IHQ). We also determine the uterine expression of Forkhead box A2 (Foxa2) protein by IHQ, which is involved in the uterine gland development. RT-PCR was performed to evaluate the expression of IGF family genes related with the cell proliferation, i.e., insulin-like growth factor-1 (IGF-1), IGF-2 and its receptor (IGF-1R). GBH treatment decreased the proliferation rate in the subepithelial stroma (7.7±0.9% vs C:12.2±1.5%, $p<0.05$) and in the luminal (7.7±1.1% vs C:16.1±1.1%, $p<0.005$) and glandular epithelium (10.8±0.8% vs C:20.6±2.0%, $p<0.005$). This alteration could not be associated with a deregulation of the IGF pathway, since no changes were detected in gene expression. Foxa2 expression, which is only detected on the glandular epithelial cells, was decreased on the GBH exposed group (2.3±0.4 vs C:3.8±0.6, $p<0.05$). Postnatal exposure to a low dose of a GBH disrupts the uterine development of prepubertal sheep, by decreasing cell proliferation and altering the expression of Foxa2 involved in uterine adenogenesis. These alterations might affect female fertility at adulthood.

Keywords: lamb uterus; glyphosate-based herbicide; cell proliferation; IGF pathway; Foxa2

(803) **NEONATAL EXPOSURE TO A GLYPHOSATE-BASED HERBICIDE DISRUPTS ANGIOGENESIS IN UTERINE DECIDUALIZATION IN PREGNANT RATS**

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The glyphosate-based herbicides (GBH) are commonly used to control weeds. Recently, it has been shown that neonatal treatment of Wistar rats with GBH alters the uterine development. These rats have reproductive problems as post-implantation failure associated with diminished decidualized area of implantation sites (IS). The aim of present work was to evaluate if decidualization failure in GBH-exposed rats could be caused by defective uterine angiogenesis. Female Wistar pups received saline solution (control, C) or an environmentally relevant dose of GBH (2 mg/kg) by sc. injection on postnatal day (PND) 1, 3, 5 and 7. On PND90 females were mated with fertile males non-exposed to GBH. Pregnant rats were sacrificed on gestation day 9 (GD9) to evaluate angiogenesis process in the IS. The newly formed blood vessels were detected by nestin immunostaining. The mRNA expression of VEGF and NOTCH1 were quantified using qRT-PCR. Macrophages were detected by immunohistochemistry and the results were expressed as volume den-

sity. GBH-exposed rats showed an increase of macrophages in the decidua, specifically in the antimesometrial zone (C: 1.2±0.1% vs GBH: 1.7±0.2%, $p<0.05$). In addition, nestin positive area decreased in GBH-exposed rats (C: 2.9±0.5% vs GBH: 1.6±0.3%, $p<0.05$). Furthermore, the mRNA expression of two angiogenic regulators VEGF and NOTCH1 was decreased in GBH-exposed rats (C: 55±3% vs GBH: 23±5% and C: 21±2% vs GBH: 12±2%, $p<0.05$, respectively). Present results demonstrate that the neonatal exposure to GBH interferes with the mechanism involved in vascular adaptation during decidualization process. The recruitment of macrophages could evidence an inflammatory process linked to decreased angiogenesis during decidualization that might be responsible for the reproductive failures found in neonatal GBH-exposed rats.

Keywords: Glyphosate, Angiogenesis, Decidualization, Uterus, Rat.

(617) **PESTICIDES IN THE SIGHT: ACTION OF HEXACHLOROBENZENE AND CHLORPYRIFOS IN TUMOR ANGIOGENESIS IN BREAST CANCER MODELS**

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Hexachlorobenzene (HCB) is an organochlorine compound found in maternal milk and in lipid foods. Chlorpyrifos (CPF) is an organophosphate pesticide used for pest control in agriculture. Breast cancer is by far the most frequently diagnosed cancer in women. HCB and CPF act as endocrine disruptors stimulating cell proliferation in different breast cancer models. Vascular endothelial growth factor (VEGF) and cyclooxygenase-2 (COX-2) promote tumor proliferation and angiogenesis. Nitric oxide (NO) and abnormal expression of nitric oxide synthases (NOS1, NOS2, NOS3) are implicated in cancer biology and tumor progression. We examined the action of HCB and CPF on angiogenesis in mammary carcinogenesis *in vivo* and *in vitro*. In a xenograft model with MCF-7(+ERα), HCB (3 mg/kg bw) and CPF (0.1 mg/kg bw) stimulated angiogenic switch (number of vessels/mm^2) (HCB $p<0.01$; CPF $p<0.05$) and increased VEGF expression by WB ($p<0.01$) in mice skin. In MCF-7, HCB (0.005μM) at 3h increased NOS1 ($p<0.05$) and NOS3 ($p<0.01$) levels by WB, while HCB (5μM) at 24h decreased NOS1 and NOS3 expression ($p<0.05$; $p<0.01$). Furthermore, both doses of HCB increased NOS2 levels at 3h ($p<0.05$) while HCB (5μM) decreased such levels at 24h ($p<0.05$). HCB also increased COX-2 and VEGF levels at both doses at 24h ($p<0.01$). CPF (50μM) increased NOS3 ($p<0.01$) and VEGF expression ($p<0.05$), while 0.05 and 50μM increased COX-2 levels at 24h ($p<0.05$; $p<0.01$). Exposure to each pesticide enhances the production of NO (0.005μM HCB at 3h, $p<0.05$, while 0.05μM CPF at 24h, $p<0.01$). Our results demonstrate that both pesticides increase angiogenesis and VEGF expression in a xenograft model and in MCF-7 cell line. At low doses, that induces proliferation, we observed an enhance in NOS expression and NO production, while at high doses that promotes apoptosis, we found a reduction in NOS expression and NO content in MCF-7 cells. Our findings suggest that HCB and CPF may be a risk factor for human breast cancer progression.

Keywords: Angiogenesis, breast cancer, hexachlorobenzene, chlorpyrifos.

(1029) **THE ARYL HYDROCARBON RECEPTOR AGONISTS REACTIVATE LINE-1 RETROTRANSPOSON IN BREAST CANCER CELLS**

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Breast cancer is the most common cancer worldwide in women and Argentina is the second country of America with the highest mortality rate. Epigenetic changes are more frequent in breast cancer cells than genetic mutations. These alterations affect retrotransposons like the long interspersed nuclear element 1 (LINE-1), which is highly activated in breast tumors. Environmental pollutants ligands of aryl hydrocarbon receptor (AhR) can induce the activation of LINE-1 and epithelial to mesenchymal transition, through the transforming growth factor-β1 (TGF-β1)/Smad signaling pathway. AhR is a transcription factor that modulates processes such as apoptosis, proliferation and migration. We worked with environmental doses of two weak ligands of the AhR: the organochlorine pesticide hexachlorobenzene (HCB) and the organophosphate chlorpyrifos (CPF). Our aim was to study the effect of these pesticides on LINE-1 expression and to elucidate whether the mechanism of action is related to the interaction AhR/TGF-β1, in the human breast cancer cell line MDA-MB-231 (- estrogen receptor α). We have reported that HCB (0.005, 0.05, 0.5 y 5 μM) activates the AhR/c-Src signaling, which subsequently induces the TGF-β1/Smad pathway, promoting MDA-MB-231 cell migration and invasion. Our results showed that LINE-1 mRNA levels, evaluated by RT-qPCR, were enhanced at 0.005 μM HCB ($p<0.01$), but reduced at 5 μM HCB ($p<0.05$) after 24 h. On the other hand, CPF (0.05, 0.5, 5 y 50 μM) increased LINE-1 mRNA content after 48 h ($p<0.01$). Western blot results showed that CPF (0.5 μM) enhanced AhR protein expression ($p<0.05$) at 24 h, as well as c-Src ($p<0.05$) and Smad 2/3 ($p<0.01$) phosphorylation after 15 and 30 min. In conclusion, we demonstrated that these pesticides modulate LINE-1 expression in MDA-MB-231, depending on the dose and the time of exposure. These findings could be linked to alterations previously observed in process like breast cancer cell proliferation, migration or invasion.

Keywords: LINE-1, HCB, CPF, breast cancer.

CELL SIGNALING 3

(1518) **CYSTIC FIBROSIS TRANSMEMBRANE REGULATOR (CFTR) IN HUMAN TROPHOBLAST HTR8/SVNEO CELLS MODULATES SEVERAL PROCESSES OF PLACENTATION**

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The expression of different channels and transporters is altered in preeclamptic placentas, including the cystic fibrosis regulatory channel (CFTR), and defective placentation is one of the main features of this gestational disorder.

Our aim was to study the expression and participation of placental CFTR in the proliferation, migration, invasion and apoptosis during placentation.

We observed by Western blot in first trimester extravillous trophoblast cell line (HTR8/SVneo) the typical band of ~160 KDa of CFTR. The silencing of the CFTR with a specific siRNA (50nM) showed a half decrease channel expression compared to control without treatment and the scramble at 48-72h ($p<0.05$, n=4). Expression and localization of the channel was confirmed by immunocytochemistry. The silencing of CFTR by siRNA resulted in the inhibition of ~30% of cell viability at 72h ($p<0.05$, n = 5) by MTT assay and similarity

decreased cell viability by Trypan blue exclusion technique ($p<0.05$, $n = 3$). We evaluated CFTR participation in the migratory capacity of the cells by wound healing assay at 5-6h. We found that cells with the CFTR siRNA covered a smaller area of the wounds (13.6±2.0%) than with the scramble (40.9±2.5%) or with the control (38.8±1.5%) ($n =9$, $p <0.05$). Matrigel-based invasion assay showed that cell invasion ability was significantly attenuated when a CFTR siRNA was used, compared to control and scramble groups ($p<0.05$, $n=3$). After staining the cells with AO and Et/Br, apoptosis cells (orange cells) were observed under the fluorescence microscope. The apoptotic percentage was increased in cells CFTR siRNA treated (46.0±6.5%) with respect to control (19.0±3.3%) and scramble (21.0±3.5%) after of the addition of peroxide for 24h ($p<0.05$, $n=12$).

We conclude that CFTR is present in HTR8/SVneo cells and participates in the proliferation, migration, invasion and apoptosis of these cells, suggesting that alterations in CFTR may be related to an abnormal placentation.

Keywords: CFTR- cell proliferation- migration- apoptosis- preeclampsia

(736) **MODULATION OF CISD1 EXPRESSION BY THE ACTIVITY OF THE CFTR CHANNEL**

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Cystic fibrosis (CF) is a frequent autosomal recessive disease caused by mutations that impair the CF transmembrane conductance regulator (CFTR) protein function, which indirectly affect the expression of a net of genes. We described a new CFTR-dependent gene downregulated in CF cells, CISD1, also referred to as mitoNEET, encoding for the first member of a family of proteins possessing a CDGSH signature. CISD1 is a dimeric mitochondrial outer membrane protein, implicated in many facets of human pathophysiology, but its molecular function remains poorly characterized. We have previously described that *CISD1* gene expression was decreased in a CF cellular model and restored in the same cells ectopically expressing wt-CFTR (CFDE and CFDE/6RepCFTR cells). Now we used another cellular model, human colon adenocarcinoma T84 cells, and by real-time PCR we determined that *CISD1* gene expression was decreased in T84 cells treated with CFTR(inh)172, compared to control cells ($p<0.05$). These results confirmed that CISD1 expression is decreased in CF cells or in cells with impaired CFTR function. We are now studying the possible mechanisms involved in this regulation. Recently, we also report an elevated expression of diverse cytokines in CF cells in culture, including IL-1β. We have previously shown that IL-1β (>2.5 ng/ml) inhibit CFTR mRNA expression in T84 cells. Here we show that externally added IL-1β (5 ng/ml) reduces the CISD1 mRNA expression. Moreover, treatments with IKK inhibitor II (NF-κB pathway) increased the CISD1 mRNA expression. In conclusion, *CISD1* gene expression is decreased in cells with impaired CFTR function. Similar effects were obtained when exogenous IL-1β was added. These results suggest that IL-1β acts as a bridge connecting the CFTR with the CISD1 expression.

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Keywords: CISD1, Cystic Fibrosis, CFTR, IL-1β.

(564) **INTRACELLULAR CHLORIDE ACTS AS A SECOND MESSENGER FOR CFTR MODULATING IL-1β EXPRESSION**

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The CFTR protein is a cAMP-regulated chloride channel. By using differential display, we have found previously several CFTR-dependent genes, including c-Src, MUC1, MTND4 and CISD1. We also reported the existence of chloride-dependent genes, such as GLRX5 and RPS27. Here, using nigericin and tributyltin to clamp the pH and the intracellular chloride concentration $[Cl^-]_i$ of IB3-1 epithelial cells, we show that IL-1β is also a Cl^--dependent gene that can be modulated by changing the intracellular chloride concentration, in a biphasic way. The IL-1β secretion measured by ELISA also showed a similar pattern of response to changes in $[Cl^-]_i$. The mechanism involves an IL-1β autocrine effect, since in the presence of the IL-1β receptor antagonist IL1RN and anti-IL-1β blocking antibody, Cl^- effects disappeared ($p<0.05$, $n=3$). Similar effects were obtained with the JNK inhibitor, c-Src inhibitor and the IKK inhibitor, suggesting that JNK, c-Src and NF-kB are important mediators of the $[Cl^-]_i$ signaling. Moreover, studying the inflammasome complex by real-time PCR and Western Blot techniques, we found that caspase-1 and NLRP3 expression mRNA and protein was modulated by Cl^- ($p<0.05$, $n=3$). Interestingly, ASC expression is not affected by Cl^-. Inhibitors of caspase-1 or NLRP3 inflammasome abolish the Cl^- stimulation ($p<0.05$, $n=3$), suggesting that Cl^- can modulate the NRLP3 inflammasome. In conclusion, the Cl^- anion act as a second messenger for CFTR, modulating the expression of NLRP3 inflammasome and the resulting secretion of IL-1β, through an autocrine IL-1β loop that involves IKK and JNK kinases. This work was supported by the ANPCYT (grant numbers PICT 2012-1278 and PICT-2015-1031), CONICET (grant PIP 11220150100227CO 2015-2017 and PUE 22920160100129CO), and UCA; also by research fellowships from CONICET.

Keywords: CFTR, Chloride, IL-1β, Caspase-1, NLRP3.

(1745) **HIV-1 TAT PROTEIN DISTURBS THE SIGNALING CASCADE THAT LEADS TO ACROSOMAL EXOCYTOSIS IN HUMAN SPERM**

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The acrosome reaction (AR) is a regulated calcium-dependent exocytosis necessary for fertilization. Fertility parameters in HIV-seropositive men are abnormal. The HIV-1 transactivating protein (Tat) is released by infected cells and extracellular Tat enters uninfected cells by endocitosis inducing toxic effects. This protein impairs neurons secretion contributing to HIV pathogenesis. The aim of this work was to determine if HIV-1 Tat was able to enter a non-endocytic cell like sperm affecting gamete function. First, we incubated spermatozoa with recombinant wild type Tat (WT-Tat). By WB and indirect immunofluorescence, we demonstrated that Tat, at physiological concentrations, pass through human sperm membranes.To elucidate the mechanisms involved in Tat internalization we challenged sperm with Tat mutants. We observed that a W11 residue is required in this process. WT-Tat strongly impaired progesterone (Pg)-induced AR as measured by exocytosis assays. Considering that HIV-1 Tat binds phosphatidylinositol (4,5)-bisphosphate (PIP_2) with high affinity and that our laboratory has shown that PIP_2 plays a key role in the sperm exocytic cascade we tested a Tat mutant unable to bind PIP_2. As expected, the mutant did not affect sperm exocytosis. To confirm that the inhibitory effect of Tat on the AR is due to its ability to bind the phospholipid, we rescued Tat-induced inhibition of secretion by adding PIP_2. This suggests that Tat is sequestering PIP_2. We assumed that Pg-induced AR inhibition will occur due to lack of IP_3 synthesis. To test this argument, we resorted to the agonist of IP_3 receptors, adenophostin that rescued Pg-induced exocytosis after Tat inhibition. These findings suggest that Tat requires the W11 residue to permeate through the plasma membrane and when inside the sperm a strong interaction with PIP_2 avoids the AR to proceed. Our findings may provide some clues to elucidate the unsolved issues concerning to male fertility in HIV patients.

Keywords: acrosomal exocytosis, Tat HIV-1, PIP_2, human sperm

(1629) **DNAJC5 Y HSC70 ARE PRESENT AND NECESSARY FOR ACROSOMAL EXOCYTOSIS IN HUMAN SPERM**

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Calcium-triggered exocytosis, which releases neurotransmitters and hormones into the extracellular medium, is a key cellular event mediated by the fusion of large and dense secretory granules with the plasma membrane.

The sperm acrosome reaction (AR) is a unique, regulated exocytosis with special characteristics that plays a central role in the fertilization process. The human sperm possesses a single, large, flat granule that is released during AR. Acrosomal exocytosis involves the opening of a large number of fusion pores, merging the outer acrosomal and plasma membranes. In this process, the monomeric SNARE proteins are assembled into *trans* complexes, causing the irreversible docking of these membranes (acrosome and plasmatic). The fusion-competent conformation of the SNARE proteins and the SNARE-complex assembly are maintained by molecular chaperones.

Molecular chaperones DnaJC5 and Hsc70 have been reported to facilitate the correct folding of polypeptides and to regulate the assembly of protein complexes involved in neurons and neuroendocrine cells exocytosis. However, the role of these chaperones in sperm remains unknown. Our goal is to determine the presence, localization and the role of these molecular chaperones in the assembly of *trans*-SNARE complexes in acrosomal exocytosis.

By western blot, immunofluorescence and electronic microscopy, we show that both DnaJC5 and Hsc70 proteins are present in human sperm. Besides, we demonstrate that DnaJC5 is predominantly membrane bound, whereas Hsc70 is cytosolic. Moreover, by functional assays, using anti-DnaJC5 or anti-Hsc70 antibodies, we show that they are functionally active in secretion since they inhibited AR in a concentration-dependent manner. These results therefore assist in our understanding of the role of DnaJC5 and Hsc70 in acrosomal exocytosis.

Keywords: chaperones, acrosomal exocytosis, sperm.

(628) **ESTRADIOL EFFECTS ON LEPTIN EXPRESSION IS MEDIATED BY THE INTERRELATIONSHIP OF DIFFERENT TRANSCRIPTIONS FACTORS.**

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Leptin is a key hormone in placental physiology. It regulates trophoblast proliferation, inhibits apoptosis, stimulates protein synthesis, and regulates fetal growth and development. The mechanisms involved in the regulation of placental leptin expression are not fully understood. Previous results from our lab demonstrated that estradiol (E_2) regulates leptin expression involving genomic and non-genomic effects. In these study we aimed to analyze the effect of Sp1 and NFκB transcription factors and cAMP/PKA signaling pathways in the induction of leptin expression by E_2 in human placental cells. BeWo cells cultured under standard conditions, as wells as human placental explants were used. Western blot, *qRT*-PCR, immunofluorescences, transfections assay with reporter constructs and expression vectors were carried out. We found that the inhibition of the NFκB factor reduced the E_2 action over the leptin expression (P£0,05), and the overexpression of the p65 subunit (Rel A) significantly increases the transcriptional activity of leptin promoter (P£0,05). On the other hand, BeWo-Sh2 cells expressing an shRNA against ERapha protein, show no effect to E_2 treatment nor with SP1 factor or with Rel A, which will suggest that theses factor requires ERalpha so it could exert its effects on E_2 leptin expression. Moreover we observed that Sp1 (P£0,05) and cAMP-PKA (P£0,05) pathway increased leptin promoter activity, instead we can counter this effect with the pharmacological inhibitors H89 (P£0,05) and SQ22536 (P£0,005). Finally by immunofluorescence we observed that there is a strong correlation between the ERalpha and the p65 subunit localization. These findings suggest that leptin expression is tightly regulated and improved the comprehension of the mechanisms where by E_2 regulates leptin expression and leptin function during pregnancy.

Keywords: Leptin, transcription factors, placenta

(455) **OXER1 ACTIVATION, BUT NOT PKA OR PKC ACTIVATION, INCREASES HUMAN ADRENOCORTICAL CELL MIGRATION**

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Lipoxygenase-dependent products of arachidonic acid metabolism act through a membrane receptor named OXER1. These compounds are produced in steroidogenic cells and are required for the activation of steroid production. We found that human adrenocortical H295R cells express OXER1 and that, in this cell type, it is involved in the PKA and PKC-dependent stimulation of steroidogenesis. Other authors have postulated that 5-oxo-ETE is a potent activator of human neutrophil migration and of prostate cancer cell proliferation. Both effects are mediated by the activation of its receptor. Here we examined the effect of PKA, PKC and OXER1 signal transduction pathways on the migration of H295R cells. The cells were cultured under different conditions and cell migration was evaluated measuring wound healing 24 h after scratch. Results are shown in arbitrary units as mean ± SEM. Treatment with 5-oxo-ETE (500 nM), agonist of OXER1, produced an increase in cell migration (control 14.3 ± 0.7 vs. 5-oxo-ETE 25.3 ± 2.5, $P < 0.003$). This result was also obtained using a cell line characterized by the stable overexpression of OXER1. In addition, H295R cells treated with 1 mM 8Br-cAMP or 0.1 mM angiotensin II did not change the migration rate (control 14.3 ± 0.7; 8Br-cAMP 10.2 ± 1.8; AII 17.5 ± 1.8). Therefore, the activation of PKA and PKC kinases might not be involved in the activation of the migration process. OXER1 activation might promote cell migration through its own signal transduction pathway. Future experiments should focus on signal transduction pathways triggered by OXER1 activation.

Keywords: OXER1 – PKA – PKC – cell migration – adrenocortical cells

(1185) **2-IODOHEXADECANAL: NEGATIVE MODULATION OF THYROGLOBULIN (TG) VIA PPARGAMMA RECEPTOR.**

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Introduction: Iodine plays an important role in thyroid physiology and proliferation (Thyroid Autoregulation). The biosynthesis of iodolipids has been observed in the thyroid gland and their participation in thyroid autoregulation has been suggested. 2-iodohexadecanal (2-IHDA) was isolated as the major iodolipid formed in the thyroid and we have shown that this iodolipid can inhibit several thyroid parameters. A possible mechanism of 2-IHDA action would involve the Peroxisome Proliferative Activated Receptors (PPARs). **Objective:** To study the potential role of PPARs in the mechanism of action of 2-IHDA. **Methodology:** In silico analysis of consensus sequences for PPAR binding in the promoter region of relevant genes for thyroid physiology was performed using the PROMO 3.0 application. We decided to study the effect of PPARs on Tg gene expression. FRTL-5 cells were cultured and treated for 24 h with increasing doses of 2-IHDA. Tg mRNA levels were assessed by RT-qPCR. The transcriptional activity of the Tg gene was determined by reporter gene assays using the pTg -688 and the pTg -688 PPAR mutated construct coupled to luciferase. The binding of PPARγ to the Tg pro-

moter was performed by chromatin inmunoprecipitation (ChIP) studies. Since the Tg promoter region has only one site for PPAR gamma, assays were performed applying rosiglitazone maleate agonist, siRNAs and inhibitors (GW9662) to this isoform. **Results:** Reporter gene assays revealed that 2-IHDA promoted the transcriptional activity of PPARγ. The PPARγ agonist mimicked the repressor effect of 2-IHDA on Tg mRNA levels. GW9662 and the PPARγ siRNA reversed the effect of 2-IHDA. ChIP analysis revealed that 2-IHDA promoted an increase in PPARγ interaction with the Tg promoter. Transfection studies showed an inhibition on Tg gene expression caused by 2-IHDA while transfection with the pTg -688 PPAR mutated construct showed no effect. **Conclusion:** 2-IHDA modulates Tg gene expression by inducing PPARγ repressor activity.

Keywords: Iodolipids, 2-IHDA, Thyroid, Thyroglobulin, PPAR.

(1082) **CHARACTERIZATION OF THE INITIATION TRANSLATION FACTOR eIF4G$_1$ AS A PKA SUBSTRATE IN *Saccharomyces cerevisiae***

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PKA has a central role in the control of the metabolism, stress resistance and proliferation in *Saccharomyces cerevisiae*. In response to nutrient consumption, *S. cerevisiae* arrests cell cycle, decreases global protein synthesis and initiates translation through internal ribosome entry sites (IRES). We have recently demonstrated that PKA connects the nutritional state of the cell with translation at different levels: through interaction with translation factors, by localization in mRNA granules and affecting the protein abundance of translation initiation factor eIF4G$_1$ by a post-translational mechanism. Phosphoproteomic assays have reported that eIF4G$_1$ is a phosphoprotein. The aim of this work was to characterize eIF4G$_1$ as a PKA substrate and to identify the phosphorylation target residues. For this purpose, the full length and different fragments of recombinant proteins derived from GST-eIF4G$_1$ were expressed and purified using a pGEX system. Using these proteins, we assayed *in vitro* phosphorylation with Tpk1 and Ca as the catalytic subunit from *S. cerevisiae* and mammals respectively. The GST-eIF4G$_1$ phosphorylation was studied by western blot using an anti-P-Ser antibody and autoradiography for the incorporation of γ-^{32}P [ATP]. The phosphorylated residues were identified by nano-LC MS/MS applying an iron trap chromatography. Our results demonstrate that eIF4G$_1$ is a PKA substrate *in vitro* and identify Thr^{934} as a novel phosphorylation target residue in eIF4G$_1$. Since, Thr^{934} residue localizes on the RNA-binding domain RNA3 at the extreme C-terminus, we will focus on studying the role of this phosphorylation site over eIF4G$_1$ protein abundance and its recruitment to the 43S preinitiation complex on the mRNA.

Keywords: PKA, eIF4G$_1$, phosphorylation, translation

BIOPHYSICS 3

(1504) **BIOPHYSICAL BASIS OF MULTIPLE CELLULAR TARGET RECOGNITION BY HPV E6 ONCOPROTEINS**

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Human papillomaviruses (HPVs) are small DNA viruses that infect mucosal or cutaneous epithelia. While “low-risk” HPVs generate only benignant proliferations, the so-called “high risk” HPVs can generate malignant proliferations, which are responsible for 5% of human cancers worldwide.

All HPV types express the E6 oncoprotein, which is a critical player in carcinogenesis induced by HPV. Most E6 oncoproteins share a common molecular property: they recognize short linear motifs containing the LxxLL consensus sequence on their cellular targets. The LxxLL motif adopts a helical conformation to dock into a hydrophobic and positively charged pocket built up by the two zinc-binding domains of E6.

Through LxxLL motif recognition one particular E6 can interact with a pool of different targets. The identity of the targeted proteins varies with the E6 proteins from different papillomaviruses, which could result in different global biological effects. Integrating affinity measurements, X-ray crystallography structures and molecular dynamics data on E6:LxxLL complexes from divergent papillomaviruses we aim to unravel the structural determinants of LxxLL motif recognition that distinguish high-risk/low-risk and cutaneous/epithelial HPV types. Here we present what we learned from two novel high resolution E6 structures from HPV18 and 49 combined with affinity determinations for their complete set of potential cellular targets.

Keywords: HPV, E6, X-ray crystallography

(1498) **EVALUATION OF THE CONFORMATIONAL CHARACTERISTICS OF THE HUMAN LYR PROTEIN ISD11.**

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Abstract: The leucine/tyrosine/arginine family (LYRMs) is a group of proteins with an adaptor like function essential for cell metabolism. One of them is ISD11 which interacts with the cysteine desulfurase NFS1, a key-component of the Fe-S cluster biogenesis machinery inside mitochondria. This interaction provides stability to NFS1 and it has a central role in the ISCU/HSPA9/HSC20 protein complex that transfers Fe-S clusters from ISCU to target proteins. Recently, it was shown that ISD11 also interacts with the mitochondrial acyl carrier protein (ACP) by electrostatic forces and exhibits alpha helical structure in which an acyl chain (linked via phosphopantetheine cofactor to ACP) is interacting with core residues of ISD11. In this context, our main objective is to understand how ISD11 acquires its structure and stability. Previous results showed that purified (isolated) ISD11 has a high tendency to aggregate. Herein, we present that the addition of the detergent n-Dodecyl β-D-maltoside (DDM) and high concentration of NaCl allows us to obtain soluble protein. By far-UV CD analysis, we observe that the protein is completed folded. We conjecture that the detergent may mimic the acyl group and its structural role, whereas NaCl diminishes the electrostatic repulsion on the surface of ISD11 when protein partners are absent. This result opens the possibility to study the role of the acyl chain on ISD11 stability and the role of ISD11 in the interactions with NFS1 and ACP among others.

To further evaluate ISD11 structure, we obtained the helical regions of ISD11 as three peptides and we evaluated the secondary structure and activity of them in the context of the NFS1-ISD11 complex. By Far-UV CD spectra we have confirmed they present an alpha-helix structure and we have determined that the N-terminal region interacts with negative charged membranes. This suggests that ISD11 may be important for the anchoring the NFS1 protein complex to the mitochondrial membrane.

Keywords: Conformational characterization, peptide-lipid interaction, desulfurase activity.

(1784) **PANNEXIN 1 EXPRESSION IN *XENOPUS LAEVIS* OOCYTES**

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Six subunits of Pannexin 1 (Panx1) form a plasma membrane channel termed ‘pannexon’. The pannexon can function as a small-conductance chloride selective channel, activated by an increase of membrane potential (Vm). Alternatively, a non selective high- conductance mode was reported, where the channel can be activated by increases in extracellular potassium concentration (K^+)e and permeate molecules less than 1.5 kD. In addition to K^+e and Vm, hypotonicity was suggested –but not thoroughly proven- to activate Panx1 activity.

In this context we used Xenopus oocytes as an expression system to express Panx1, and characterize the modulation of Panx1 currents by K^+e and hypotonicity.

Expression of Panx1 was assessed by two-electrode voltage

clamp technique. We employed a voltage step protocol to evaluate the small conductance mode or a continuous pulse protocol to observe the high conductance mode. By applying a voltage step protocol in Panx1 injected oocytes we observed voltage sensitive outward currents that were absent in water injected oocytes (I_{panx1} at 60 mV = 0.60 ± 0.12 μA, n=10 vs I_{water} at 60 mV 0.12 ± 0.01μA, n=7, p<0.05), while addition of CBX (a Panx1 inhibitor) suppressed these currents. Assessment of Panx1 by $(K^+)e$ stimulation was performed in a continuous pulse protocol. A 5-fold increase of currents was evidenced in Panx1 injected oocytes, suggesting the presence of high conductance mode. On the other hand, hypotonic medium (50 mOSM, 1.5 min), as compared to isotonic control, did not alter the magnitude of currents (I_{iso} 60 mV = 0.31 ± 0.12 μA vs I_{hypo} 0.33 ± 0.12 μA, n=5, NS). Results suggest that $(K^+)e$, but not hypotonicity, was able to activate Panx1 into the high conductance mode of pannexon. This data could be relevant in the understanding of the Pannexin 1 role during cell volume changes.

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Keywords: Pannexin 1, Voltage Clamp, Xenopus oocytes, Potassium, Osmotic stimuli.

(1376) **PROCYANIDIN DIMERS INTERACTION WITH PORCINE PANCREATIC LIPASE**

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The aim of this work was to study the effect of procyanidin dimers enriched fractions from peanuts skins on porcine pancreatic lipase (PPL) activity and characterize this interaction. Extracts were obtained from skin peanuts (type Runner from Argentina, crop 2012) subjected to blanching (90ºC, 10 min), extracted with ethanol-water (70:30 v/v) following by an ethyl acetate extraction to obtain three fractions: purple (PF), brown (BF) and yellow (YF). Interaction with PPL was studied in terms of enzymatic activity and quenching of fluorescence. Enzymatic activity was tested by monitoring the cleavage of 1,2-O-dilauryl-rac-glycerol-3-glutaric acid (6-methyresorufin) ester to produce methylresorufin previous incubation of PPL with 0–2.5 mg/ml of fractions. Orlistat and epigallocatechin gallate were used as positive controls. PPL inhibition order was YF ≈ BF > PF with IC_{50} of 1.0±0.1 mg/ml, 1.2±0.1 mg/ml and >2 mg/ml, respectively. Quenching of fluorescence (λ_{exc}= 295 nm) showed a decreased in Intensity without changes in the maximum peak position, suggesting that the microenvironment of the tryptophan residues is not changed. Data obtained from double logarithmic Stern-Volmer equation was applied to calculate binding constants of YF and BF at different temperatures obtaining the thermodynamic parameters. The binding process was spontaneous (ΔG at 298K = -22.0 and -20.8 kJ/mol for YF and BF, respectively), endothermic (ΔH=13.7 and 39.5 kJ/mol for YF and BF, respectively) and with an increase of entropy (ΔS=121 and 203 J/K mol for YF and BF, respectively).

Based on the fractions composition, analyzed by HPLC-ESI-MS/MS, the inhibitory effect was associated to the presence of procyanidin dimers type A, and in a less extent to procyanidin dimers type B. Experiments with purified procyanidin dimers A1, B1 and B2 confirm the results.

These results suggest that compounds isolated from peanut skin can be a useful food ingredient with the potentiality of decrease fat absorption in humans.

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Keywords: flavonoides; food ingredients; lipid absorption; thermodynamics

(1379) **SPECIFICITY AND REACTIVITY OF MTB PROTEIN KINASES PKNB AND PKNG**

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Tuberculosis is one of the most important infectious diseases in the world, causing the death of about 2 million people per year, mainly in developing countries. The Pkns are the central protein kinase family of the bacillus, which is composed of 11 proteins, two particularly interesting: pknB and pknG. The cellular processes in which pknG and pknB participate are diverse. However, the axis of this work will be the study of the phosphorylation of garA, a regulator of the tricarboxylic acid cycle. T21 and T22 residues of the garA N-terminus are susceptible to phosphorylation, an event that regulates the activity of the protein. When garA is phosphorylated, phosphorylated residues interact with residues of FHA domain, preventing interaction with other proteins. PknG phosphorylates garA at threonine 21, adjacent to the residue phosphorylated by pknB (T22), and these two phosphorylation events are mutually exclusive. However, until now the reason for the specificity and the functional relevance remains unclear. In the present work, we use Molecular modeling, molecular dynamics simulations, and kinase activity assays to explain the differences in the selectivity on garA phosphorylation. We finally found that amino acid differences at the garA n-terminal are responsible for specificity. We concluded that there are two hydrophobic residues which anchor garA in the pocket of the active site of pknG and one polar residue that anchors in the pocket of pknB.

Keywords: Tuberculosis, Molecular Dynamics, pknG, pknB, garA

(407) **STRUCTURAL CHARACTERIZATION OF *Arabidopsis thaliana* GRF3-GIF1 INTERACTION**

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Plant development is a process that is finely regulated by the action of various molecular mechanisms. One of these mechanisms comprises the activation or repression of the expression of certain genes, through the action of specific proteins. The Growth-Regulating Factors (GRF) – GRF-Interacting Factors (GIF) system is formed by two families of activators and transcriptional coactivators conserved in terrestrial plants. A range of studies have demonstrated the function of the combined action of these proteins on plant development, but there is no biophysical evidence of such protein-protein interaction so far.

Here we present the first structural study of *Arabidopsis thaliana* GRF3 and GIF1. The proteins show a high degree of intrinsic disorder throughout the sequence. To perform a detailed evaluation of their structural features, we designed protein constructs with the regions genetically mapped as essential for the interaction between GRF3 and GIF1, the QLQ and SNH domains, respectively. Using biophysical techniques such as Circular Dichroism and Nuclear Magnetic Resonance, we gathered information on the degree of disorder and the residual structure in these domains. Further information at a residue-specific level allowed us to begin the structural characterization of both the individual GRF3 and GIF1 and the GRF3-GIF1 complex.

Keywords: GRF, GIF, protein-protein interaction, CD, NMR

(45) **STRUCTURE AND FUNCTION STUDY OF PEPTIDES DERIVED FROM *E. Coli* ALPHA HEMOLYSIN FOR THE CONSTRUCTION OF AN IMMUNOTOXIN**

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Abstract: Escherichia coli alpha hemolysin (HlyA) is a pore-forming protein which belongs to the family of 'Repeat in toxins'(RTX). On the basis of experimental data and structural predictions, four

peptides derived from HlyA were synthesized: PEP 1: transmembrane domain described as hemolytically active; PEP 2: also a transmembrane domain which sequence corresponds to a cholesterol binding domain (CARC); PEP3: similar to PEP2 but with residue Y^{347} substituted by A and PEP4: similar to PEP2 but with a CRAC sequence ("cholesterol recognition/interaction amino acid consensus domain"). The aims of this work were to study the participation of CRAC and CARC in the stabilization of HlyA monomers in membranes by their interaction with cholesterol, to evaluate the role of Y^{347} in the interaction with membrane, and finally to find a cytotoxic peptide for the construction of an immunotoxin. Peptides were synthesized by the solid phase peptide synthesis method (Fmoc strategy), purified by HPLC (C-18 column), the molecular mass was determined by mass spectrometry and peptide structure by circular dichroism. The hemolytic activity of peptides was measured using human erythrocytes, and cytotoxicity using the MTT reduction method on the breast cancer cell line MCF-7. Results describe PEP2 as hemolytic and cytotoxic, which is promising and encourage us to use it in the design of immunotoxins. PEP3 was found not to be cytotoxic suggesting residue Y^{347} is fundamental for the interaction of HlyA with lipidic membranes. PEP4 was found not to be hemolytic nor cytotoxic, which implicates the CRAC sequence added was unfavorable for peptide activity.

In conclusion, we synthesized, and study the structure and activity, of four peptides derived from HlyA of *E. coli*. Also, we found a peptide that it is both cytotoxic and hemolytically active, which leads our group to the design and construction of an immunotoxin comprising PEP2 and an antibody that specially recognizes tumoral cells.

Keywords: immunotoxins, cancer, *E. Coli.*

(79) **THE INTERFACIAL PROPERTIES OF THE HIV-1 GAG MATRIX DOMAIN ARE MODULATED BY PROTEIN MYRISTOYLATION AND NUCLEIC ACID BINDING**

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Abstract: The specific traffic and assembly of the HIV-1 GAG protein at the plasma membrane (PM) is tightly regulated by the myristoylation of its matrix domain (GAG_{MA}) and its binding to RNA. When GAG reaches the PM a perfect protein monolayer is formed. Understanding how this layer is constituted and stabilized is relevant to develop new strategies aiming to interrupt the gemmation of new viral particles at the membrane. For this reason, we studied the stability, thermodynamics and the topography if this first protein layer from Langmuir monolayers composed of the myristoylated or unmyristoylated versions of GAG_{MA} in the presence or the absence of a single strand DNA (ssDNASel25), an analog of the Sel25 RNA sequence, that tightly binds GAG_{MA}. We also used a similar approach to study monolayers of GAG_{MA} -derived peptides (first 21 resides of GAG_{MA}) in their myristoylated and unmyristoylated forms. Our results show that the protein/peptide films are destabilized by the presence of ssDNASel25, inducing solubilization of the film components into the bulk phase. In addition, the oligonucleotide affects the protein-protein or peptide-peptide lateral interactions provoking interfacial topography changes of the monolayers, visualized by Brewster angle microscopy. Additionally, we show how the myristoyl group has major effects on the lateral stability and the elasticity of the monolayers. Altogether, here we propose a general model for GAG_{MA} interfacial association, considering the effect of myristoylation and the interaction with oligonucleotides. The model proposes a new role of GAG_{MA} based on the lateral stabilization of protein monolayers.

Keywords: Human immunodeficiency virus, HIV-1 GAG matrix domain, Oligonucleotide interaction, Brewster-angle microscopy, Langmuir monolayers.

(881) **THERMAL STABILITY AND INACTIVATION OF THE CATALYTIC DOMAIN FROM THE COPPER ATPASA OF THE THERMOPHILIC *ARCHAEOGLOBUS FULGIDUS***

Santiago Agustín Martinez Gache
IQUIFIB

Abstract: The copper (I) ATPase from *Archaeoglobus fulgidus* (*Af* CopA) is an integral membrane protein composed of the following cytoplasmic domains, catalytic (ATP-PN), actuator and two metal binding domains. The independently folding catalytic domain is responsible for the ATPase activity at 70 ^{0}C. We evaluate the thermal inactivation of ATP-PN as a function of the time at several temperatures, through activity, intrinsic fluorescence and circular dichroism measurements. In all the temperatures tested, high amounts of residual secondary structure after the decrease or even full loss of the ATPase activity where found. Comparison between *Af* CopA and his isolated ATP-PN shows more resistance of the former to thermal inactivation than *Af* CopA. Thermal stability was evaluated through bioinformatics and structural analysis of ATP-PN and his orthologs in hyperthemophilic, thermophilic, mesophilic and pscychrophilic microorganisms. Our results show similar hydrophobic, aromatic, glycine and proline amino acid composition among the different microorganisms evaluated. Increased number of charge residue and decrease number of polar residue was observed from psycrophilic to hyperthermophilic. Structural analysis reveals different distributions and numbers of electrostatics and hydrogen bond interactions between the microorganisms evaluated.

Keywords: thermal stability, inactivation, functional, structural, bioinformatics.

(1801) **HIS-TAG β-GALACTOSIDASE IN HETEROGENOUS SYSTEM**

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The *Escherichia coli* β-galactosidase (β-Gal) is a widely used enzyme for basic and applied research. Usually, the enzyme activity is measured in heterogeneous media. In our laboratory, we demonstrated that β-Gal can interact with lipid/water interfaces. In this condition we observed an enhanced catalytic activity, an increase in thermostability and the prevention of β-Gal proteolysis. Currently we are producing a recombinant $\beta\text{-Gal}_{His}$ (6 histidine residues at the carboxy terminus). These additional histidines (His-tag) decreases significantly (circa 50%) the catalytic activity of the protein. On the other hand, in the presence of lipid interfaces an important superactivation (up to 100%) is also observed. Since at the working pH 6.8 an important population of histidine residues are dissociated and conferred additional positive charges to the enzyme, we evaluated the ability of negatively charged interfaces to modulate $\beta\text{-Gal}_{His}$ activity. Interestingly the superactivation exerted by DOPC:DOPG (80:20, molar ratio) MLVs is even greater than that induced by zwitterionic bilayers of pure DOPC. The molecular organization of those lipid mixtures at the air-water interface served as membrane models and were studied through surface pressure–mean molecular area compression isotherms. Monolayers were composed of pure DOPC, pure DOPG or DOPC:DOPG mixtures at different molar ratios (80:20, 60:40, 40:60 and 20:80). Mixtures showed a non-ideal behavior with positive deviation from ideality (repulsive interaction between the components). Our results suggest that, beyond the attractive electrostactic interactions between the positively charged His tag and the negatively charged surface, the higher sensitivity to the negative interface is being favored by the reduction of the surface free energy associated to the $\beta\text{-Gal}_{His}$–membrane binding. This contributes to an increased surface density of $\beta\text{-Gal}_{His}$ at the membrane which translates into an increased specific activity.

Keywors: β-galactosidase, interfaces lipidic, monolayer

STRUCTURAL AND FUNCTIONAL BIOCHEMISTRY 3

(76) **STUDIES ON THE LIPID A STRUCTURE OF *BRUCELLA SUIS* AND *BRUCELLA ABORTUS* LIPOPOLYSACCHARIDE**

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Abstract: Brucellae are Gram-negative bacteria that cause brucellosis, one of the most worldwide distributed zoonosis, transmitted to humans by contact with either infected animals or their products. The lipopolysaccharide (LPS) exposed on the cell surface is considered a major virulence factor of *Brucella,* and it induces a strong antibody response during infection when whole cell vaccines are used. LPSs present three regions with different chemical and biological properties: the lipid A, the core oligosaccharide and the O-antigen. In the last years although some studies go deeper into the structures of the core and the O-antigen of these LPS, no analysis of the lipid A has been performed. In this context, we have analyzed in depth the structure of the lipid A isolated from *Brucella suis* and *Brucella abortus* LPSs. The lipid A moieties were released by acid hydrolysis from both LPS and analyzed by MALDI-TOF mass spectrometry in the positive and negative ion modes, using different matrices. Interestingly, a new feature was detected: the presence of a pyrophosphorylethanolamine residue (PPEtN) substituting the backbone. LID-MS/MS analysis of some of the detected ions allowed to assure a Lipid A structure composed by a diGlcN3N disaccharide, mainly hexa-acylated and penta-acylated, bearing one phosphate and one PPEtN residue. As one mechanism of antimicrobial peptide resistance is to modify the cell surface with positively charged moieties, the finding that *B. abortus* and *B. suis* contain one PPEtN on their lipid A moieties may open new possibilities for exploring into the not fully elucidated set of mechanisms involved in Brucellae virulence that include the ability to escape prompt detection by innate immunity during the initial stages of infection.

Keywords: Brucella, lipopolysaccharide, Lipid A, MALDI-TOF MS.

(502) **INHIBITION BY PRODUCT. A DIFFERENTIAL MODE OF REGULATION BETWEEN FERREDOXIN-NADP⁺ REDUCTASES**

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Ferredoxin-NADP⁺ reductases (FNR) are flavoenzymes involved in biologically relevant processes. FNR catalyzes the reversible electron transfer between NADP(H) and ferredoxin or flavodoxin. The reaction involves a hydride exchange with the pyridine nucleotide and then an electron transfer to or from the other substrate. We have previously identified that *Escherichia coli* FNR is purified with the substrate/product NADP⁺ tightly bound. The nucleotide binding produces an inhibition of the enzyme activity which is lost when NADP⁺ is released. Our findings suggest that this inhibition may have a regulatory function on this bacterial FNR and would implicate a different catalytic mechanism than the one reported for the plastidic enzymes. *E. coli* FNR belongs to one of the three plant-type FNR groups in which these enzymes can be classified. These groups have well-identified structural differences. One of the most relevant is located in the carboxyl terminus of FNR, where NADP⁺ binding and catalysis occur. We have comparatively studied the catalytic properties of FNR from different pathogenic bacteria: *E. coli*, *Pectobacterium carotovorum*, *Brucella abortus* and *Leptospira interrogans*, in order to determine if the phenomenon observed in *E. coli* reductase is a unique process of bacterial FNR. We found that all the aforementioned reductases, except *L. interrogans* FNR, contain NADP⁺ bound. Moreover, kinetic analyses of these enzymes allowed establishing a direct correlation between the ability of high-affinity binding of NADP⁺ and a decrease of their catalytic efficiences. It is important to note that *L. interrogans* possesses a plastidic FNR. We propose that this regulation would be an important aspect of the functionality of the enzymes and, therefore, in the regulation of redox homeostasis in bacteria. From these results, we propose using this high-affinity binding as a differential target for the inactivation of metabolic pathways in which FNR participate in pathogenic bacteria.

Keywords: Ferredoxin-NADP⁺ reductase, inhibition, electron transfer, catalytic mechanism

(505) **MOLECULAR AND BIOCHEMICAL CHARACTERIZATION OF PYRUVATE DECARBOXYLASE IN THE FRUCTOPHILIC NON-*SACCHAROMYCES* YEAST *STARMERELLA BACILLARIS***

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S. bacillaris is a fructophilic non-*Saccharomyces* yeast ubiquitously present in grapevine ecosystems. *S. bacillaris* is a promising species to be used in mixed alcoholic fermentations with *S. cerevisiae* to reduce alcohol content of wines. We have recently proposed that pyruvate decarboxylase (PDC), a key enzyme at the branch-point of the respiratory versus fermentative pathways, may show genetic and phenotypic diversity among grapevine yeasts evolving in different *Vitis* ecosystems. In this work we present our preliminary characterization of the structural gene encoding PDC and the PDC enzyme from *S. bacillaris* strains isolated from *V. vinifera* and *V. labrusca* fermenting grape musts. PDC activities were determined on total cell protein extracts, coupled with alcohol dehydrogenase and measuring the oxidation rate of NADH at 340 nm. Kinetic parameters (i.e., substrate activation, $K_{0,5}$, V_{max} and K_{cat}) of *S. bacillaris* PDC were established on two selected indigenous strains. PDC activity in *S. bacillaris* and *S. cerevisiae* strains, isolated from *V. vinifera* and *V. labrusca* ecosystems, was determined in cells growing in media supplemented with glucose or fructose as carbon sources. Similar PDC activities were observed in *S. bacillaris* cells growing in both media. *S. cerevisiae* cells, on the other hand, showed a much higher PDC activity when cells were grown in media containing glucose versus fructose. These results could highlight the underlying metabolic control of PDC activities in a fructophilic (*S. bacillaris*) versus a glucophilic (*S. cerevisiae*) yeast species. This preliminary study contributes to the recognition of the biodiversity of PDC in wine yeast species, evolutionary adapted to alternative *Vitis* ecosystems.

Keywords: Vitis, yeast, *S. bacillaris*, *S. cerevisiae*, pyruvate decarboxylase

(784) **BIOCHEMICAL AND PHYSIOLOGICAL CHARACTERIZATION OF A 3-METHYLCROTONYL-COA CARBOXYLASE COMPLEX FROM *Xanthomonas***

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The phytopatogen *Xanthomonas axonopodis pv. citri* (Xcc) produce canker, pathology that affects citric. Xcc, as an obliged plant parasite, is unable to survive out of its host for long periods of time. Nevertheless, during the epiphytic survival state, over the leaf, many metabolic and nutritional aspects remain unknown. Branched-chain aminoacids catabolism prevents their over-accumulation, facilitates branched-chain fatty acids biosynthesis and provides energy for the cell. The enzymatic complex 3-methyl-crotonyl-CoA carboxylase (MCC) is essential for leucine degradation pathway. MCC carboxylates 3-methyl-crotonyl, as a substrate, to finally generate acetyl-CoA and acetoacetate, which are latter incorporated into different metabolic pathways. MCC complexes belong to the biotin-dependent acyl-CoA carboxylases (ACCases) group. The objective of this work was characterized a MCC complex from Xcc. We identified by bioinformatics two genes that encode two subunits of an ACCase complex in *Xanthomonas*, and others gram negative bacterial pathogens. We purified the two ACCases subunits, AccC and AccD, by affinity columns and reconstituted the enzymatic activity *in vitro* using different acyl-CoAs as substrate. For this we used two methods, including fixation of radiolabeled bicarbonate and a coupled assay. We determined the kinetic parameters and confirmed that these proteins conform a MCC complex. Furthermore, we had shown, by using a mutant analysis, that this MCC complex is relevant for the survival of Xcc *in planta*.

Keywords: Xanthomomas, Methyl-crotonyl-CoA carboxylases, phytopatogen, leucine catabolism, canker

(878) **LIPOATE BIOSYNTHESIS IS ESSENTIAL FOR *CAENORHABDITIS ELEGANS* DEVELOPMENT**

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Lipoic acid is a universally distributed sulfur containing cofactor. It is derived from octanoic acid and it is essential for the functioning of multienzymatic complexes involved in oxidative and one-carbon metabolism. It is also a potent antioxidant. The ways in which prokaryotes proteins become post-translationally modificated with LA are very well understood. On the contrary, eukaryotes lipoylation pathways are not clearly characterized, even in simple organisms such as yeast. There are several reports of human patients defective in protein lipoylation who died in early life or survived but with severe neurological disorders. Treatment for those patients was just to alleviate symptoms. Our goal is to elucidate how proteins become lipoylated in the nematode *Caenorhabditis elegans* in order to understand the process in humans and develop new therapies. By complementation assays we have already demonstrated that *C. elegans* M01F1.3 protein is the worm lipoate synthase. We also reported that its depletion by interference RNA (RNAi) caused a larval arrest phenotype in the second generation of N2 worms. Here we show that this experiment carried out in a RNAi hypersensitive strain also generates a larval arrest phenotype, but in the first generation ($p<0.01$). Notably, the addition of exogenous lipoate was unsuccessful to restore the normal life cycle. Worms subjected to RNAi treatment that were incubated in presence of Fe (II) exhibited a lower resistance to oxidative stress compared to control animals ($p<0.05$). RNAi experiments were also performed with strains that allow tissue-specific knockdown, and we found that M01F1.3 expression is necessary in neurons for the correct development of worms. These results support that M01F1.3 is essential for the correct development of the worms and suggest that its expression is crucial in neurons.

Keywords: lipoic acid, nematode, RNA interference

(909) **STUDY OF THE SYNTHESIS AND DEGRADATION OF THE CARBON RESERVE POLYMER IN *EUGLENA GRACILIS***

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Euglena gracilis is a fresh water protist able to grow photosynthetically or heterotrophically using a diverse array of organic compounds as the sole source of carbon and energy for growth. *E. gracilis* is a suitable source for the generation of several compounds used for the production of cosmeceuticals, nutraceuticals, food, paramylon and wax esters. When grown aerobically it produces an insoluble β1,3glucan as storage polymer. Synthesis of carbon reserve polymer (paramylon) occurs via UDP-glucose, a key metabolite of the carbohydrate pathways present in most organisms, generated in a reaction catalyzed by UDPglucose pyrophosphorylase (EC 2.7.7.9; UDP-GlcPPase).The enzyme from *E. gracilis* was expressed in *Escherichia coli* cells. The purified enzyme was kinetically characterized, catalyzing UDP-glucose synthesis with a V_{max} value of 3350 U/mg, and affinity for substrates of 0.24 mM and 0.17 mM for glucose1P and UTP respectively. We have also performed, for the first time, confocal microscopy assays to visualize the intracellular location of UDP-GlcPPase.

On the other hand, degradation of paramylon is catalyzed by β-1,3-glucanases. We found several transcripts corresponding to endo-β-1,3-glucanase (EC 3.2.1.39) belonging to different glycoside hydrolases (GH) families. Activity β-1,3-glucanase were measured achieving 0.05 U/mg from soluble fractions of crude extract and 0.03 U/mg from membrane fractions solubilized with detergent. In order to deepen the knowledge in the degradation of the paramylon, we performed the novo synthesis and molecular cloning of two genes coding for two endo-β-1,3-glucanase, one from GH 17 family and the other one from GH 64 family. These genes were used for recombinant expression in *Escherichia coli* cells.These results obtained allow us to improve our understanding about carbon reserve metabolism in *Euglena gracilis* for biotechnological purpose.

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Keywords: Euglena gracilis, paramylon, UDP-glucose pyrophosphorylase, endo-β-1,3-glucanase.

(914) **ENZYMATIC CHARACTERIZATION OF A BETA-D-GLUCOSIDASE FROM *THERMOANAEROBACTER BROKII.***

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Abstract: Beta-glucosidases (EC 3.2.1.21), catalyze the hydrolysis of glycosidic bonds to terminal non-reducing residues in beta-D-glucosides and oligosaccharides, with release of glucose. These enzymes are powerful tools for degradation of lignocellulosic residues in biofuels production, as well as in a number of other biotechnological applications. The gene coding for the β-D-glucosidase (*cglT*) of *T.brokii* was obtained by *de novo* synthesis, after which it was cloned into a pET22b(+) expression vector. The gene was heterologously expressed in Escherichia coli BL21 (DE3) cells and the recombinant enzyme fused to a His-tag (C-term) was chromatographically purified by a His Trap™ IMAC HP column. The purification yield was of 14.7 mg of electrophoretically pure recombinant protein per litre of culture. The pure enzyme was biochemically characterized. Its enzymatic activity was optimal at pH 6.0 and between 65-70°C. The enzyme showed thermal stability, retaining 73% of the activity after incubation at 70 °C during 1 h. The enzyme kinetics was analyzed by using 4nitrophenyl-β-D-glucopyranoside and cellobiose as substrates, obtaining the respective kinetic parameters of V_{max} 63.9 U/mg and 177 U/mg; $S_{0.5}$ 0.10 mM and 1.12 mM. These properties make this enzyme an attractive candidate for being used as a component in a pilot process of enzymatic degradation of lignocellulosic residues to obtain ethanol or hydrogen based bioenergy. Molecular modeling of this protein is underway to determine structure to function relationships useful for the design of specific modification to obtain an enzyme optimized for application in biorefinery processes.

Keywords: Beta-glucosidase, hydrolysis, biofuels.

(933) **ON THE METABOLISM OF GLYCERALDEHYDE-3-PHOSPHATE IN *NITROSOMONAS EUROPAEA***

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Nitrosomonas europaea is a chemolithoautotroph that obtains energy through the oxidation of ammonia to nitrite in the presence of oxygen and fixes CO_2 through the Benson-Calvin cycle (BCc). In the BCc, ATP and NADPH are utilized to reduce 3P-glycerate (3PGA) to glyceraldehyde-3-phosphate (Ga3P); this being opposite to what occurs in glycolysis, where Ga3P is oxidized to 3PGA to produce energetic or reductive power. In this metabolic enclave, Ga3P dehydrogenases (Ga3PDHases) play a critical role. In plants, four Ga3PDHases were identified: GAPA/B (EC 1.2.1.13, chloroplastidic), GAPC (EC 1.2.1.12; both, plastidic or cytosolic) and GAPN (EC 1.2.1.9, cytosolic). Based on sequence homology we identified in the *N. europaea* genome the genes *Neu0327* and *Neu2000* as coding for putative GAPC and GAPN, respectively. To further explore the probable role of these two genes in triose-P metabolism in the bacterium we cloned and expressed them in *E. coli*; then we purified the proteins and characterized them kinetically. *Neu0327* expression produced a typical GAPC like Ga3PDHase, catalyzing the reversible oxidation of Ga3P to 1,3bisP-glycerate, using specifically NAD^+ as cofactor and with ~6-fold higher activity in the glycolytic direction of reaction. On the other hand, the protein product of *Neu2000* irreversibly oxidized Ga3P to 3P-glycerate using NAD^+ or (preferentially, with 33-fold higher catalytic efficiency) $NADP^+$, thus resembling a GAPN enzyme. However, the activity of the latter with Ga3P was negligible when compared to that assayed using succinate semialdehyde (SSA) as a substrate; which suggests that *Neu2000* codes for an SSA dehydrogenase (EC 1.2.1.16, SSADHase). Altogether, our results support a scenario for *N. europaea* where only one NAD^+/NADH dependent Ga3PDHase is involved in the metabolism of triose-P (via the BCc as well glycolysis/gluconeogenesis); while the protein product of *Neu2000* would preferentially act as an SSADHase with low potential to have a GAPN function.

Key words: glyceraldehyde-3-phosphate, succinate semialdehyde, *Nitrosomonas europaea*, Benson Calvin cycle, glycolysis.

(1465) **RE-ROUTING GLUCOSAMINE-1P FATE TOWARDS ALTERNATIVE ENZYMATIC STEPS IN *Rhodococcus jostii***

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The amino sugar glucosamine (GlcN), 2-amino-2-deoxy-D-glucose, is an essential component of glycosaminoglycans and glycoproteins. Together with *N*-acetylglucosamine (GlcNAc) are among the most prevalent sugars in the biosphere and a major component in cell envelopes of fungi and bacteria. Consistent with the ubiquity of these sugars, pathways for their metabolism (including catabolic and anabolic processes) are conserved in bacteria. GlcN is biosynthesized from fructose6-P and is further acetylated to GlcNAc. The balance between GlcN and GlcNAc is essential for bacterial growth and involves GlcN-1P as a mere intermediary. Curiously, we recently discovered that GlcN-1P is efficiently used as a substrate by enzymes related to glycogen metabolism in *Rhodococcus jostii*. ADP-glucose pyrophosphorylase (ADP-GlcPPase, EC 2.7.7.27) activates Glc-1P to ADP-Glc which acts as glucosyl donor for glycogen synthase (GlgSase, EC 2.4.1.21). GlcN-1P is a substrate of ADP-GlcPPase (mainly after activation), which represents a unique feature among this enzyme from different organisms. Also, the actinobacterial GlgSase uses ADP-Glc and Glc-1P to synthesize maltose-1P as a key metabolite in these organisms. We found that a *R. jostii* GlgSase accepts GlcN1P (but not other hexose-1P) to the same extent that Glc-1P. The fact that the two enzymes catalyzing consecutive metabolic steps in the classical GlgAC pathway are able to efficiently utilize GlcN-1P suggest a role for the amino sugar different than the routing towards GlcNAc synthesis in *R.jostii*. Even more, we found that one of the annotated UDP-GlcPPases (EC 2.7.7.9) reacts 10-fold faster with GlcN-1P than Glc-1P supports the above assertion. Results presented in this work revisit GlcN metabolism in this biotechnological relevant organism. In addition, they are an important source to study the evolution of carbohydrate related enzymes and, most importantly, a source for in vitro synthesis of new compounds.

Keywords: Promiscuity, glycogen, carbohydrates, hexoses-1P partitioning, alosterism

(1805) **AN ENDOGENOUS GALECTIN EXPRESSED BY ADIPOSE TISSUE PROMOTES GLYCAN-DEPENDENT ANGIOGENESIS**

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The continuous advance of metabolic syndrome and its associated pathologies (type 2 diabetes and cardiovascular disease) brought adipose tissue (AT) research back to the spotlight. In response to caloric excess, adipose tissue remodeling leads to chronic inflammation and persistent stress. In turn, these phenomena result in adipocyte hypertrophy, increased angiogenesis, immune cell infiltration, and extracellular matrix overproduction. The mechanisms and clinically relevant pathways underlying systemic chronic inflammation associated with obesity are not completely understood, Galectin-12 (Gal-12) is a tandem-repeat member of the galectin family, preferentially expressed in lipid droplets of adipocytes, with known ability to suppress lipolysis. To further understand its biological significance, we have cloned and expressed recombinant murine Gal-12 (mGal-12) in *Escherichia coli*, biochemically characterized its lectin activity and identified with preferential affinity towards Lewis X structures. Here we demonstrated the pro-angiogenic role of this lectin in transwell migration assays using human endothelial cells (HUVEC), an effect which was prevented by competition with specific saccharide ligands. Expression of m-Gal12 was stimulated in 3T3L1 adipocytes by hypoxic microenvironments. Altogether, these data indicate that mGal-12 not only regulates adipogenesis but also angiogenesis in adipose tissue, highlighting a new potential target for development of specific therapies for obesity-related disorders.

Keywords: adipose tissue, Galectin-12, angiogenesis, Lewis X, hypoxia.

ONCOLOGY-ONCOIMMUNOLOGY 7

(190) **DOWN-REGULATION OF GALECTIN-8 AND ITS LIGAND ALCAM ("*ACTIVATED LEUKOCYTE CELL ADHESION MOLECULE*", CD166) REDUCES *IN VIVO* TUMOR GROWTH IN A MURINE MODEL OF BREAST CANCER.**

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Galectin-8 (Gal-8) is a '*tandem-repeat*'-type galectin, with two carbohydrate recognition domains. We previously showed that the *activated leukocyte cell adhesion molecule* (ALCAM), which is a cell adhesion molecule, serves as a Gal-8 ligand. The aims of this work were: 1) To analyze cell surface effects of the Gal-8/ALCAM axis on adhesion and migration of human MDA-MB-231 breast cancer cells; 2) To investigate Gal-8 and ALCAM effects on cell proliferation and spheroid formation; 3) To evaluate Gal-8 and ALCAM effects in a murine model of breast cancer. We established MDA-MB-231 cell lines in which Gal-8 (MDA-shGal8) and/or ALCAM expression (MDA-shALCAM) were stably knocked-down with specific shRNA; scrambled-shRNA was used for controls (MDA-shControl). Our results showed that cell adhesion onto immobilized Gal-8 was significantly reduced after ALCAM silencing in MDA-shALCAM (26.3±7.0%) and MDA-shALCAM-shGal8 (25.6±6.8%) as compared to MDA-shControl cells (100%), $p<0.05$. In migration assays onto immobilized Gal-8, wound-closure percentages were significantly reduced after ALCAM silencing in MDA-shALCAM (23.2±1.3%) and MDA-shALCAM-shGal8 (22.8±1.2%) as compared to MDA-shControl cells (40.8±2.5%), $p<0.001$. ALCAM down-regulation, but not Gal-8 silencing, significantly reduced cell proliferation by MTT (MDA-shALCAM: 0.39±0.19 and MDA-shALCAM-shGal8: 0.38±0.03 versus MDA-shControl cells: 0.48±0.31, $p<0.001$) and spheroid volume (80%, $p<0.001$). Tumor growth in *nude* mice indicated that tumors generated by MDA-shALCAM, MDA-shGal8 and MDA-shALCAM-shGal8 cells were significantly smaller than those produced by MDA-shControl cells at 60 days post-inoculum ($p<0.05$); tumors derived from MDA-shALCAM-shGal8 (291.2±89.8 mm^3) showed smaller volume than those from MDA-shALCAM cells (1075.8±388.9 mm^3) ($p<0.05$) at 90 days. Our results demonstrate that there are ALCAM-Gal-8 interactions at the cell surface, and that depletion of Gal-8 and ALCAM delays primary tumor growth.

Keywords**:** Galectin-8, ALCAM, breast cancer.

(1188) **FIRST EVIDENCE OF CHANGES IN THE TCRB REPERTOIRE FROM A CUTANEOUS MELANOMA PATIENT IMMUNIZED WITH THE CSF-470 VACCINE**

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We have developed the allogeneic vaccine CSF-470, tested in a Phase-II study for post-surgery adjuvant treatment in stage IIB-III patients (CASVAC-0401, NCT01729663). The aim of this work was to analyze the peripheral and tumor-associated immune infiltrate and the TCRβ immune repertoire in a cutaneous melanoma patient (#006) immunized with the CSF-470 vaccine that developed a cutaneous metastasis at the end of the 2-year protocol. *Methods:* PBMC at 0, 12, 24 and 48 months after protocol start, primary tumor and metastasis biopsies were obtained. Peripheral and tumor-associated immune populations were analyzed by flow cytometry and immunohistochemistry. CDR3-TCRβ sequencing of PBMC and metastasis was performed by NGS. *Results:* from immunization, a sharp increment of NK cells followed by an increase in $CD4^+$ and $CD8^+$ cells by the end of the protocol were detected in blood. Analysis of pre- and post-CSF-470 vaccine biopsies revealed a marked increase in $CD8^+$, $CD4^+$ and $CD20^+$ lymphocytes, with low Tregs. Regarding the TCRβ immune repertoire in blood throughout CSF-470 immunization, an increase in diversity and clonality was observed, as shown by a shift with expansion of selected pre-existing and new arising clones while diminution of others (n=125836). In tumor infiltrating lymphocytes (n=1382), the prevalent clones were the ones which were expanded after CSF-470 immunization, both new and pre-existing ones (50%). These clones persisted in time, since 2-years after completing immunization, 51% of the clones present in the resected metastasis were still detected in blood. *Conclusion:* this is the first report of the TCRβ repertoire from a cutaneous melanoma patient immunized with the CSF-470 vaccine. The immune population changes observed in peripheral blood as well as in the local compartment following immunization of this patient suggest that the CSF-470 vaccine induced a specific anti-tumor adaptive immune repertoire that reached the tumor lesions.

(1758) **IMMUNE CELLS ASSOCIATED TO LUNG METASTASIS CAN BE MODIFIED BY THE CONSUMPTION OF A PROBIOTIC FERMENTED MILK**

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Lung metastases (MTS) are common from breast cancers. Immune cells associated to MTS can help tumor cell growth or can response against them. Previous studies showed that milk fermented by probiotic *Lactobacillus casei* CRL431 (PFM) modulated the immune response in mice bearing breast tumor and decreased tumor growth and MTS. The aim of this study was to evaluate the effect of PFM (administered after tumor surgery) against immune cells associated to lung MTS. Female BALB/c mice were injected 4T1 cells to induce breast tumors and when tumors reached volume near to $0.3cm^3$ were surgically removed. After that, mice were divided into 2 groups: one received PFM and the other, milk (M). Healthy mice given milk were used as control (C). Mice were euthanized (60d after tumor injection); the lungs were removed for histology and for the isolation of immune cells. Cells were cultured to measure cytokine releases after the in vitro stimulation with 4T1 cells or they were analyzed by flow cytometry to evaluate macrophages and $CD4^+$ and $CD8^+$ T lymphocytes. PFM administration diminished the nodes in the lungs compared to M group. Cells from lung of PFM group decreased significantly ($p<0.05$) the concentration of IL-10, TNFα and IL-6 (71.3±0.3; 363.7±20.2; 766.07±100) in culture supernatants compared to M group (118.4±6.5; 489.4±40; 2899.9±300). Macrophages decreased significantly ($p<0.05$) in PFM group (26.6%±3.6) compared to M group (50.5%±16.8), maintaining percentages similar to C group (27.5%± 2.14). PFM group showed the lowest percentage of $IL\text{-}10^+$ macrophages. The percentages of $IL\text{-}12^+$ macrophages and $CD8^+$ cells were similar in both PFM and M group. The highest percentages for $CD4^+$ and $CD4^+CD8^+$ cells were observed in PFM group. Conclusion: Consumption of PFM after tumor surgery decreased lung MTS by modulating the immune response in the lungs. The decrease of macrophages has a key role in this modulated response, especially through the reduction of IL-10 producing macrophages.

(911) **IMMUNE-CHECKPOINT INHIBITORS AND ANTI-TUMOR VACCINES SUPPORT THE IMMUNOSTIMULATORY THEORY OF CANCER**

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Immune-checkpoint inhibitors and antitumor vaccines produce tumor-inhibitory and stimulatory effects on growing tumors depending on the stage of tumor growth at which treatment was initiated. These paradoxical results can be understood by the immunosurveillance postulates but might be explained by the Immune Stimulatory Theory of Cancer, that was originally proposed on the basis that the immune response (IR) induced by immunogenic murine tumors was not monotonic but biphasic, with strong IR producing inhibition and weak IR inducing stimulation of tumor growth. In a previous work we have demonstrate that most spontaneous murine tumors (ST) studied grow in an accelerated way in pre-immunized hosts and more in immunodepressed mice; that the interaction of specifically-immune T cells and target tumor cells at low stimulatory ratios enhanced the production of rantes and MIP1α and when Winn tests were carried out in indomethacin-treated tumor bearing mice or TLR4KO tumor bearing mice, the stimulatory effect was not observed. We extended those observations to understand better the mechanism. We observed in ST an increase of PDL1 and an activation of TLR4 and p38 signaling pathways, which recruit more macrophages and other inflammatory cells that would produce pro-inflammatory cytokines, TNFα ($p<0.02$); IL1β ($p<0.01$) and IL6 ($p<0.02$), leading to an accelerated tumor growth ($p<0.05$). At medium and large-sized tumors, different anti-tumor immunological schedules were attempted therapeutically against a strongly-immunogenic MCC (low PDL1) and a weakly-antigenic LB (high PDL1): a) *Vaccines and immune-depressors p<0.05*, b) antiCTLA4 and/or antiPDL1 $p<0.01$, and c) *Counteraction of the tumor-immunostimulatory effects* (indomethacin or anti-p38), $p<0.01$. This produced a significant inhibition of tumor growth. These results were encouraging and could be an interesting contribution to the use and management of different types of immunological treatments against the tumor. **Key words:** murine tumors, immunostimulatory theory, immunosurveillance, anti-tumor vaccines, immune-checkpoints inhibitors.

(842) **IMMUNOMODULATORY ROLES OF HISTAMINE H4 RECEPTOR IN BREAST CANCER**

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Histamine regulates the growth and development of different types of tumors. Although the role of histamine H4 receptor (H4R) in immune cells is extensively investigated, its immunomodulatory function in cancer is completely unknown. This study aimed to investigate the role of H4R in antitumor immunity in a model of triple negative breast cancer (TNBC). We evaluated growth parameters, histological characteristics of tumors and the composition of tumor, splenic and tumor draining lymph nodes (TDLN) immune subsets in a syngeneic model TNBC developed orthotopically with 4T1 cells in H4R knockout (H4R-KO) and wild-type (WT) mice.

Mice lacking H4R show reduced tumor size and weight (1.6±0.2 g vs. 0.9±0.1 g, P=0.003) and decreased number of lung metastases. Tumors of H4R-KO mice exhibit a more differentiated histopathological pattern (grade 2) compared to WT mice (grade 3, highly aggressive potential) and display a significant decrease in the mitotic index

and angiogenesis. H4R-deficency is associated with a decrease in the percentage of $CD4^+$ tumor infiltrating T cells ($p<0.001$) and an increase of $CD19^+$ lymphocytes ($p<0.01$) and NK cells ($p<0.05$). A positive correlation was detected between the percentage of $CD8^+$ infiltrating T cells and tumor weight ($R=0.8924$, $p<0.05$) while MDSC negatively correlated with tumor growth ($R= -0.8862$, $p<0.05$) just in H4R-KO mice. Likewise, TDLN of H4R-KO mice show decreased CD4+ T cells ($p<0.05$), but increased percentage of NK cells ($p<0.05$). Tumor weight was negatively correlated with NK cells ($R=-0.8874$, $p<0.05$) as well as B lymphocytes ($R=-0.8604$, $p<0.05$), but positively correlated with CD8+ T cells ($R=0.7730$, $p<0.05$) in TDLN of H4R-KO mice.

These results suggest an interplay between H4R expressing immune cells in tumor microenvironment and cancer cells, which has implications in breast cancer progression.

Keywords: histamine H4 receptor, breast cancer, antitumor immunity

(935) **IMMUNOTHERAPY BASED ON THE TLR3 LIGAND POLY A:U MODIFIES THE TUMOUR-ASSOCIATED MYELOID COMPARTMENT UPREGULATING PDL1 SPECIFICALLY ON $MHCII^-$ MONOCYTES ON A TYPE I IFN-DEPENDANT MANNER**

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Poly A:U (pAU) is a synthetic dsRNA which signals through TLR3 to modulate an immune response by inducing the release of type I IFNs on a broad range of cells including cancer cells and tumour-associated myeloid populations. Relying on this, pAU can be exploited as an immune-adjuvant on cancer therapy to improve an antitumor immune response. In this work, we evaluated the effect of pAU treatment on a murine B16 melanoma cancer model focusing on the myeloid compartment, including tumour-associated macrophages (TAMs - $CD45^+$; $CD11b^+$; $F4/80^+$; $LyC6^-$; $CD24^-$), tumour-infiltrating DCs ($CD45^+$; $CD11b^+$; $CD24^+$; $CD11c^+$; $MHCII^+$), together with tumour-associated monocytes ($CD45^+$; $CD11b^+$; $Ly6C^+$; $CCR2^+$) which can be divided into MHCII+ and MHCII- monocytes. We demonstrated that a three-dose regime of intratumoral treatment with pAU (100µg/tumour) significantly decreases tumour size from 0.33g to 0.15g ($p<0.05$) compared to PBS-treated mice. Within tumour-infiltrating leukocytes, plasticity and cell polarization of TAMs are key aspects to define a final antitumoral (M1-like) or protumoral (M2-like) outcome and here we observed that pAU significantly decreases the total number of M1-like macrophages ($MHCII^{high}$; CD206-) and M2-like macrophages ($MHCII^{low}$; CD206+) the latter being the population accounting for most of the intratumoral IL10 production as seen by FACS. We determined that both MHCII+ and MHCII- monocytes were a key source of intratumoral TNFα, which was upregulated after pAU treatment ($p<0.05$). Strikingly, when we evaluated the expression of PDL1, expressed by APCs and tumour cells, we observed a significant upregulation ($p<0.05$) of this ligand specifically on MHCII- monocytes after the treatment with pAU and very little impact on other tumour-infiltrating cells. Finally, most of the effects described after pAU administration on the myeloid compartment were lost in IFNAR-/- mice, suggesting that pAU-induced type I IFNs directly modulate myeloid cells in the tumour.

Keywords: Immunotherapy, TLR3, PDL1, Monocytes

(619) **IMPACT OF BRAF INHIBITORS ON THE IMMUNE MICROENVIRONMET IN MELANOMA**

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Abstract: BRAF is mutated in 50% of melanoma patients. BRAF is a component of the RAS/RAF/MEK/ERK pathway and BRAF or MEK inhibitors increase progression-free and overall survival in BRAF- mutant patients. However, most patients relapse with acquired resistance and ~20% of patients present intrinsic resistance. Preclinical and translational studies have shown that targeting the RAS/BRAF/MEK/ERK pathway has effects on the expression of immunomodulatory pathways. Macrophages are key players within the tumor microenvironment and their functional profiles control responses to a variety of therapies. Whereas M1 macrophages efficiently present antigens to T cells and promote tumor destruction, M2 macrophages promote tumor progression, immune evasion and angiogenesis. Most patients who develop resistance to targeted therapies derive little benefit from anti-CTLA-4 and anti PD-1 based immunotherapies. Our preliminary findings based on glycophenotyping, transcriptomics data analysis, western blotting and functional studies show changes in the galectin/glycan axis in melanoma resistance to BRAF inhibitors (BRAFi). Particularly we found in patient tumor biopsies collected prior to and on/following treatment with the BRAFi vemurafenib that M2 macrophage density (as defined by CD163+/CD68+) was increased after treatment ($p=0.02$, $n=7$). Initial *in vitro* data supports a direct interaction between tumor cells and peripheral blood monocytes, with BRAF inhibitor-resistant cells driving monocyte differentiation towards a CD163+ phenotype. Moreover, the glycosylation profile of BRAFi resistant cells show an increase in asialo core 1 O-glycans and augmented levels of polylactosamines. Additionally, resistance to BRAFi decreases α(2,6) sialylation levels ($n=3$, $p<0,05$). We hypothesize that there are specific galectin/glycan interactions that could be the drivers of an M2-macrophage immunosuppressive microenvironment in the resistant tumors preventing response to immunotherapy treatment.

Keywords: melanoma, macrophages, glycobiology, immunotherapies, targeted therapies.

(1418) **NON-NEURONAL CHOLINERGIC SYSTEM MODULATE THE CROSS-TALK BETWEEN THE IMMUNE SYSTEM AND GLIOBLASTOMA CELLS**

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Glioblastoma multiforme (GBM) is the deadliest and most common type of human primary brain tumor. This tumor is defined by the hallmark features of uncontrolled cellular proliferation, diffuse infiltration, robust angiogenesis, resistance to apoptosis and genomic instability. Acetylcholine is a neurotransmitter which can also modulates cell survival, proliferation and differentiation in neuronal and non-neuronal cells such as immune cells, which has been referred to as a "non-neuronal cholinergic system". The aim of this work was to elucidate the relevance of the non-neuronal cholinergic system in the interaction between immune and GBM cells. We first evaluated the expression of acetylcholine receptors in human GBM cell lines by fluorescence microscopy. We found that both U251 and U373 human GBM cells express acetylcholine muscarinic receptors M1 and M3. In order to evaluate whether the cholinergic system affects the cross-talk with immune cells, human U251 cells were co-cultured

with human dendritic cells (DC) in the presence of cholinergic agonists (carbachol and muscarine). Mononuclear cells were isolated from buffy coats of healthy adult nonsmoker volunteer and CD14+ cells were then isolated by positive selection and then were cultured with GM-CSF and IL-4. The co-cultures were incubated in the presence of carbachol 10^{-8} M) and muscarine (10^{-8}M). We found that U251 cells upregulated the expression of CD86 in DCs as assessed by flow cytometry in presence of carbachol and muscarine with respect to control co-cultures ($p<0.05$). Human U251 and U373 GBM cells were cultured in presence of a cholinergic agonist (carbachol) to evaluate their expression of the coactivation marker ligand OX40 ligand (OX40L) as assessed by flow cytometry, but no differences were observed between the cells treated with carbachol and controls. Conclusions: our findings suggest that the non-neuronal cholinergic system is present in GBM cells and could modulate their cross-talk with the immune system.

(142) **ROLE OF RET RECEPTOR TYROSINE KINASE AT THE POST-LACTATIONAL TRANSITION, A WINDOW OF NORMAL MAMMARY GLAND DEVELOPMENT WITH HIGH INFLAMMATION AND CANCER POTENTIAL**

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Under the influence of hormones, the mammary gland undergoes repeated cycles of proliferation, differentiation at lactation, and regression to involution, a process through the gland reverts back to virgin females. Those changes permanently alter the morphology/molecular characteristics of the breast and lead to important, yet poorly understood changes in cancer risk. Her2, a member of the receptor tyrosine kinases (RTK) family is well known to promote aggressive breast cancers. We have reported that Ret, another RTK member, is overexpressed in about 40% of human tumors. It is well known that RTKs, e.g. the Her2, also have roles in normal breast, but nothing is known about the function of Ret. We found that Ret is highly expressed in the mouse glands during mid-lactation. Blocking Ret activity in lactation by in vivo administration of Ret inhibitor reduces pup size, suggesting its potential role in this period.

We generated a mouse strain (Ret/MTB) with the MMTV mammary gland specific promoter controlling Ret expression in an inducible system, allowing induction of Ret by feeding mice with doxycycline. We found that abnormal Ret overexpression outside of the lactation, leads to the development of tumors that recapitulates human luminal carcinoma, with an inflammatory component and an active Stat signaling (phospho-Stat1/3). Since Ret expression can be turned-on in an inducible manner, we used this model to express Ret at different developmental stages. Indeed, Ret appears to have a role in the post-lactation transition to involution. When Ret is induced early in lactation we observe enhanced kinetics of involution. The involution period is well known to drive cancer progression. By RNA-seq we found that Stat signature is increased in Ret-overexpressing glands, which was confirmed by several techniques. Thus, our results suggest that if Ret expression is deregulated during the post-lactational transition this might contribute to breast cancer development.

Keywords: tyrosine kinase receptor, mammary gland, post-lactation, cancer

(1787) **"TLR2 STIMULATION PROMOTES AUTOPHAGY AND MODULATES FLUDARABINE-INDUCED CELL DEATH IN CHRONIC LYMPHOCYTIC LEUKEMIA CELLS"**

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Chronic Lymphocytic Leukemia (CLL) is a disease characterized by the clonal proliferation and accumulation of mature, typically CD5-positive B-cells within the blood, bone marrow, lymph nodes, and spleen. Leukemic transformation is initiated by alterations that impair apoptosis of clonal B-cells and the pathways engaged in programmed cell death involve several Bcl-2 family proteins. It has been described that Bcl-2–family proteins may regulate autophagy. This degradative pathway has dual role in cancer depending of the type of tumor. Therefore, autophagy can be exploited for promote survival, or cell death, as well. Whereas autophagy can be regulated by Toll like receptors (TLRs), and these receptors participate in the CLL progressive pathogenesis, we hypothesize that TLR2 activation modulate autophagy in CLL cells. This effect may influence the expression of genes and proteins involved in CLL pathogenesis. We analyzed LC3B expression in peripheral blood mononuclear cells isolated from CLL-patients. Pam3CSK4 (TLR2 ligand) induced increased LC3B II expression in CLL cells and this effect was potentiated by co-stimulation with Pam3CSK4 plus Fludarabine. Interestingly, Pam3CSK4 modulated CLL cell death induced by Fludarabine.

On the other hand, MDP (ligand for the innate immunity receptor NOD2) induced similar effects on CLL cells.

These preliminary results suggest that innate receptors may affect autophagy and leukemia cell survival.

Keywords: Toll like receptors; autophagy; Chronic Lymphocytic Leukemia

ONCOLOGY-ONCOIMMUNOLOGY 8

(298) **ANTI-TUMOR ACTIONS OF PACLITAXEL PLUS CARBACOL ON HUMAN TRIPLE NEGATIVE BREAST CANCER CELLS**

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Abstract: The administration of low doses of cytotoxic drugs alone or combined with repurposing drugs scheduled with short inter-dose intervals is called metronomic therapy (MT). MT is a new strategy in cancer treatment, since it exhibits high effectiveness and low incidence of side effects. The most aggressive type among human breast tumors is triple negative (TN), probably due to the absence of a specific target for pharmacological treatment. Here, we analyzed the effect of a combination of subthreshold concentrations of carbachol (CARB) (10^{-12}M) with paclitaxel (PX) (10^{-8}M) on MDA-MB231 cell line derived from a TN breast cancer patient during 40 h. By MTT assay we observed that the addition of 10-6M PX, a concentration similar to that used in conventional chemotherapy increased tumor cell death by 40±2% ($p<0.001$ vs. control) but also increased by 33±2% ($p<0.001$ vs. control) cell death in non-tumorigenic mammary cells MCF-10A. On the other hand, the combination of CARB with PX increased cytotoxicity by 27±3% ($p<0.01$ vs. control) without affecting MCF-10A viability. In addition, CARB plus PX reduced MDA-MB231 tumor-induced neovascularization (N°vessels/mm^2 skin) in nude mice skin (skin: 3.17±0.05; tumor: 4.14±0.23 $p<0.001$ vs. skin; tumor+CARB+PX: 3.16±0.35 $p<0.001$ vs. tumor) and the expression of vascular endothelial growth factor-A in tumor cells ($p<0.05$). Using a xenogeneic model, nude mice were inoculated with MDA-MB231 tumor cells ($3x10^6$) in the left flank and after tumor palpation, animals were treated with CARB plus. PX (i.p.). The combination significantly reduced tumor growth in relation to control (untreated mice; $p<0.001$). These results suggest that CARB plus PX at low doses could be a new strategy to treat TN breast tumors in humans.

Keywords: cancer; metronomic-therapy; angiogenesis

(1522) **ANTITUMORAL AND ANTIMETASTATIC ACTIVITY OF MAITAKE D-FRACTION IN TRIPLE-NEGATIVE BREAST CANCER CELLS.**

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D-Fraction is a proteoglucan extracted from *Grifola frondosa* (Maitake) mushroom. Previously, we reported that D-Fraction decreases breast cancer (BC) cell viability regardless of hormone receptors and HER2 status of cells. Furthermore, D-Fraction reduces tumor burden and lung metastases in a murine model with hormone-independent LM3 cells. In triple-negative (TNBC) MDA-MB-231 cells, we also demonstrated that D-Fraction decreases their migration and invasion capacity. The purpose of the current study is to identify the cellular and molecular mechanisms by which D-Fraction decreases the migratory/invasive potential of MDA-MB-231 cells. In addition, we propose to evaluate the antitumoral effect of D-Fraction in 4T1 cells, another cell line representative of TNBC subtype. By western blot, we found that D-Fraction increases E-cadherin expression in MDA-MB-231 cells compared to vehicle treatment ($p<0.05$). By immunofluorescence, we detected that D-Fraction decreases the presence of β-catenin in the cytoplasm/nucleus ($p<0.001$) and promotes its membrane localization ($p<0.01$). Also, we found that D-Fraction increases the adhesion of MDA-MB-231 cells to substrate ($p<0.05$). By zymography, we detected that D-Fraction decreases MMP-2 and MMP-9 activity by 53.59 % ($p<0.001$) and 27.31 % ($p<0.05$) respectively, compared to vehicle treatment. On the other hand, manual cell counting and WST-1 assay were performed in TNBC 4T1 cells. D-Fraction decreases the viability of 4T1 cells in a dose- and time-dependent manner ($p<0.05$). Wound healing assay demonstrated that D-Fraction decreases the migratory capability of 4T1 cells ($p<0.001$). By transwell Matrigel assay, D-Fraction reduces the invasive capability of these cells ($p<0.001$). In conclusion, our results suggest that D-Fraction decreases the viability and metastatic potential of TNBC cells: promoting an epithelial phenotype; reducing the capability of tumor cells to degrade extracellular matrix and increasing cell-substrate adhesion.

Keywords: D-Fraction, Maitake, triple-negative breast cancer, antitumoral, antimetastatic.

(1332) **CDC42 REVERTS AGGRESSIVENESS OF A TRIPLE NEGATIVE BREAST CANCER CELL LINE TROUGH METHYLATION OF ID4 PROMOTER**

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Breast cancer constitutes a group of diseases characterized by different morphologies and biological behaviors. Molecularly they can be classified as luminal A, luminal B, human epidermal growth factor receptor 2, and triple-negative breast cancers (TN); lacking ER/PR/HER2. Clinically, the TN subtype has an aggressive nature, higher rates of relapse and shorter overall survival. TN tumors affect younger patients and are more prevalent in African-American and Latin women. Cdc42 is a plasma membrane-associated small GTPase which is involved in the regulation of several cellular functions and its expression is dysregulated in several tumor types. Here we show that Cdc42 overexpression reduced TN aggressive phenotype through the methylation of ID4 (inhibitor of Differentiation) promoter. Briefly, MDA-MB321 cell lines were transfected with a Cdc42-GFP vector and afterwards ID4 methylation status was measured by droplet digital and conventional Methyl Specific PCR. MS-MLPA assay revealed that ID4 methylation increased significantly in the Cdc42 transfected cells and interestingly, the methylation values of other genes remained unchanged. WB assay revealed that ID4 protein expression decreased after cdc42 transfection. Since ID4 inversely regulates BRCA1 expression in breast tumors, we evaluated BRCA1 status after transfection. Our results show that BRCA1 protein expression increased in the Cdc42 transfected cells. Migration and Apoptosis assays by wound healing and flow cytometry determined that the transfected cells migrated less ($p<0,001$) and had increased apoptosis ($p<0.05$), in line with a reduction in aggressive behavior. In silico studies from TCGA data revealed that Cdc42 expression was significantly associated with the expressions of: HDAC3, MBD2, MBD1 and YY1. Remarkably, all these genes participate in the regulation of gene transcription. Taken together our results show that cdc42 induces ID4 methylation and reverts aggressiveness in a TN cell line.

Key words: Triple negative breast cancer; Cdc42; Methylation; ID4

(1857) **CROSS-TALK BETWEEN ANDROGEN RECEPTOR AND ErbB-2 SIGNALING PATHWAYS IN TRIPLE-NEGATIVE BREAST CANCER**

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Triple negative breast cancer (TNBC) encompasses tumors without clinically significant levels of estrogen/progesterone receptors and membrane ErbB-2 (MErbB-2) overexpression or gene amplification. TNBC tumors have poor prognosis and neither established biomarkers nor therapeutic targets. On the one hand, we and others have shown that MErbB-2 (tyrosine kinase receptor) migrates to the nucleus of BC cells (nuclear ErbB-2, NErbB-2) where it binds to promoters/enhancers of target genes to regulate BC proliferation and migration. Interestingly, we have previously shown that NErbB-2 is required for in vitro and in vivo TNBC growth. On the other hand, several reports have proposed the androgen receptor (AR), another member of the steroid receptor superfamily, as a new target in TNBC. AR is expressed in 10-53% of TNBC and was proved to be critical for BC proliferation. We and others have shown a functional interplay between growth factors and steroid hormone receptors signaling pathways in BC. We propose the existence of a cross-talk between AR and ErbB-2 signaling pathways which regulates the expression of genes involved in TNBC growth. The experimental model used was the human TNBC cell line MDA-MB-453 which displays high expression levels of AR and NErBb-2. By Western Blot, we evidenced that dihydrotestosterone (DHT) treatment for short times (minutes) induced tyrosine phosphorylation of ErBb-2 (at residues 877, 1221/1222 and 1248). By confocal microscopy, we observed that DHT also induced ErBb-2 and AR nuclear translocation and co-localization. Finally, both DHT and HRG (heregulin, one of the ErbBs' ligands) up-regulated Erk5 protein levels, an ErBb-2 target gene that we have previously shown to be involved in BC proliferation. Our findings evidence that DHT-activated AR induces ErbB-2 rapid activation, nuclear translocation and co-localization, suggesting a functional cross-talk between both receptors which drives Erk5 regulation.

Keywords: androgen receptor, NErBb-2, cross-talk, TNBC.

(1794) **SUBCELLULAR DISTRIBUTION OF BETA-CATENIN IN CISPLATIN-TREATED TRIPLE NEGATIVE BREAST CANCER CELLS**

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(1) IMBECU. (2) QUIANID.

Triple negative breast cancer subtype (TNBC) accounts for about 15-20% of all invasive breast tumors. TNBC lacks expression of estrogen receptor (ER), progesterone receptor (PR) and Epidermal Growth Factor type 2 Receptor (HER2). To date not a single therapy has been applied, conventional cytotoxic chemotherapy is currently the only treatment option for TNBC. Unfortunately, some patients respond but others become resistant or eventually relapse. New therapeutic approaches are focused in the search of other molecular target and anticancer drugs not classically used for breast cancer treatment, such as platinum analogues (cisplatin). Aberrant expression of beta-catenin has been linked to tumor progression and has become a promising target for cancer therapy. The aim of this work was to study the functionality of beta-catenin exposed to cisplatin in human cell lines, MDA-MB231 (TN) and MCF10A (non-

tumorigenic epithelial cell line). Cisplatin toxicity was evaluated by MTT. The localization of total and active beta-catenin was analyzed by confocal microscopy and the expression levels by WB. The IC50 doses for MDA-MB231 and MCF10A were 91,48μM and 55,80μM, respectively. Cisplatin treatment induced localization of total and active beta-catenin from the plasmatic membrane/cytoplasm to the nucleus in MDA-MB231, but no changes were observed in MCF10A cells. Expression levels of total and active beta-catenin decreased significantly ($p \leq 0,05$) with cisplatin exposure in MDA-MB231 cells whereas the levels of these proteins were not significantly modified in MCF10A cells. Our preliminary data show altered subcellular localization and expression levels of beta-catenin by cisplatin in TNBC cells.

Key words: cisplatin, beta-catenin, triple negative breast cancer, subcellular distribution

(1643) **RUNX1 GENE EXPRESSION REGULATION IN TRIPLE NEGATIVE BREAST CANCER**

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Triple negative breast cancer (TNBC) is a heterogeneous group of diseases associated with early recurrence and low survival rates. Treatment options are limited due to the lack of specific therapeutic targets and are consequently managed with standard chemotherapy. Additionally, TNBC is associated with epithelial-mesenchymal transition (EMT). EMT is considered a process implicated in the metastatic cascade involving cellular conversion toward an invasive cell type and recent evidences strongly suggest that EMT process might be involved in tumor chemoresistance. It was found that RUNX1 protein expression correlates with poor prognosis in TNBC patients. Results from our group demonstrate that RUNX1 protein is able to up regulate the expression of RSPO3 (oncogene) and down regulate GJA1 (tumor suppressor gene) on TNBC cell lines. We demonstrate that RUNX1 is necessary for mammary tumor cell migration. Our main goal was to evaluate how RUNX1 gene expression is regulated in TNBC. Here we show that RUNX1 gene expression and transcriptional activity is significantly up regulated in Py2T after TGFβ treatment ($p<0.05$), and could be responsible of EMT in TNBC. To study how RUNX1 gene expression could be regulated we explored and found several DNA binding sites for the glucocorticoid receptor (GR) in silico. RUNX1 expression was significantly up regulated after dexamethasone (GR agonist) treatment on Py2T and MDA-MB-231 ($p<0,05$). Furthermore, RUNX1 gene expression was significantly downregulated after mifepristone (GR antagonist) treatment on the same cells ($p<0,05$). Interestingly, mifespristone has been described as a potential adjuvant in TNBC treatment. Here we propose a novel way to regulate RUNX1 expression, aiming at sensitizing tumor cells to known synthetic drugs. These data strongly suggests that **RUNX1 could be responsible of EMT and a potential target of GR** and, after further research**,** we could be able to find **a new therapeutic target to control TNBC progression.**

Keywords: TNBC, RUNX1, GR, EMT

(462) **RUNX2 REGULATES THE HORMONE REQUIREMENT AND THE METASTATIC / POTENTIAL OF T47D BREAST CANCER CELLS *IN VIVO*.**

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Abstract: We have previously shown that FGF2 increases the proliferation of MCF-7 and T47D breast cancer cells activating FGFR2, progesterone and estrogen receptors alpha (PR and ERα respectively). It has been reported that FGF2 increases RUNX2 expression in bone cells and few reports suggest that this might also occur in breast cancer cells. Since high RUNX2 levels were detected in murine mammary carcinomas which acquired a hormone independent phenotype, we are currently exploring the role of the FGF2-FGFR-RUNX2 axis in breast cancer progression.

The aim of this study was to evaluate if RUNX2 overexpression was able to bypass the hormone requirement of non metastatic T47D breast cancer xenografts which express ERα and PR. We stably transfected T47D cells with an expression plasmid carrying RUNX2 (RUNX2 cells) or with the empty vector (control cells). RUNX2 cells showed an increase in FGFR2 and FGF2 expression, supporting the hypothesis that FGF2 increases RUNX2, and RUNX2, in turn increases FGF2 expression, maintaining a positive loop. Both cell types were s.c. inoculated with Geltrex into the flank of NSG mice in the presence or absence of 17-β-Estradiol (E2 pellets; 0.5 mg). In untreated mice, only RUNX2 cells proved to be tumorigenic, although tumors remained with small sizes during the experiment. In E2-treated mice, RUNX2 xenografts grew faster and with a more aggressive phenotype as compared with control tumors ($p<0.001$). Lung metastases were only encountered in mice bearing the RUNX2 xenografts. To evaluate endocrine responsiveness, RUNX2 xenografts growing in E2-treated mice, were treated with Fulvestrant (0.5mg/week) or MFP (6 mg pellets). RUNX2 tumors did not respond to endocrine therapy and all animals showed lung metastasis. We conclude that RUNX2 promotes tumor progression and it may play a role in the acquisition of endocrine resistance. Further studies are required to elucidate the role of FGF2/FGFR2 in the establishment of this phenotype.

Keywords: RUNX2, FGF2/FGFR2 axis, metastasis, breast cancer.

(685) **THE FLAVONOID 2'NITROFLAVONE SUPPRESSES HUMAN TRIPLE-NEGATIVE BREAST CANCER CELL GROWTH *IN VITRO***

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Abstract: In addition to the human epidermal growth factor receptor 2 (HER2)-positive subtype, triple-negative breast cancer (TNBC) is one of the most aggressive subtype of mammary tumor. Owing to its lack of estrogen receptor, progesterone receptor and HER2, neither endocrine nor anti-HER2 molecular targeting therapy yield promising results, and standard chemotherapy is the backbone of systemic treatment. Flavonoids are a group of polyphenolic compounds considered potential chemopreventive candidates for cancer treatment. Previously, we synthesized the flavonoid 2'nitroflavone (2'NF) and it was demonstrated to induce LM3 murine mammary adenocarcinoma cell apoptosis and to act as an anti-tumor agent *in vivo*. Our aim was to evaluate 2'NF effect on human TNBC cell growth *in vitro*. 2'NF exerted a dose- and time-dependent inhibitory effect on MDA-MB-231 and MDA-MB-468 cells (MTT assay). The half maximal inhibitory concentration (IC_{50}) was 3.13±0.39 and 6.97±1.37% μM at 72h for MDA-MB-231 and MDA-MB-468 cells, respectively. Maximal inhibition of MDA-MB-231 cell growth (66.27±5.24%, vehicle control: 100% $p<0.001$) was obtained at 10 μM after 72h-treatment with 2'NF (higher concentrations produced a similar effect). For MDA-MB-468 cells maximal inhibition of cell viability (94.16±1.14% $p<0.001$) was obtained after incubation with 40 μM 2'NF for 72 h. Using clonogenic survival assays in MDA-MB-231 and MDA-MB-468 cells treated with 1.25 μM 2'NF for 10 days, colony formation rendered 66±21 and 65±2% respectively, as compared to the corresponding control cells (100%). In conclusion, 2'NF inhibited MDA-MB-231 and MDA-MB-468 cell growth *in vitro*. Thus, we propose 2'NF as a novel potential candidate for TNBC therapy.

Supported by CONICET (PUE-046).
Keywords: MDA-MB-231 cells, MDA-MB-468 cells, flavonoids

(237) **TRISTETRAPROLIN (TTP) PROMOTES SURVIVAL OF HC11 MAMMARY EPITHELIAL CELLS**

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A common feature among relevant RNAs in mammary gland cancer is the presence of sequences enriched in adenine and uracil (AREs) situated in 3´untranslated regions (3´UTR). The stability of

RNAs with AREs is regulated by proteins that bind these regions, like tristetraprolin (TTP). This protein promotes degradation of mRNAs with AREs in their 3´UTR such as proinflammatory cytokines and invasiveness-associated factors.

We have previously reported that TTP is expressed in the mammary gland, reaching the highest levels during lactation. More recently, in conditional TTP-***knockout*** mice in which expression of this protein is down-regulated specifically during lactation, we found increased cell death and augmented expression of inflammatory cytokines in the secretory epithelium. We concluded that TTP expression is required for lactation maintenance.

Then, to determine whether this protein is also relevant for the survival of undifferentiated mammary cells, we analyzed cell viability of HC11 clones that were stably transfected with specific TTP-shRNAs (TTP-KD cells). Using the 3-(4,5-dimethylthiazol-2-yl)-5(3-carboxymethonyphenol)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay, we determined that TTP-KD cells were less viable ($p<0,001$) than HC11 control cells, stably transfected with sh-scramble sequences (control cells). Similar results were obtained in the presence or absence of epidermal growth factor (EGF) or steroids in the culture medium. In addition, it was determined that the diminished viability could be, at least partially explained by the increase ($p<0,001$) of spontaneous apoptosis (analyzed by TUNEL assay) associated with higher levels of Bax protein ($p<0,001$), tested by Western Blot, in TTP-KD vs. control cells.

Taking together, these results indicate that TTP expression is relevant for both differentiated and undifferentiated mammary cell survival. We are presently analyzing the mechanisms underlying this effect.

Key words: HC11, tristetraprolin, cell survival, apoptosis

(1388) **ANTIPROLIFERATIVE EFFECT OF THE COMBINATION OF 2´NITROFLAVONE AND SAFINGOL IN MAMMARY TUMOR CELLS**

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In a previous work, we have demonstrated that the synthetic flavonoid 2´nitroflavone (2´NF), obtained in our lab, inhibited tumor growth by inducing apoptosis *in vitro* and *in vivo* in the murine mammary tumor model LM3. Some authors have proved that certain flavonoids induce apoptosis through an increase in the amount of ceramides. It has also been reported that the sphingosine kinase inhibitor safingol prevents ceramide catabolism, contributing to tumor cell death. Based on these results, in the search of new drug combinations that enhance the effect of each drug, we decided to explore the antiproliferative effect of the combination of 2´NF and safingol in LM3 cells. Cells were incubated with different concentrations of 2´NF and safingol alone or combined for 48 or 72h. IC_{50} values were 21±3 µM (48h) and 8±1 µM (72h) for 2´NF, and 1.4±0.5 µM (48h) and 1.1±0.4 µM (72h) for safingol. Proliferation of NMuMG normal murine mammary cells was not affected after 72h of incubation with concentrations of 50 µM (2´NF) or 5 µM (safingol). In order to quantitatively characterize the interaction between 2´NF and safingol, dose-effect curves were analyzed by Compusyn software. Results obtained showed that combination indexes were 0.72±0.06 (48h) and 0.71±0.01 (72h), indicative of synergism, in cells incubated with 5 µM of 2´NF and 0.6 µM of safingol. Additionally, when cells were treated with the same drug combination for 24h, an increase in the number of cells with apoptotic characteristics was observed by fluorescence microscopy after ethidium bromide and acridine orange staining. Furthermore, an increment of hypodiploid cells was detected by flow cytometry analysis. In conclusion, we demonstrated that a specific combination of safingol and 2´NF synergistically inhibited mammary tumor cell proliferation. This finding encourage us to study the molecular mechanisms involved in this effect and to consider this drug combination as a potential therapy for mammary cancer treatment.

Keywords: flavonoids, safingol, mammary tumor, antiproliferative effect, synergism

GENETICS AND MOLECULAR BIOLOGY 7

(318) **TRANSMEMBRANE-DOMAIN SHAPE IS A NOVEL ENDOCYTOSIS SIGNAL FOR SINGLE-SPANNING MEMBRANE PROTEINS**

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Endocytosis is crucial for all cells as it allows them to incorporate material from the extracellular space and control the availability of transmembrane proteins in the plasma membrane. The classical model for endocytosis of membrane proteins involves cytosolic signals that interact with adaptor proteins, driving active concentration of cargo in endocytic vesicles. In yeast, endocytosis followed by recycling to the plasma membrane results in a polarised distribution of membrane proteins by a kinetic mechanism. Here we report that increasing the volume of the residues that constitute the exoplasmic half of the transmembrane domain in the yeast SNARE Sso1, results in its polarised distribution at the plasma membrane. Expression of this chimera in strains affected in either endocytosis or recycling revealed that this polarisation is achieved by endocytic cycling. A bioinformatics search of the *Saccharomyces cerevisiae* proteome identified several proteins with high-volume exoplasmic hemi-TMDs. Our experiments indicate that TMDs of these proteins can confer a polarised distribution to the Sso1 cytoplasmic domain, indicating that the shape of the TMD can act as a novel endocytosis and polarity signal *in yeast.* Additionally, high-volume exoplasmic hemi-TMDs can act as an endocytosis signal in mammalian cells in culture.

(556) **ATP RELEASE DEPENDS ON AUTOPHAGIC STIMULATION AND RAB21 IN HELA CELLS**

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ATP exocytosis has emerged as an important autocrine/paracrine signal to trigger responses regarding platelet aggregation, inter-astrocytes communication, cell migration, differentiation, etc. The molecular mechanisms underlying this process have not been well defined so far. In addition, autophagy-dependent ATP release was shown to be an important process involved in immunogenic cell death and inflammation. Autophagic exocytosis has been also implicated in IL-1β, IL-18, galectin-3 secretion and presentation of antigens to the major histocompatibility complex. The regulation of this process is poorly understood. Our group has previously shown that there is an important role of the v-SNARE protein VAMP7 in the autophagic ATP exocytosis. Since there is a close connection between VAMP7, the small GTP-ase Rab21 and autophagy we decided to investigate the role of this Rab protein, its guanine-nucleotide exchange factor VARP and another Rab related to VARP (Rab32) in the autophagic exocytosis of ATP. HeLa cells were transiently transfected with GFP-Vector, YFP-Rab32wt, YFP-Rab32T39N (negative dominant mutant), GFP-Rab21wt, GFP-Rab21T33N (negative dominant mutant) and/or RFP-LC3. Then we incubated this cells in full medium, resveratrol 50nM and rapamycin 50nM for 4 hours or starvation medium for 2 hours. We used LC3B or VARP antibodies to detect endogenous proteins. Lysotracker red was put 30 minutes before the incubation finished. For ATP release assays, we used the luciferin-luciferase kit to measure the amounts of ATP in the extracellular medium. We took samples at 0, 30, 60, 90, 120, 180 and 240 minutes after the autophagic stimulus began. We found that autophagy leads to a redistribution of Rab32 or Rab21 molecules to the cell periphery, where they colocalized with LC3 and VARP. ATP release was significantly increased in starvation and in less content

in resveratrol condition. Our results suggest a role for Rab21, Rab32 and VARP in the autophagic exocytosis of ATP.

Keywords: Rab21, ATP release, Autophagy, Rab32, VARP

(759) **CAMP REGULATES PHAGOSOMAL MATURATION DURING *STAPHYLOCOCCUS AUREUS* INFECTION**

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Staphylococcus aureus is a microorganism that causes serious diseases in humans and it is known to induce an autophagic response in the host cell upon infection. We have previously demonstrated that the virulence factor a-hemolysin (Hla) is responsible of this autophagic response and it is used by the microorganism for escaping from its containing phagosome labelled with the autophagic protein LC3. We have further demonstrated that the autophagy induced by this bacterium is independent of the canonical PI3K/Beclin1 pathway and, instead, it is regulated by an AMPc/Epac/Rap2b pathway. In the current work we studied the implication of this pathway in the maturation of the *S. aureus* phagosome. We have found that treatment of infected cells with the second messenger cAMP, besides of regulating the autophagic response induced by *S. aureus,* also regulates its phagosome maturation by altering the recruitment of the small GTPase Rab7. We further found that the cAMP analogue, 8pCPT, which is able to specifically activate Epac1 also alters Rab7 association to the *S. aureus* phagosome. Likewise, overexpression of YFP-Epac1 itself has the same inhibitory effect. However, when we analyzed the effects of Rap2b, a downstream target of Epac1, the recruitment of Rab7 wasn't impaired. Therefore, our results indicates that cAMP regulates the maturation of the *S. aureus*-containing compartment in an Epac dependent but Rap2b independent-manner suggesting that other effectors might be involved.

Keywords: Staphylococcus aureus, phagosome, Rab7, cAMP.

(793) **THE *C. elegans* DAUER LARVA UNCOVERS A ROLE FOR CHD7/8 IN AUTOPAGHY REGULATION**

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Abstract: In harsh environments, the nematode *C. elegans* develops into a dauer larva, a stress-resistant, metabolically altered and long-lived variant of the L2-stage larva. The nuclear hormone receptor DAF-12 plays important roles in development and aging and is required for dauer formation. We identified DAF-12 target genes by chromatin immunoprecipitation. To address the relevance of these genes, we conducted an RNAi screen for dauer suppressors. Inhibition of *chd-7* (chromodomain helicase DNA-binding protein) leads to developmentally arrested, abnormal dauers that are sensitive to SDS and have impaired fat accumulation. Notably, the allele *chd-7(gk290)* forms abnormal dauers with these same features, validating our screen. Longevity of *daf-2(e1370)* and *glp-1(e2144)* mutants is significantly impaired by *chd-7(gk290)* and *chd-7* overexpression increases lifespan. Loss of *chd-7* function resembled mutations in autophagy genes allowing us to uncover roles for *chd-7* and its mammalian ortholog in this process. Specifically, both *chd-7(gk290)* worms expressing the autophagy sensor GFP::LGG-1 and electron micrographs of *daf-2(e1370);chd-7(gk290)* mutants display abnormal autophagosome accumulation. Hela cells expressing the related autophagy sensor GFP-LC3 also show abnormal autophagosomes upon Chd7 knockdown, suggesting a conserved role for Chd7 in autophagy regulation. Based on structural and sequence conservation, CHD-7 shares more that 60% homology with human CHD7 and CHD8, which are related with a spectrum of human disease phenotypes. Mutations in Chd7 are associated with CHARGE syndrome, a neurodevelopmental disorder with no known treatment and disruptive mutations in Chd8 have been related with autism spectrum disorders (ASDs). Our ability to exploit *C. elegans* to analyze *chd-7* in the context of dauer formation creates an opportunity to identify relevant pathways misregulated by this class of evolutionarily conserved chromatin modifiers.

Keywords: Caenorhabditis elegans, *chd-7*, autophagy, dauer, longevity.

(1619) **FOCAL CALCIUM RELEASE DURING ACUTE UNFOLDED PROTEIN RESPONSE**

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The Endoplasmic Reticulum (ER) is a multi-functional organelle that plays a critical role in a variety of processes, where the ER Ca^{2+} acts as a key messenger. Under resting conditions, the luminal Ca^{2+} concentration reflects a balance between active uptake by Ca^{2+}-ATPases and passive efflux pathways of which the translocon can play a prominent role). The translocon is an aqueous pore, primarily formed by the Sec61α core spanning the ER lipid bilayer,that is blocked by the ribosome on the cytosolic side and by the ER chaperone, BiP, on the luminal side. We hypothesize that during the acute phase of the UPR (Unfolded Protein Response), immediately after accumulation of unfolded protein in the lumen, the Ca^{2+} ER eflux through the translocon is increased. To test this mechanism of action, we performed cytosolic Ca^{2+} measurements in primary cultures of human astrocytes, expressing the Ca^{2+} indicator GCaMP6 tethered to the ER membrane, after induction of the UPR by Tunicamycin (Tm, glycosylation inhibitor). We observed focal release of Ca^{2+} (microdomains) in stressed astrocytes that was significantly inhibited by translocon blockers (emetine or anisomycin). In addition, the Tm-induced Ca^{2+} signal was amplified by pre-treatment either with AB5 cytotoxine, which specifically hydrolyses BiP, or with the translocon opener puromycin. The effect of these pharmacological tools was corroborated by co-immunoprecipitations that showed changes in the interactions either between Sec61α and BiP or Sec61α and the ribosomal protein S6. Important to note that the likehood of obtained Tm-induce local Ca^{2+} events, increase by using either the slow chelator EGTA-AM or Xestospongin C and Ryanodine ($InsP_3$ and Ry Receptors inhibitors, respectively). Taken together, these data, strongly suggest that the chaperone BiP and the ribosome are dissociated from the translocon increasing Ca^{2+} permeability.

Keywords: Endoplasmic Reticulum Stress, Translocon, Calcium.

(1638) **INDUCTION OF AUTOPHAGY PROMOTES CRUZIPAIN PROCESSING DURING *Trypanosoma cruzi* DIFFERENTIATION.**

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T. cruzi is the causative agent of Chagas' disease. During its life cycle, this parasite present four developmental stages: epimastigotes (epi), metacyclic trypomastigotes (MT), bloodstream trypomastigotes and amastigotes. Reservosomes are organelles of epi that accumulate proteins and lipids ingested by endocytosis. This content is digested by hydrolases to supply energy during the differentiation of epi to MT (metacyclogenesis). The autophagic pathway of *T. cruzi* is activated during starvation and differentiation. Cruzipain (Cz) is the major cysteine protease of *T. cruzi* and a key enzyme for nutrition, differentiation and host cell infection. Cz is synthesized as a zymogen, processed and delivered to reservosomes. The aim of this work was to evaluate the participation of autophagy in the distribution and function of Cz during metacyclogenesis. Epimastigotes (Y strain) were kept in BHT medium (control) or subjected to nutritional stress under TAU medium for 2 h followed by dilution in TAU3AAG medium for 72 h. By confocal microscopy, we observed that starvation induces the accumulation of Cz in compartments located at the posterior region of the parasite. Colocalization of these compartments with Atg8 and DQ-BSA (**autophagic and hydrolyt-**

ic markers respectively) increased by nutritional stress. Western blot analysis showed that the Cz zymogen was recognized in both control and starvation conditions (2 h), although the latter had decreased zymogen levels at 72 h. We also detected the mature form of Cz and a band corresponding to the carboxyl-terminal fragment only under starvation condition (72 h). When we used autophagy inhibitors, Cz did not accumulate in reservosomes. K777, a Cz inhibitor, induced a Cz distribution at the cell surface, avoiding their accumulation in the reservosomes and the differentiation into TM. These findings indicate that induction of autophagy by starvation triggers cruzipain activation and processing, and promotes *T. cruzi* differentiation.

Key words: CHAGAS; T. CRUZI; METACYCLOGENESIS, AUTOPHAGY; CRUZIPAIN

(991) **THE EFFECTS OF AN EXCESS OF AMINO ACIDS ON LIFESPAN EXTENSION IN PROTOTROPHIC YEASTS**

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Lifespan extension has been a human challenge since ever. Aging is a multifactorial and complex process characterized by a progressive damage of cellular functions that inevitably leads to death. It is regulated by numerous nutrient-sensing pathways. Amino acids levels are sensed by at least two evolutionarily conserved and intersecting mechanisms: one involving Gcn2 kinase and the other involving the TORC1 kinase. Dietary interventions are effective strategies for preventing aging and diseases and many of them are linked to amino acid and protein levels and their regulation. The aim of this work was to study how an excess of amino acids affect longevity in prototrophic yeast cells and to determine which nutrient-pathways are involved. Chronological lifespan (CLS) was measured using the colony forming unit spot assay in cells grown in the absence and in the presence of all amino acids. We used wild type cells and cells deficient in the kinase Tor1 and in the transcription factor, Gcn4, a key regulator of amino acid biosynthesis and the main target of the kinase Gcn2. Longevity was affected neither by growth conditions nor by mutant genotypes assayed. When tolerance against thermal stress was analysed during the aging process, we found that cells lacking *GCN4* were less resistant to thermal stress than the other strains used and that amino acids had a protective effect; however, no differences during aging were observed when cells were subjected to oxidative stress. Also, we determined stress tolerance, autophagy and the activity of the unfolded protein response (UPR) pathway in cells at the exponential and stationary growth phases. Differences found before aging started did not explain the similar CLS behaviour observed. Altogether these results allow us to conclude that lifespan extension does not result solely from increased stress resistance, UPR or autophagy activation, supporting the idea of the complexity of the aging process.

Keywords: lifespan, yeast, amino acid

(1865) **DUPLICATION AND POSITIVE SELECTION DROVE UGGT-2 NEOFUNCTIONALIZATION IN *CAENORHABDITIS***

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UGGT (UDP-Glc::glycoprotein glucosyltransferase) discriminate folded from misfolded glycoproteins, is the key component of the quality control mechanism of glycoprotein folding that ensures that only properly folded proteins exit the ER. In vertebrates and in the nematode genus *Caenorhabditis* there are two homologous genes encoding UGGT-like proteins. Expression of both *Caenorhabditis* genes in *S. pombe* mutants devoid of UGGT activity showed that UGGT-1 is active while UGGT-2 although being an essential protein in worms apparently does not display a canonical UGGT activity. The aim of this study was to explore the origin and evolution of both *Caenorhabditis* UGGTs. Bayesian phylogenetic analyses were carried out in 189 UGGT sequences representing all major eukaryotic groups. The *Caenorhabditis* and the vertebrate clades are reciprocally monophyletic, and both split into two identical subtrees, revealing that UGGT went through independent duplications in these two lineages. Conservation and selection analyses were performed within the *Caenorhabditis* clade applying codon-based and lineage-based approaches analyzing the two protein domains separately, reflecting a generalized background of purifying selection in both UGGTs, suggesting that the two gene copies have been subjected to functional constrains. The catalytic domain is highly conserved as a consequence of a strong negative selective pressure, and shows almost no evidences of positive selection. In contrast, the recognition domain has undergone a positive diversifying selection process, wich showed evidences of Episodic Diversifying Selection in UGGT 2. A probable outcome of the observed selective scenario is that UGGT 1 retained the canonical function, while UGGT 2 may have retained only the catalytic domain and consequently may recognize a different substrate. In this sense, UGGT 2 would have experienced a neofunctionalization driven by diversifying (positive) selection.

(578) **LEUKEMIA: INTRACELLULAR CHANGES IN LRP-1 RECEPTOR TRAFFIC AND AUTOPHAGIC RESPONSE.**

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Modification of cell membrane proteins is an important event during erythropoiesis. Under maturation stimulus, erythoid cells active intracellular process such as, nuclear extrusion, autophagy of non-necessary organelles and regulating membrane protein. In leukemia cells this maturation response is impaired. Low density lipoprotein related-receptor protein-1 (LRP1) is a transmembrane receptor involved in numerous mechanism of cancer cells like migration, survival and apoptosis regulation. We recently demonstrated that hemin (a maturation inductor) can stimulate erythroleukemia cells and activate autophagy and elimination of mitochondria. LRP1 is responsible of hemin cellular uptake. The aim of our work is to study whether hemin is able to stimulate LRP-1 traffic and by this regulate important intracellular changes in chronic leukemia cells through LRP1 signal. For this we performed an intracellular localization of LRP1 by immunofluorescence of K562 leukemia cell line. Different vesicles of endocytic and autophagic pathway were tagged with specific proteins such as Rab5, Rab7, Rab11, CD63, lysosomes and autophagosomes. Also a Golgi marker was used. We evaluate both early and late times. Moreover we study if hemin stimulates autophagy though LRP1 by western blot using silencing RNA against them and measuring levels of LC3-II protein (specific for autophagy). Importantly our results showed that hemin significantly modifies LRP1 normal intracellular localization from early endosomes and recycling vesicles to late endosomes and lysosomes in a time dependent manner. Likewise LRP1 is associated to autophagic vesicles under hemin incubation and its silencing downregulate over 50% the autophagic response. Taken together this results led us to think that hemin could regulate LRP1 activity and that this receptor is involved in crucial process as autophagy in leukemia cells. LRP1 modification may be important in cancer malignancy prognosis and therapeutics advances.

Key words: LRP1, leukemia, autophagy, cancer.

(990) **THE ROLE OF RAB24 IN AUTOPHAGY**

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Autophagy is a highly-conserved eukaryotic degradation pathway in which cytoplasmic materials, such as molecules and organelles, are sequestered by autophagosomes and transported to the lysosome for digestion. The autophagic pathway maintains the cells' basal homeostasis and supports adaptation during stress; thus, deregulation of this process contributes to several human diseases, including cancer, bacterial or viral infections, and neurodegeneration. It is therefore critical that autophagy is properly regulated. This process requires the Rab GTPases for accurate membrane trafficking, since they control vesicle budding, targeting and fusion. Our laboratory has already shown that Rab24 participates in central vesicle trafficking events like endosome degradation and autophagy. Besides the fact that Rab24 is essential for the endosome-lysosome fusion process, little is known about the role and molecular mechanism of Rab24 in autophagy. Consequently, in the present study, we explore, in cellular models, the participation of Rab24 in autophagosome formation and maturation. For this purpose, we performed several techniques, such as protein transfections and RNAi knockdown, Western blot-based assays to monitor LC3 lipidation, and fluorescence microscopy-based methods to study the distribution of autophagosomal protein markers and molecular tandem probes. Overall, our findings reveal that Rab24 is a novel regulator of autophagy and elucidate significant insights about the role of Rab24 in specific stages of this process.

Keywords: autophagy, vesicle trafficking, Rab GTPases, Rab24.

GEN BM 8 / GENETICS AND MOLECULAR BIOLOGY 8

(1525) **CHARACTERIZATION OF MICRORNAS IN *Neospora caninum* ISOLATES WITH DIFFERENTIAL PHENOTYPE BY QUANTITATIVE REALTIME PCR.**

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Neospora caninum, a major cause of abortion in cattle, is an obligate intracellular parasite phylogenetically related to *Toxoplasma gondii*. A wide range of *N. caninum* isolates with specific host preferences and differential virulence has been characterized. Small non-coding RNAs play an important role in parasites, as regulatory molecules associated with physiology, infection and manipulation of immune response. Previous studies in *T. gondii* revealed the existence of a large repertoire of miRNAs, some of which were associated with virulent strains. In this regard, our aim was to explore the presence of miRNAs in *N. caninum* and the potential association with different strain phenotypes.

Four families of miRNAs (miR-15, miR-40, miR-60 and miR-61) were selected from *T. gondii* transcriptomic data. For reverse-transcription reaction, miRNAs specific stem-loop primers were designed. On the other hand, RNA and cDNA were obtained from *N. caninum* tachyzoites: Nc-6 Argentina (moderate virulent isolate) and Nc-1 (highly virulent isolate). Quantitative Real-time PCR was performed using SYBR Green master mix, 4 miR-specific forward primers and a universal reverse primer from a consensus sequence within the stem-loop.

The results showed that all the selected miRNAs were expressed in both *N caninum* isolates. Particularly, differential expression was detected for miR-15 and miR-60, being highly transcribed in the less virulent isolate (Nc-6 Argentina). On the contrary, the higher expression of miR-60 has been previously associated with increased virulence in *T. gondii* isolates.

To our knowledge, our work is the first description of miRNAs in *N. caninum* isolates. Additionally, we demonstrated the differential expression of two of the targeted miRNAs in the phenotypically diverse isolates. These results represent a promising starting point for further research on the study of regulatory role of non-coding RNAs in *N. caninum* virulence strategies.

Keywords: *Neospora caninum*, microRNAs, RTqPCR.

(1469) **CODON USAGE IN THREE SPECIES OF THE GENUS *Echinococcus.***

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Species of the genus *Echinococcus* are Platyhelminthes parasites causatives of hydatid diseases in animals and humans all over the world. They have complex life cycles which comprise an intermediated and a definitive host, and such hosts infections specificity or preference seems to depend on the *Echinococcus* species.

The codon usage bias is the non-random usage of synonymous codons for coding amino acids. The synonymous sites are under weak selection, and codon usage bias is kept by the equilibrium in mutational bias, genetic drift and selection pressure. The differential codon usage choices are also relevant to human infecting by many pathogens organisms.

Previously we have sequenced and annotated the genomes of three *Echinococcus* species which have allowed to perform several analyses such as orthology studies, genetic variability and microRNA/target genes repertory. Here we performed codon usage analyses using a whole genome datasets for comparative genome analysis of *E. canadensis*, *E. multilocularis* and *E. granulosus* by applying codonW, CodonO softwares and principal component analysis (PCA) on 3000-4000 genes and we evaluated the nucleotide content in each position of the codons.

As demonstrated by PCA and Enc analyses many genes have non-random selection of codon for coding proteins. Indeed genes with strong codon bias seem to be expressed at a higher level compared to other genes. However most of the genes appear not to have a biased codon usage and the frequencies of codons among the *Echinococcus* species are similar and exhibit a strong RSCU correlation among each other. On the other hand the use of UAG as a stop codon signal is absent for all the genes, otherwise the stop codons UAA and UGA comprises the 46.4% and 53.6% of termination signal respectively.

Comparative analysis of codon usage among the *Ehinococcus* species could reveal genes responsible for specie-specific phonotypes and host preference infections.

Keywords: *Echinococcus,* codon usage, genes

(1799) **DEVELOPMENT OF SIMPLIFIED INDUCIBLE EXPRESSION SYSTEMS FOR TRYPANOSOMATIDS.**

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In trypanosomatids, inducible expression systems are based on the sequential use of two types of genetic manipulation vectors. First, wild type strains have to be transfected with plasmids that code for regulatory elements such as bacterial repressors and polymerases. It is only after establishing these cell lines that is possible to modulate the expression of sequences of interest carried in the second type of plasmid that make up the system. In addition, since the discovery of highly processive RNA transcription of bacteriophage polymerases in *T. brucei*, these enzymes have been included in almost all inducible expression systems developed so far, although endogenous promoters that allow constitutive as well as regulatable expression have been described.

In this work we conceived a simplified inducible expression system based entirely on endogenous promoters with operators for bacterial repressors. It is worth noting that along with reporter genes and selectable markers the latter represent the only included foreign elements. This reduced set, made possible to accommodate the entire system together with the sequence of interest in a single vector and

thus, the obtention of an inducible cell line can be accomplished by one step transfection of a wild type strain.

Due to the fact that *T. brucei* is more amenable to genetic manipulation, initial prototypes were designed for this organism and transfected in procyclic Lister-427 trypomastigotes. After selection, Western blot analysis revealed a 35-fold expression induction of the reported GFP gene compared to non-induced cells.

The system described here will represent a useful molecular genetic tool that, independently of the strain background, will enable tightly-regulated protein expression and stem-loop based RNAi strategies. Once established, it will constitute the starting point for the development of equivalent systems for other trypanosomatids.

Keywords: genetic manipulation, inducible expression, trypanosomatids.

(1672) **THE HYPERVARIABLE REGIONS OF kDNA MINICIRCLES IN THE 6 MAIN LINEAGES OF *Trypanosoma cruzi*: A NEXT GENERATION SEQUENCING APPROACH**

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The kinetoplastic DNA of *Trypanosoma cruzi* consists of dozens of maxicicles (analogous to the mitochondrial DNA of other eukaryotes) and about 30,000 minicircles. Each minicircle (≈ 1.4 kb) is organized into four conserved regions (≈ 120 bp) located 90° apart and intercalated by an equal number of hypervariable regions (≈ 240 bp) known as mHVR (minicircle hypervariable regions). There are few known mHVR sequences, possibly due to technical difficulties for sequencing this kind of DNA regions in the past. Nowadays, the Next Generation Sequencing (NGS) techniques open the possibility of a deeper knowledge of the mHVR sequence diversity. In the present work, we describe our general approach for the study of the diversity of mHVR sequences in the main 6 DTUs of *T. cruzi*, the generation of libraries of these regions and the sequence diversity obtained by NGS. These are the first results in the framework of a broader work that aims to study the diversity of mHVRs and their evolution, as well as the searching for lineage specific sequences in order to be used in genetic typing.

Keywords: *T. cruzi,* DTUs, mHVR, NGS.

(233) ***TRYPANOSOMA CRUZI* LONG READS BASED ASSEMBLY: REVEALING THE REAL EXTENT OF GENOME COMPLEXITY**

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Although the genome of the *Trypanosoma cruzi* (CL Brener strain) is available since 2005, and those from some additional strains were later reported, very high-quality genomic sequences are not yet available due to the intrinsic genome complexity of this parasite. This limitation poses a number complications for diverse types of analyses that require high degree of precision. Third-generation sequencing technologies are particularly suitable to address the challenges associated with the high degree of repetition of *T. cruzi*̂s genome since they allow obtaining sequencing reads of 15 kb in length and many larger than 30 kb. This opens the possibility to directly determine the full sequence of large clusters of repetitive sequences (without collapsing them), as well as determining the single copy sequences that surround both sides of these clusters. As a consequence assembly fragmentation is largely avoided. Furthermore this approach allows separated assembly of homologous chromosomes, namely haplotypes are retrieved as separate contigs/scaffolds instead of a unique mosaic sequence. We present the genome of the hybrid *T. cruzi* strain TCC, sequenced using PacBio SMRT technology. Its final assembly consists in a diploid genome of 86.7Mb, the 9% of which being composed by a well known satellite of 195pb. The remaining genomic regions are distributed in only 692 contigs with an N50 of 300Kb, the longer ones being larger than 1 MB and correspond to entire chromosomes. New tandem and disperse repetitive sequences were identified, including some located inside coding sequences. A total of 30947 genes were identified, 62% having an assigned function. The quality of this new *T. cruzi* genome allows one to separate the two parent halpotypes as well as to identify the centromeres, the real extension of repetitive elements and genes.

assembly, pacbio, Trypanosoma cruzi, haplotypes, repetitive elements

(1599) **CRISPR-CAS9 TARGET VALIDATION OF *Trypanosoma cruzi* BROMODOMAINS**

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Trypanosoma cruzi is a protozoan parasite and the causative agent of Chagas´ disease. This parasite has a complex life cycle with two intermediate hosts, a triatomine insect and a mammalian vertebrate. Nuclear gene organization in *T. cruzi* is atypical from other eukaryotic cells. Genes arrange in long tandems that are transcribed polycistronically from a small number of initiation sites. The transcription is driven by a limited number of basal nuclear factors and its regulation depends on chromatin structure. Among the usual proteins that participate in epigenetic regulation, a small number of genes coding for acetylases, deacetylases and bromodomains are present in the *T. cruzi* genome. Bromodomains are conserved protein modules capable of binding acetylated lysines and found in many proteins associated with chromatin. *T. cruzi* has at least six genes that encode for bromodomain-containing factors (TcBDF1-6). The overall objective of the project is to explore bromodomains as drug target in *T. cruzi*, by assaying their essentiality using a CRISPR/Cas9 gene deletion approach. Briefly, target sequences (SgRNA) were cloned into the expression plasmid pTREX-Cas9-neo. Parasites were transfected by electroporation and drug selected. Deletion of the target genes were determined by PCR. We obtained mutated parasites for all the genes tested but only for Bdfx we got the homozygous population, which mean that this gene is not essential for epimastigotes growth. For the rest of the genes we detected less amount of protein for mutated cultures but we couldn´t get homozygous null mutants, and only one of them showed a detrimental growth rate. We are evaluating the essentiality of these genes in the other life cycle stages as well as testing the expression of dominant-negative mutants approach for some of the genes. Altogether these results suggest that except Bdfx, bromodomains genes could be crucial for *T. cruzi* normal growth.

Keywords: *T. Cruzi*, CRISPR/Cas9, Bromodomain, Chromatin, Acetylation

(1828) **IDENTIFICATION OF A RARE DORMANT SUB-POPULATION OF *TRYPANOSOMA CRUZI* AMASTIGOTES ABLE TO REASSUME PROLIFERATION, INFECTION AND GENERATION OF NEW QUIESCENT FORMS**

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Benznidazole (BZ), the primary drug used to treat Chagas disease, fails to provide parasitological cure in the majority of cases, despite long treatment times (up to 60 days). In a number of infections, drug treatment failures have been linked to the presence of subpopulations of pathogens that become physiologically inactive or dormant. These so-called persister cells may be stochastically generated in pathogen populations and have been hypothesized to serve as a survival mechanism against possible catastrophic events that could eliminate an entire metabolically active population. In our

analysis of persisting parasites in mice with chronic *T. cruzi* infection, we consistently observed the existence of rare amastigotes not undergoing active proliferation, as assessed by the incorporation of the thymidine analog 5-ethynyl-2´-deoxyuridine (EdU) into amastigote DNA. Quiescent amastigotes were also evident *in vitro* even after prolonged incubation with EdU (72hr). TUNEL assays determine that these dormant parasites were not dead within host cells. Incorporation of EdU into DNA can impact DNA replication, so we used labeling with fluorescent dyes as an alternative protocol to monitor amastigote replication. We not only confirmed *in vivo* and *in vitro* results, but also found that non-proliferating amastigotes were able to resume proliferation: differentiate to trypomastigotes, infect new host cells, replicate and generate again a progeny of dormant forms. Remarkably, the screening of adipose tissue from infected mice treated with BZ revealed the resistance capacity of dormant parasites. Replicating amastigotes are sensitive to drug and could not been detected. The cause of this arrest and the link between these parasites and drug treatment failures *in vivo* will be the subject of future studies.

Keywords: Trypanosome; dormancy; quiescence; proliferation; amastigotes.

(1902) **NUCLEAR ARCHITECTURE AN CHROMOSOMOC INTERACTIONS IN T.CRUZI**

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Trypanosoma cruzi es un parásito protozoario responsable de la enfermedad de Chagas en las Américas, desde el sur de Estados Unidos hasta el cono sur Sudamericano. La enfermedad afecta con más dureza las zonas rurales y pobres, donde el insecto vector hematófago aparece asociado a viviendas precarias. La transmisión ocurre principalmente a través de insectos de la familia *Reduvidiae*, de forma congénita de madres portadoras y por tranfusiones sanguíneas y trasplantes de órganos o tejidos. La enfermedad crónica tiene a menudo como manifestación la miocarditis chagásica, que es la forma de falla cardíaca de origen infeccioso más común a nivel global. En tripanosomátidos se considera que el principal mecanismo de control de la expresión génica es post- transcripcional. No se han identificado secuencias promotoras de la ARN pol II (con la excepción del promotor del gen del spliced leader o SL). El genoma de *T. cruzi* (de 60.3 Mb) consiste en 41 cromosomas (con hasta 50% de secuencias repetidas) en los que los genes se organizan en clusters de transcripción policistrónicos. A diferencia de los policistrones procariotas, éstos contienen genes no necesariamente relacionados funcionalmente. Estas unidades transcripcionales pueden estar presentes en una u otra hebra y la transcripción tiene lugar de forma bidireccional entre dos clusters divergentes adyacentes. Las regiones de cambio de hebra codificante se denominan SSR (Strand Switch Regions). Los mRNA se generan a partir del procesamiento de los transcritos policistrónicos mediante trans-splicing y poliadenilación. Nuestra hipótesis es que la regulación génica en *T. cruzi* involucra, entre otros, aspectos epigenéticos a nivel de la conformación de la cromatina. Es razonable predecir que exista una arquitectura nuclear dinámica en *T. cruzi* que incluya interacciones a distancia de las

SSR entre sí y/o con otros elementos reguladores. Para definir las regiones de interacción genómica con SSRs, estamos utilizando como aproximación técnica la captura de conformación de la cromatina (C4). Las SSRs pueden ser bidireccionales divergentes, bidireccionales convergentes, o unidireccionales. Para sistematizar nuestro análisis, seleccionamos una SSR correspondiente a cada categoría. Generamos moléculas circulares cerradas de ADN conteniendo las regiones interactuantes correspondientes a cada una de las regiones *bait* seleccionadas. Valiéndonos de una base de datos con las regiones de cambio de hebra de *T. cruz*i construida previamente, diseñamos oligonucleótidos específicos para las regiones *bait*, y amplificamos los productos interactuantes para cada categoría. Los productos están siendo analizados por secuenciación masiva.

(309) **MOLECULAR DISSECTION OF THE APICOMPLEXA CENTROSOME; A SMALL ORGANELLE WITH BIG POTENTIAL**

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The centrosome is a key organelle orchestrating many cellular processes. It is involved in coordinating chromosome segregation, intracellular vesicle trafficking, signaling cascades, and the assembly of the cortical cytoskeleton and of the basal body and its associated flagella. In previous work we demonstrated that the centrosome in Apicomplexan parasites, a phylum which encompasses several human and veterinary pathogens, is responsible for coordinating the progression of the division cycle through the assembly of multiple cell-cycle specific physical tethers. These tethers ensure that nuclear mitosis and cytokinesis occur synchronously; their individual ablation leads to futile division cycles, and parasite death. In addition we, and others, have shown that the structure and organization of the ApiCentrosome is unique and divergent. Moreover, the ApiGenomes do not appear to encode for the canonical regulators of centrosome biogenesis found in the mammalian hosts. Thus, we propose that the ApiCentrosomes constitute a relatively unexplored rich source of potentially drugabble targets. Here, we set out to explore the molecular composition of the ApiCentrosome by identifying its proteome, and the interactomes of previously identified centrosome components in the model Apicomplexa *Toxoplasma gondii*. Our results will shed light onto the biology of a critical organelle for parasite survival, and will broaden our knowledge on the molecular composition and evolutionary history of this important organelle.

(1934) **MDR1 POLYMORPHISMS IN HIV INFECTED INDIVIDUALS. ITS INFLUENCE IN PORPHYRIA CUTANEA TARDA TRIGGERING**

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A high association of Porphyria Cutanea Tarda (PCT) with HIV infection (17%) is found in our country. To date slight evidence exists about if triggering factors of PCT, a rare toxicogenetic disease, in HIV patients are related to the infection and/or therapy. The Multidrug Resistance protein (MDR1) is involved in the transport of xenobiotics and antiretroviral drugs. Some polymorphisms in MDR1 gene are of clinical importance, among them: exon 12 (c.1236C>T), 21 (c.2677G>T/A) and 26 (c.3435C>T) with high incidence in Caucasians. The frequency of these SNPs was previously studied in control, PCT and PCT-HIV individuals. The aim was to complete this research analyzing HIV patients without PCT by PCR-RFLP assay. The analysis of 1236T-2677T/A-3435T haplotypes was performed. The polymorphic allelic frequencies were 0.45 (exon 26), 0.36 (exon 12) and 0.37 (exon 21). Genotypic frequencies were as follows: exon 26: CC 25.0%, CT: 59.4%, TT 15.6%; exon 12: CC 28.1%, CT 71.9%, TT 0%; exon 21: GG 40.0%, GT 43.3%, TT 13.3%. In HIV population, the frequency of T allele for exon 26 was lower than PCT and PCT-VIH groups, although differences were not significant. For exon 21, T allele frequency was significantly minor ($p<0.05$) in HIV than PCT-HIV group. For exon 12, the mutant allele in HIV group had a less frequency ($p<0.05$) respect to PCT cohort. The genotypic analysis showed that the allele T of exon 26 and 21 is presented in homocigosis with a minor frequency than PCT-HIV group; the same observation was found for exons 26 and 12 in HIV cohort but in comparison with PCT group. Our previous results showed the same conclusions suggesting that the polymorphism of exon 21 could be involved in the manifestation of PCT in HIV individuals related to the antiretroviral therapy used in these patients; while in exon 12, T allele could be associated with other risk factors in the onset of PCT.

INATA 3 / IMMUNOLOGY (INNATE IMMUNITY) 3

(111) EXPRESSION, INTRACELLULAR LOCALIZATION AND PTMS IN DIFFERENT CELL LINES OF THE MRNA DECAY REGULATORS OF THE ZFP36 FAMILY

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Abstract: 3 nucleo-cytoplasmic shuttling, mRNA binding, CCCH zinc finger, PPPP containing, low-abundant phosphoproteins (zfp36, zfp36L1, zfp36L2) compose the zfp36 family. In the cytosol trough 2 central tandem zinc fingers, they bind to AU-rich elements (ARE) in the mRNA´s 3'UTR resulting in mRNA instability/ degradation by other proteins. Several ARE-containing mRNAs, especially of inflammatory mediators, are stabilised by the p38 pathway through inactivating phosphosites in these proteins. To understand the elusive particular role(s) of the L1 member, different cell lines were studied assessing their expression by WB and qPCR. Several antibodies were tested. Zfp36 protein was detected for the first time in HeLa by an improved WB and IP. L1 and L2 were KD in HeLa by RNAi. The effects on p38-regulated inflammatory mediator expression were examined. L1 function was investigated in L1 -/- cell lines (n=17). L1 or L2 did not contribute to post-transcriptional regulation of inflammatory mediators by the p38 pathway in HeLa or L1 null cells. L1 null cells over-expressed IL-6 protein/mRNA but L1 was not regulating IL-6 mRNA stability. Instead, IL-6 underwent fastest transcription in the L1´s absence suggesting that the IL-6 transcript is an indirect target through some factor being itself a L1 target. On the other hand, L1 and Zfp36 showed reproducible ($p \leq 0.01$, n=19) inverse basal protein expression levels in macrophages, with L1 increasing and Zfp36 decreasing during differentiation, suggesting that L1 has actually a role in sustaining a Mc-MΦ differentiation switch. Concerning PTMs, the phosphorylation extent was regulating the intra-cytosolic localization with hyperphosphorylated forms of Zfp36 and L1 becoming insoluble in the cytosol and putatively associated with the cytoskeleton. Other results suggest roles for Pro isomerisation and for electrostatic interactions in the fine-tuning, rheostatic regulation of the activity, level and localization of these proteins.
Keywords: mRNA

(169) GLYCAN RESIDUES ARE INVOLVED IN THE RECOGNITION AND IMMUNOGENICITY OF S-LAYER GLYCOPROTEIN FROM *Lactobacillus kefiri*

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Surface layers are (glyco)-proteinaceous cell envelopes ubiquitously found in different bacterial species, including potentially probiotic *Lactobacillus kefiri*. In previous studies, we showed that S-layer glycoprotein from *L. kefiri* CIDCA 8348 (SLP-8348) was not able to induce the activation of macrophages by itself, but it favored the LPS-induced response, since there was a significant increase in the expression of co-stimulatory molecules, IL-6 and IL-10 in comparison with LPS-stimulated cells. In this study, we aim to investigate the role of glycan residues in SLP-8348 internalization by murine macrophages as well as in its immunogenicity. SLP-8348 was removed with 5 M LiCl and exhaustively dialyzed against PBS. Atto-647N-labeled SLP-8348 was incubated with RAW 264.7 macrophages both at 4°C (to evaluate binding) and at 37°C (to evaluate internalization), and fluorescence intensity was measured by flow cytometry. RAW 264.7 cells internalized the SLP-8348 in a process that was mediated by carbohydrate-receptor interactions since it was inhibited by glucose, mannose or EGTA, a Ca+2 chelating agent. These results correlated with the recognition of SLP-8348 by ConA. Moreover, EGTA completely abrogated the effect of SLP-8348 on LPS-stimulated RAW 264.7 cells. To test the SLP-8348 immunogenicity, one dose (10 μg/mouse) of SLP-8348 or oxidized SLP-8348 (SLPOx-8348) in combination with incomplete Freund´s adjuvant was subcutaneously injected into the right flank in front of the hind leg on BALB/c mice. After ten days, cells from inguinal lymph nodes were labeled with CFSE and stimulated *in vitro* with SLP-8348 (10 μg/ml) for five days. Proliferation index of $CD4^+$ T cells as well as secretion of IFN-γ were significantly lower in the group of mice treated with SLPOx-8348 respect to SLP-8348-treated mice. This is the first report showing that glycan chains are involved in the uptake of a SLP by macrophages and are crucial in the immunogenicity of this glycoprotein.
Keywords: S-layer glycoprotein; C-type lectin receptors; Lactobacillus; Macrophage activation

(527) DEVELOPMENT OF INNATE T CELLS IN THE THYMUS UNDER INFECTIOUS/INFLAMMATORY SYSTEMIC CONDITIONS

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Our previous work demonstrated that during the acute stage of certain infections with a strong Th1 component (*Trypanosoma cruzi* or *Candida albicans*), SP CD8 thymocytes alter their differentiation from "conventional" to "innate" lineage. Innate $CD8^+$ cells express a particular phenotype ($CD44^{hi}$ $CD122^{hi}$ $EOMES^{hi}$), produce high levels of IFNγ and have high cytotoxic activity. The switch in the SP CD8 lineage occurs by the inflammatory process since we obtained similar data when we induced systemic expression of IL-12 and IL-18 by hydrodynamic injection of their cDNAs. The aim of our work is to determinate the stage at which innate $CD8^+$ T cells develop in the thymus and the molecular mechanisms that are involved in this differentiation. Our data demonstrate that the innate phenotype give rise as early as the double positive (DP) stage since when we co-cultured DP or SP CD8 $CD45.1^+$ control thymocytes with $CD45.2^+$ *T. cruzi*-infected thymocytes we observed that $CD45.1^+$ cells adopt the mentioned innate features ($p<0,05$).

The canonical Wnt/β-catenin signaling pathway is involved both in the differentiation of CD8 T cells and the generation of functional CD8 T cell memory. To investigate if this particular pathway is involved in the generation of innate $CD8^+$ cells, we co-cultured $CD45.1^+$ control thymocytes with $CD45.2^+$ control or *T. cruzi*-infected thymocytes in the presence of Lithium chloride, an inductor of canonical Wnt pathway and we observed up-regulation of CD44 and EOMES regardless of the infection ($p<0,05$), while in the presence of iCRT, an inhibitor of β-catenin signaling, $CD45.1^+$ control thymocytes down-regulate CD44 and EOMES in both control or infected conditions ($p<0,05$).

The development of innate $CD8^+$ T cells in the thymus under Th1 systemic processes may have implication in the output and the repertoire of T cells in secondary immune organs. Canonical Wnt pathway can be implicated in this deviation, but more experiments are necessary.

Keywords: Thymus; innate T cells; inflammatory/infectious processes; IL12 plus IL18; Wnt/β-catenin.

(672) EXTRACELLULAR ACIDOSIS DRIVES THE DIFFERENTIATION OF HUMAN MONOCYTES INTO DENDRITIC-LIKE CELLS

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Differentiation of monocytes into macrophages and dendritic cells (DCs) is likely determined by the properties of the inflammatory milieu, however, little is known about the identity of the factors that control the polarization of monocytes toward each of these fates. Extracellular acidosis is a common feature of inflammation and the tumor microenvironment. Here, we analyzed the ability of extracellular acidosis to modulate monocyte's differentiation profile. Culturing PBMCs or isolated monocytes(>85% purity) for 7 days in medium supplemented with 10% FCS adjusted to pH 6.5, in the presence of PHA (2 μg/ml) or allogeneic lymphocytes (monocyte-lymphocyte

ratio 1:4), resulted in the appearance of an adherent spindle-shaped population of cells displaying a DC-like phenotype, characterized by the expression of CD1a, CD1c, DC-sign, HLA-DR and low/undetectable CD14. This effect was not observed in cells cultured at neutral pH. Taking the expression of CD1a as a marker of DCs, we found that isolated monocytes cultured with PHA for 7 days, at pH 6.5 or 7.3, resulted in 70±10% vs 0.8±0.6% of CD1a+ cells respectively (mean±SE,n=6,p<0.001). A similar phenotype was observed for isolated monocytes cultured for 7 days at pH 6.5 in medium supplemented with 0.1% BSA, in the absence of any other stimulus: 40±10% vs 2±1% of CD1a+ cells, (pH 6.5 vs 7.3 respectively, n=15, p<0.001). These CD1a+ cells significantly (p<0.01) up-regulated the expression of HLA-DR and CD86 upon LPS stimulation, suggesting the acquisition of a DC-like mature phenotype. Moreover, they were shown to be able to stimulate the proliferation of allogeneic CD4 T cells with much more efficiency than monocyte-derived macrophages obtained by treatment with M-CSF (p<0.01), and in a similar fashion than DCs differentiated under traditional conditions (in the presence of IL-4 + GM-CSF). Our results suggest that local acidosis might be a relevant trigger of DC differentiation during the course of inflammatory processes.

(696) **DIFFERENTIAL EFFECTS OF LACTIC ACID BACTERIA PEPTIDOGLYCANS ON LUNG EPITHELIAL CELLS AND ALVEOLAR MACROPHAGES**

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We demonstrated that peptidoglycan from *Lactobacillus rhamnosus* CRL1505 (Pg05) is as effective as viable strain for improving systemic and respiratory humoral immune response against *Streptococcus pneumoniae* in malnourished mice. These immunomodulatory capacity cannot be extrapolated to peptidoglycans (Pgs) from other strains. The aim of this work was to study the interactions of Pgs obtained from different lactobacilli, with mice lung epithelial cells (LEC) or alveolar macrophages (AM), in order to elucidate the mechanisms involved in the differential effect of Pg05. We compared Pg05 with Pgs from the non-probiotic *L. rhamnosus* CRL534 (Pg534) and from the probiotic *L. plantarum* CRL1506 (Pg06). AM or LEC were cultured 24h or 24-48h with different concentrations of Pg05, Pg06 or Pg534, in the absence or presence of *S. pneumoniae*. In addition, we studied the cytokines production of LEC with conditioned medium from AM pre-stimulated with Pgs. The results showed that 90% of LEC and AM remained viable after the treatments with Pgs. Pg05 (50 ug/mL) and Pg06 were able to differentially modulate the cytokines production in AM and LEC. Unlike other Pgs, Pg05 significantly modulated the activation of AM by an increase of IL-1β, KC and IL-10 with a decrease of TNF-α (p<0.05). In the same way, although Pg05 and Pg06 induced an increase of IL-17 in LEC, only Pg05 was able to increase IL-10. Stimulation of LEC with AM conditioned medium showed a diferential production of cytokines between pgs, with an pro-inflammatory profile to Pg06 and Pg534, and an immunomodulatory profile to Pg05.Pam-3-cys (TLR1/TLR2 agonist) increased the effect of the Pgs, suggesting the participation of TLR2 in the interaction Pg-LEC. In conclusion, the immunomodulatory effect of Pg05 is a strain specific property and it could act inducing the activation of epithelial cells and macrophages, which could be responsible to improve the immune response against pathogens.

Key words: peptidoglycan, Lactobacilli, lung epithelial cells, alveolar macrophages.

(839) **EFFECTS OF SAGS OF THE EGC OPERON ON INNATE IMMUNE CELLS**

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Bacterial superantigens (SAgs) are enterotoxins that bind to MHC-II and TCR molecules, activating as much as 20% of T cells and promoting a cytokine storm which enhances endotoxic shock and produces immunosuppression, hindering the immune response against bacterial infection. The egc operon reported in autochthonous S. aureus strains includes natural variants of the SAgs genes seg, sei, sem, sen and seo. We have previously characterized the complementary activity of SEG, SEI, SEO and SEM on phagocytic cells, and reported a M1 response tendency.

In the present work, we investigate if other cells of the innate immunity are responsible for this pro inflammatory profile. Thus, we evaluated the SAgs effect on human purified γδ T cells from healthy donors by flow cytometry and ELISA. We found that, in contrast to the effect on αβ T cells, SAgs activated γδ T cells from 0.1 μM (p<0.05), but became toxic at 10 μM. We also found a significant production of IFN-γ and TNF-α since 0.1 μM (p<0.05). Such activity was not related to the αβ TCR binding site, since mutant SAg which not bind to this TCR, activated γδ T cells as much as wild type SAgs. Therefore, SAgs display a differential activity on γδ T cells, compared to αβ T cells.

Given that CD1 molecules are targets of γδ TCR we analyzed their expression on human mononuclear cells upon SAgs stimulation, by flow cytometry. We found a differential expression of monocytic CD1a and b in response to these SAgs (p<0.1).

The previously described M1 profile induced by SAgs on monocytes and macrophages and the present demonstration that SAgs stimulate γδ T cells to produce IFN-γ and TNF-α, strongly suggest an early pro inflammatory activity of SAgs on the innate immune response. In addition, the reduction of the pool of phagocytic and effector cells and the generation of a non-efficient Th1 profile would conspire against the successful eradication of an extracellular bacteria.

(941) **DECTIN-1 PATHWAY-MEDIATED IMMUNOMODULATION IN THE LOCAL RESPONSE DURING *CANDIDA ALBICANS* BRAIN INFECTION.**

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b-glucans of the fungal wall are recognized by Dectin-1 receptor. This interaction is essential for immune response to *C.albicans* in the periphery, but little is known about its role during Central Nervous System infections. b-glucans are widely recognized as immunomodulators, and it has been proved *in vitro* that the activation of microglia through Dectin-1 attenuates the proinflammatory response. Our aim was to evaluate the role of Dectin-1 recognition in brain, during *C.albicans* systemic infection. Male C57BL/6 (WT) and Dectin-1 deficient (KO) mice were injected intravenously with *C.albicans* SC5314 ($2.5x10^6$ yeast), and at 4, 12, 24 and 48h post-infection (pi) the animals were sacrificed. The fungal burden (CFU) was studied in blood, kidney and brain homogenates. An early colonization was observed in the periphery as well as in brain(p_{4h}<0.05). Brain innate immune response was assessed at early times, in order to study the contribution of resident cells. At 4 and 12h, Dectin-1 and IL-1b expression (qPCR), *in situ* pro- and anti-inflammatory cytokines secretion (IL-1b,IL-6,TNFa,IL-10,TGFb)(ELISA), histopathological and microgliosis/astrogliosis studies were performed. The fungal burden was higher in WT than KO mice(p<0.05). Interestingly, infection in WT mice does not induce proinflammatory cytokines secretion at 4h, while a significant increase was observed in KO mice with sustained levels at 12h. Local IL-1b and TNFa secretion in KO mice was significantly higher than in WT animals at 4h(p<0.05). Moreover, the IL-1b mRNA decreased at 12h pi in WT(p<0.05) but not in KO mice. TGFb production was downregulated after the infection in WT($p_{4h,12h}$<0.05) but not modified in KO mice. When TGFb secretion between groups was compared, significant differences were observed(p<0.05).This study provides new evidences about the role of Dectin-1 in immunomodulatory mechanisms developed in the brain in order to moderate the delicate balance between inflammation and fungal control.

Keywords: *C.albicans*, Dectin-1, b-glucans.

(1287) **EFFECT OF IL-10 ON RECRUITMENT OF PMN-LIKE MYELOID CELLS (PLMCs) DURING THE ESTABLISHMENT OF BACTERIAL ENDOTOXIN-INDUCED TOLERANCE**

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In sepsis by Gram-negative, endotoxin -LPS- is capable of inducing a tolerance state to LPS (TOL), the initial step of immunosuppression, the main cause of death. We have previously demonstrated that the TOL in absence of IL10 could improve innate immune response mainly mediated by PMN recruitment. The aim of this work was to evaluate the IL-10 effect on the recruitment of PLMCs with high levels of CD11b+/GR1+ and CD11b+/Ly6G+ in TOL. For this, BALB/c wild type(WT) and IL-10 deficient(KO) mice are used. The TOL establishment is achieved with a regimen of increasing doses of LPS (0.25-10ug/mouse) for 10 days. Glucocorticoids (GC) levels are measured by RIA. Flow cytometry was applied to measure PLMCs. During the establishment of TOL an increase in the percentage of recruitment of PLMCs is shown, this increase is greater in KO: %cells-spleen: WT-TOL:10.99±1.24, KO-TOL:32.26±4.93*, *p<0.001; %cells-femur: WT-TOL:45.02±2.14, KO-TOL:73.85±4.33*, *p <0.0001 (these data correlate with the increase previously seen in blood and peritoneum). At the same time, GC levels remain elevated in TOL mainly in KO: [GC](pg/ml) day 3 (WT-TOL:1800±591, KO-TOL:9092±1654*, *p<0.05), day 7 (WT-TOL:1696±523, KO-TOL:11203±518*, *p<0.001) and day 11 (WT-TOL:1725±219, KO-TOL:9992±2492*, *p<0.0001). At the end of the TOL schedule, mice received a lethal dose of LPS(200ug/mouse) and survival was evaluated up to 48hs: % survival: WT-TOL:100%(5/5), KO-TOL:0%(0/4). Our results indicate that the IL-10 is involved in the recruitment of PLMCs into TOL and this action could be regulated by the relationship between IL-10 and GC. On the other hand, the increase of PLMCs along with the high levels of GC in the TOL in the KO mice does not seem sufficient to protect them from a LPS shock. These facts will be further developed in the future.

Keywords: Sepsis, LPS, IL-10, GC, PMN.

(1294) **DIFFERENTIAL RESPONSE OF DOPAMINE MEDIATED BY β-ADRENERGIC RECEPTORS IN HUMAN KERATINOCYTES AND MACROPHAGES: POTENTIAL IMPLICATON IN WOUND HEALING**

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Dopamine (DA) is a neurotransmitter modulator of the immune system. Against external agents, keratinocytes and macrophages of the cutaneous immune system produce pro-inflammatory cytokines and metalloproteinases (MMP), which are involved in wound healing. Both cells have a catecholaminergic system thought to modulate several processes in the skin such as inflammation and tissue repair. In this work, we evaluated the effect of dopamine (10^{-6}, 10^{-5}, 10^{-4}M) in the presence or absence of the β-adrenergic antagonist propranolol (10^{-5}M) on wound closure (wound scratch assay), MMP activity (MMP-9, zymography), cytokine production (IL-8, IL-1β, ELISA), IκB/NFκB pathway activation (western blot), in a cell line of human keratinocytes HaCaT and THP-1 macrophages (PMA-differentiated cells). We previously demonstrated that DA increased IL-6 and IL-8 production by β-adrenergic receptor in human keratinocytes. In this work, we demonstrated that DA (10^{-5}) did not modify the wound closure in keratinocytes, but decreased the propranolol stimulatory effect ($p<0.05$) delaying the cell migration. MMP-9 activity ($p<0.001$) and propranolol-induced MMP9 activity were decreased by DA ($p<0.05$). In addition, DA (10^{-5}) increase NFκB-p65 levels in nuclear extracts ($p<0.01$), this effect was reduced in presence of propranolol ($p<0.05$), suggesting NFκB pathway is involved in the effect of DA on keratinocytes. On the other hand, DA (10^{-5}) increased MMP-9 activity ($p<0.01$) without affecting the propranolol-induced MMP-9 activity in THP-1 macrophages. DA increased IL-8 production ($p<0.01$), effect that was reduced in presence of propranolol ($p<0.05$). However, DA did not modify NFκB-p65 in nuclear extracts, evidencing the latter pathway is not implicated in the effect of DA in human macrophages. In conclusion, these results suggest a differential effect of DA, via β-adrenergic receptors, that depends on the physiological condition and the cell type involved that could affect the wound healing process.

Keywords: dopamine, beta-adrenergic receptors, keratinocytes, macrophages.

(1736) **CHARACTERIZATION OF THE SUBCELLULAR COMPARTMENT INVOLVED IN HUMAN NEUTROPHIL INTERLEUKIN-1**B **(IL-1 B) SECRETION**

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IL-1β is a major proinflammatory cytokine synthesized in the cytoplasm as an inactive precursor that is activated by proteolytic cleavage. It is a leaderless cytosolic protein that is secreted by unconventional mechanisms different from the classical RE-Golgi pathway. We previously determined that human neutrophil IL-1β processing is dependent on caspase-1 and elastase and/or proteinase-3. Our studies also indicated that neutrophil IL-1β is secreted by an unconventional secretory autophagic mechanism. Here we aim to get insights of the subcellular mechanisms that control IL-1β secretion from human neutrophils by employing immunofluorescence staining and confocal microscopy. We determined that after 3.5 h post-LPS+ATP stimulation part of the neutrophil IL-1β colocalized with elastase and myeloperoxidase in a vesicular compartment (n=3). Neutrophil stimulation with LPS+ATP also induced colocalization of caspase-1 with IL-1β (Mander´s coefficient -MC-: 0.52; n=2; at least 80 cells analyzed/experiment), and this effect was potentiated when neutrophils were also subjected to starvation (MC: 0.59). We also found colocalization of IL-1β with the chaperone protein HSP90 (MC: 0.55 and 0.47; full nutrient and starvation, respectively; n=2). Noteworthy, wide vesicles with IL-1β overlapping with HSP90 signals were found protruding from the cell membrane. By employing the fluorescent probe FLICA to track the presence of activated caspase-1 in live cell imaging assays, we detected FLICA signal as defined spots inside cells with a pattern similar to that observed with caspase-1. Additionally, IL-1β did not colocalize with LAMP-2 at either 3.5 or 5 h post-LPS+ATP stimulation (MC: 0.21 and 0.22; n=4, at least 50 cells analyzed/experiment). Our results suggest that IL-1β is packaged in vesicular compartments which can also include caspase-1 and/or elastase where it might be processed or degraded. HSP90 might contribute to IL-1β entry to vesicles different to lysosomes.

Keywords: Neutrophil, autophagy, inflammation, interleukin-1β

IMMUNOLOGY (CLINICAL IMMUNOLOGY) 2

(1436) **CD32 EXPRESSION IDENTIFIES FUNCTIONALLY DISTINCT NEONATAL CD4+ T CELLS**

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Receptors for the Fc portion of IgG (FcγR) represent a critical link between innate and adaptive immunity. They enable IgG to trigger effector functions such as cytotoxicity and complement activation. In addition to acute disease, Respiratory Syncytial Virus (RSV) bronchiolitis is associated with asthma during infancy. Although the expression and activity of FcγRII (CD32) on neonatal CD4+ T cells could influence their function, it has not been characterized.

Our aim consisted in identifying CD32 expressing T cells in cord blood mononuclear cells (CBMCs) by flow cytometry; as well as to determine whether cross-linking of FcγR by immobilized IgG modulated the pattern of secreted cytokines. Moreover, we also analyze the frequency of CD4+CD32+ T cells in RSV infected infants.

Our data demonstrated that CD4+CD32+ T cells represent ~3.9% ± 0.6 of CD4+ T cells (n=10). In contrast with CD4+CD32- T cells, this subset mainly consists of naive CD45RA+ and CCR7+ cells ($p<0.01$). We also found that CD4+CD32+ T cells showed a higher frequency of CD25+, FOXP3+, and CD16+ (FcγRIII) markers, compared with CD4+CD32- T cells. We then analyzed whether immobilized IgG, could modulate the production of cytokines by CD4+ T cells. Functionally, culture on immobilized IgG did not change the production of IL-2 and IL-8, but significantly enhanced the secretion of IL-5 ($p<0.01$) in comparison with IgG non-stimulated CBMCs. Interestingly, we detected a significant higher frequency of circulating CD4+CD32+ T cells in severe RSV-infected children compared with CBMCs ($p<0.01$; n=15).

Our data suggest that CD32 expression by neonatal CD4+ T cells modulates their functional profile by promoting Th-2 like response. Because, severe RSV infection in early life is associated with asthma, the fact that RSV children expressed higher frequency of CD4+CD32+ T cells might play a role in the harmful immune response during disease.

Keywords: CD4+ T cells, CD32, CBMCs, RSV

(1664) **EXPRESSION OF DOPAMINE RECEPTORS IN PBMC FROM PATIENTS WITH PULMONARY OR PLEURAL TUBERCULOSIS. A POTENTIAL ADVERSE PROFILE**

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Tuberculosis (TB) is the second cause of death worldwide, mostly affecting the lungs (pulmonary TB, PTB), although pleural tuberculosis (PLTB) can be a frequent extrapulmonary manifestation. The immune system receives and sends messages to the central nervous system modulating each other through the Hypothalamus Pituitary Adrenal axis and the autonomous nervous system (ANS). When studying patients with PTB or PLTB we have recently shown that both patients groups had increased dopamine plasma levels. To extend this observation and mainly because dopamine effects are strongly dependent on the types of receptors expressed by target cells, we have now analyzed the expression of transcripts (qRT-PCR) for the dopamine receptors (DRs) of type 1 (DR-1 and DR5, both mediating inhibitory effects) and type 2 (DR-2, DR-3 and-DR4; mostly stimulating) in peripheral blood mononuclear cells (PBMC) of patients with PTB (n = 18) and PLTB (n = 12) and healthy controls (HCo; n=16). Both patient groups showed a significant decrease in the transcripts for DR-3 and DR4 ($p<0.05$ for both). Concerning DR, its expression was slightly increased but statistically insignificant in both TB patient groups. The expression of DR5 and DR2 was nearly undetectable precluding any between-group comparisons. Since the ultimate effect of DA on cells is a consequence of the combined action of the DRs, we calculated the DR1/DR3 and DR1/DR4 ratios (inhibition/stimulation) which appeared both increased in PTB and PLTB patients ($p<0.03$, $p<0.001$, respectively). Therefore, that DRs expression profile from PBMCs of TB patients is more compatible with a predominant inhibitory ability respect HCo. These findings along with the previously shown increased dopamine levels, point out to a detrimental effect of the peripheral dopaminergic system on the development of cellular immune responses in TB.

(94) **CYTOTOXIC CD8+CD127- T CELLS LEVES IN HIV INFECTED CHILDREN**

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Introduction: homeostatic expansion of naive and memory T cells subsets require normal IL-7 alpha chain (CD127) expression levels. This molecule is exclusively expressed on T cells, and in HIV (+) adults patients, its down-regulation is associated with increase CD8+ T cells apoptosis rate.

Objective: to determine CD8+CD127- T cells levels in HIV infected children

Material and methods: percentage of CD8+CD127- T cells levels in 66 patients with HIV infection by vertical transmission, in antiretroviral therapy, was studied by flow cytometry (FACSCalibur, BD) and specific monoclonal antibodies. Plasma HIV RNA viral load (VL) was determined by PCR NASBA. A control group (Co) of 10 healthy children was also studied.

Results: according to immunologic categories of Classification System for HIV infected children: LTCD4+percentage levels ≥ 25% (no evidence of suppression), < 24 % (evidence of moderate or severe suppression), and VL: detectable or not (> or < 50 IU/ml), children were divided in four groups. Percentage levels of CD8+CD127- T cells in different groups were: A: (n: 8) (CD4<24%, VL<50 IU/ml): 13±7, B (n: 37) (CD4≥25%, VL<50 IU/ml): 7±4, C

(n: 10) (CD4<24%, VL> 50 IU/ml): 14±2, D (n: 11) (CD4≥25%, VL> 50 IU/ml): 10 ± 4. Co group showed a percentage of LTCD8+CD127- of 5±2 %. A significant increase ($p\leq 0.05$) of CD8+CD127- T cells levels in patients of A, C and D groups, against B and Co children were observed. A significant negative correlation between LTCD4+ and LTCD8+CD127- percentage levels (r: - 0.448, $p\leq 0.05$) was recorded. A significant positive correlation (r: 0.272, $p\leq 0.05$) between VL values and LTCD8+CD127- percentage levels, was also observed.

Conclusion: the increase of loss of CD127 expression on cytotoxic CD8+ T-cells in children with moderate or severe immunosuppression, and its correlation with viral replication and disease progression markers, could be another feature of HIV-associated immune perturbation.

Keywords: CD8+ T cells, CD127, HIV,children.

(968) **FERTILE HYDATIDS AND CO-INFECTION WITH FASCIOLASIS MODULATES THE CYTOKINE PROFILE IN CATTLE WITH CYSTIC ECHINOCOCCOSIS**

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Introduction: During Cystic Echinococcosis (CE), the immune interplay between the parasite and the host is complex, encompassing effective parasite-killing immune mechanisms developed by the host, which in turn are modulated by the parasite. However, there is no report that describes the cytokine profile in animals with different CE fertility status and co-infection with Fasciolasis (FAS). **Material and Methods:** Blood samples were collected from 38 cattle before slaughtered and classified in 5 categories: Healthy, CE fertile, CE infertile, CE infertile + FAS, CE cyst <1cm. IL-18, IL-10 and IFN-γ serological concentration was determined by MULTIPLEX (Millipore™) assay. **Results:** IL-18 serological concentration in animals

with CE fertile had a mean of 0.22 ng/mL, which is almost the double than the other groups. The mean concentration of IL-10 in animals with CE fertile was 0.21 ng/mL, while the rest of the groups were lower. In the other hand, the concentration of IFN-y in CE infertile (0.99 ng/mL) and CE infertile + FAS (1.0 ng/mL) was slightly higher than the other groups. **Conclusion:** The results suggest that fertile hydatids and co-infection with Fasciolasis modulates the cytokine profile in cattle with Cystic Echinococcosis. **Acknowledgments.** Fondecyt-Chile 1161475; Universidad Andrés Bello (DI-1398-16/I, DI-1249-16/RG).

Key Words: Hydatidosis, Echinococcus, fertility, Distomatosis.

(1284) **HUMAN NEUTROPHIL ELASTASE DEGRADES THE THERAPEUTIC MONOCLONAL ANTIBODIES EFFECTIVE IN IBD**

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Therapeutic monoclonal antibodies which are effective in other inflammatory diseases such as rheumatoid arthritis are less effective inflammatory bowel disease (IBD). Human Neutrophil Elastase (HNE) is highly expressed in IBD mucosa, especially in ulcerative colitis (UC). The aim of this study was to determine if HNE degrades biologics, rendering them ineffective, and whether its action can be reversed by its natural inhibitor, Elafin.

Biologics (Infliximab, Adalumimab, Etanercept, Vedolizumab) were digested using different concentrations of recombinant HNE, in the absence or presence of Elafin, overnight. Neutrophils where isolated from human blood (4 UC patients and 2 healthy donors) by gradient centrifugation with Na-Dextran solution. Neutrophils where lysed and elastase activity was quantified. Antibody integrity after recombinant or natural HNE digestion was then analysed by western blot, and the functional capacity of the antibodies to neutralise TNF-alpha was tested using recombinant human TNF-alpha and a TNFR reporter cell line. Recombinant and HNE from blood cells fully degrades all anti-TNF-alpha agents and Vedolizumab (anti-α4β7 integrin specific mAb) in a dose-dependent manner (HNE 0.125μg/ml to 5 μg/ml degrades biologics from 6% to 99.9%, respectively). This activity is significantly inhibited by recombinant Elafin ($p<0.001$). Treatment with HNE also partially prevented the ability of the biologicals to inhibit TNF-alpha bioactivity (HNE at 10μg/ml causes 90% reduction of the anti-TNF›s neutralizing ability, while HNE at 5 μg/ml diminishes only 25% their neutralizing activity).

These results may explain some of the reasons for primary non-responsiveness to anti-TNF alpha therapy in IBD patients.

Keywords: Inflammatory Bowel Disease, Human Neutrophil Elastase, Elafin, Therapeutic Monoclonal antibodies.

(1387) **HLA- DQ2/ DQ8 AND ANTIBODIES ASSOCIATED TO CELIAC DISEASE IN RISK INDIVIDUALS**

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About 90% Of Celiac Disease (Cd) Patients Carry Hla-Dq2 Or Dq8 Heterodimers. Hla Typing Provides Additional Data In Uncertain Cases And Risk Groups Such As Relatives, Autoimmune Diseases, And Genetic Syndromes. Serological Tests For Anti-Transglutaminase (Tg), Anti-Endomysium (Ema) And Anti-Deamidated Gliadin Peptide (Dgp) Antibodies (Abs) Are A Useful Diagnostic Tool For Its High Sensitivity And Specificity.

Aim: To Determine Frequency Of Hla Dq Alleles Associated With Cd And Its Relation To The Presence Of Specific Abs In Risk Individuals.

Materials And Methods: 83 Patients, Ẋ Age: 7 Years Old (Range: 1-40). Group1:55 Cd Relatives, Group 2: 28 With Associated Diseases Or Clinical Suspicion. Hla -Drb1, Dqa1 And Dqb1 Genes By Polymerase Chain Reaction-Sequence Specific Primers (Pcr-Ssp) (Biodiagene®Typing). Serum Abs Tg And Dgp By Elisa; Ema By Ifa And Iga Levels By Nephelometry.

Results: Alleles Total Frecuency: 62.7% Dqb1*02 (Dq2); 39.8% Dqb1*0302 (Dq8); 45.8% Dqa1*05 (Dqx.5). 78/83 (94%) Patients(P) Carried One Allele Associated With Increased Risk Of Cd And 16/83 (19%) Had Some Specific Abs. **62P Had High-Risk Alleles: 9**P With Double Doses Of Dqb1*02, 8 Had Complete Haplotype Dr3 Dqa1*05Dqb1*02, And Only 3 Had Increased Levels Of Specific Abs; **20**P Showed Dqa1*05Dqb1*02 (One Dose),8 Had Specific Abs (1 With High Titer); **22**P Carried Dqa1*03Dqb1*0302, 2 With Specific Abs; **6**P Had Dr3dqa1*05Dqb1*02/Dr4 Dqa1*03Dqb1*0302, 1 Had High Level Specific Abs; **5**P Showed Dqb1*02/Dqa1*03Dqb1*0302, Without Abs. **16P Had Low-Risk Alleles**: **12**P One Dose Dqb1*02 (3P Had Specific Abs And 1 With High Titer); **4**P Dqa1*05 Without Other Allele Or Abs. We Didn't Find Difference In Abs Or Allele Distribution Between Groups. Clinical Symptoms Were Found In 23/43P, 20P With High-Risk Alleles, Only 6 With Positive Abs.

Conclusions: 74.7% Of Our Patients Carried High-Risk Haplotypes For Cd, With Higher Frequency Of Dq2. Only 6 P Had High Serum Levels Of Cd Specific Abs.

This May Suggest Cd Diagnosis.

Keywords: Celiac Disease, Hla Dq2/Dq8

(1803) **GALECTIN-1–DRIVEN TOLEROGENIC PROGRAMS AGGRAVATE *YERSINIA ENTEROCOLITICA* INFECTION BY REPRESSING ANTIBACTERIAL INMUNITY**

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Yersinia Enterocolitica (Ye) Is An Enteropathogenic Bacterium That Causes Gastrointestinal Disorders. To Subvert The Host's Immune Response, Ye Uses A Type Iii Secretion System And Effector Proteins, Called Yersinia Outer Proteins (Yops), That Modulate Activation And Survival Of Immune Cells. In This Study, We Show That Galectin-1 (Gal-1), An Immunoregulatory Lectin Widely Expressed In Mucosal Tissues, Contributes To Ye Pathogenicity By Undermining Protective Antibacterial Responses. We Found Higher Expression Of Gal-1 In The Spleen And Peyer's Patches (Pps) Of Mice Infected Orogastrically With Ye Compared With Noninfected Hosts ($P<0.01$ In Spleen At Day 5, $P<0.001$ In Pps At Day 5, $P<0.05$ In Spleen And Pps At Day 7). This Effect Was Prevented When Mice Were Infected With Ye Lacking Yopp ($P<0.01$) Or Yoph ($P<0.05$), Two Critical Effectors Involved In Bacterial Immune Evasion. Consistent With A Regulatory Role For This Lectin, Mice Lacking Gal-1 Showed Increased Weight ($P<0.05$) And Survival, ($P<0.001$) Lower Bacterial Load At 5 And 14 D Postinfection ($P < 0.001$, $P < 0.05$, Respectively), And Attenuated Intestinal Pathology Compared With Wild-Type Mice. Thus, Lack Of Endogenous Gal-1 confers protection against Ye infection. Supplementation of recombinant Gal-1 in mice lacking Gal-1 or treatment of wild-type mice with a neutralizing anti-Gal-1 mAb confirmed the immune inhibitory role of this endogenous lectin during Ye infection. Finally, a possible functional interaction between YopP and Gal-1 via YopP glycans is suggested. Thus, targeting Gal-1-glycan interactions may contribute to reinforce antibacterial responses by reprogramming innate and adaptive immune mechanisms.

Keywords: Yersinia enterocolitica, virulence factors, Galectin-1

(1820) **FOLLOW UP DURING 6 YEARS OF 52 PATIENTS WITH SUBCUTANEOUS IMMUNOGLOBULIN TREATMENT BY PUSH AS REPLACEMENT AND IMMUNOMODULATORY THERAPY**

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Introduction: Several studies have shown that subcutaneous Immunoglobulin (SCIG) is as good as Intravenous Immunoglobulin (IVIG) preventing infections in primary immunodeficiencies (PID), also SCIG has been proposed as an alternative in the immunomodulatory treatment (IT) of many neurological diseases and other diseases and it seems to be as efficient as IVIG.
Objetive: To describe the follow up of 52 patients (p) with SCIG treatment during 6 years in a single center in Argentina. Results: We reviewed the clinical history of two Groups (G) of patients followed in our center, G1: 44p with PID with IG replacement treatment, G2: 8p with IT for different diseases. Mean age: G1 19.4yo (1.25 – 63.4), G2 43 yo (12.5 – 77.2). Among the G1 6p are under 5 yo (mean age 3.2) 23 are between 5 and 18 (mean 10) and 15 are over 18 yo (mean 40.2). The mean time of follow up was 2,98 years. 13 patients were follow for more than 5 years and 26 patients between 1 and 5 years. SCIG was administered at 1 or multiple injection sites by push, in each site a maximum of 20 ml in children and 35 ml in adults. G1: The mean dose was 144 mg/kg/w.G2: Dose was 300 mg/Kg/w. G1: The mean serum IgG level was 1143 mg/dl. Levels were stable over the years. Efficacy: Among the G1 p, the annual rate of infection was 0,19 infections/patient/year. G2: All patients presented remission. Tolerance: 28,8% presented mild episodes related with the injection site and only three p presented 3 systemic adverse reactions in a total of 10,449 infusions. Conclusion: SCIG therapy is safe and effective for replacement treatment in PID patients, even in children under 5 years old. SCIG may be as effective as IVIG in the maintenance therapy in CIDP and other autoimmune diseases. Further systemic clinical studies are needed to better define combination therapy, optimal dosage and application intervals of SCIG.
Keywords: primary immunodeficiencies, immunoglobulin.

(1913) **HORMONAL IMBALANCE DURING HIV-TB CO-INFECTION: SIMULTANEOUS DETERMINATION OF DEHYDROEPIANDROSTERONE AND ITS BIOLOGICALLY ACTIVE OXYGENATED METABOLITES IN HUMAN PLASMA**

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Abstract: An estimated one third of the world's population is affected by latent tuberculosis (TB), which once active represents a leading cause of death among infectious diseases. Human immunodeficiency virus (HIV) infection is a main predisposing factor to TB reactivation. Individuals HIV-TB co-infected develop a chronic state of inflammation associated with several metabolic disorders, as hypothalamic-pituitary-adrenal (HPA) axis dysregulation. This results in a hormonal imbalance, disturbing the physiological levels of cortisol and dehydroepiandrosterone (DHEA). DHEA and its oxygenated metabolites androstenediol (AED), androstenetriol (AET) and 7-oxo-DHEA are immunomodulatory compounds that may regulate physiopathology in HIV-TB co-infection. In order to study possible changes in plasma levels of these hormones, we developed an approach based on high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS). To our knowledge, this represents the first report of their simultaneous measurement in HIV-TB individuals and the comparison with healthy donors, obtaining statistically higher plasma levels of DHEA, AET and 7-oxo-DHEA in patients. Moreover, we found that concentrations of 7-oxo-DHEA positively correlated with absolute CD4+T cell counts, nadir CD4+T cell values and with individuals who presented TB restricted to the lungs. This research contributes to understanding the role of these hormones in HIV-TB and emphasizes the importance of deepening their study in this context.
Keywords: HIV-TB co-infection, 7-oxo-DHEA, HPLC-MS/MS

(610) **ANALYSIS OF PELVIC PAIN DEVELOPMENT, MAST CELL INFILTRATION AND PROSTATE INFLAMMATION IN AN ANIMAL MODEL OF CHRONIC PELVIC PAIN SYNDROME**

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Pain and inflammation in the absence of infection are hallmarks in Chronic Pelvic Pain Syndrome (CPPS) patients. In this set, mast cells have been pointed out as key players in pain induction and central sensitization. Although its etiology remains unclear, autoimmunity has been proposed as a cause and animal models of Experimental Autoimmune Prostatitis (EAP) models have long been used for studying CP/CPPS.

Herein, we analyzed prostate inflammation induction, mast cell infiltration and chronic pelvic pain development in EAP in three common laboratory mouse strains presenting with differential susceptibility to autoimmune prostatitis: the NOD mice (highly susceptible), the C57BL/6 mice (moderately susceptible), and the BALB/c mice(resistant).

Chronic pelvic pain development, evidenced by increased tactile allodynia responses, was similar between immunized NOD and C57BL/6 mice, although the severity of leukocyte infiltration and inflammation was greater in the first case. In fact, prostate tissue from NOD mice revealed markedly increased expression levels of inflammatory cytokines and chemokines. Similar results, but to a lesser extent, were observed when analyzing prostate tissue from C57BL/6 mice. However, similarly increased numbers of mast cells, mostly degranulated, were detected in prostate samples from either NOD or C57BL/6 mice. Conversely, minimal prostate leukocyte infiltration and inflammation was observed in immunized BALB/c mice. Besides, they showed no pelvic pain development and the lowest prostate tissue mast cell total counts and in a resting state.

Our results provide new evidence indicating that NOD, C57BL/6, and BALB/c mice develop different degrees of chronic pelvic pain, type and amount of prostate inflammation and mast cell infiltration. Remarkably, chronic pelvic pain development correlated with mast cell infiltration, suggesting that mast cells are inflammatory cells involved in mediating pelvic pain induction.

Keywords: pain, inflammation, prostatitis, mast cells, chronic pelvic pain syndrome.

ANIMAL BIOLOGY 4

(806) **EXOGENOUS YAMANAKA FACTORS (h-OKSM) MAINTAIN PLURIPOTENCY OF BOVINE AND PORCINE IPSC-LIKE CELLS OBTAINED WITH STEMCCA DELIVERY SYSTEM**

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Abstract: The use of induced reprogramming of somatic cells to produce transgenic animals requires the development of a biotech-

nological platform. In our study, different strategies for the generation of induced pluripotent stem cells (iPSC) of bovine and porcine cells were evaluated. Episomal and lentiviral vectors were used for delivering reprogramming human factors OCT4, SOX2, KLF4 and c-MYC (h-OKSM) into bovine (BEF) and porcine (PEF) embryonic fibroblasts. Cells were cultured in different media containing different supplements: DMEM/F12 with KSR + FGF, E8 medium for culturing on feeder and serum free conditions and finally SB43 medium in which MAPK/ERK, Wnt and TGF pathways are modulated. Episomal vectors (1x, 2x and 4x runs of transfections) failed to generate iPSCs in both species. In these cultures, we detected a gradual loss of expression of exogenous factors. On the other hand, protocols based on the integrative lentiviral vector, STEMCCA, produced porcine iPSC-like cells more efficiently than from bovine cells. The iPSC generated maintained stem cells features and expression of exogenous factors. Inactivation of STEMCCA is still a bottleneck for establishing bonafide iPSC, since culture media tested did not probably maintain endogenous OKSM expression.

Keywords: iPSC-like cells, bovine and porcine fibroblasts, episomes, STEMCCA, lentiviral vectors

(303) **PEROXIDATION OF CANINE ERYTHROCYTE MEMBRANES: RELATION WITH THE OXIDATIVE STRESS CAUSED BY EXERCISE**

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Exercise is considered as a condition of excessive generation of reactive oxygen species (ROS), which can produce oxidative stress and pose potential problems, inducing the loss of membrane integrity and cellular dysfunctions. In relation to blood cells, circulating erythrocytes are regularly exposed to stress conditions and are especially vulnerable as they have no membrane repair mechanism or regenerative capacity. The present investigation was carried out to determine the presence of oxidative alterations in the erythrocyte membrane in dogs submitted to a high intensity exercise test by estimating the degree of peroxidation by chemiluminescence in a suspension of lysates erythrocytes incubation with t-butyl hydroperoxide (t-BHP). Samples were taken with the animal at rest before exercise (T0 or rest), at the maximum exercise time (T1 or exercise) and at the end of the recovery (T2 or recovery). Suspension of lysates erythrocytes with hypotonic phosphate buffer were incubated at a final concentration of 0.25 mg/ml total hemoglobin in an in vitro system for 60 min. at 37°C in the presence of 2 mM of t-BHP. Identical aliquots of the preparation were incubated without additions as the control experiment. Peroxidation was measured by monitoring light emission with a liquid scintillation analyzer Packard 1900 TR. Differences were observed in the total values of chemiluminescence throughout the exercise routine, with observed values of 339.620 cpm, 275.290 cpm and 311.630 cpm for T0, T1 and T2 respectively The conclusions of this study are the existence of changes in the erythrocyte membranes of the dogs subjected to physical exertion, probably associated with the release of ROS caused by the exercise.

(47) **SPERM BINDING TO THE OVIDUCT FOR RESERVOIR FORMATION INVOLVES SCAVENGER RECEPTOR CYSTEINE-RICH (SRCR) DOMAINS OF THE GLYCOPROTEIN DELETED IN MALIGNANT BRAIN TUMOR 1 (DMBT1).**

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DMBT1 is present in two forms in the lumen and cells of pig oviduct. The membrane form participates of sperm binding for the formation of a reservoir, in which sperm stay quiescent until ovulation. DMBT1 contains several domains: Scavenger Receptor Cysteine-Rich (SRCR), CUB y Zona Pellucida (ZP), all of which may specifically bind different cells and proteins. It exposes Galβ1-3GalNAc and it is generally accepted that carbohydrate recognition is involved in reservoir formation. The aim of this work was to determine which of the domains of DMBT1 are important for reservoir formation. Sperm binding to oviductal cells was challenged *in vitro* after treatment with antibodies specific for each domain. Anti-SRCR was developed against polypeptide aa 203-689 obtained by cDNA cloning, expression in *E. coli*, purification and rabbit immunization. Anti-CUB was developed similarly in previous works for aa 578-803; and anti-ZP was developed using ZP isolated from porcine oocytes. Primary cultures of oviductal epithelial cells were treated with dilutions of the antibodies and pre-immune serum. Sperm were added and bound spermatozoa were counted (quintuplicate). The rate of sperm bound upon antibodies treatment/sperm bound with pre-immune serum was expressed as percentage, using sperm bound without any serum treatment as 100%. Assumption of normality was determined by Shapiro-Wilks test. Linear mixed Models with Heterogeneous Variance or ANOVA and LSD Fisher (software infoSTAT) were used. Only anti-SRCR antibodies inhibited sperm binding (41%, $p<0.05$). Glycosylation prediction (GlycoEP Predicton Service) shows scarce potential sites in SRCR, being the main O- glycosylation motifs at the scavenger interspersed domains that separate SRCRs. The present work does not allow distinguishing if SRCR polypeptide itself or carbohydrates potentially bound to it are responsible for sperm binding, but DMBT1's SRCR motifs are main domains in porcine sperm reservoir formation.

Keywords: sperm reservoir, DMBT1, SRCR, pig

(361) **THE ANTIOXIDANT EFFECT OF LUTEIN PROTECTS AGAINST OXIDATIVE DAMAGE TO PORCINE SPERMATOZOA**

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Boar sperm is especially susceptible to peroxidative damage generated by oxygen free radicals (ROS). Chemiluminescence (CL) was started incubated porcine sperm in an in vitro system ascorbate $-Fe^{+2}$, a technique that allows to assess oxidative stress in these cells. Lutein is known for its antioxidant effect, it is chemically a dihydric derivative of α-carotene and belongs to the group of xanthophylls. The main objective of this study was to investigate the antioxidants effects of lutein on the boars sperme. The effect of lutein was analyzed by two methods: 1) by addition to in vitro system ascorbate $-Fe^{+2}$ and 2) incubating lutein with porcine spermatozoa. Porcine sperm were incubated in an in vitro system ascorbate $-Fe^{2+}$ dependent, for 120 min. at 37°C in the presence of increasing amounts of lutein (50 μg, 150 μg and 250 μg) per mg of protein, in addition, samples of porcine semen previously were incubated with lutein (0.15 and 0.25 mg / ml) during 24 h at 15 ° C. Peroxidation was quantified in a liquid scintillation counter Packard 1900 TR by chemiluminescence in cpm (counts per minute). Analyzing the effect of lutein by the two previously mentioned methods, was observed that the total cpm/mg protein originated from light emission: chemiluminescence, was lower in samples obtained from lutein group than in the control group (without lutein), with a significance $p < 0,005$. The percentages of inhibition of peroxidation were not concentration dependent. These results show that lutein may act as an antioxidant protecting sperm membranes from oxidative damage.

(1256) ***TRIBOLIUM CASTANEUM*** **(COLEOPTERA: TENEBRIONIDAE): EPICUTICULAR HYDROCARBONS AND A VOLATILE SEX PHEROMONE.**

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The red flour beetle*, Tribolium castaneum* is a model organism for insect development studies and a major stored product pest of agriculture relevance. Insect surface is covered by hydrocarbons (HC) protecting them against chemical and physical aggression. The aim of this study was to identify and quantify the changes in the epicuticular HC of *T. castaneum* through the development cycle, and to investigate the potential presence of a volatile sex pheromone. Component identification was performed by capillary gas chromatography (CGC) coupled to mass spectrometry (MS); adult volatiles were extracted by solid phase microextraction (SPME) and analyzed by CGC-MS. The HC amounts increased significantly from larva to adult. In immature stages, saturated HC are predominant, with chain lengths of 23 to 43 carbons. A significantly different HC phenotype was observed in the adult stage, with large amounts of branched chains of 27 to 31. In this study, small amounts of a novel aldehyde component were detected in males. Females, but not males, were attracted to this chemical in bioassays. The MS analysis revealed a molecular weight of 210, a twelve carbon unsaturated backbone and at least one methyl-branch. Further analyses are ongoing to fully determine the structure. Here we show that *T. castaneum* HC undergo substantive changes through development, suggesting a precise regulation of the biosynthetic enzymes. The complex metabolic pathway for both, the HCs and the sex pheromone probably takes place in different tissues and is initiated by the action of two fatty acid synthases (FASs), with different affinity to incorporate methyl branches in the elongating chain. Five genes encoding FAS were identified in the *T. castaneum* genome. Studies will be started in order to identify the FAS genes responsible for HC and pheromone synthesis. This knowledge will help understand HC biosynthesis and its regulation. The discovery of a sex pheromone is of interest in pest control.

Keywords: Tribolium, Hydrocarbons, Pheromone, CGC-MS.

(684) **FUNCTIONAL AND MORPHOLOGICAL ASSESSMENT OF GLANDS OF PATAGONIAN FROGS: MOLECULAR CHARACTERIZATION OF PREPROPEPTIDES AND HISTOLOGY OF THE SKIN OF *Odontophrynus occidentalis* AND *Pleurodema somuncurense***

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Antimicrobial peptides present in amphibian skin are important functional molecules of the innate immune system and play defensive roles against external risk factors. The emergence of multiple strains of pathogenic bacteria resistant to conventional antibiotics has become a serious problem to public health, requiring the search of novel therapeutic molecules. We identified and characterized AMPs in the skin of adult specimens of *P. somuncurense (Pso)* and *O. occidentalis (Ooc)*. RNA from cephalic-scapular and dorsal skin regions was isolated and their cDNA was obtained. These cDNA were linked to the pCR™4-TOPO vector to transform *E.coli* DH5α into competent cells. We analyzed and sequenced positive clones, obtaining 11 prepropeptides that codified mature peptides with promising physicochemical features and novel structures. Several presented positive net charge, α-helix structure predicted by 3D software and amphipatic arrangement of the α-helix, which promote their mechanisms of action. Two peptides were similar to the thaulin family peptides, previously described by our group in the skin of the Patagonian frog *Pleurodema thaul*, while the remaining showed 32-53% percent of similarity when compared with other amphibian AMPs. The peptide synthesis in progress aims at characterizing and evaluating their antimicrobial, hemolytic and cytotoxic activity. Histological analysis of Ooc skin revealed the presence of ordinary mucous glands (OMG) and ordinary serous glands (OSG) in the six studied body regions. Both types of glands shared structural traits common in anurans. OMGs were homogeneously distributed, whereas OSGs showed an heterogeneous distribution and were more numerous in the scapular region. The discovery of these peptides is a promising result in the search of new antimicrobials and highlights the potential of Patagonian species as a novel source of AMPs.

Keywords: Antimicrobial peptides, cDNA, frog skin, glands

(135) **EVOLUTIONARY TRENDS IN *Pomacea* EGG CAROTENOPROTEINS INVOLVED IN A BIOCHEMICAL DEFENSE SYSTEM**

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The evolution of structure-function relationship of hyperstable proteins is poorly understood. Studying *Pomacea* snail carotenoproteins as a model, we observed that those involved in embryo nutrition and defense have changed during genus evolution: while brightly colored ones of the most derived clade (*P. canaliculata* and *P. maculata*) act as antinutritive defenses against predators; those of *P. scalaris*, pale, additionally possess a strong lectin activity. The aim of this work was to analyze the evolutionary trends of *Pomacea* carotenoproteins, studying PpPV1, the carotenoprotein of *P. patula*, a basal member of the genus. PpPV1 absorption spectrum shows a 380 nm maximum indicating a blue-shift from the basal clade compared with the derived species (430 – 510 nm). Electrophoretic mobility also shifted from a mobility equivalent to a 450 kDa protein in native PpPV1 towards lower MWs in the most derived carotenoproteins (293 – 380 KDa). Despite these differences, PpPV1 have similar subunits MW to those of its derived species homologues. Derived clades are kinetically stable (a high energetic barrier that not allow passing from native to denaturized state). This characteristic was evaluated in PpPV1 hypothesizing that was acquired in derivative clades in species with notable reproductive success. However, PpPV1 oligomer withstands boiling and is resistant to SDS-induced denaturation, highlighting that carotenoproteins of *Pomacea* eggs are already thermal and kinetically stable in the most basal species. Functional analysis of hemagluttinating activity revealed that *P. patula* periviteline fluid surrounding the embryo has a moderate lectin activity that is not mainly ascribed to PpPV1. In conclusion, the comparative analysis shows that, while carotenoprotein color and size changed, subunits and structural stability was conserved. The later may have increased tolerance to mutations that accelerated the evolution of new functions.

Keywords: perivitelines, mollusca, kinetic stability

NEUROSCIENCE 7

(1047) **THIOL REDOX HOMEOSTASIS IN VISUAL CORTEX IN AN EXPERIMENTAL GLAUCOMA MODEL**

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Abstract: Alterations in thiol redox homeostasis have been implicated in the pathology of neurodegenerative diseases. Recent evidence has detected neurodegenerative lesions in glaucomatous optic nerve, lateral geniculate nucleus and visual cortex, suggesting that glaucoma should be considered a neurodegenerative disorder.

The aim of this work was to evaluate thiol redox homeostasis in visual cortex in a glaucoma rat model. Female Wistar rats (3 months) were divided in two groups (n=12): glaucoma in which rats were operated under a microscope by cauterized two of the episcleral veins (G) and control which received a sham procedure (C). Seven days after surgery rats were euthanized, brains were removed and visual cortex was separated.The following markers were evaluated: glutathione reductase (GR), glutathione peroxidase (GPx), reduced glutathione (GSH), oxidized glutathione (GSSG), redox index (GSH/GSSG), glucose-6-phosphate deshidrogenase (G6PD) and thioredoxin reductase (TrxR)

Comparing glaucoma to control: GR diminished 52% (C: 13.0 ± 1.0 nmol/min.mg protein *p<0.001*), GPx increased 56% (C: 0.079 ± 0.007 μmol/min.mg protein *p<0.05*), GSH decreased 46% (C: 7.02 ± 0.39 **μmol/g** *p< 0.001*), GSSG increased 26 % (C: 0.35 ± 0.020 μmol/g, *p< 0.05*), redox index diminished 57% (C:20.0 ± 1.8

p< 0.001), G6PD decrease 45% (C: 0.18 ± 0.02 mmol/min.mg protein *p<0.01*) and TrxR decreased 34% (C:14.9 ± 0.8 nmol/min.mg protein *p<0.001*).

The decrease in GR activity as well as the increase in GPx activity would lead to a decrease in GSH and an increase in GSSG. Moreover, this situation is exacerbated by the decrease in G6PD activity which provides the NADPH needed by GR to regenerate GSH. Besides thioredoxin is recycled at the expense of NADPH by TrxR, though the decrease in NADPH and in TrxR activity could lead to a decrease in thioredoxin which reduces disulphide bonds of proteins. Results allow concluding that thiol redox homeostasis is altered in visual cortex in a glaucoma model.

Keywords: glaucoma, glutathione, redox homeostasis, visual cortex, oxidative stress

(778) **SEARCHING FOR THE MOLECULAR PATHWAYS INVOLVED IN CERAMIDE-INDUCED DEATH OF RETINA PHOTORECEPTORS**

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Programmed cell death of photoreceptors (PhR) is a hallmark of neurodegenerative diseases of the retina, irrespective of their cause. Identifying the mediators and molecular mechanisms involved is essential for designing new treatments. Ceramide (Cer), a simple sphingolipid, signals cell death in many cell types. Our laboratory demonstrated that it induces PhR death in culture and Cer was then shown to participate in different retina pathologies. We now investigated the molecular mechanisms involved in this death.

Rat retina neuronal cultures were treated at day 3 with or without 10 μM C2-Ceramide (Cer) for 6 hours. Cer decreased neuronal viability to 50%, as determined by MTT assays. Cer selectively increased PhR with pyknotic nuclei to 50 %, compared to 5 % in controls, without augmenting their TUNEL labeling, whereas amacrine neurons were not affected. Cer decreased PhR mitochondrial functionality and promoted AIF translocation from mitochondria to nuclei. Noteworthy, no cleaved-caspase 3 increase was observed; pretreatment with Z-VAD-FMK, a caspase pan inhibitor, failed to prevent PhR death, suggesting this death was caspase-independent. Cer did not increase LDH release and a necroptosis inhibitor did not protect PhR, implying programmed necrosis was not involved. Autophagy was also ruled out, as the autophagy inhibitor 3-MA did not prevent Cer-induced death. Next, we investigated the involvement of cathepsins and calpains. Pretreatment with ALLN, a calpain and cathepsin inhibitor, significantly increased viability from 50% in Cer-treated cultures to over 80%, decreasing the percentage of pyknotic PhR. Of note, inhibiting both cathepsins (Pepstatin A, CA-074) and lysosomal activity (Bafilomycin A1) did not block PhR death.

As a whole, our results suggest that Cer promotes programmed cell death in PhR in a caspase-independent manner, activating calpain proteases, and inducing mitochondrial permeabilization followed by translocation of AIF and nuclear pyknosis.

Keywords: ceramide, retina, photoreceptors, cell death.

(467) **ROLE OF THE PURINERGIC RECEPTOR P2X7 IN THE REGULATION OF THE PROLIFERATIVE, VASCULAR AND INFLAMMATORY RESPONSE IN THE INJURED RETINA OF ADULT ZEBRAFISH**

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Unlike mammals, zebrafish can regenerate retinal neurons to replace those lost by injury or disease. Müller glia in mammals responds with hypertrophy and scarring. Injury-responsive Müller cells in zebrafish show limited gliosis and cell cycle reprogramming to repair the retina. The P2X7 purinergic receptor (P2RX7) has been involved in the genesis of retinopathies in rodents and humans. We characterized a hypoxia-like injury by delivering $CoCl_2$ intraocularly that virtually kills all photoreceptors in the zebrafish retina. So we examined here the *in vivo* regenerative response of $CoCl_2$-injured retinas in the presence or absence of a specific P2RX7 antagonist (A740003). We quantified the number of proliferative progenitor and microglial cells. We labeled endothelial cells and assessed GFAP immunoreactivity. Quantitative mRNA expression of different hypoxia-induced genes and cell proliferation-related genes was also analyzed by RT-qPCR.

Lesioned retinas treated with A740003 showed a significant increase in the number of proliferative progenitors including a larger number of dividing nuclei of the GFAP-positive Müller glia. The number of microglial cells around vessels and proliferation-related gene expression were also significantly enhanced. Vascular endothelial growth factor and its receptor and Hypoxia-induced factor genes were differentially expressed in the antagonist-treated retinas. Likewise, apyrase-treated injured retinas exhibited a larger number of proliferative cells while the number of apoptotic cells increased in all retinal layers and that of bipolar cells displayed a significant decrease.

The antagonist of P2X7R enhanced overall injury severity. Similar results were observed when extracellular nucleotides were eliminated by an excess of intraocular apyrase. These findings suggest that the P2X7R plays a crucial neuroprotective role in the zebrafish retina since the selective blockade of its activity had a deleterious impact on the injured tissue.

Keywords: Purinergic signalling system, zebrafish, retina regeneration, P2RX7, hypoxia-like injury

(1180) **RETINAL NEUROPROTECTION AGAINST ACUTE RETINAL ISCHEMIA INDUCED BY ISOLATED VISUAL STIMULATION IN ADULT RATS**

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Enriched environment (EE) is a complex combination of inanimate and social interaction. EE boosts exploratory conduct, voluntary physical exercise, sensorial and cognitive functions, and social interaction. We have previously shown that EE induced neuroprotection against unilateral retinal ischemia in adult rats. However, the relative contribution of each component to the effects of EE is still controversial. Our aim was to dissect the individual contributions of the EE repertoire components in its protection against retinal ischemia. Adult male *Wistar* rats were subjected to unilateral or bilateral retinal ischemia and afterwards housed in stardard environment (SE), EE, social environment (SoE), novelty environment (NE), or visual environment (VE). SE consisted in stardard laboratory cages housing 2 animals/cage. EE consisted in big cages (100 x 50 x 82 cm) housing 6 animals/cage with a variety of objects, tunnels and running wheels daily relocated and weekly replaced. SoE and NE consisted in SE housing 5 animals/cage, and EE objects respectively. VE consisted in SE cages surrounded by monitors showing black/white contrast (or grey patterns as controls) during the 12 h light phase. An antagonist for brain-derived neurotrophic factor (BDNF) Trk-b receptor (ANA-12) was administered to animals with unilateral retinal ischemia exposed to black/white contrast patterns. Retinal function (electroretinography) and histology were analyzed 3 weeks after ischemia. Retinal ischemia induced functional and histological alterations ($p<0.01$), which were not protected by SoE or NE. The retinal protection achieved by EE against unilateral ischemia ($p<0.01$) was abolished when ischemia was induced bilaterally, regardless of motor activity. The exposure to VE achieved retinal protection against unilateral ischemia ($p<0.01$), which was prevented by ANA-12. These results suggest that visual stimuli, likely in a BDNF dependent manner, could account for the retinal protection induced by EE.

Keywords: retina, ischemia, enriched environment, neuroprotection, vision

(800) **MIGRATION OF RETINAL PIGMENT EPITHELIUM CELLS IS REGULATED BY SPHINGOSINE-1- PHOS-**

PHATE

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Abstract: Retina proliferative diseases, such as diabetic retinopathy, are common causes of blindness. They are characterized by increased migration of two cell types that normally support retinal function: retinal pigment epithelium (RPE) and Müller glial cells (MGC). We previously found that sphingosine-1-phosphate (S1P), a bioactive lipid, increases MGC migration (Simon et al; 2015). Now we analyzed if S1P also regulates RPE cell migration.

Cultures of ARPE19 cells, a human retinal pigment epithelial cell line, were supplemented with 5 μM S1P and migration was evaluated by scratch-wound assays. To investigate whether ARPE19 cells synthesized S1P to promote migration, cultures were treated with 30 μM sphingosine kinase 1 inhibitor 2 (SphKI2), a SphK1inhibitor. To analyze the role of PI3K and ERK/MAPK signaling pathways in cell migration, cultures were pre-incubated with10 μM LY294002 and 10 μM U0126 -a PI3K and ERK/MAPK inhibitor, respectively- before S1P supplementation.

S1P addition significantly enhanced RPE cell migration; after 24hours, migration in S1P- supplemented cells doubled compared to control conditions. Pre-treatment with SphKI2 reduced by 30% the migration observed in controls, implying endogenous synthesis of S1P promoted RPE cell migration. Addition of exogenous S1P to SphKI2- treated cultures partially restored cell migration, suggesting S1P acts as an intra and extracellular cue. Finally, S1P activated both PI3K and ERK/MAPK signaling pathways to induce ARPE migration. Cultures pre-treated with LY294002 or U0126 and then with S1P barely showed 3% and 0.1%, respectively, of the migration observed in S1P-supplemented cultures.

Our results suggest that RPE cells synthesize S1P, which activates PI3K and ERK/MAPK pathways to induce migration and the increase in S1P levels exacerbates this migration. Since deregulation of thisprocess is involved in several retinal pathologies, modulation of S1P signaling emerges as a potential tool for treating these diseases.

Key words: migration, retinal pigment epithelium, retina, sphingosine-1- phosphate

(463) **MICROARRAY ANALYSIS OF GENE EXPRESSION IN HUMAN MÜLLER CELLS IN RESPONSE TO HYPOTONICITY**

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Müller cells are involved in controlling extracellular homeostasis in the retina, where neural activity alters osmotic gradients, leading to cell swelling and consequently to a regulatory volume decrease (RVD) response. We previously demonstrated in human Müller cells (MIO-M1) that RVD depends on the efflux of KCl, Taurine and Glutamate, followed by water outflow through Aquaporin-4 (AQP4). Hypotonic shock (HS) also induces a slight transient increase in intracellular Ca^{2+} levels and membrane depolarization and repolarization. However, the mechanisms for long-term adaptation to hypotonicity are largely unknown. Thus, the aim of this study was to identify HS-related changes in gene expression triggered by cell swelling. Gene transcription by MIO-M1 cells exposed to control or HS (ΔOsm:100 mOsm) for 30 minutes was compared using Affymetrix microarray for 10000 genes. Microarray data analysis was performed with GenArise software and DAVID Bioinformatics Resources 6.8 Database. HS induced the upregulation (Z-score>2SD) of 241 genes and downregulation (Z-score<-2SD) of 264 genes. Among HS-upregulated genes were those involved in membrane transport, glucose homeostasis, intracellular signaling events and neurotransmitter secretion. In particular, several members of the solute carriers SLC familiy involved in transport of neurotransmitters and organic/inorganic compounds were upregulated. Genes which decreased their expression were mainly those involved in cell growth regulation, cell proliferation and apoptosis. AQP4 was also downregulated, probably to prevent further swelling. Metabolic pathways affected by HS, including up and downregulated genes, were mainly those concerning neuroactive ligand-receptor interactions and Glutamine/Glutamate metabolism. HS-responsive genes regulation, which probably lead to adaptive changes in Müller cells physiology, may be important for maintaining retinal extracellular environment homeostasis under conditions of sustained hypotonicity.

Keywords: Müller cells – hipotonicity – gene expression

(89) **LIGHT-INDUCTION OF THE ENZYME ARALKYLAMINE N-ACETYLTRANSFERASE (AANAT) IN THE CHICKEN INNER RETINA AND ITS POTENTIAL PHYSIOLOGICAL ROLE**

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The neurohormone melatonin is synthesized from serotonin through two steps of which serotonin is converted first to N-acetyl-serotonin (NAS) by the enzyme Aralkylamine N- Acetyltransferase (AANAT). AANAT is present mainly in the pineal gland, retina and other regions while NAS can activate the TrkB receptor to generate neuroprotective effects and neurogenesis. Melatonin synthesis is controlled by light (L) and the circadian clock. In photoreceptor cells, AANAT activity peaks during the dark and at subjective night while activity is significantly decreased by L exposure. By contrast, melatonin synthesis, AANAT expression and activity are high during the subjective day or L phase in chicken retinal ganglion cells (RGCs) (Garbarino et al 2004). Here we investigate the expression of AANAT and of nonvisual opsins in highly enriched RGC cultures obtained from embryos by a discontinuous BSA gradient, and exposure to different L conditions. Cultures expressed melanopsins, Opn3 and Opn5 which may confer intrinsic photosensitivity. In fact, cultures exhibited blue L induction of AANAT immunoreactivity as compared with dark or red L treated cells. In addition, expression of this enzyme was significantly increased by forskolin (10 uM), an adenylate cyclase activator, in the dark. Results suggest that AANAT is a blue L-induced enzyme in RGCs controlled by cAMP. Further studies will investigate the cascade controlling AANAT expression in RGCs and its effects on retinal cells.

Palabras clave: melatonin, retina, acetyl-serotonin, light induction, neuroprotection

(1739) **LACK OF GALECTIN-1 PREVENTS BIOCHEMICAL AND FUNCTIONAL ALTERATIONS IN A MOUSE MODEL OF OXYGEN-INDUCED RETINOPATHY**

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Abstract: Neovascular retinopathies are leading causes of irreversible blindness. Although vascular endothelial growth factor (VEGF) inhibitors have been established as the mainstay of current treatment, clinical management of these diseases is still limited. Previously we showed that Galectin-1 (Gal1) is involved in vascular and non-vascular alterations associated with retinopathies, using the oxygen-induced retinopathy (OIR) mouse model. Postnatal day 17 OIR mouse retinas showed the highest neovascular profile and VEGF levels and exhibited neuro-glial injury as well as retinal functional loss, which persisted until P26 OIR. Concomitant to VEGF up-regulation, Gal1 was highly expressed in P17 OIR retinas. Whereas VEGF returned to baseline levels at P26, increased Gal1 expression persisted until this time period. Here, we evaluated vascular and non-vascular alterations in Gal1-deficient (*Lgals1*$^{-/-}$) OIR mice. Briefly, C57BL/6 wild type (WT) and *Lgals1*$^{-/-}$mice were exposed to 75% O_2 from P7 to P12, after which they were brought to room air for additional five (P17) or nine days (P26). At P17 and P26

mice were sacrificed. Some eyes were fixed to obtain cryosections and retinas were isolated from others to analyze VEGF, glutamine synthase (GS) and glial fibrillary acidic protein (GFAP) expression by Western blots. Finally, retinal function was analyzed by electroretinography. GraphPad Prism program was employed for statistical analysis. Results showed that *Lgals1*$^{-/-}$ P17 OIR had less VEGF protein levels than WT P17 OIR retinas. Besides, *Lgals1*$^{-/-}$ P26 OIR showed a reduction in GFAP expression compared with WT P26 OIR retinas. Moreover, *Lgals1*$^{-/-}$ OIR retinas did not show any alterations in GS expression and the OIR retinal functional defects were partially prevented in these mice. Thus, Gal1 play a key role in the pathogenesis of neovascular retinopathies and could be postulated as a novel therapeutic target.

Keywords: Neovascular retinopathies, Vascular endothelial growth factor, Galectin-1.

(1064) **ENRICHED ENVIRONMENT PRESERVES THE VISUAL FUNCTIONS AND REDUCES THE STRUCTURAL DAMAGE OF THE OPTIC NERVE IN AN EXPERIMENTAL MODEL OF OPTIC NEURITIS**

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The therapeutic potential of enriched environment (EE) against damage induced by neuroinflammation has been scarcely studied. Optic neuritis (ON) is an inflammatory, demyelinating and neurodegenerative disease of the optic nerve that can cause irreversible blindness. We studied the effect of EE housing on visual pathway damage induced by experimental ON. Vehicle or LPS were micro-injected into the optic nerve of adult male *Wistar* rats. After injection, one group of animals was housed in EE and another group remained in standard laboratory cages (SE) for 21 days. EE prevented ON-induced decrease in consensual pupillary reflex (PR), visual evoked potential amplitude, and anterograde transport. Moreover, EE prevented the decrease in phosphorylated neurofilament-immunoreactivity, microglial reactivity and astrocytosis, and reduced demyelination and prevented retinal ganglion cell and axon loss induced by LPS injection ($p<0.01$). EE prevented the increase of oxidative damage and protein levels of nitric oxide synthase-2 and cyclooxygenase-2, as well as the levels of interleukin-1β mRNA and TNFα induced by experimental ON ($p<0.01$). In addition, EE housing increased brain-derived neurotrophic factor levels ($p<0.01$). When EE exposure started at 4 (but not 7) days post-injection of LPS, a protection of RP was observed at 21 days post-LPS, which was blocked by daily administration of ANA-12 (a Trk-B receptor antagonist) from day 4 to day 7 post-injection of LPS ($p<0.01$). Exposure to EE from day 4 to day 7 post-injection significantly preserved the PR at 21 days post-injection ($p<0.01$).These results indicate that EE housing reduces neuroinflammation of the optic nerve and preserves visual functions.

(1470) **DEGENERATIVE AND ANGIOGENIC EFFECTS OF HYPERTHYROIDISM ON THE ADULT ZEBRAFISH RETINA**

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Alterations of the neuro-glio-vascular unit underlie different pathologies of the CNS, including retina pathologies. Adult zebrafish (ZF) are able to regenerate tissues and organs being a suitable model to study the regulation of cell genesis and differentiation.

We examined the effect of hyperthyroidism induced by exposure to elevated levels of T4 on repairing the neuro-glio-vascular circuit during retina regeneration in the ZF.

Retinas of a group of ZF were injured with an intravitreal injection of ouabain whereas control groups were injected with saline. Then, the animals were separated into four groups: 2 groups (injured and saline) were treated with T4 (300 µg /L)and other two groups (injured and saline)were treated with vehicle (0.1 M NaOH) in the tank water up to 25 days after injury (dpi). Euthanasia was done and retina sections were obtained at 25 dpi. Double immunofluorescence was performed to detect different cell populations and vascularization in the retinal tissue. mRNA levels of Thyroid hormone receptors (TRs), VEGF receptors (VEGFRs) and cell-type markers in the retina were detected by quantitative RT-PCR.

Retina neovascularization was observed in the groups exposed to elevated levels ofT4 either injured or not injured. Analysis of virtually all cell populations and their synaptic connections showed a significant decrease in cell number as well as in the dimension of synaptic layers in lesioned ZF. mRNA relative expression of TRs and VEGFRs showed significant changes depending on the receptor type. mRNAs of cell markers correlated with the observed morphological changes.

In conclusion, an exacerbation of the TH signaling system induces severe inhibition of retina cell regeneration and tissue growth alterations. Moreover, high T4 levels induced retina neovascularization. The changes observed in both injured and T4-treated retinas in the long term are compatible with processes described in neurodegenerative and neovascularizing retinopathies.

Keywords: Retina regeneration, Thyroid Hormone, retina degeneration

BIOTECHNOLOGY AND BIOINFORMATICS 1

(44) **THE CONFORMATION OF AN ANTIBODY FRAGMENT IS MODIFIED BY AN UNUSUAL CYSTEINE V_L87 WITHOUT INTERFERING WITH THE DISULFIDE BOND FORMATION**

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The Cys residues are almost perfectly conserved in all antibodies. They contribute significantly to the antibody fragment stability. The relevance of two natural contiguous Cys residues of an antirecombinant human follicle stimulation hormone (rhFSH) in a format of single-chain variable fragment (scFv) was studied. This scFv derived from a monoclonal antibody (mAb) that naturally contains 5 Cys residues: VH22 and VH92 in the variable heavy chain (VH) and VL23, VL87 and VL88 in the variable light chain (VL). The influence of Cys at positions VL87 and VL88 was studied by considering the wild type fragment and mutant variants: C88S, C87S, and C87Y. The analysis was carried out using antigenbinding ability measurement by indirect specific ELISA and a detailed molecular modeling that comprises homology methods, long molecular dynamics simulations (MD) and docking. We found that Cys VL87 affected the antibody fragment stability and its ability to bind the antigen without interfering with the disulfide bond formation. In the C88S mutant, the CysVL87 was not able to replace the Cys VL88 role and the antibody loss its ability to bind rhFSH. Nevertheless, C87S and C87Y mutants increased the ability of the antibody to bind rhFSH. From the molecular dynamics study we found that the mutant C87Y caused a conformational change in the VH-CDR3 loop, increased flexibility of the V_H-CDR2 and V_HCDR3 loops and a relative rotation of the VH and VL domains. These changes also comprised the increment in the total number of interchain hydrogen bonds that brings more stability to the multidomain structure. The docking simulations of the wild type scFv, C87S and C87Y mutants on hFSH proposed that the last variant has the larger probability to bind the target. The docking studies also suggested that the paratope loops with larger interaction with the epitope are V_HCDR2, V_HCDR3 and therefore are prone to be affected by the conformations or mobility of these zones.

Keywords: Single-chain variable fragment; Cysteine contiguous residues; Molecular modeling; Mutating cysteine

(187) **NOVEL SENSITIVE MONOCLONAL ANTIBODY BASED COMPETITIVE ENZYME-LINKED IMMUNO-**

SORBENT ASSAY FOR THE DETECTION OF RAW AND PROCESSED BOVINE BETA-CASEIN

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Abstract: Cow milk protein allergy (CMPA) is the most common childhood food allergy, which can sometimes persist or can newly develop in adulthood with severe symptoms. CMPA's treatment is complete dietary avoidance of milk proteins. To achieve this task, patients have to be aware of milk proteins found as "hidden allergens" in food commodities. In regard to milk proteins, it has been reported that allergenicity of caseins remains unaffected upon heat treatment. For these reasons, we aimed to obtain monoclonal antibodies (mAbs) against native and denatured β-casein, one of the most abundant and antigenic caseins, in order to develop an indirect competitive ELISA (icELISA) to detect and quantify traces of this milk allergen in raw and processed foodstuffs. We developed two specific hybridoma clones, 1H3 and 6A12, which recognized β-casein in its denatured and native conformations by indirect ELISA (iELISA). Cross-reaction analysis by Western blot and iELISA indicated that these mAbs specifically recognized β-casein from bovine and goat milk extracts, while they did not cross-react with proteins present in other food matrixes. These highly specific mAbs enabled the development of sensitive, reliable and reproducible icELISAs to detect and quantify this milk protein allergen in food commodities. The extraction of β-casein from foodstuff was efficiently carried out at 60°C for 15 minutes, using an extraction buffer containing 1% SDS. The present study establishes a valid 1H3 based-icELISA, which allows the detection and quantification –0.29 ppm and 0.80 ppm, respectively– of small amounts of β-casein in raw and processed foods. Furthermore, we were able to detect milk contamination in incurred food samples with the same sensitivity as a commercial sandwich ELISA thus showing that this icELISA constitutes a reliable analytical method for control strategies in food industry and allergy prevention.

Keywords: β-casein, food allergens, cow milk protein allergy, monoclonal antibodies, ELISA

(586) **NANOBODY AND F(ab')$_2$-BASED IMMUNOCAPTURE ASSAYS FOR DIAGNOSIS OF STEC-ASSOCIATED HEMOLYTIC UREMIC SYNDROME**

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Human infection with Shiga toxin-producing *Escherichia coli* (STEC) is a major cause of postdiarrheal hemolytic uremic syndrome (HUS). The ability to produce Shiga toxins (Stx1 and/or Stx2) are the key virulence trait of STEC strains, and the O157:H7 and non-O157:H7 STEC strains can produce both or only one of the two types of toxins in their different variants (Stx1: Stx1a, Stx1c and Stx1d; Stx2: Stx2a, Stx2b, Stx2c, Stx2d, Stx2e, Stx2f and Stx2g). Therefore, Shiga toxins detection assays are considered the most suitable methods for diagnosis of STEC-associated diseases. In this work, we developed two capture ELISAs for Stx1 and Stx2 detection from broth culture and stool samples. In a first approach, we exploited nanobody technology for the development of a double nanobody capture ELISA for Stx2a detection, the most prevalent variant of Stx2. Using this immunocapture nanobody-based ELISA, we were able to detect the native toxin from culture supernatants of STEC strains expressing Stx2a and from stools samples obtained from HUS patients. In order to detect the entire repertory of Shiga toxin variants, we developed a capture ELISA based on a F(ab´)$_2$ fragments that specifically recognize Stx1 and Stx2. The F(ab´)$_2$ fragments were obtained from the peptic digestion of IgGs obtained from horses immunized with the recombinant proteins BLS-Stx1B and BLS-Stx2B (Brucella Lumazine Synthase-B subunit of Stx1 and 2). Using this approach we were able to detect with high sensitivity Stx1a and Stx1c as well as the Stx2 variants Stx2a, Stx2b, Stx2c and Stx2f greatly expanding the Stx detection spectrum. Both immunocapture assays are novel diagnostics that allow a sensitive and specific detection of Shiga toxins and may be of great value for diagnosis of HUS associated with STEC. Furthermore, the nanobodies and F(ab')$_2$ fragments evaluated in this work may constitute excellent diagnostic tools for the development of simple, rapid and accurate lateral flow immunoassays.

Keywords: Hemolytic Uremic Syndrome, Shiga toxins, STEC, diagnostics, nanobodies.

(679) **GENERATION OF EIAs (ENZYME IMMUNEASSAYS) FOR LOCAL AND REGIONAL VIRAL DISEASES**

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The aim of the laboratory is establish a workflow that converges in enzyme immuneassays (EIAs) for serological identification of several viral infectious agents that have local and regional importance, with implications in diagnostic and epidemiological surveillance. It is very important to recognize the relevant antigens of the viruses of interest. In this way, we determine the more important epitopes using immunobioinformatics tools, and proceed to work with versions of recombinant proteins, produced in a bacterial system. These proteins are: HT-NS1SLEV, HT-NS1DENV-1, HT-NS1WNV, HT-NS1ZIKV, HT-NS1YFV, HT-NS1-EpSLEV, HT-NS5SLEV, HT-VP1B19, and HT-L1HPV. Alternatively, we have an antigen presentation design based on Z protein of JUNV for mammal's cells that can be use complementarily. The entire sequence of NS1SLEV was included to this system, obtaining VLPs that are being analyzed in their composition. Therefore, the HT-NS1SLEV protein was used to inoculate mice and evaluate the humoral response α-NS1SLEV, and start the protocol for monoclonal antibody generation, a fundamental component that ensures the specificity and quality of the kits. To the EIAs is necessary to purify the IgGs or MAbs. For this, we are working in an affinity chromatography that replaces the commercial acquisitions, reducing the production costs. The first step is the production of the affinity protein in a bacterial system, and then this protein will be couple to the matrix. The local EIAs kits development is an important work area to be cover in our countries. The availability of these tools at health institutions gives access to a correct diagnosis and to the registration of epidemiological data useful for planning plague control strategies, between other things.

(989) **BIOINFORMATIC IMMUNOGENOMIC PROFILING TO REVEAL BIOMARKERS OF RESPONSE TO TARGETED THERAPIES AND CHECKPOINTS BLOCKADE IN MELANOMA PATIENTS**

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Abstract: Melanoma is the deadliest form of skin cancer. There

has been a paradigm shift in the treatment of melanoma based on the development of targeted therapies and immunotherapies showing unprecedented survival benefits. However, responses are not universal and patients who initially respond develop acquired resistance. Critically, treatment is associated with inflammatory toxicity and high cost. Therefore, determining which patients would derive clinical benefit from immunotherapy is a compelling clinical question. The aim of this study was to determine predictive molecular biomarkers of response to immunotherapy and to quantify the cellular composition of the immune response in order to investigate its relationship with response to treatment. We analyzed the gene expression profiles of melanoma tumor samples from databases with clinical annotation such as The Cancer Genome Atlas (TCGA, n=470), The Cancer Immunome Atlas (TCIA) and public datasets from Gene Expression Omnibus (GEO). For this meta-analysis we implemented an established computational approach (CIBERSORT) based on a deconvolution method to infer the proportion of 22 immune cell types from gene expression profiles. Bioinformatics and statistical analysis were performed with the R programming language and public web tools. Our preliminary findings show a differential molecular signature involved in shaping the immune infiltrate in melanoma tumors sensitive and resistant to targeted therapies (BRAF and/or MEK inhibitors), and also in melanoma tumors responding and non-responding to anti PD-1 blockade. These molecules could be potential biomarkers of response as well as new actionable targets for the development of combination therapies. We also observed differences in the cellular composition of the immune infiltrate in melanoma tumors that are likely to be important determinants of both prognosis and response to treatment.

Keywords: bioinformatics, immunotherapy, genomics, immunoprofiling, melanoma

(1434) **OBTAINING AND CHARACTERIZING OF SINGLE DOMAIN ANTIBODIES DERIVED FROM CAMELIDS (NANOANTIBODIES OR VHH) AGAINST FOOT-AND-MOUTH DISEASE VIRUS FOR DIAGNOSTIC APPLICATION**

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Foot-and-Mouth Disease (FMD) is a viral disease of livestock with serious economic repercussions. Development of innovative diagnostic methods for the FMD control is of concern due to the susceptibility of species of economic interest. Camelids present heavy chain only antibodies (IgG 2 and 3), so the variable domain consists of a single domain (VHH or Nanoantibody). These antibodies represent a novel tool with unique characteristics conferring great potential in the development of new more sensitive diagnostic methods. For this reason, the present work aimed to obtain and preliminary characterize VHH specific against FMDV for its application in the diagnostic innovation.

Two llamas (*Lama glama*) were immunized with the strains O1/Campos, A24/Cruzeiro (A24), A/Arg/2001 (A2001) and C3/Indaial (C3). Humoral immune response (total IgG and subtype titers) was assessed throughout the immunization schedule obtaining high levels of heavy chain-only antibodies against the 4 serotypes by ELISA. Serum reactivity against viral proteins was confirmed in Western Blot. Two Nanoantibody libraries were constructed starting from llama peripheral blood and VHH against O1C strain were selected by Phage Display. Even more, 119 out of 182 selected clones recognized the 4 serotypes in ELISA. From these clones, 17 which showed very high reactivity in ELISA were selected for diversity analysis by enzymatic digestion. As a result, it was shown that 16 out of 17 clones presented different fingerprinting pattern.

In conclusion, two VHH libraries were constructed from immunized llama with the O1C, A24, A2001 and C3 strains. Both llamas had a high immune response and their sera were able to recognize the 4 FMDV serotypes. Furthermore, the two llamas had high levels of heavy chain only antibodies, mainly IgG3 subtype. At least 16 different cross-reactive Nanoantibodies were obtained against the 4 strains. Thus, these antibodies represent a potential tool for diagnostic innovation of FMDV.

Keywords: Foot-And-Mouth Disease; Vhh; Nanobodies; Diagnosis, Virus.

(1451) **OPTIMIZATION OF THE PRODUCTION PROCESS OF AcrA-O157,
A RECOMBINANT GLYCOPROTEIN FOR THE SERODIAGNOSIS OF HEMOLYTIC UREMIC SYNDROME.**

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Escherichia coli O157:H7 represents the most important etiological agent of Hemolytic Uremic Syndrome associated with Shiga toxin-producing *Escherichia coli* (STEC) worldwide. Recently, our group have developed a high-performance test for the serodiagnosis of human infection with *E. coli* O157 employing a new recombinant glycoprotein (AcrA-O157), consisting of the O157 polysaccharide attached to a carrier protein (AcrA), as antigen. Characterization and optimization of AcrA-O157 production process is important for increasing productivity and for scaling up. In this work, we evaluated AcrA-O157 production in different culture media and defined 2xYP as the optimal culture medium. In order to maximize the specific productivity of AcrA-O157, we analyzed the relationship between the specific product synthesis rate (qp) and the growth rate (μ). Exponential fed-batch cultures were designed using a pre-defined exponential feeding strategy based on the desired μ and the biomass yield. We analyzed glycosylation rate, product synthesis rate and production kinetics at three different specific growth rates: 0,32 h^{-1} (equal to μ_{max}), 0,21 h^{-1} and 0,14 h^{-1} by Western Blot and ELISA. We observed maximum glycosylation degree of AcrA at highest μ indicating that the glycosylation rate is affected by the specific growth rate. Specific rate of product formation was also influenced by specific growth rate, increasing 3-fold and 7,2-fold at 0,21 h^{-1} and 0,32 h^{-1}, respectively, when compared to 0,14 h^{-1}. Additionally, we observed a shorter lag phase to reach the maximal specific productivity when working at higher μ. Based on these results, we conclude that μ_{max} is the optimal specific growth rate allowing us to achieve the highest productivity with a shorter lag phase.

Keywords: optimization, recombinant glycoprotein, SUH serodiagnosis

(1511) **CONSTRUCTION OF IMMUNE VHH LIBRARIES AND SELECTION OF SPECIFIC NANOBODIES AGAINST HUMAN IGG4 FOR DIAGNOSTIC APPLICATION IN SYSTEMIC LUPUS ERYTHEMATOSUS**

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Systemic Lupus Erythematosus (SLE) is a chronic and inflammatory autoimmune disease. The prevalence of SLE in Argentina is 58.6 per 100 thousands inhabitants. Due to the heterogeneity of the clinical manifestations and the wide profile of autoantibodies developed by the affected individuals, it is a challenge to diagnose specifically the disease. On one hand, it has been reported a correlation between IgG4 levels and severity of the disease. For this reason, IgG4 has been proposed as a putative biomarker for SLE diagnosis. On the other hand, camelids present heavy chain only antibodies, so the variable domain consists of a single domain (called VHH or Nanoantibody). The use of VHH has revolutionized the field of monoclonal antibodies due to their unique and superior properties compared to conventional antibodies. They have a great potential for the development of more sensitive diagnosis methods. For this reason, our goal was to construct two VHH libraries and obtain specific nanobodies against human IgG4 for their application in SLE diagnosis.

In this work, two llamas were immunized with human IgG4 obtaining a high immune response measured by ELISA. Starting from llama peripheral blood, the heavy chain variable regions of conventional antibodies (VH) and only heavy chain antibodies (VHH) were amplified. In a Nested-PCR, only the VHH fragment were amplified and subsequently cloned in the pHEN4 phagemid vector. Then, TG1 *E. coli* bacteria were electroporated to obtain two Nanobody librar-

ies. Phages expressing VHH that specifically recognized IgG4 biomarker were selected by Phage Display methodology. Even more, reactivity of selected Nanobodies was confirmed by Phage-ELISA.

In conclusion, two immune VHH libraries were obtained with the expected size and high percentage of full length clones. Furthermore, specific Nanobodies against human IgG4 were selected by Phage display. Thus, these VHH constitutes an innovative tool for potential application in SLE diagnosis.

Keywords: Systemic Lupus Erythematosus (SLE), VHH, IgG4, Nanobody, Diagnosis.

(1562) **BOVINE TUBERCULOSIS, PRODUCTION OF MONOCLONAL ANTIBODIES AGAINST BOVINE IFN GAMMA USING A *IN SITU* METHOD OF SCREENING**

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Bovine tuberculosis (TBB) is one of the major zoonotic diseases in our country that causes large economic losses. SENASA employs a control of TBB eradication based on the tuberculin skin test; however, several countries use an alternative and complementary indirect ELISA technique based on bovine IFN detection. Our objective is to obtain bovine anti-IFN monoclonal antibodies (MAb) which can be used for the development of a national ELISA test. Since one of the critical stages of obtaining MAb is the detection of positive clones between the generated hybridomas, two techniques were compared: serial dilution of the positive cells and *in situ* screening using nitrocellulose membranes pre-adsorbed with the antigen (Ag). For this, BALB/c mice were immunized with recombinant bovine IFN and after reaching a titer of 10^4, they were sacrificed, the spleen removed to obtain splenocytes and fused to the NS0 cells following Milstein method. The resulting hybridomas were cultured in selective medium and at 10 days after fusion a first screening by indirect ELISA was performed. They were expanded and then tested according to the *in situ* screening method described by Orii (2013) with modifications. For this, nitrocellulose discs pre-absorbed with bovine IFN were placed on the cells and incubated for 4 hours. After incubation, the discs were mixed with 5% milk TPBS buffer contained anti-mouse IgG conjugated to alkaline phosphatase detecting positive cells with BCIP/NBT substrates. Using as reference the location of the points on the discs, the cells which originated them were transferred to 96 wells plates for expansion and subsequently subcloning by limiting dilutions. 96% of the cells selected with *in situ* screening were positive for MAb production whereas only 3% were obtained using limiting dilution technique. The gain of positive clones was significant using the *in situ* method of screening. KEYWORDS: Bovine Tuberculosis – MAb – bovine IFN

(633) **PHYSICOCHEMICAL AND BIOLOGICAL CHARACTERIZATION OF THE RITUXIMAB BIOSIMILAR RTXM83**

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Biosimilars are biologic drugs designed to be similar to existing biopharmaceutical products for which patents have expired. Even though there are already approved Biosimilars in Europe and USA, the biosimilar manufacturing industry is also flourishing in emerging markets such as LatAm, Russia and Asia, where biosimilar adoption represents a strategic avenue to provide high quality and clinically effective medications at reduced cost. Rituximab is a chimeric monoclonal antibody approved for Non-Hodgkins lymphoma, chronic lymphocytic leukemia, and some autoimmune conditions. It is targeted against the pan-B-cell marker CD20 and exerts its therapeutic action through three different mechanisms of cell destruction: direct signaling of apoptosis, complement-dependent cytotoxicity and antibody-mediated cellular cytotoxicity. We herein present the development and physicochemical and functional characterization of the Rituximab biosimilar RTXM83. In accordance with the guidelines established by International Regulatory Agencies such as the EMA and the FDA, the critical quality attributes of RTXM83 were evaluated in a comparability exercise to its reference product (RP) using an orthogonal approach. Some of the methodologies employed in this study are: peptide mapping and MS analysis, CD spectroscopy, SDS-PAGE, SE HPLC and glycan and charge variants analysis by chromatography, SPR binding assays and cell-based potency bioassays. The results indicate that the primary and higher order protein structures as well the post-translational modifications and impurity profiles of RTXM83 are similar to the RP. RTXM83 also showed similarity to the RP in terms of ADCC, CDC and apoptosis potency and binding affinities to CD20, C1q and a complete panel of Fc Receptors. Overall, these data reveal that RTXM83 can mimic the described mechanisms of action of Rituximab and provide confidence that it will match the potency and safety of the reference product in ongoing clinical trials.

Keywords: Biosimilar, Rituximab, Analytical methods, Comparability, Regulatory Agency.

CARDIOVASCULAR Y RESPIRATORIO 4 / CARDIOVASCULAR AND RESPIRATORY SYSTEMS 4

(1855) **ARRHYTHMIC MECHANISMS OF TICAGRELOR IN MURINE MODELS OF CARDIOVASCULAR RISK FACTORS.**

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Ticagrelor (TICA) inhibits the P2Y12 receptor and interferes with cellular adenosine uptake. Increased adenosine levels during ischemia and reperfusion protect against ischemia-reperfusion injury. This work aims to prevent cardiovascular remodeling and ventricular arrhythmias with chronic administration of TICA in SHR rats fed with fructose to induce metabolic syndrome. We studied male rats from risk factor models: 1) WKY, normotensive control; 2) FFR, fructose-fed rat 10% (w/v) fructose solution during all six weeks; 3) SHR, spontaneously hypertensive rats; 4) FFHR fructose-fed hypertensive rats. These four groups were divided into TICA: (30 mg/kg intraesophageal for six weeks) or vehicle (n=8 each group). Metabolic variables and systolic blood pressure were compared at weeks six and before sacrifice at week twelve. Macroscopic and microscopic measurements evaluated cardiac remodeling. Langendorff-perfused hearts underwent 10 min of regional ischemia and assessed for reperfusion arrhythmias. Connexin-43 and PKC-epsilon expression were determined by western blot. The FFHR experimental model presented metabolic syndrome criteria and cardiac remodeling. Chronic treatment with TICA reduced ventricular fibrillation incidence in WKY, SHR and FFHR groups but failed to protect FFR. In all groups, TICA increased connexin 43. However, PKC-epsilon phosphorylated form of connexin 43 did not increase in FFR+TICA. We conclude that antiarrhythmic effects of TICA could be blunted in some risk factor models regarding connexin 43 phosphorylation by PKC. Further studies TICA effects on intercellular communication or hemichannel function of connexin 43 seem highly worthwhile.

Key words: ticagrelor, arrhythmias, connexin 43, PKC, metabolic syndrome

(583) **ASSOCIATION BETWEEN GENE EARLY B-CELL FACTOR 1 (EBF1) RS4704963 SNP AND CARDIOVASCULAR RISK FACTORS**

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High blood pressure (HBP), obesity, smoking and type II diabetes

(DBT) are cardiovascular risk factors (CRFs) in which genetic susceptibility plays an important role.

Transcription factor early B-cell factor 1 (EBF1) is expressed in adipocytes and olfactory neuroepithelium, and participates in immune system development, adipogenesis and insulin resistance. The presence of the single nucleotid polymorphism (SNP) rs4704963: T<C in the EBF1 gene has been associated with early onset of coronary disease and a higher incidence of central obesity in patients suffering stress.

Our objective was to study the relationship between the presence of the SNP rs4704963 of EBF1 gene and the above-mentioned CRFs in patients with multiple CRFs with or without acute myocardial infarction (AMI).

To this end, 104 patients having two or more CRFs were evaluated. Characteristics of the population: mean age 59 years, 60.6% male, 63.5% with AMI, 36.5% with CRFs without AMI, 51% diabetic, 57.7% hypertensive and 59.6% smokers. Genotyping was performed on DNA obtained from blood samples of patients through nested PCR technique and subsequent sequencing. Statistical test CHI2 and logistic regression (OR) were done.

Results: The SNP rs4704963 was present in 16.3% patients in the heterozygous T/C and none in the homozygous T/T haplotype.

Considering the dominant genetic model, no correlation was detected between the SNP presence and AMI occurrence.

In our population, neither HBP, waist diameter nor DBT correlated with the development of AMI, although obesity increase measured by BMI ($p<0.05$) and smoking ($p<0.05$) did positively correlate.

The presence of the SNP did not correlate with an increase in BMI, waist diameter, DBT or HBP, but a significant correlation ($p<0.05$) was found with smoking.

Conclusion: The presence of SNP rs4704963: T<C in the EBF1 gene is not related to the development of obesity, DBT, and HBP, but it can be a predisposing factor for smoking.

Keywords: diabetes, obesity, gene EBF1, polimorfism, myocardial infarction.

(1224) **HIGH FAT DIET-INDUCED METABOLLICALLY OBESE AND NORMAL WEIGHT RABBIT MODEL SHOWS EARLY VASCULAR DYSFUNCTION. ROLE OF CYCLOOXYGENASE-2 IN NORMAL OXIDATIVE STATUS**

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Association between obesity and vascular dysfunction has been widely probed. However, mechanisms of vascular changes in individuals with normal weight and clinical characteristics of metabolic syndrome (MS) have not been described so far. The aim of the present work was to evaluate endothelial function and vascular reactivity in a metabolically obese and normal weight (MONW) rabbit model developed by feeding animals on a high fat diet (HFD). **Methods:** male rabbits were fed either regular diet (CD) or 18% fat in regular diet (HFD) by 6 weeks. **Results:** HFD induced glucose intolerance (CD:140±5 mg/dl vs HFD:163±4 mg/dl), increased fasting glucose (CD: 102±6 mg/dl v HFD: 126±5 mg/dl), triglycerides(CD: 112±6 mg/dl v HFD: 193±5 mg/dl), C reactive protein (CD: 5.1±0.9 mg/dl v HFD: 22±3 mg/dl), visceral abdominal fat (CD: 0.29±0.05 % vs HFD: 2.3±0.1 %) , TyG index (product of the fasting blood glucose and TG levels, CD: 8.27±0.22 vs HFD: 9.28±0.11) and decreased HDL-cholesterol (CD: 54±4 mg/dl vs HFD: 23±3 mg/dl) and plasma nitrites (CD: 1752±784 nmoles/l vs HFD: 324±109 nmoles/l). No differences were found in body weight, TBARS and Glutathione serum levels between the two diet groups. In aortic rings, isometric contractions measurement showed that HFD: a) reduced the acetylcholine relaxation, effect reversed by NS398 (cyclooygenase-2 inhibitor) and SC560, (cyclooxygenase-1 inhibitor); b) increased the contractile response to norepinephrine and KCl; c) improved the angiotensin II-potency, effect reversed by NS398 and SQ 29538 (TP receptor blocker). Immunohistochemistry and western blot showed cyclooxygenase-2 expression only in arteries from HFD rabbits. **Conclusions:** HFD induced vascular dysfunction in a model of MONW. Cyclooxygenase-2 up regulation may account for these functional alterations. Considering that normal oxidative status was found in our MONW model inflammatory process may account for the metabolic alterations in early stages of the MS.

(1088) **IL-6 REGULATES INSULIN RESISTANCE AND CARDIOVASCULAR DISEASE DURING *TRYPANOSOMA CRUZI* EXPERIMENTAL INFECTION**

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Oxidative stress generation is proposed as the common pathogenic factor mediating the appearance of insulin resistance while producing increased cardiovascular risk. We have recently reported a potent anti-oxidant effect of IL-6, so we hypothesize that IL-6 could be involved in insulin sensitivity and cardiovascular function during *T. cruzi* infection. We observed that infection induces increased frequency of nitric oxide (NO)-producing monocytes in peripheral blood from IL-6-deficient mice (KO) in comparison with C57BL/6 (WT) mice at all days post-infection (dpi) studied (0 dpi p=0.0301, 4 dpi p=0.0006, 14 dpi p=0.0007, 21 dpi p=0.0165). Among the metabolic parameters assayed in plasma, we observed increased glucose (p=0.0120) and insulin (p=0.0286) levels, with the consequent augmented HOMA-IR index (p=0.0197) at 14 dpi in KO mice compared to WT mice. These results suggest that IL-6-deficiency induces acute insulin resistance. The fatty acid transporter and scavenger receptor CD36 is implicated in the pathogenesis of insulin resistance and associated cardiovascular complications. Considering that KO mice showed higher frequency of CD36+ circulating monocytes (p=0.0045) in comparison with WT mice at 14 dpi, we analyzed if IL-6 could be regulating insulin sensitivity by modulating this scavenger receptor. IL-6 stimulation of *T. cruzi*-infected bone marrow-derived macrophages (BMDM) diminished the frequency of CD36+ BMDM and increased the percentage of insulin receptor+ BMDM compared to unstimulated-infected cells. Considering that cardiovascular dysfunction is a complication of metabolic syndrome, we observed that KO mice showed increased creatin-kinase (CK) MB/total CK ratio (p=0.0016) and creatinine plasmatic levels (p=0.0003), biomarkers of cardiac and kidney damage respectively, in comparison with WT mice. Altogether, the data obtained show that IL-6 protects mice from *T. cruzi*-induced oxidative stress and the consequent insulin resistance and kidney dysfunction.

Keywords: IL-6, cardiovascular disease, oxidative stress, insulin resistance, CD36.

(1176) **NEBIVOLOL IN COMBINATION WITH TEMPOL IMPROVES METABOLIC AND HEMODINAMIC PARAMETERS IN A MODEL OF METABOLIC SYNDROME.**

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The metabolic syndrome (MS) is a constellation of metabolic abnormalities such as glucose intolerance, hipertrigliceridemia, hypertension and inflammation that predispose to type 2 diabetes and cardiovascular diseases.

The therapeutic arsenal of hypertension is composed by several pharmacology groups. Beta blockers have a lower benefit in the treatment of essential hypertension compared with other drugs. However in the last years, have been arouse the third generation beta blockers, like nebivolol, which enhances of NO dependent va-

sodilatation.

Tempol is an antioxidant that mimics the action of the superoxide dismutase enzyme, that catalyses the conversion of O2˙- into H2O2, and the consequent reduction of blood pressure.

The aim of this work was to evaluate the effects of nebivolol, alone or combined with tempol, on systolic blood pressure (SBP), the echocardiography and metabolic parameters in a model of metabolic syndrome.

Male Sprague Dawley rats (250-300g) with or without fructose (F) (10% w/v) in tap water during 12 weeks, were distributed in 4 groups (n=6): control, Nebivolol (15 mg/kg/day/ i.g.), Tempol (60 mg/Kg/day/ i.g) and nebivolol + tempol. All groups were evaluated for indirect SBP by tail-cuff method, echocardiography Doppler and oral glucose Tolerance test (OGTT).

In comparison with normotensive rats, F overloaded rats showed an increase of indirect SBP (mmHg, C: 120±2 vs F: 138±4, $p<0.05$). Treatment with nebivolol, alone (116±1.20 mmHg) or combined with tempol (118±2.8 mmHg), was able to reduce SBP when compared with F rats. The Isovolumetric relaxation time and echocardiography hypertrophy parameters were improved with the combinated therapy. Also the OGTT test was improved in the combined treatment.

In the experimental model of MS induced by F, chronic treatment with nebivolol was able to reduce SBP. The combination of nebivolol with the antioxidant tempol improves ventricular left hypertrophy, diastolic function and the OGTT in the fructose group.

Keywords: Hypertension, Nebivolol, Antioxidantes, Metabolic Syndrome.

(1121) **NITRIC OXIDE AS SIGNALING MOLECULE INVOLVED IN EARLY STAGES OF CARDIAC MITOCHONDRIAL DYSFUNCTION IN DIABETES MELLITUS**

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Previous results from our laboratory have shown that sustained hyperglycemia (28 days) leads to heart mitochondrial dysfunction with increases in H_2O_2 and NO steady-state concentrations. This mitochondrial impairment was detected in the absence of heart hypertrophy and of changes in resting cardiac performance, suggesting that mitochondrial dysfunction precedes the onset of diabetic cardiac failure. In this scenario, H_2O_2 and NO could be molecules involved in mitochondrion-cytosol signaling. The aim was to study the role of NO and H_2O_2 in early stages of the mitochondrial dysfunction, in an experimental model of Type I Diabetes Mellitus (DM). Diabetes was induced by a single dose of Streptozoticin (60 mg/kg, ip.) in male Wistar rats. Glycemia was determined 72 hours after injection (C:117±6 mg/dl; DM:502±27 mg/dl). The animals were sacrificed at day 10 and heart mitochondrial fraction was isolated. State 3 respiration sustained by malate-glutamate and by succinate was 18% and 13% lower in diabetic than in control rats. Because of resting mitochondrial O_2 consumption rates did not modify, respiratory control ratios mainly declined when malate-glutamate was used as substrate. These results agree with complexes I-III and II-III activities that were lower (15 and 11%) in diabetic than in control animals. However, no difference was observed in complex IV activity. Moreover, mitochondrial GSSG/GSH ratio was higher (25%) in diabetic animals suggesting an imbalance in cellular redox state. Preliminary results have shown that while mitochondrial NO production rate was 18% higher in diabetic rats, H_2O_2 generation did not modify. These results show an early mitochondrial dysfunction, after 7 days of sustained hyperglycemia, accompanied by an increase in NO generation suggesting that this molecule could be involved in the triggering of heart mitochondrial biogenesis in an initial phase of DM.

Keywords: Heart mitochondria Type I Diabetes Nitric oxide Mitochondrial nitric oxide synthase (mtNOS) Hydrogen peroxide

(1157) **PLATELET AND FIBRIN NETWORK MORPHOLOGY ALTERATIONS IN A RABBIT MODEL OF DIET INDUCED CARDIOMETABOLIC SYNDROME**

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The cardiometabolic syndrome (CMS) is a constellation of interrelated risk factors of metabolic origins that together promote the increased risk of cardiovascular disease (CVD). Central obesity with the associated CMS components promotes proinflammatory and prothrombotic states. The aim of the present work was study whether platelet and fibrin network-morphology or coagulation profiles was altered in a rabbit model of CMS. Male rabbits were fed either a control diet (CD) or atherogenic high fat diet (18 % fat and 1 % cholesterol, HFD-HC) during 6 weeks. Scanning electron microscopy was used to study platelet- and fibrin network-morphology by preparing platelet-rich plasma (PRP). Ten µl of PRP was used to study platelet morphology and 10 µl of PRP was mixed with 5 µl of bovine thrombin (2,7UI/ml) to study fibrin network morphology. Activated Partial Thromboplastin Time (APTT), thombin time and platelets count were performed. Platelets of the CD appeared spherical with few pseudopodia present while the platelets of the CMS presented with numerous pseudopodia and spreading, indicating activation. The fibrin networks of the CD consist of thick and thin fibers that form an organized network of fibers. The fibrin networks of the CMS appeared less organized with less taut fibers. Fibrin fiber thickness was found to be significantly increased in the CMS group (p-value <0.05) when compared to CD. The thicker fibers formed irregular networks with thick masses of fibrin fibers. The hemostatic profiles of the CMS did not differ significantly (p-value>0.05) from the CD, indicating a normal functioning coagulation cascade. The findings indicate that premature activation of platelets (as a result of chronic inflammation) that in turn causes altered fibrin formation but not alterations in the coagulation cascade may account for the prothrombotic status in the CMS.

Keywords: Cardiometabolic Syndrome, fibrin network-morphology, platelet

(870) **STEVIA´S CARDIOPROTECTIVE EFFECTS**

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Stevia rebaudiana bertoni, an herbaceous plant widely distributed and used in Paraguay and Brazil for its sweetener character, has several medicinal properties including antitumor, antihypertensive and anti-inflammatory actions. The aim of the present study was to investigate the cardioprotection exerted by oral stevioside (E, 168 mg/kg for 15 days), a major component of stevia, and its relationship with the activation of protein kinase B (PKB/Akt) in Langendorff perfused rat hearts subjected to ischemia (I)-reperfusion (RP). Hearts from female Wistar rats (200-250g) fed ad libitum were used. Wortmannin (W, 100nM), PI3K/Akt inhibitor was added 15 min before I. The contractility was evaluated by determining the left ventricular developed pressure (LVDP), rate-pressure product (RPP) was determined by multiplying heart rate (HR) by LVDP, peak rate of contraction and peak rate of relaxation (±dP/dt), and the left ventricular end-diastolic pressure was measured (LVEDP). The creatine kinase (CK) release was determined in the coronary effluent during the first 10 min of RP and the myocardial infarct size (MIS) was determined by staining with triphenyltetrazolium chloride (TTC) dissolved in phosphate buffer. ANOVA, n=8/group. E improved postischemic recovery and W annuled the cardioprotective actions mediated by E (at 30 min RP RPP (%): C 55.5±8.8, C+W 52.0±5.1, E 87.2±14.8*, E+W 57.0±14.8; *p<0.05 vs C, W, E+W). These results were accompanied with similar changes in ±dP/dt. LVEDP developed lower values in the E group (LVEDP (%) at 10 min of RP: C 15.5±2.0, C+W 29.0±2.2, E 2.2±0.9*, E+W 24.0±3.6; *p<0.05). E decreased CK release and infarct size (CK (UI/g wet weight) C 40.6±3.5, C+W 47.0±4.7, E 24.8±3.2*, E+W 33.0±3.8; MIS (%) C 62.1±1.5, C+W

68.2±2.1, E 41.6±3.5*; E+W 58,2±2.1; *p<0.05 vs C, W, E+W). These results suggest that oral administration of E presents cardioprotective effects that could be partly mediated by Akt activation.

Keywords: stevioside, ischemia, reperfusion.

(1641) **TABAQUISM AND CARDIOMYOPHATIES. EFFECTS OF TOBACCO CONSUMPTION ON CARDIAC CALCIUM HANDLING IN *Drosophila melanogaster***

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Tabaquism is a chronic disease caused by nicotine addiction, being responsible for about 40.000 deaths/ year in Argentine. Cardiovascular effects include coronary heart disease, acute myocardial infarction and congenital cardiomyopathy. The fruit fly *Drosophila melanogaster* is a well-known model used to study several human diseases and addictions. The possibility to edit its genome, to produce transgenic lines and to study the interaction between genes and the environment, make this organism an alternative to study a possible relationship between genes and tobacco effects. We developed an experimental approach of “chronic consuming-cigarette” flies simulating active human smoking. We compared the cardiac function of smoking flies (S) vs. non-smoking controls (C). Tobacco increased heart rate (beats/min: S: 155.5 ±14.6 n=6, C: 105.8±6.9 n=12), reduced Ca2+ transient amplitude (arbitrary units of fluorescence: S: 1.27±0.2 n=10, C: 0.25±0.05 n=7), time of to peak contraction (TTP S: 0.16±0.01 sec n=12, S: 0.12±0.01 sec n= 5) and relaxation time (t1/2 S: 0.12±0.01 sec n=12, C: 0.07±0.02 sec n=5). Similar results were obtained with nicotine alone, in semi-intac preparation. Together, our evidences suggest that nicotine is the principal component of tobacco that induces cardiac changes in the fly’s adult heart.

Current experiments are exploring receptors and signaling pathways involved in nicotine action on heart function. *D. melanogaster* presents glutamatergic and beta like-adrenergic receptors, associated to nervous terminals reaching the heart. Moreover, nicotinic receptors (nAChR) could be present in cardiac *Drosophila* muscles. We detected nAChR1 in adult total extract of protein of *D. melanogaster* using the antibody against human nAChR1. Assays in course are orientated to determinate the contribution of these three types of receptor activation to the nicotine effects on cardiac activity.

Key words: Tabaquism; Nicotine; Drosophila melanogaster; Cardiac alterations; Nicotinic receptors.

REPRODUCTION AND FERTILITY 4

(280) **DIETS ENRICHED IN OLIVE OIL REGULATES THE EXPRESSION OF PPAR TARGET GENES IN THE FETAL LIVER IN A RAT MODEL OF GESTATIONAL DIABETES MELLITUS (GDM) INDUCED BY INTRAUTERINE PROGRAMMING.**

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In a rat model of GDM induced by intrauterine programming, we previously found sex-dependent alterations in PPAR target enzymes involved in lipid accretion or oxidation as well as increased prooxidant/proinflammatory markers in the fetal liver. **Aim**: To address whether maternal diets enriched in olive oil prevent the alterations in lipid metabolizing enzymes in the fetal liver and whether there are changes in the expression of nuclear factor erythroid-derived-2 like 2 (NRF2), a PPAR target gene relevant in the regulation of antioxidant enzymes. **Methods**: Pregestational diabetic rats (F0) were obtained by neonatal streptozotocin administration. F0 rats were mated with healthy males. No treatments were performed in the F1 offspring until the 3rd month of age, when the rats were mated with healthy males. During F1 pregnancies the rats were fed with a standard diet supplemented or not with 6% olive oil. Maternal metabolic parameters and the mRNA levels of the genes codifying fatty acid synthase (FAS), acyl CoA oxidase (ACO) and NRF2 in the fetal liver were evaluated (by RT-PCR) on day 21 of pregnancy. **Results**: Maternal olive oil treatment did not prevent maternal hyperglycemia but reduced maternal triglyceridemia (21%, p<0.05). The increased FAS mRNA levels in the liver of male fetuses of GDM rats were prevented with the olive oil treatment (92% reduction, p<0.001). ACO levels, increased in the liver of female fetuses of GDM rats, were reduced with the olive oil treatment (95% reduction, p<0.001). Moreover, NRF2 expression was reduced in the liver of female fetuses of GDM rats fed with standard diet compared to controls (64% reduction, p<0.01), an alteration prevented by the olive oil treatment (100% increase, p<0.05). **Conclusion:** Maternal olive oil treatment in the rats that develop GDM, do not prevent GDM induction, but regulate fetal liver enzymes involved in lipid metabolism and antioxidant pathways, probably improving liver function in the offspring´s later life.

Keywords: Gestational Diabetes Mellitus, Olive oil, Fetal Liver, Intrauterine Programming, Maternal Treatment

(1177) **EFFECT OF NITROSATIVE STRESS ON AQP9 EXPRESSION IN HUMAN TROPHOBLAST**

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Preeclamptic placentas are characterized by an abnormal trophoblastic invasion of the spiral arteries resulting in an inadequate utero-placental blood flow. Consequently, an intermittent perfusion is established which leads to an ischemia/reperfusion injury in the syncytiotrophoblast. Fluctuations in O_2 tensions may increase the generation of reactive nitrogen species (RNS) by promoting the formation of peroxynitrite (ONOO-) which could damage lipids, proteins and DNA. Recently, we reported that placental explants exposed to hypoxia/reoxygenation showed an overexpression of AQP9 with a lack of functionality similar to that observed in preeclamptic placentas. However, the mechanisms by which it occurs are still unknown.

Our aim was to evaluate the effect of nitrosative stress mediated by $ONOO^-$ on AQP9 expression. This study was approved by the ethics committee of the Hospital Nacional Dr. Prof. A. Posadas. Placental explants were cultured in complete DMEM-F12. $MgSO_4$ was added to neutralize the effect of ONOO-, since it is used extensively for *prevention* and *treatment* of eclampsia, associated with a reduction in maternal and fetal morbidity. Lipid peroxidation (TBARS) was determined. Expressions of superoxide dismutase 1 (SOD-1), as a measure of the antioxidant enzyme defense system, and AQP9 were also studied.

In the presence of 100 μM of $ONOO^-$, AQP9 expression significantly increased 75.42 ± 0.75% compared to control (*n=7; p<0.05*). In this condition, TBARS increased 3-fold (*n=5, p<0,002*), while SOD-1 showed a 70.35 ± 16.51% decrease (*n=5, p <0.02*). The addition of $MgSO_4$ restored SOD-1(*n=3, p<0.02*) and AQP9 (*n=5, p<0.05*) expressions to control levels.

Our results show that the production of RNS dramatically increases AQP9 expression. However, further studies are required to elucidate whether the high nitric oxide levels could nitrate the tyrosine residues of AQP9, resulting in a non-functional protein and the implications in the pathogenesis of preeclampsia.

Keywords: AQP9, trophoblast, human placenta, nitrosative stress.

(1823) **EFFECT OF RESVERATROL ON ACTIVE MITOCHONDRIA AND LIPID PEROXIDATION OF VITRIFIED BOVINE EMBRYOS**

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Despite being a new method for embryo cryopreservation, vitrification has been reported to increase reactive oxygen species (ROS) production and to diminish reduced glutathione content leading to deleterious effects on cells. To prevent oxidative damage, several antioxidants have been added to vitrification media. The aim of this study was to determine the effect of resveratrol on active mitochondria and lipid peroxidation of vitrified bovine embryos. Cumulus-oocyte complexes were cultured in medium 199 with the addition of fetal calf serum and gonadotropins at 39°C, 5% CO_2 in humidified air for 22h and then co-incubated with a suspension of $2x10^6$ motile spermatozoa/ml for 20h in IVF-mSOF. Then, presumptive zygotes were transferred to IVC-mSOF medium with or without the addition of 0.5μM resveratrol. At day 7, blastocysts were vitrified by a minimum volume method in the presence or absence of 0.5μM resveratrol. A group of non-vitrified blastocysts was used as control. Active mitochondria and superoxide anion ($O_2^{\cdot-}$) production were determined in the control and in different groups of vitrified blastocysts by Mitotracker Green FM/MitoSOX Red dual stain, while lipid peroxidation was determined by Bodipy. Blastocysts subjected to vitrification/warming presented higher active mitochondria and $O_2^{\cdot-}$ production than fresh blastocysts (P<0.05). The addition of resveratrol to IVC or vitrification medium reduced the increase in active mitochondria but not the increase in $O_2^{\cdot-}$ production (P<0.05) compared to blastocysts cultivated in non-suplemented IVC and in vitrification media. However, lipid peroxidation presented no significant changes in any case. In conclusion, these results might suggest that resveratrol would be scavenging other ROS during the propagation phase but not in the initiation phase, thus explaining its lack of effect on $O_2^{\cdot-}$ levels produced in vitrification/warming.

Keywords: resveratrol, vitrification, bovine embryos, ROS, superoxide anion.

(328) **MATERNAL OVERWEIGHT ALTERS THE SEXUAL MATURATION IN MALE OFFSPRING BY MODIFYING THE TESTICULAR HISTOLOGY AND TESTOSTERONE LEVELS**

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An appropriate maternal body weight is needed for a normal fetal programming. Previous studies showed that maternal overweight affects the size and body weight, ano-genital distance, sperm count and morphology of the germ cells. Now, we studied whether the maternal overweight affects the testicular morphology and the levels of testosterone. To this end, male offspring from rats fed with standard (OSD) or cafeteria (OCD) diet, with high lipid content, were used. Considering the percentage of maternal overweight, the OCD were divided into two groups: offspring from rats with 25% and higher than 35% of overweight (OCD25 and OCD35, respectively). Offspring always were fed with standard diet and were euthanized at 60 days of age to obtain testes and blood serum. Seminiferous tubular diameter (STD), thickness of the seminiferous epithelium (SE) and tubular lumen diameter (TLD), expressed in μm, and the serum levels of testosterone, expressed in ng/ml, were examined. All data are expressed as mean±SEM and statistical analysis was performed by one-way ANOVA with Dunnett´s multiple comparison test or Kruskal-Wallis test with Dunn´s multiple comparison test. Compared with OSD (STD: 59±2, SE: 15±1), both OCD groups exhibited decrease in STD and SE, although these changes were only significant for OCD35 (STD: 41±4; P<0.001; SE: 8±1, P<0.01). Likewise, OCD35 showed higher testosterone levels (5.2±0.6, P<0.05) than OSD (2.7±0.3). No significant difference was found in that of OCD25 group. In conclusion, our results suggest that an intrauterine environment with high fat content may alter the reproductive ability of the male offspring, at least in part, altering the morphology of the testes and testosterone levels, likely as a result of altering the organogenesis.

Keywords: Overweight, cafeteria diet, testis morphology, testosterone.

(1125) **EFFICIENCY OF EMBRYO TRANSFER IN SHEEP UNDER DIFFERENT NUTRITIONAL STATUS OF DONOR AND RECIPIENT EWES**

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The objective of this study was to evaluate the effect of the nutritional status of embryo donors and recipients on embryo survival after direct transfer of embryos in sheep. The experimental study was carried out at the Reproduction Laboratory Facilities of INTA Bariloche. A total of 36 donor and 75 recipient Merino ewes received either 1.5 (S, Supplemented) or 0.5 (R, Restricted) times daily maintenance requirements (S donors n = 19, R donors n = 17, S recipients n = 36, R recipients n = 39), from the beginning of the estrus synchronization treatment until day 30 post estrus. On day 7 post estrus, one transferable embryo of donor females S or R was transferred into a recipient female S or R, defining the following groups: SS (n = 11), SR (n = 11), RS (n = 25) and RR (n = 28). The concentration of progesterone and metabolic hormones in donor and recipient ewes was determined according to nutritional status. The pregnancy rate on day 17 of gestation was similar between groups (73, 45, 72 and 64% for SS, SR, RS and RR, respectively; P > 0.1). However, embryo losses between days 17 and 35 of gestation were higher in the SR group (18%) than in the other three groups (9, 8 and 7% for SS, RS and RR, respectively; P < 0.1). Moreover, pregnancy rate on day 35 of gestation was lower in the SR group (27%) compared to the other groups (64, 64 and 57% for SS, RS and RR, respectively; P < 0.1). Donors and recipients under nutritional restriction had lower concentrations of IGF-1 (P < 0.01). In conclusion, failure to establish and maintain pregnancy may be related to an asynchrony between an unfavorable uterine environment in restricted recipient ewes and embryos produced by donors in a high nutritional condition.

Keywords: embryo losses, undernutrition, embryo transfer, sheep

(184) **FOXO1 TARGET GENES ARE ALTERED IN THE HEART OF THE OFFSPRING FROM DIABETIC RATS**

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Cardiovascular alterations in the adult can be developmentally programmed by maternal diabetes. FoxO1 participates in cellular oxidative homeostasis, metabolism and survival of cardiomyocites and its overactivation is related to cardiac dysfunction in diabetes. We have previously found increased FoxO1 levels in the offspring's heart from diabetic rats. FoxO1 activation can be inhibited by serum glucocorticoid kinase 1 (sgk1) phosphorylation that induces FoxO1 nuclear exportation. Connective tissue growth factor (CTGF, regulates fibrosis) and MMP2 (related to inflammatory processes when it is in excess) are FoxO1 target genes. Our **objective** is to evaluate serum markers of heart damage, mRNA levels of CTGF and MMP2 and phosphorylation status of sgk1 in the offspring's heart from control and diabetic rats. **Methodology**: Pregestational diabetic rats were obtained by neonatal streptozotocin administration and were mated with healthy males. Adult male offspring from control and diabetic rats were evaluated. Serum levels of lactate dehydrogenase (LDH) and myocardium creatine kinase (CK-MB) were determined by a commercial kit. In the heart, CTGF and MMP2 mRNA levels were evaluated by qPCR and phosphorylation status of sgk1 by western blot. **Results**: LDH and CK-MB, serum markers of heart damage, were found increased in diabetic offspring compared to

control (1.8 and 1.5 fold, p<0.05). mRNA levels of CTGF and MMP2 were increased (2.8 and 2.4 fold, p<0.05 and p<0.01 respectively) and Sgk1 phosphorylation status and ratio of phospho-sgk1/total sgk1 were decreased (p<0.05) in the heart of diabetic offspring compared to controls. **Conclusion:** The increased mRNA levels of FoxO1 target genes involved in profibrotic and proinflammatory processes are probably related with the increased levels of active FoxO1. These alterations could be involved in the increased levels of serum markers of heart damage found in male offspring from diabetic rats.

Keywords: Diabetes, intrauterine programming, FoxO1, heart

(1694) **DIFFERENTIAL GENE EXPRESSION AND HORMONAL REGULATION OF BONE MORPHOGENETIC PROTEIN INHIBITORS IN BOVINE OVIDUCT EPITHELIAL CELLS**

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Bone morphogenetic proteins (BMPs) play important roles in mammalian reproduction and female fertility. Previous studies have demonstrated that BMP ligands and receptors are expressed in an anatomically and temporally regulated fashion in the bovine oviduct. However, the molecular mechanisms that regulate BMP signaling in the oviductal context are still unknown. The present study aims to explore the regulatory context of BMP signaling in the bovine oviduct by focusing attention on their negative modulators. For this purpose, the mRNA expression of five antagonists of the BMP signaling pathway (*FST*, *BAMBI*, *NOG*, *CHRD* and *SOSTDC1*) were evaluated by RT-qPCR in bovine oviduct epithelial cells (BOECs) obtained from ampulla and isthmus at different stages of the estrous cycle. In addition, to evaluate if the expression of these genes respond to hormonal stimulation, cell suspension cultures of BOECs derived from both anatomical regions were stimulated with estradiol-17β (0.1, 1 and 10 nM) or progesterone (1, 10 and 100 nM) and analyzed by RT-qPCR. The expression of *FST*, *BAMBI*, *NOG*, *CHRD* and *SOSTDC1* was detected in BOECs from ampulla and isthmus during the estrous cycle. Except for *NOG*, regional and temporal differences were observed in the expression patterns of the genes analyzed. Particularly, higher mRNA levels were observed for *FST* in ampulla (P<0.001) and for *BAMBI* in isthmus (P<0.05) during the luteal phase. In the case of *CHRD* and *SOSTDC1*, a similar regulation pattern to *FST* was observed, but anatomical differences were statistically less significant (P<0.05). Under *in vitro* culture conditions, mRNA levels of *FST* and *BAMBI* increased in response to progesterone treatment while estrogen (10 nM) tended to suppress their expression. The region specific and cycle dependent expression differences together with the susceptibility to steroid hormones provide evidence to suggest a functional importance of BMP inhibitors in the regulation of oviductal physiology.

Keywords: BMP inhibitors, oviduct, bovine, steroid hormones

(732) **GLUCOSE UPTAKE IS REGULATED BY VIP THROUGH MTOR-MEDIATED PATHWAYS IN TROPHOBLAST CELLS**

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Adequate trophoblast (Tb) metabolism is crucial to allow nutrients transport from the mother to the fetus required for fetal growth and development. A dysregulation of this process may cause inappropriate fetal growth inducing Intrauterine Growth Restriction among other complications. Glucose is the main energy substrate for the placenta and the fetus and its transfer depends on maternal levels, placental glucose metabolism and facilitated transport through GLUT carrier proteins. GLUT1 appears to be one of the primary transporters of the placenta and is found at both maternal and fetal-facing Tb membrane. The vasoactive intestinal peptide (VIP) is a pleiotropic neuropeptide that was shown to inhibit glucose oxidation in rat enterocytes. We have previously demonstrated that VIP is synthesized by human Tb cells having autocrine and paracrine effects on these cells and favors Tb cell migration and invasion. Objectives: to study the role of VIP in the regulation of glucose uptake in Tb cells, placenta weight and fetal growth. Methods: we used two human Tb cell lines, Swan-71/BeWo, and implantation sites of C57BL/6 WT females mated to VIP-/- or WT males. Swan-71 VIP knocking-down was carried out using a VIP siRNA. GLUT1 and mTOR expression were evaluated by qRT-PCR and glucose uptake by flow cytometry using the fluorescent analogue 2-NBDG. Results: VIP induced glucose uptake in Tb cells through mTOR activation. An increase of GLUT1 and mTOR expression in siVIP vs. scramble transfected Tb cells and a dysregulation of glucose uptake were observed. On the other hand, VIP deficient embryos at day 14,5 showed reduced weight (WTxWT vs. WTxKO, p<0.001) without changes in placenta weight. An increased expression of GLUT1 and mTOR was observed in placenta from VIP WTxKO mice. Conclusion: Results of these *in vitro* and *in vivo* models are consistent with a role of VIP as a local factor modulating glucose uptake by the placenta and support its relevance in fetal growth.

Keywords: VIP-Glucose-Trophoblast cell-Fetal growth-Nutrient transport

(1366) **HYPOXIA INDUCIBLE FACTOR-1 (HIF-1) IMPAIRS FUSION OF HUMAN TROPHOBLAST BEWO CELLS: INFLUENCE OF ENDOCANNABINOID SIGNALING.**

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The syncytiotrophoblast (STB) form a multinucleated layer that is maintained and regenerated by the fusion of underlying cytotrophoblast cells (CTBs). This structure is responsible for specialized functions of the human placenta and disturbance in this process may result in pregnancy-associated diseases such as preeclampsia and intrauterine growth restriction. Hypoxia modifies human placental villous syncytialization, however these mechanisms remains unknown. Previously we demonstrated that hypoxia inducible factor-1 alpha (HIF-1α) deregulated the endocannabinoid system (ES) in human placenta. In the present study, we investigated if HIF-1 and ES perform pivotal roles in trophoblast syncytialization.

BeWo cell line was cultured with 100μM forskolin (FSK) to induce cell fusion. CTB (BeWo) and STB (BeWo + FSK) cells were treated with and without cobalt chloride ($CoCl_2$), a hypoxia-mimetic agent that stabilizes (HIF-1α); or R-(+)-Methanandamide (Met-AEA), a stable anandamide analogous. Cell viability was evaluated by MTT assay. Markers for trophoblast syncytialization: syncytin-1 and glial cells missing-1 (GCM-1), were analyzed by western blot and RT-PCR. The location of the E-cadherin was studied by immunocytochemistry.

Incubation with $CoCl_2$ and Met-AEA significantly reduced cell viability (p<0,05; n=5). Treatment with 100μM $CoCl_2$ significantly increased HIF-1α protein levels (p<0,01; n=4). Forskolin-induced cell fusion identified by increased levels of GCM-1 mRNA and syncytin-1 protein expression was reversibly suppressed with $CoCl_2$ and Met-AEA treatment (p<0,05; n=5). Localization of E-cadherin confirmed that forskolin induced cell fusion. In the presence of $CoCl_2$ there was a significant decrease in BeWo cells fusion rate compared to forskolin treatment only.

The above results suggest that increased levels of HIF-1 may disturb trophoblast syncytialization and that endocannabinoids could play an important role in this critical process of human placenta.

Keywords: Trophoblast, HIF-1, Syncytialization, Endocannabinoids.

(761) **EFFECTS OF DEHYDROEPIANDROSTERONE ON THE UTERINE STROMA OF PERIPUBERTAL RATS**

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In a previous study we have shown an increase of the subepithelial stroma and myometrium thickness associated with a decrease in cell density in the uterus of hyperandrogenized rats. Here, we investigate aquaporin (AQP) expression, collagen content and cell proliferation of the uterine subepithelial stroma and myometrium in androgenized peripubertal rats. Wistar rats were injected sc with sesame oil (control group) or dehydroepiandrosterone (DHEA) 6 mg/100 g body weight from postnatal day 21 for 20 consecutive days to induce hyperandrogenism. Before sacrifice, the animals received an ip injection of Bromodeoxyuridine (BrdU, 6 mg/100 g body weight). One uterine horn was frozen in liquid nitrogen and used to evaluate the mRNA expression of AQP1 to 9 by quantitative real-time PCR. The other uterine horn was fixed in 10% buffered formalin and embedded in paraffin for histological studies. Collagen content was measured as integrated optical density (IOD) using picrosirius red staining and polarization microscopy. Cell proliferation was evaluated by determining BrdU incorporation by immunohistochemistry. The uterus of control rats expressed AQPs 1-5 and 7-9. In the DHEA group, AQP3 and 8 expressions were increased while AQP7 expression was decreased, compared to controls rats. Collagen content was increased in both the subepithelial stroma (control:1392±251 vs DHEA:4055±341; $p<0.05$) and myometrium (control:691±92 vs DHEA:2764±128; $p<0.05$) of DHEA-treated rats. Cell proliferation was decreased only in subepithelial stroma of DHEA-treated animals (control:7.3±2.4% vs DHEA:1.5±0.4 %; $p<0.05$). The results suggest that DHEA modifies the water content and the extracellular matrix of the uterus evidenced by changes in AQPs expression and collagen content. These results, together with a lower cell proliferation index may explain the increase in thickness and the decrease in cell density observed in the uterine subepithelial stroma and myometrium of hyperandrogenized animals.

Keywords: Dehydroepiandrosterone, rat, collagen, AQP, uterus.

ENDOCRINOLOGY 4

(1008) **BONE ACTIONS OF THE FORGOTTEN ESTROGEN, ESTRONE**

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Estrogens play an important role in bone remodeling. Although estradiol action has been the subject of intensive investigation, little is known about the contribution of the other major endogenous estrogen, estrone (E1). The aim of this work was to evaluate the effect of E1 on bone cells, particularly the modulation of osteoblasts (OB) maturation. Murine calvarial pre-OB were cultured for 3-15 days and exposed to E1 (10^{-8} – 10^{-10}M) in the last 48h. OB characterization was performed by the measurement of bone markers, using RT-PCR technique. mRNA levels for BMP-2 and Runx-2 were detected in all time of culture (3-15 days). Indeed the expression of both types of ER (α andβ) was also detected. In 15 days OB culture, E1 significantly increased cell proliferation (0,68±0,09 vs 0,49±0,09, E1 vs C, $p<0,001$ in MTT assay). These results were confirmed by cell counting (41% above C, $p<0,05$). Since mature OB exhibited high level of alkaline phosphatase activity (ALP) and enriched extracellular calcium deposition, we evaluated the effect of E1 on both parameters. E1 enhanced ALP (15% a/C, $p< 0,01$) and increased the number and size of calcification nodules in the extracellular matrix (one fold $p<0,005$, Alizarin staining), with major effect at longer period of cell culture. Accordingly with this, markedly decreased in calcium content was detected in aliquots of cultured medium (205±25 vs 118±23, C vs E1, $p<0,001$). Indeed E1 modulates extracellular collagen deposition visualized by Sirius red staining. On genes related with OB maturation, E1 treatment elicited 2 fold increase in Runx-2 mRNA levels, with higher effects at 3-5 days of culture ($p<0.05$). No changes were observed in BMP-2 expression. Similar results as that elicited by E1 were obtained when OB treatment were performed with the selective estrogen receptor modulator, raloxifene compound. In summary the results presented suggest that, E1 exhibits a positive action on bone, either on OB and extracellular matrix.

Keywords: osteoblasts, estrone, bone markers, proliferation, differentiation

(1508) **DIFFERENTIAL ACTIONS OF TESTOSTERONE ON PROSTATE SMOOTH MUSCLE CELLS IN ACCORDANCE WITH THE SUBCELLULAR LOCALIZATION OF THE ANDROGEN RECEPTOR**

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Testosterone (T) actions in prostate smooth muscle cells (pSMCs) are critical for prostate homeostasis and the development of pathological conditions. T can operate through the classical cytoplasmic androgen receptor (AR) eliciting genomic signaling, or via receptors located at the plasma membrane for nongenomic signaling. Here we aimed to evidence nongenomic T signaling in pSMCs and their role in cell proliferation, differentiation, and modulation of lipopolysaccharide (LPS)-induced response. For this purpose rat pSMCs were stimulated with T or BSA-conjugated T (T-BSA). To analyze modulation of LPS-response, pSMCs were stimulated with LPS, LPS+T or LPS+T-BSA. ANOVA-Tuckey was used for statistical analysis

Membrane localization of AR in pSMCs was determined by confocal microscopy and flow-cytometry with anti-AR antibody, and verified by western blot (WB) of isolated cell surface proteins. Furthermore, we showed that pSMCs can respond to T or T-BSA by nongenomic activation of ERK1/2 and Akt phosphorylation, as evidenced by WB. Membrane stimulation with testosterone resulted in AR- and pERK- dependent increase in cell proliferation (Ki67 and total cell count) which was determined by the use of specific inhibitors ($p<0.05$). Besides, pSMCs stimulated with T-BSA presented higher proliferation as compared to T ($p<0.05$). Membrane stimulation also favored smooth muscle phenotype by increasing their markers (calponin, α-smooth muscle actin) and decreasing vimentin (WB) ($p<0.05$). In the contrary, we showed that the anti-inflammatory effects of T are promoted only by the cytosolic AR which decreased LPS-induced TNFα and IL-6 levels (ELISA) ($p<0.05$), and NF-κB nuclear translocation (immunofluorescence) ($p<0.05$).

We postulate that T might perform pro-homeostatic effects in pSMCs by modulating cell proliferation and inflammation through classical intracellular mechanisms, while pathological, hyperproliferative actions would be induced by membrane-initiated nongenomic signaling.

Keywords: Prostate, Androgen receptor, Nongenomic signaling, Smooth muscle cells, Testosterone.

(1422) **ESTRADIOL WOULD FAVOR INDIRECTLY MAMMARY GLAND ELONGATION AND SIDE-BRANCHING BY STIMULATING PROGESTERONE RECEPTOR EXPRESSION IN PREGNANT *LAGOSTOMUS MAXIMUS* (RODENTIA: CHINCHILLIDAE).**

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Prolactin (PRL), progesterone (P) and estradiol (E2) are major regulators of mammary gland (MG) tissue remodeling throughout the repeated cycles of pregnancy-lactation-regression. Considering

the atypical modulation of the hypothalamus-hypophysis-ovary axis of *Lagostomus maximus* we studied the relation among P and E2 serum values, their MG-receptor expression levels (PR, ER and PRLR) and their relation with the MG-cell proliferation and MG-vascularization. P and E2 serum levels (ELISA) and mammary PRLR-, PR-, VEGF- and PCNA expressions (PCR and immunohistochemistry) were determined in 4 groups of adult vizcachas (resting, pregnant, lactating, regressing). Maximal levels of E2 and P were recorded at pregnancy ($p<0.005$) when the GM reaches a great development evidenced by high levels of both VEGF and PCNA. In order to establish the importance of the ovarian steroids over PRLR, PR, VEGF and PCNA expressions, adult non-pregnant females were ovariectomized (OVX) and treated with either E2 (OVX_E) or P (OVX_P). OVX animals were used as controls. Mammary PRLR immunoreactive area in OVX_E dropped approximately to half of that detected in OVX group ($p<0.005$) suggesting that the expression of this receptor is P dependent. On the contrary, the absence of changes in the expression of VEFG and PCNA among the experimental groups could indicate that the regulation of these markers is not under the direct and unique control of ovarian steroids but rather that they would coordinate their action with other factors (i.e. PRL) in MG-tissue remodeling. Interestingly, both PR gene expression and PR immunoreactive area were notably lower in MG of OVX_P compared to both OVX_E and OVX groups ($p<0.005$) indicating the role of E2 in PR expression. Our data suggest that the increased PR expression associated to the branching and elongation of the MG ductal tree at pregnancy may be indirectly stimulated by E2. Fundación Científica Felipe Fiorellino, CONICET-PIP110/14, MINCyT-PICT1281/2014

(722) **ESTROGEN AND PROGESTERONE MEDIATE DENDRITIC SPINE FORMATION THROUGH THE ACTIN REGULATORS CORTACTIN AND WAVE-1**

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Pyramidal neurons from cortex and hippocampus have thousands of dendritic spines (DS) that form excitatory synapses. As a consequence, failures in DS formation can lead to an improper cognition, a common feature in neurodegenerative diseases like dementia. Actin nucleation is a fundamental process in DS formation and it depends on actin regulators. Among them, WAVE1 and Cortactin are key activators of the Arp2/3 complex, ultimate responsible for DS formation. Our previous research has established that 17β-estradiol (E2) and progesterone (P4) modulate several signaling pathways leading to WAVE1 phosphorylation and DS formation. However, the precise molecular mechanisms of this regulation remain to be elucidated. Therefore, we asked ourselves firstly, if Cdk5 Kinase and PP2A phosphatase regulate WAVE1 phosphorylation (their target protein) in response to rapid treatments with E2 and P4. Secondly, we evaluated if Cortactin phosphorylation was affected by the same treatments. To test our hypothesis, we employed primary culture of embryonic rat cortical neurons, immunofluorescence and western blot analysis. Our results showed that, after treatments with E2 or P4 (10nM, 20min), the number of DS was significantly increased (over a 50% vs. CON, $p<0,05$), indicating that both hormones rapidly modified neuronal morphology. The increase in the number of DS was prevented by Roscovitine, a Cdk5 inhibitor, and mimicked by Okadaic Acid, a PP2A inhibitor. These changes were accompanied by a significantly increase of the phosphorylation patterns of $Cdk5^{Y15}$, $PP2A^{Y307}$ and $WAVE1^{S397}$ after E2 and P4 stimulation. Furthermore, we also determined that Cortactin mediates DS formation via a Rac1/PAK1 cascade, after E2 and P4 treatment. As a conclusion, our results suggest that E2 and P4 exert a dynamic regulation of neurons morphology by inducing a rapid activation of Cortactin and WAVE1. E2 and P4 can promote DS formation and therefore they contribute to synaptic plasticity processes.

Keywords: E2, P4, WAVE1, cortical neurons, dendritic spines

(1417) **ESTROGEN RECEPTORS ARE INVOLVED IN LUTEINIZING HORMONE (LH) PULSATILITY DURING PREGNANCY IN THE VIZCACHA, *LAGOSTOMUS MAXIMUS*.**

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The South American plains vizcacha shows ovulatory around mid-gestation enabled by a hypothalamic-hypophyseal-ovarian axis activity with elevated levels of serum estradiol (E_2) and gonadotropin-releasing hormone (GnRH). Considering that E_2 is involved in hypophyseal regulation, the aim was to study the action of E_2 receptors (ER) on luteinizing hormone (LH) secretion in the vizcacha during gestation. We used hypophyses of early- (EP), mid- (MP), and term-pregnant (TP) vizcachas to study GnRH receptor (GnRHR), ER alpha (ERa), ER beta (ERb), and LH expression by Western-blot and immunohistochemistry, and LH pulsatility by RIA (n=6 / group). In order to evaluate the action of ER on LH secretion, LH pulsatility was studied in hypophyses of ovulating females incubated with ERa and ERb antagonists, or ERa and ERb agonists supplementation. Hypophyses without supplementation were used as control (CTL). ANOVA followed by Bonferroni was used to determine significant differences among groups ($p<0.05$). A significant decrease in LH pulsatility was observed in TP vs MP and EP, and this was concordant with variations in serum LH. Hypophyseal expression of GnRHR and ERa showed significant increments at MP vs EP and TP. In addition, ERb expression levels did not change during gestation. The colocalization analysis of ERa with GnRHR showed a decrease in MP vs EP and TP, whereas the colocalization of ERa with LH showed a concordant increment with pregnancy progression. Regarding to the involvement of ER in LH secretion, LH pulsatility showed significant decrease with ERa or ERb specific agonists vs CTL whereas the combination of both ER antagonists showed a decrement vs agonists effects. These results suggest that ER would enable LH pulsatillity in the vizcacha at mid-gestation as result of a hypophyseal sensibilization to GnRH input. Moreover, LH secretion during ovulation may require the action of both ERa or ERb isoforms. Fund. Científ. Felipe Fiorellino, PIP110/14, PICT1281/2014.

Keywords: estrogen, hypophysis, vizcacha, LH, GnRH.

(726) **mRNA RELATIVE EXPRESSION OF ESTROGEN RECEPTORS IN PERIPHERAL BLOOD MONONUCLEAR CELLS (PBMC) OF HEALTHY DONORS.**

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Estrogens regulate and play an important role in several physiological and pathological processes. The cellular effects produced by estrogens are mediated by two receptors: ERα and ERβ. The analysis of the ERs expression in PBMC may provide a useful tool to understand the responsiveness of these cells to estrogens and its association with certain pathologies, like chronic lymphocytic leukemia (CLL). In this study we investigated the relative expression of mRNA of both ERs in PBMC from healthy males and females between 25-90 years old. After informed consent, peripheral blood samples were obtained and PBMC isolates using Ficoll's gradient. RNA was obtained with Trizol reagent, and reverse transcribed. Real-Time PCR was performed using L19 as a housekeeping gene and statistical analyses were carried out using R software. We studied two groups of healthy donors for both genes: men and women younger than 40 and older than 60 years old. With regards to the ERs expression in women we found a significant difference only in the ERβ expression ($p=0,0312$), being higher levels for those donors older of 60. In males samples, there were significant differences in the two receptors and the higher levels for both were in donors <40 ($p=0,0105$ ERβ and $p=0,0330$ ERα respectively). When we compared both sexes the results obtained showed a marked difference in the expression of ERα, being higher for women >60 than men of the same

age (p=0,0037). Moreover, ERβ showed higher expression in men <40 (than women <40, p=0,0413), while women >60 showed higher levels than men of the same age (p=0,0043). These results demonstrate that the ERs expression in PMBC is a dynamical process, and the age and sex are important variables that influence the mRNA quantity. In the future, we are going to study mRNA expression of ERs splice variants and proteins in individuals with CLL taking into account that CLL is more frequent in people older than 50 years, and being men more susceptible than women.

Keywords: estrogen receptors; peripheral blood mononuclear cells

(453) **NOVEL ROLE OF PITUITARY MEMBRANE PROGESTERONE RECEPTOR α (mPRα) IN THE FUNCTION OF LACTOTROPH POPULATION**

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In the pituitary, progesterone (P4) actions remain controversial because its effect depends on the hormonal milieu and the receptor types involved. Our previous results demonstrated that, besides the nuclear progestin receptors (nPRs), all membrane progestin receptors (mPRs) subtypes (α, β, γ, δ, ε) are also expressed in the anterior pituitary of female Sprague Dawley (SD) rats, being the mPRα the most abundant. In the present work, we observed by IF and Flow citometry that 65% of lactotrophs express mPRα. Focusing on the role of mPRα in the lactotroph physiology, we first performed a single-point competitive binding assay to demonstrate that P4 and the mPRα specific agonist Org OD 02-0 (02), significantly displaced [^{3}H]-P4 binding in plasma membranes of GH3 cell line, whereas the nuclear PR agonist R5020 was ineffective. Next, we demonstrated, in *ex vivo*-incubated SD-female pituitaries, that P4 100nM and 02 100nM, but not R5020 10nM decreased PRL secretion in medium (measured by RIA) and increased PRL content in the pituitary. We also observed the inhibitory effect of P4 and 02 on PRL release in GH3 cells, and again, R5020 was ineffective. We next assayed putative second messengers involved. We found that both P4 and 02 induced a significant decrease in cAMP accumulation. The inhibition of 02 on PRL release was blocked by pre-treatment with pertussis toxin (PTX 2,5ug/ml). In addition, 15min-treatment of P4 and 02 induced a significant increase in ERK phosphorylation, while R5020 did not. Finally, since TGFβ1 is one of the main inhibitors of lactotroph function, we evaluated whether this system could be regulated by mPRα, mediating in part, the effects of P4 on PRL secretion. In fact, P4 and 02, but not R5020, increased active TGFβ1 levels, measured by ELISA, in *ex vivo*-incubated SD-female pituitaries, and also in *in vitro* GH3 cell-culture. All these results provide the first evidence of specific mPRα involvement in the control of lactotroph function.

Keywords: pituitary, progesterone, membrane receptors, lactotroph, prolactin

(1493) **A "YIN YANG" RELATIONSHIP BETWEEN ESTROGEN RECEPTOR A AND B ON PTEN/PI3K/AKT IN THE PITUITARY TUMORAL PROLIFERATION**

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Abstract: Previously it was shown that estrogen receptor (ER) β increased the tumoral suppressor PTEN expression to modify the lactotroph and somatotrophs population growth and adequately respond to cyclic PRL y GH requirement during the estrous cycle. Expanding these investigations, the aim of this work was to analyze the ERα and β effects on PTEN/PI3K/Akt in the pituitary tumoral growth regulation mechanisms.

To carry out this objective, female rats treated with estradiol benzoate during 20, 40 and 60 days (hyperplasic/adenomatous pituitary model) and transfected GH3 tumoral cells (GH3 ERα+/ERβ- and GH3 ERα+/ERβ+) stimulated with E2 or ERα (PPT) and β (DPN) agonists were used. PTEN, Akt, p85α of PI3K, D1 cyclin and CDK4 expression was determined by western blot, the subcellular localization was visualized using confocal microscopy, the cell cycle progression was analyzed by flow cytometry and the cellular proliferation was quantified by BrdU technique. Statistical analysis: ANOVA-Tukey.

During the hyperplastic/adenomatous process development the PTEN protein expression showed cytoplasmatic distribution with significant reduction in its levels: at 20 days of tumoral development a reduction of approximately 50% was observed, being almost undetectable at 60 days. Akt, p85α and cell cycle regulator proteins D1 cyclin and CD4 significantly increased in relation with tumoral development, in agreement with the rise of proliferative phase cell number (S+G2/M). In GH3 ERα+/ERβ-, PTEN was found mainly in the peripheral cytoplasm with Akt phosphorylation and cellular proliferation increase. In contrast, in GH3 ERα+/ERβ+ cells a nuclear PTEN signal was found, reinforced with DPN stimulation, with decreased of mitogenic activity.

These observations suggest that ERα and β induce opposite effects on PTEN/PI3K/Akt signaling. Both ER stimulate PTEN import or export to and from the nucleus, activating or inhibiting PTEN/PI3K/Akt signaling regulating tumoral pituitary cell growth.

Keywords: PTEN, estrogen receptors, pituitary tumor, proliferation

(1077) **TESTOSTERONE EFFECTS ON THE THERMOGENIC PROGRAM OF THE INGUINAL ADIPOSE TISSUE FROM THE ADULT RAT**

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Little is known regarding the effects of androgens on the thermogenic process of beige adipocytes. We previously demonstrated an inhibitory action of Testosterone (T), *in vivo* and *in vitro*, in the thermogenic process of retroperitoneal adipose tissue (AT) cells. In the present study we evaluated the effect of T on the thermogenic activity of the subcutaneous AT (Inguinal AT: IAT). For this aim, three groups of male S-D rats were used: control (CTR), pre-pubertally (27 day-old) orchidectomized (ODX) and control pair-fed (CTR-PF). At 60 days of age, the IAT pad was dissected and adipocyte precursor cells (APCs) were isolated. APCs were cultured and differentiated (3 days) with a classic pro-beige cocktail. On differentiation day (Dd) 8, cells were processed to quantify UCP-1 gene expression (qPCR). Additionally, APCs from IAT from adult CTR rats were isolated and differentiated with the classic pro-beige cocktail in the absence or presence of 0.1 μM T (basal: B or T, respectively), thereafter cells remained in culture without or with T. On Dd 8, 10 μM FSK was added for 4 h to subsets of B and T cells: B without FSK (B-B), B with FSK (B-FSK), T without FSK (T-B) and T with FSK (T-FSK). Cells were then processed to quantify UCP-1 mRNA levels. APCs from ODX animals differentiated *in vitro* showed a higher UCP-1 mRNA expression ($p<0.01$, ODX vs CTR and CTR-PF). On the other hand, *in vitro* T treatment inhibited UCP-1 gene expression in differentiated adipocytes ($p<0.001$, T-B vs B-B). As expected, FSK increased UCP-1 gene expression in IAT adipocytes ($p<0.001$, B-B vs B-FSK and T-B vs T-FSK); however, the inhibitory effect of T on UCP-1 expression remained after FSK stimulation ($p<0.01$, T-FSK vs B-FSK). We conclude that pre-pubertal androgens depletion leads to a greater thermogenic activity of differentiated IAT beige adipocytes, an effect largely confirmed *in vitro* after T added throughout cell differentiation. PICT2015-2352. (1951). PIP 0198.

Keywords: adipose tissue, androgens, UCP-1, adipocyte precursor cells

(1090) **EFFECT OF ESTRADIOL ON THE ADIPOGENIC PROCESS IN RETROPERITONEAL AND INGUINAL ADIPOSE TISSUE OF FEMALE RATS**

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Abstract: The mechanism by which estradiol (E2) regulates adipose tissue (AT) development are largely unknown. Previously, we have shown an *in vitro* E2-dependent decreased adipogenic potential in retroperitoneal adipose tissue (RPAT) adipocyte precursor cells. In this study, our aim was to assess the role of E2 deficiency on RPAT and inguinal AT (IAT) pads in female rats. S-D rats were divided into three groups: non-ovariectomized (Sham operated, CTR), ovariectomized (OVX) and ovariectomized pair-fed (OVX PF), latter ones castrated prepubertally. CTR and OVX food consumption was *ad libitum*, while for OVX PF was the same to CTR, due to a higher food consumption of OVX rats. Food intake and body weights were recorded daily from surgery (27-day-old) to experimental day (60-day-old). At this time, trunk blood was collected to measure plasma metabolites. RPAT and IAT pads were dissected, weighed and histological analysis were performed. Moreover, stromal vascular fraction (SVF) from both tissues was isolated to test mRNA levels of PPARγ (adipogenic marker) by qPCR. OVX PF animals show a decrease in RPAT mass and %RPAT (OVX PF P<0.001 vs. CTR and OVX), while the opposite was observed in IAT, having more %IAT (OVX PF, P<0.001 vs. CTR and OVX). Plasmatic leptin levels were lower in OVX PF group (P<0.01 vs. CTR) in line with smaller adipocyte size in RPAT (P<0,001 vs. CTR) were seen, while for IAT no differences were found. IAT undifferentiated SVF cells from OVX rats showed increased PPARγ expression (OVX, P<0.01; OVX PF, P<0.001 vs. CTR), probably indicating an increased IAT adipogenic capacity. Similar expression levels were observed in RPAT mRNA in all groups. Our preliminary results may indicate that E2 has differential effects depending on the AT depot. Subcutaneous AT (IAT) seem to be benefited by the absence of E2 increasing its adipogenic capacity (PIP0198; PICT2015-2352).

Keywords: Adipose tissue, adipogenesis, estradiol.

HEMATOLOGY 2

(396) **ACTION OF MODIFIED ERYTHROPOIETINS ON ENDOTHELIAL CELLS IN A PROINFLAMMATORY ENVIRONMENT**

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Erythropoietin (Epo), the erythropoiesis growth factor, is also known as an antiapoptotic agent. In this context, carbamylated Epo (cEpo) maintains the neuroprotective effect but fails to stimulate erythropoiesis. Despite the benefit of Epo treatment to overcome anemia associated to different pathologies, a significant number of patients, particularly those with cardiovascular or chronic renal diseases, fail to respond. This may be related to the simultaneous presence of other independent risk factors, such as hyperhomocysteinemia. This study was aimed at identifying Epo structural changes due to carbamylation and *N*-homocysteinylation, and investigating whether these changes could affect Epo activity on EA.hy926 endothelial cells in a proinflammatory environment. cEpo was prepared by reaction with potassium cyanate while homocysteine thiolactone was incubated with Epo to yield the *N*-homocysteinylated protein. Analysis by gel and capillary electrophoresis revealed structural changes with respect to the native protein. Wound healing assays showed a stimulatory effect of TNF-α on cell migration which was significantly increased by TNF-α+Epo combination (C 21±2%, *TNF-α 44±3%, Epo 30±2%, *EpoTNF-α 66±4%, *P<0.05, n=6),mediated by a mechanism involving EpoR induction. A similar effect of TNF-α+Epo was found on VCAM and ICAM mRNA expression (Real Time PCR, respect to C=1; VCAM: *TNF-α 22±3%, Epo 2±1%, *EpoTNF-α 36±2%, *P<0.01; ICAM: *TNF-α 76±9%, Epo 3±1%, *EpoTNF-α 123±5%, *P<0.05, n=3) as well as in monocytic cell adhesion assays (Fluorescence microscopy and fluorometric quantification with monocytic THP1 cells, n=6).The fact that no such activities were shown by the modified Epos suggests altered Epo function due to structural changes. In summary, the proangiogenic ability of Epo, enhanced in the presence of proinflammatory factors, might favor its action as a vascular protectant in ischemia and a mediator of lymphocyte migration dependent of adhesion molecules.

Keywords: Erythropoietin, TNF-alpha, V-CAM, cell adhesion, cell migration

(801) **AUTOANTIBODIES IN IMMUNE THROMBOCYTOPENIA AFFECT THE PHYSIOLOGICAL INTERACTION BETWEEN MEGAKARYOCYTES AND BONE MARROW EXTRACELLULAR MATRIX PROTEINS**

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Abstract: Although autoantibodies from immune thrombocytopenia (ITP) interfere with megakaryocyte and platelet production, their effect on several aspects of megakaryocyte (MK) physiology has not been completely elucidated. After approval by the institutional Ethics Committees and written informed consent, blood samples from 14 ITP patients and controls were obtained and recalcified plasma, and IgG fractions, were prepared. Plasmatic autoantibodies were detected using PAKAUTO kit. Normal mature MKs were obtained after 13-day culture of human cord blood-derived CD34+ cells and incubated with recalcified plasma, or purified IgG, on type I collagen, fibrinogen and von Willebrand factor-coated surfaces to study different MK functions: MK adhesion by counting non-adhered cells, MK spreading by immunofluorecent staining with FITC-CD61, downstream signal activation of specific molecules by western blot and proplatelet formation by direct count on an inverted microscope. Data were analysed by Mann-Whitney test or, when the number of patients' samples were too small, individual values were compared to the normal range. All parameters evaluated were significantly affected in the presence of ITP plasmas bearing specific autoantibodies against the megakaryocytic glycoproteic (GP) receptors for type I collagen, fibrinogen and von Willebrand factor: GPIaIIa, GPIIbIIIa and GPIbIX, respectively. Purified IgG fractions reproduced the effect observed in the presence of plasma samples. Our results demonstrate that autoantibodies block the physiological interaction between MKs and these extracellular matrix proteins, and suggest they could alter normal MK functions within the bone marrow microenvironment, contributing to thrombocytopenia in ITP.

Keywords: Autoantibodies; Extracellular matrix proteins; Glycoproteins; Immune thrombocytopenia; Megakaryocytes.

(1538) **INFLUENCE OF THE TYPE OF TREATMENT ON PLATELET SIZE IN PATIENTS WITH MYELOPROLIFERATIVE NEOPLASMS**

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Myeloproliferative neoplasms (MPN) comprising essential thrombocythemia (ET), Polycythemia vera (PV), and primary myelofibrosis (MF) are frequently associated with thrombotic complications. Current treatments for these entities include hydroxyurea (HU), μ-interferon (IFNa), anagrelide (Ana) and, especially in MF, the JAK inhibitor, ruxolitinib (Ruxo). Considering that large platelets are more reactive and linked to thrombotic events, in this study we investigated possible differences in platelet size in 69 MPN patients grouped according to their diagnosis, mutational status and treatment.

Taking into account that cell counters underestimate platelet volume, máximum platelet diameter (MPD) was measured by optical microscopy on May-Grünwald-Giemsa–stained blood films and by software image analysis in at least 100 platelets in each sample. MPN patients had increased platelet size (t test, p=0.0347) but no differences were seen when patients were grouped according to their diagnosis or their mutational status. However, MPD was increased in patients without treatment, 2.67μm(2.19-3.44), n=24, as well as those treated with IFNa, 3.06μm(2.69-3.28), n=8, Ana 2.99μm(2.32-3.35), n=10, or Ruxo, 3.14μm(2.80-3.52), n=7, compared to patients treated with HU, 2.44(2.19-2.83), (n=22); MPD from IFNa, Ana and Ruxo groups were also higher than normal controls, 2.55μm(2.24-2.98), n=26 (ANOVA followed by Tukey´s test, p<0.0001).

In orden to deepen in possible causes underlying these differences, we studied platelet microtubular structure (FITC-anti-tubulin staining and epifluorescent observation). Preliminary data obtained in 11 MPN samples showed a direct correlation between % of platelets with abnormal microtubular rings and MPD (Pearson, r: 0.684, p=0.029).

Our results suggest that the type of treatment influence platelet size. The decrease in MPD observed under HU treatment could represent another factor contributing to the beneficial effect of this drug on thrombosis prevention.

Keywords: Myeloproliferative neoplasms; Platelet size; Thrombosis; Treatment; Microtubular structure.

(1480) **PATHOGENIC MECHANISMS LEADING TO THROMBOCYTOPENIA IN SYSTEMIC LUPUS ERYTHEMATOSUS**

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Systemic lupus erythematosus (SLE) is characterized by the production of a wide variety of autoantibodies. In 15-20% of cases, patients develop thrombocytopenia which appears to be autoimmune. This study was aimed at investigating possible causes of low platelet count, including platelet apoptosis and abnormal platelet production. Blood samples from 15 SLE patients (P) (platelet count, median, 92x10^6/L, range 2-290x10^6/L) and 10 normal controls were obtained, and platelets as well as plasma samples were separated. Platelets were analyzed for apoptosis parameters and SLE plasma samples were incubated with normal mature megakaryocytes (obtained after 12 day-culture of CD34+ progenitors from normal human cord blood) to assess their effect on proplatelet formation (PPF) (direct count under microscopic observation). Platelet apoptosis was increased in SLE (n=8): phosphatydilserine (PS) exposure (FITC-Anexin-V binding), P 9.3%(0.7-80.2), controls (C) 3.6%(1.9-7.6); loss of mitochondrial inner membrane potential (DΨm) (JC-1), P 26.6%(9.6-71.3), C 12.4%(3.6-18.6); active caspase-3 (FITC-anti active caspase-3 antibody binding), P 3.1%(0.5-34), C 2.7%(1.1-4.6), although not reaching statistical significance (Mann-Whitey test). However, 4/8 patients showed values higher than the normal range, all of them presenting thrombocytopenia. Response to calcium ionophore-induced apoptosis was normal. Basal platelet activation was not observed in our population as evaluated by PAC-1 binding, P 3.4%(0.5-15.8), C 2.8%(1.1-7.4), and P-selectin expression, P 19.9%(11.4-58.2), C 18.7%(5-36.6), indicating that PS exposure is not due to activation but to apoptosis in SLE platelets. PPF was decreased in the presence of SLE plasma samples (n=15), 0.42%(0.14-1.79) compared to C,1.06%(0.51-2.16), p=0.0367 (Mann-Whitney test). In conclusion, peripheral platelet clearance due to platelet apoptosis and inhibition of platelet production could contribute to thrombocytopenia in SLE.

Keywords: Systemic lupus erythematosus; Thrombocytopenia; Platelet apoptosis; Platelet activation; Proplatelet formation.

(1542) **ANTIPLATELET ACTIVITY OF *St*STBc-3, A SUBTILISIN-LIKE SERINE PROTEASE OF *Solanum tuberosum***

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Cardiovascular disease is one of the leading death causes worldwide. Antiplatelet and anticoagulant therapies improved in the last decades but the search of new therapeutic possibilities it´s still a necessity.

The aim of the present work is to characterize the antiplatelet activity of a subtilisin-like protease of potato (*St*STBc-3).

Washed platelets in HEPES Tyrode buffer with human fibrinogen and $CaCl_2$ were incubated with thrombin and the percentage of clot size relative to initial suspension volume was determined. *St*STBc-3 inhibits clot retraction. We observed complete lack of clot retraction at all times assayed. Fibrinogen was added exogenous and is not limiting.

To further investigate the possible action mechanism, platelet aggregation was measured using three different agonists (A23187, convulsin and thrombin) at different concentrations. All the experiments were repeated three times. *St*STBc-3 inhibits platelet aggregation induced by all agonists tested. These results suggest *St*STBc-3 blocks different pathways of the aggregation process.

*St*STBc-3 has potent antiplatelet activity and it´s an interesting protease to be evaluated for antiplatelet therapy. We´ll perform other analysis to further characterize the therapeutic potential of this enzyme.

Keywords: antiplatelet activity, plant serine proteases, clot retraction, platelet aggregation

(1396) **SPLENIC IRON PROTEINS RESPONSE IN MICE MODELS BY MEANS OF IRON EXCESS AND ERYTHROPOIESIS INDUCED BY ERYTHROPOIETIN SIGNALS**

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Introduction: Iron and erythropoiesis are closely related since the iron required for erythropoiesis is derived from the recycling of heme-iron by splenic macrophages. Hence, the spleen is susceptible to iron-related disorders for example the iron excess. **Objective:** To evaluate the effect of Erythropoietin in spleen when two states coexist, it was used an animal model of iron-excess and also an erythropoiesis induced by EPO. **Materials and Methods**: CF1 mice (25±5g; 3 months-old) were divided into groups (n=4/group): 1)*Iron-adequate* (control); 2)*Iron-overload* (iron saccharate; *days 0,4,8,12* ip;1800mg/kg); 3)*EPO (days17,18,19) ip; 20000UI/kg)*; 4)*Iron-overload+EPO*. Immunohistochemistry: anti-prohepcidin, L-ferritin and DMT1 (divalent metal transporter1). Perl´s staining. Erythropoietic response was assessed by hematological

tests. The Protocol was approved by Committee on Experimental Animal Use and Care, UNS. **Results:** Prohepcidin expression was intense in Iron-overload respect to control, while it was weak in EPO and Iron-overload+EPO. DMT1 and L-ferritin expressions were increased in Iron-overload and decreased in EPO and Iron-overload+EPO compared to control. Hb (g/dl) and HCT (%) in Iron-overload+EPO (16,8±0,4;54±1) and EPO (17,1±0,7;56±2) showed a significant rise respect to Iron-overload (14,8±0,23;49±1) and control (15,5±0,5;45±1). Hemosiderin was intense in Iron-overload, while it was weak in EPO and Iron-overload+EPO. Plasma iron levels showed a significant increase in Iron-overload respect control, EPO and Iron-Overload+EPO states (p<0, 05). **Conclusions:** The decrease on splenic L-ferritin and DMT1 in iron-overload with EPO demonstrates that erythropoiesis activity induced by EPO depends on splenic iron. To conclude, we consider that EPO could have a protective effect on iron excess.

Key words: ERYTHROPOIESIS, IRON-OVERLOAD, MICE MODELS, SPLEEN.

(395) **THE PRESENCE OF HOMOCYSTEINE AND ADENOSINE EXACERBATED THE TNF- α CYTOTOXICITY IN HUMAN ERYTHROLEUKEMIA CELLS**

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Some factors are known to influence cell survival regulation, including TNF-α and cellular hypomethylation, which can be induced by high levels of homocysteine and adenosine. S-adenosylhomocysteine (SHA) production is accelerated by the reversible enzyme SAH hydrolase during the methionine metabolism. Both, hyperhomocysteinemia (hHcy) and elevated SAH has been described in end stage renal disease (ESRD) and associated with several cellular abnormalities in these patients. Other negative prognosis factors in ESRD are inflammatory conditions leading to anemia. The aim of this study was to investigate if hHcy and the accumulation of adenosine could affect the survival of human erythroleukemia K562 cells in an inflammatory environment and whether the growth factor erythropoietin (Epo) could reverse this effect.

Cells were incubated (2 h) with Hcy (H 500 μM) and adenosine (A 250 μM) in free serum medium and then exposed (24 h) to TNF-α (T 60 ng/mL). Cell viability measured by the MTT assay and expressed as OD was: C 1.10±0.17; *HAT 0.43±0.18; *P<0.01, n=13. Apoptosis was evaluated by annexin V-positive cells (Flow cytometry): C 16.8±2.7%; *HAT 43.0±6.4%; *P<0.01, n=7 and cell shrinkage: C 100%; *HAT 68.6±4.2%; *P<0.01, n=6. We then investigated possible disruption of the mitochondrial membrane potential (MitoTracker dye, MMP: C 100%; *HAT 52.0±5.2%, *P<0.01, n=6). Previously, we demonstrated that Epo acted as an antiapoptotic factor in these cells, yet incubation with Epo could not reverse the increased sensitivity of cells to TNF-α citotoxicity. Treatments with TNF-α, H, or A did not show significant cytotoxic effects.

In conclusion, the combination of H and A modulates the sensitivity of erythroleukemia cells towards the cytotoxic action of TNF-α by a mechanism mediated by disruption of the mitochondrial membrane potential. The results suggest a possible relationship between hHcy and anemia which could be especially important in patients with cardio-renal syndrome.

Keywords: hyperhomocysteinemia; anemia; TNF-α; cytotoxicity, human K562 cells.

(1916) **THROMBIN GENERATION IN PATIENTS TREATED WITH APIXABAN**

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Apixaban is given at fixed doses without laboratory adjustment but in particular situations it is necessary be measured. Coagulation assays, prothrombin time(PT) and activated partial thromboplastin time(APTT), are not reliably for determining apixaban anticoagulation. Chromogenic anti-Factor Xa activity(aFXA) using specific calibrator is the preferred clinical assay. Thrombin generation(TG) a new global assay, may be a useful tool to know the anticoagulant activity. Aims: determine the influence of apixaban on TG parameters and to assess the effects of this drug on routine coagulation tests. Ten patients taking 2.5 or 5 mg of apixaban twice daily had two blood samples taken: at peak and at trough. Plasmas were tested for PT, APTT, dRVVT(diluted Russell viper venom test) and aFXa using Apixaban commercial calibrator. TG was assessed with CAT method (Thrombinoscope,Bv). As activator we used tissue factor(5pM) and phospholipids (4μM) and normal plasma samples as control. There was a significant difference in apixaban concentrations, determined by aFXA activity, between peak and trough (p≤ 0,0001). PT and APTT were barely affected by apixaban concentration and still within the normal reference range. dRVVT screen being higher than the upper limit of the local range also at the trough (57.1±9.7) and at peak (70.47±13.31).The lag time was the most affected TG parameter (trough vs normal control p≤0,004; peak vs normal control p≤0,0001). By contrast, endogenous thrombin potencial(ETP) did not differ to normal control neither at trough nor at peak(trough vs normal control p≤0,86; peak vs normal control p≤0,26). Conclusions: routine tests were insensitive to apixaban but dRVVT could be used to rule out significant levels of drug. Lag time appeared to be the most sensitive parameter to assess apixaban since it was clearly prolonged at all drug concentration indicating a delay on thrombin generation. Although the ETP that represents the net amounts of thrombin generated did not differ to normal controls.

(1845) **RELATIONSHIP BETWEEN LIPID PROFILE, ERYTHROCYTE MEMBRANE CHOLESTEROL CONTENT AND ERYTHROCYTE DEFORMABILITY IN ADOLESCENTS WITH OVERWEIGHT OR OBESITY**

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Obesity and overweight have been associated with abnormal plasma lipid profile (LP). This could modify the erythrocyte membrane cholesterol content (EMc) since it results from exchange between plasma lipoproteins and cell. We analyzed the relationship between nutritional category, LP and EMc, and their possible influence on erythrocyte deformability (ED). We evaluated 74 volunteers from Rosario city, with ages between 11-19 years old. They were divided into 3 groups: 1-normalweights = 30, 2-overweights = 17 and 3-obeses = 27; according to their BMI. We excluded adolescents who suffered from a disease or were under pharmacological treatment. Plasma assays: cholesterol (Cho), triglycerides, high-density lipoprotein Cho (HDL) and low-density lipoprotein Cho by enzymatic methods. Blood assays: ED estimated by rigidity index (RI) by filtration method, EMc measured by lipid extraction with solvents and colorimetric analysis, and osmotic fragility (OF) using photometry at 540 nm. Statistics: ANOVA. Results: (mean ± SD). We observed: higher EMc in group 3 (0.96 g/L ± 0.51) than in groups 1 (0.64 g/L ± 0.34) and 2 (0.66 g/L ± 0.24) (p<0.01); lower HDL value in group 3 (45 mg% ± 10) than in group 1 (54 mg% ± 14) (p<0.05), without significant variations in the other parameters of LP. We noted higher OF in groups 2 and 3 than in group 1 (p<0.05), and no significant differences in RI. However, we found an upward trend of RI in groups 2 and 3. These results suggest that although overweight and obese adolescents present normal LP, they have alterations in the EMc, that could be explained by the changes in HDL. The increased EMc could change fluidity and elastic properties of the erythrocyte membrane and might be responsible for the increase in OF, and thus contribute to the tendency to decrease ED. We propose to continue studying hemorheological parameters as possible contributors of morbidity and emphasize the importance of take care of healthy nutritional status in adolescents.

Keywords: overweight, obesity, adolescence, cholesterol membrane content, erythrocyte deformability.

PHARMACOLOGY 5 (PHYTOPHARMACOLOGY)

(1912) ACTION OF NATURAL PRODUCTS IN AN EXPERIMENTAL MODEL OF ALLERGIC INFLAMMATION OF UPPER AND LOWER AIRWAYS

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Introduction: Allergic inflammation is a pathophysiological feature of numerous medically important processes including allergic asthma. These types of conditions deteriorate the quality of life of those affected and so far there are no effective treatments. In previous work we have shown that dehydroleucodine (DhL), active ingredient of *Artemisia douglasiana* Besser (regional medicinal plant popularly known as "matico") exhibits antiulcerous, anti-inflammatory and antioxidant activity.

Objective: To determine the action of the lyophilized extract (LE) of matico in the prevention / treatment of allergic and inflammatory pathologies.

Materials and methods:

Adult male rats, Wistar strain. Group (G) ref: without any treatment. Control G (-): received intraperitoneal injection and nebulization with PBS. Control G (+): sensitized with an intraperitoneal injection of birch pollen and nebulized with the same substance. LEG (A): nebulization with a low concentration of LE. GLE(B): nebulization with a higher concentration of LE. BuG: nebulized with Budesonide (anti-inflammatory of the airways). Nebulizations were performed 25 days after sensitization. After the treatment, peripheral blood was removed and the leukocyte formula was analyzed. Statistics analysis: ANOVA-1 followed by Tukey-Kramer test.

Results:

Segmented neutrophils: increased in LEG (A) compared to CG (-) $P < 0.05$; also increased in LEG (B) with respect to LEG (A) $P < 0.001$. Basophils: decrease in GLE (B) with respect to GC (+) $P < 0.05$. CG (+) shows a decrease of the same with respect to CG (-) $P < 0.01$. Monocytes: increase in ELG (B) compared to CG (-) $P < 0.05$.

Conclusions:

The usefulness of the model used has been demonstrated since there are obvious signs of inflammation in animals sensitized with pollen. The leukocyte formula is modified in the animals exposed to the lyophilized matico extract when compared to control groups.

Keywords: allergic inflammation, upper and lower airways, pollen, *Artemisia douglasiana* Besser

(1708) ALOE VERA: IN VITRO IMMUNE-MODULATORY ACTIVITY OF DECOLORIZED LEAF JUICE AND INNER LEAF JUICE AND CHEMICAL CHARACTERIZATION

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Aloe vera leaf juice, when intended for human ingestion, is purified of its latex constituents *via* a process known as decolorization. Limited information is available regarding aloe's putative health effects after decolorization. Previous work showed higher antioxidant and immune-modulatory activity from *A. vera* decolorized leaf juice (DL) than inner leaf juice (InL). Our present aim was to assess the preparations' activity against a panel of human cytokines and to chemically characterize both DL and InL. **Methods:** Human peripheral blood mononuclear cells were treated with 2 g/l of DL or InL. The cultures were then treated with lipopolysaccharide (LPS) or polyinosinic:polycytidylic (poly I:C), to mimic bacterial or viral insult, and the culture supernatants were tested against a panel of human cytokines. Data was analyzed by ANOVA repeated measures. The major chemical constituents in DL and InL were measured using a variety of analytical methods. **Results:** Cytokine analysis showed that DL alone significantly increased secretion of IL-1β, IL-6, IL-10, IFN-γ, TNF-α and MCP-1/CCL2 while no significant effect was observed for InL. In the presence of LPS, DL significantly increased secretion of TNF-α while InL had no effect, both preparations decreased secretion of MCP-1, and InL increased secretion of RANTES while DL had no effect. In the presence of poly I:C, DL increased secretion of IL-6 and TNF-α and decreased secretion of RANTES, while InL had no effect. We found several chemical differences between DL and InL, notably major mineral (8.32% InL to 17.70% DL), organic acid (17.05% InL to 45.26% DL) and major oligosaccharide (9.20% InL to 21.00% DL) content. **Conclusions:** The decolorized aloe vera leaf preparation showed higher immune-modulatory activity than the inner-leaf preparation as measured by the cytokines tested, and differs chemically from the inner leaf preparation in potentially significant ways.

This work was funded by Herbalife International of America.

Keywords: Aloe vera; chemical characterization; immune modulation; inflammation

(549) ANTIOXIDANT PROPERTIES OF *Vaccinium myrtillus L.* STEM AGAINST ETHANOL-INDUCED GASTRIC MUCOSAL INJURY IN RATS

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The role of oxygen derived free radicals in the development of acute experimental gastric lesions induced by ethanol is well-known. *Vaccinium myrtillus* L. (*Vm*) is an emerging crop in the province of Tucuman, its fruits being the main objective of the harvest. Until now, there is no data from the use of their stems for therapeutic purposes. In a preliminar study, we demonstrated an enhanced secretion of gastric mucus and effective gastroprotective effect in ethanol-induced gastric ulcers model in rats pretreated with aqueous extract of *Vm* stem (150 mg/Kg body-weigth, orally).

The present study was designed to evaluate the antioxidant effect of the AE of *Vm* stems and to examine their participation in the gastroprotective activity. Phytochemical screening of the AE (infusion 5%) of *Vm* stems was carried out. The *in vivo* experimental groups were the following: 1- control group, 2- positive control group (sucralfate 100mg/kg) and 3- AE of *Vm* treated group (150mg/kg) (n= 6 adult Wistar rats/group). The levels of thiobarbituric acid (TBA, as index of lipid peroxidation) and the activities of reduced glutathione (GSH) and catalase (CAT) were measured in homogenates of gastric mucosa. The pretreatment of the animals with the AE of *Vm* increased activity of the CAT enzyme and decreased GSH and TBA levels in gastric mucosa homogenates. In addition, the morphological observations at macro and microscopic level also supported a protective effect of the AE. The present results indicate that the gastroprotective effects of AE of *Vm* stem may be related to their content of phenolic compounds, mediated by its antioxidant properties. We suggest that the AE of the stem of *Vm* may represent an attractive therapeutic option for protecting against the pathogenesis of gastric ulcers.

Keywords: *Vaccinium myrtillus* L, antioxidant, gastroprotective effects

(245) ANTI-PHOSPHOLIPASE A2 ACTIVITY OF *Eclipta prostrata* EXTRACTS ON YARARÁ AND CASCABEL VENOM

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Phospholipase A2 enzyme (PLA2) present in ophidian venoms is able to hydrolyze membrane phospholipids and fatty acid esters,

while exhibiting also other activities as indirect hemolysis, edematogenic, myotoxicity, cardiotoxicity, neurotoxicity, anticoagulant and platelet aggregation Paraguay and Brazilian Amazonia are regions where are found ethnomedicine references to the use of *Eclipta prostrata* (L.) L., as antivenom. Both extracts and isolated wedelolactone, have been reported to inhibit phospholipase A2 activity of *Lachesis muta* and *Calloselasma rhodostoma* venoms from Brazil and Thailand respectively.

In the present work, the action of different extracts against PLA2 from the main snakes responsable of accidents in the Argentinean northeast, *Bothrops diporus*, *B. alternatus* and *Crotalus durissus terrificus*, was studied to validate this action with autochthonous species.

Aerial parts (AP) and roots (R) of native plants collected in Corrientes were dried and ground to prepare by maceration aqueous (1), ethanolic (2) and hexanic (3) extracts. The extracts were dried under vacuum.

Inhibition of PLA2 from the different venoms was demonstrated by the *in vitro* inhibition of indirect hemolytic activity on blood agar plates phosphatidylcholine, incubating the minimal hemolytic doses (doses producing a hemolysis halo of 10 mm after the plates were incubated by 20 hours at 37 °C), the plant extracts and the preincubated venom with the plant extracts 30 minutes at 37ºC in 1:40 ratio (venom: extract).

The results showed that both extracts, A2 and R2, were active: A2 inhibited 33% of *B. diporus* PLA2 activity, 28% of *B. alternatus* and 31% of *Crotalus*, while R2 inhibited 31%, 25% and 100% of the activity respectively. The rest of extracts did no show significant activity.

The results here found showed the *E. prostrata* ethanolic root extract as an efficient inhibitor of PLA2 activity present in *Crotalus durissus terrificus* venom. Thus validating its ethnopharmacological use.

Key words: alexiteric activity; ethnobotany; anti-PLA2 activity; *in vitro* test

(429) **COMPARATIVE STUDY OF THE ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITIES OF TWO SPECIES OF *Urera aurantiaca* FROM DIFFERENT GEOGRAPHICAL AREAS: THEIR RELATION TO POLYPHENOLS CONTENT**

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Urera auratiaca is an Argentinean species that has been traditionally used to treat symptoms of inflammation. The aim of this study was to determine comparatively the antioxidant and anti-inflammatory effects of two species of *Urera aurantiaca* coming from different geographical areas of Argentina: Salta (S) and Misiones (M). Methanol extracts were prepared to obtain polyphenolic compounds. Both extracts showed antioxidant activities and *in vitro* anti-inflammatory activities, results represent mean ± ESM of three experiments made in triplicate: DPPH radical scavenging activity S EC_{50}: 245 **± 40** µg/ml, M EC_{50}: 977 **± 20** µg/ml; Reducing power S EC_{50}: 126 **± 3** µg/ml , M EC_{50}: > 1000 **± 50** µg/ml; Inhibition of lipid peroxidation S EC_{50}: 1862 **± 10** µg/ml, M EC_{50}: 2000 **± 20** µg/ml; SOD like activity S EC_{50}: 537 **±** 37 µg/ml, M EC_{50}: 363 **±** 24 µg/ml; Inhibition of albumin denaturation S EC_{50}: 812 **±** 59 µg/ml, M EC_{50}: > 1000 **±** 25 µg/ml; Proteinase inhibitory action S EC_{50}: 10 **± 2** µg/ml, M EC_{50}: 42 **± 4** µg/ml. They also were able to reduce the augmented NO release in activated macrophages but S extract showed the largest inhibition: 76.6% at 500 µg/ml and was able to restore LPS to the basal levels. S extract showed the best antioxidant and anti-inflammatory properties in almost all the tested experiments probably due to the highest polyphenols content (S: 30.9 ± 1.8; M: 14.1± 2.6 GAE/g) and specifically due to the highest flavonoids content (S: 2.10 **± 0.02**%; M: 1.49 ± 0.02%). These differences between the extracts are probably related to ambient diverse conditions: temperature, rain, soil composition and ecosystem.

(1156) **EFFECT OF THE SESQUITERPENE LACTONE DEOXYMIKANOLIDE *ON TRYPANOSOMA CRUZI* INFECTED MICE AND EVALUATION OF ITS IMMUNOMODULATORY ACTIVITY ON MACROPHAGE CELLS**

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Chagas' disease is a parasitic disease caused by the protozoan parasite *Trypanosoma cruzi*. It is considered, among others, a Neglected Tropical Disease. The drugs currently in use for the treatment of this parasitosis are not at all effective and have severe drawbacks. Nature has proved to be a rich source of bioactive compounds. Sequiterpenes lactones, present mainly in Asteraceae species, are interesting compounds due their pharmacological properties.

Deoxymikanolide, isolated from *Mikania* spp.*,* was active and selective against *Trypanosoma cruzi* epimastigotes, trypomastigotes and amastigotes (IC_{50}% of 0.08, 1.5 and 6.3 *µ*g/ml, respectively). Since deoxymikanolide presented significant *in vitro* activity and good selectivity on the different *T. cruzi* stages, the aim of this study was to evaluate its effect in an *in vivo* model of *T. cruzi* infection and its immunomodulatory activity on macrophage cells.

Balb/c mice were infected with a lethal dose of *T. cruzi* (RA strain) and treated with deoxymikanolide or benznidazole (1 mg/kg of body weight/day) for five consecutive days by the intraperitoneal route. Cytokine production was evaluated using RAW 264.7 macrophage cell line.

Infected mice that received deoxymikanolide presented a lower blood parasitemia, as compared to control. In deoxymikanolide-treated mice, a significant decrease in the mortality caused by *T. cruzi* infection was observed. While nearly 70% of deoxymikanolide treated mice survived the acute phase of infection, 100% mortality was observed in control mice by day 22 post-infection.

In vitro macrophage stimulation with 25 *µ*g/mL deoxymikanolide produced a significant increase in the secretion of TNF-α and IL-12.

This finding makes it an interesting lead molecule which may be useful for the development of new drugs for the treatment of Chagas disease.

Keywords: *Mikania* spp.; *Trypanosoma cruzi*; sesquiterpene lactone

(54) **EVALUATION OF THE ANTI-INFLAMMATORY ACTIVITY OF *Clinopodium gliesii* (muña muña) IN ANIMAL INFLAMMATION MODELS**

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The chemical machinery of a vegetable, offers a great diversity of biological properties occupying a fundamental role in the field of pharmacology. The search for new anti-inflammatories with fewer adverse effects posses a challenge for researchers.

The objectives of the present study were to evaluate the antiinflammatory activity and to initiate the chemical toxicological study of extracts and essential oil of aerial parts of *Clinopodiun gilliesii* (muña muña).

The extractions were done in serial form, using 96º alcohol (EE) and boiled distilled water (EA). The essential oil (Ac) was obtained by pulling steam. For the pharmacological studies, the carrageenan-induced plantar edema method (acute inflammation) and the cotton disc induced granuloma formation method (chronic inflammation) at doses of 250 and 500 mg / kg were used and the Ac was evaluated by via topical. The chemical study of the extracts was performed by preliminary phytochemical screening and the Ac was analyzed by gas chromatography. Toxicity was assessed using an

acute toxicity model in vivo.

The results reveal that EE and Ac show a significant acute anti-inflammatory activity, compared with positive patterns. As for chronic anti-inflammatory activity, EE (500 mg / kg), ibuprofen (100 mg / kg) and meprednisone (5 mg / kg) significantly reduced the weight of exudate and granuloma induced by the cotton disc (24, 17, 45.56 and 57.17%, respectively). The extracts revealed the presence of reducing compounds, polysaccharides, tannins, triterpenes, sterols and coumarins as major phyto-constituents. The chromatographic profile of volatile compounds of Ac showed a great wealth in terpene substances. Rats showed no obvious symptoms of toxicity.

These findings are encouraging to continue the studies necessary for the development of a phytopharmaceutical with medicinal utility.

Keywords: antiinflammatory activity, phytochemical studies, acute toxicity, *Clinopodiun gilliesii*

(41) **PRELIMINARY STUDY ON THE APPLICATION OF NATURAL LECTINS AS IMMUNOHEMATOLOGICAL REAGENTS**

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It is been suggested that, recognition between proteins and carbohydrates is of crucial importance in various biological processes, such as viral, bacterial and parasitic infections, in the typing of blood groups, cancer and growth and differentiation. Lectins are the model system of choice to study the molecular basis of these recognition events because they are easy to purify in large quantities, but also exhibit a wide variety of carbohydrate specificities. Because lectins offer broad perspectives as tools diagnosis and therapeutic use, the objective of this work was to investigate the agglutinating activity of seeds of seven different amaranth cultivars. For this, suspensions of red blood cells from adult donors were used (NaCl 1%). The agglutination activity was evaluated by means of classic immunohematological techniques. After the necessary time had elapsed, the degree of agglutination was observed with the naked eye, in different periods of time (5-30 min), the interpretation of the results was performed using the following indices by crosses: (-) non-agglutination, (+) weak agglutination, (++) medium agglutination, (+++) strong agglutination, (++++) full agglutination. In this study were studied seeds from *Amaranth* plants, grown in INTA-Anguil in the province of La Pampa, according to the norms of good practices. Extracts obtained in saline were used, according to known methods. The protein content of each extract was determined by the method of (Kit AA, Wiener lab.). In order to characterize the proteins present in the amaranth extracts, the protein gel electrophoresis technique was applied. All extracts analyzed showed protein activity, and the same protein profile. Tests showed that, *Amaranthus* extracts strongly agglutinated all blood samples from adult donors. The results indicate the need to expand the studies on the activity of the different extracts of the lectins, for their use as cheap hemoclasifiers reagents in the immunohematological laboratory.

Key words: Lectins, Amaranthus, blood groups, agglutination.

(813) **SYNERGISTIC EFFECT BETWEEN CLOXACILLIN AND ESSENTIAL OIL OF *Melaleuca armillaris* AGAINST *Staphylococcus aureus***

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The increasing antibiotic-resistance impulse us to investigate new therapeutic alternatives.

Cloxacillin (CLOX) is an antibiotic widely used in veterinary medicine against *S. aureus*. Our objective was to evaluate the effect of the combination CLOX with *Melaleuca armillaris*'s essential oil (EO) as an alternative.

EO was obtained by steam distillation. Three wild type *S. aureus* strains were isolated from Holstein cows. *S. aureus* ATCC 29213 strain was used as reference.

The Minimum Inhibitory Concentration (MIC) of CLOX and EO in combination was determined by microdilution in broth at pH 7.4; 6.5 and 5. The checkerboard technique evaluated the interaction between CLOX and EO, the Fractional Inhibitory Concentration Index (FIC) was calculated:

(A): CLOX MIC in combination with EO; (B): EO MIC in the mixture with CLOX, (MIC)a and (MIC)b: MIC of the antimicrobial and EO alone, respectively. Synergism was considered if FIC ≤ 0.5; Partial synergism if 0.5 <FIC <1; Indifference if 1 ≤ FIC <2 and antagonism when FIC ≥ 2. From those combinations yielded the lowest FIC values, we evaluated the index of antibacterial activity (E), established as the difference between the Log10 values of the number of viable bacteria initial (nt_0) and at the end of the test (nt_{24}) according to: E = nt_{24} - nt_0. For this purpose mixtures of EO / CLOX (Control without antimicrobials, 0.5, 1, 2, 4 and 8 times the MIC of the mixture, respectively) were prepared, incubated at 37 ° C and colonies counted. It was considered: a) Bacteriostatic effect: E = 0; B) Bactericidal effect: E = -3 and c) Effect of virtual eradication of bacteria: E = -4.

By combining EO with CLOX, it was possible to decrease the concentration of antibiotic necessary to inhibit *S. aureus*, even by modifying the pH of the medium. This is also reflected in the bactericidal activity achieving an effect close to virtual eradication. Promising results were obtained against the treatment of staphylococcal infections.

(1144) **THE AQUEOUS EXTRACT OF *SMILAX CAMPESTRIS* DIMINISHED RESISTANT ACID PHOSPHATASE POSITIVE CELL NUMBER INDUCED BY RANKL, AND INCREASE APOPTOSIS ON RL-INDUCED OSTEOCLAST-LIKE CELLS, ACTING THROUGH ER ALPHA**

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Smilax campestris Griseb. (SM), is the most widely distributed Smilax specie in the north of Argentina and it is used as antirheumatic folk medicine due to its anti-inflammatory and anti-oxidant properties. Our lab is studying the possible therapeutic effect of SM on oestrogen-induced bone resorption. Previously, we demonstrated that the treatment of RAW cells with aqueous extract of SM diminished the *in vitro* osteoclastogenesis induced by RANKL. Also, NFATc1 nuclear translocation was inhibted by SM in a dose-dependent manner, acting at level of oestrogen receptors alpha (ER alpha). This work aimed to analyze the mechanisms involved in the inhibitory effect of SM on the *in vitro* osteoclastogenesis induced by RANKL. Specific objectives developed were: 1) to compare the effect of SM and oestrogen on the differentiation to tartrate resistant acid phosphatase (TRAP) positive cells, 2) to determine whether SM induce apoptosis on RANKL-induced osteoclast-like cells by TUNEL assay, and 3) to analyze the effect of SM and oestrogen on the metalloproteinase-9 (MMPs) activity by Zymography.

RAW cells were cultured with RL (100 ng/mL) in the presence or absence of SM (10-100-1000 ng/mL) and oestrogen (10-8, 10-7, 10-6 M) during 5 days for TRAP and Zymography, or during 2 days for TUNEL assay. To analyze the role of ER, RAW cells were incubated for 1 h with ER alpha or ER beta antagonist previous to SM treatment. SM 1000 ng/ml inhibited the RANKL effect on TRAP expression and MMP activity ($p<0.0001$) similarly to inhibition induced by oestrogen. Inhibitory activities for TRAP of SM were reversed by ER alpha antagonist ($p<0.01$). In the apoptosis studies, we observed that SM 10 ng/mL induced the cell apoptosis ($p<0.0001$). ER alpha antagonist revert the apoptotic effect of SM ($p<0.001$).

We conclude that aqueous extracts of SM are capable to significantly diminish TRAP expression and induce apoptosis on RL-induced osteoclast-like cells, acting through ER alpha.

Keywords: Smilax campestris, RANKL, ER alpha, osteoclastogenesis

INFECTOLOGY 4

(1536) INSIGHTS INTO THE INTRACELLULAR LIFESTYLE OF *Bordetella pertussis*

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Bordetella pertussis (*Bp*), the causative agent of whooping cough, *survives inside* macrophages in mildly acidic compartments. *In this study we show the role of several proteins involved in nutrient acquisition by Bp inside these cells. By shotgun proteomics we found that intracellular Bp has an overexpression of t*he outer membrane receptors BfrD and BfrE, involved in iron uptake from transferrin via host catecholamines. *We further found that a ΔbfrDE mutant strain had a reduced intracellular survival inside THP-1 macrophages,* suggesting that *Bp* may use catecholamines as a shuttle to acquire iron from transferrin. Confocal studies showing transferrin inside *Bp* phagosomes seem to support this hypothesis. *The main periplasmic iron binding proteins of Bp are AfuA that binds Fe^{+3} and IRP1-3 that binds Fe^{+2}, the latter ion being more stable at the phagosomal pH. Shotgun proteomics showed that the expression of AfuA is switched off in intracellular bacteria and this downregulation could be induced in vitro in bacteria growing at mildly acidic pH. Although our proteomic study failed to identify IRP1-3 in intracellular bacteria, this protein is induced under acidic in vitro conditions suggesting that Bp adapts to the phagosomal pH by expressing the best suited periplasmic iron binding proteins. We further found that* MgtC promotes the growth of *Bp* at low pH as well as under magnesium starvation. *A ΔmgtC* strain had a diminished intracellular survival inside THP-1 cells, suggesting that this protein is involved in the *adaptation to the conditions found by Bp inside the phagosome.* This defect could be rescued by the addition of the vesicle pH neutralizing reagent Bafilomycin A1 or an excess of Mg^{+2} to the cell culture medium. Altogether, these results show that through its evolution *Bp* has acquired an array of factors involved in nutrient uptake in the intracellular location, allowing this bacterium to adapt an intracellular lifestyle that promotes its persistence inside the host.

Keywords: *Bordetella pertussis*, macrophages, intracellular survival

(1710) EFFECT OF HYPEGLYCEMIA AND CORTISOL-MEDIATED STRESS ON INFLAMMATORY RESPONSE AND PPARγ EXPRESSION OF MACROPHAGES DERIVED FROM THP1 LINE STIMULATED WITH *Mtb*

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Tuberculosis (TB) is the second leading cause of death by an infectious agent, *Mycobacterium tuberculosis (Mtb).* In 2015, WHO reported 10.4 million new TB cases, 15% of them attributed to co-morbidity TB plus type 2 diabetes mellitus (T2DM). It is estimated that 10% of the population is infected with *Mtb* and diabetes may increase 3 times the possibility of developing active TB. Previously we reported that patients with the TB+T2DM comorbidity showed a more pronounced adverse immune-endocrine profile than those with TB alone.

In light of these results we decided to investigate the effect of hyperglycemia [D-Glucose –Glc- 5 mM (physiological dose) or 10, 20 or 40 mM (supraphysiological doses)] and cortisol-induced stress (0.1 or 1 μM) on innate immune responses, for example the production of IL-1β (ELISA) and the expression levels of mRNA PPARγ (RT-qPCR) in 24 h cultured macrophages (Mø) derived from THP1 cell line stimulated with *Mtb* (strain H37Rv killed by γ radiation – *Mtbi*). *Mtbi* stimulation significantly increased IL-1β levels regardless of Glc doses added to the cultures ($p<0.0001$). As regards PPARγ (relevant for controlling the expression of genes involved in Glc metabolism, lipid storage and inflammatory responses) its expression level was significantly increased only in cultures exposed to the highest Glc dose ($p=0,008$ vs Mø+Glc 40mM)., Cortisol treatment (0.1 or 1 μM) decreased IL-1β levels ($p<0.0001$, vs. stimulated cultures), but increased significantly PPARγ levels ($p=0.0008$, vs. stimulated cultures) in a Glc dose dependent fashion. Moreover, an inverse correlation between PPARγ and IL-1β ($p<0.04$) was observed. Interestingly Glc concentrations greater than 5 mM were positively correlated with PPARγ expression independently of cortisol treatment ($p<0.01$). In stress situations the Glc-associated increase in PPARγ expression levels may play an unfavourable role in the anti-TB response in light of the inverse relation between PPARγ expression and IL-1β production.

(1714) CHITOSAN AND CLOXACILLIN COMBINATION IMPROVE ANTIBIOTIC EFFICACY ON DIFFERENT LIFESTYLE GROWTH OF BACTERIAL ISOLATES FROM CHRONIC BOVINE MASTITIS

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Bovine mastitis is the most common pathology that affects dairy cows around the world. *Staphylococcus* spp are the most frequently bacteria isolates in intramammary infections (IMI). In our work, we selected seven isolates from chronic IMI, refractory to all protocols for bovine mastitis. We found that 85%, 71% and 57% of isolates present resistance to more than two, three and four antibiotics, respectively. The higher cure-rates to mastitis antibiotic therapy are during the dry period, in which cloxacillin (Clx) are the most frequently used. By other side, chitosan (Ch) exhibit important properties as antimicrobial and pharmaceutical agent. The aim of this work was focused on the design of a new formulation that can improve conventional antibiotic therapy. For that, combinations of Clx and Ch were applied to different lifestyle of bacterial growth. In the present work, we analyzed Clx effect on pre-stablished biofilm and invasion of bovine mammary epithelial cells by bacteria from chronic isolates. Accordingly, we found that biofilms and intracellular survival disruption needed between 16-128 and 4-32-folds more Clx concentration that their planktonic counterparts, respectively. Ch incorporation to antibiotic therapy can improve antibiotic efficacy in planktonic, biofilms and intracellular survival, reducing Clx concentration in 16-64, 5-16 and 4-32-folds, respectively. In fact, Ch addition to conventional therapy cannot only reduce bacterial viability, but also inhibits a new biofilm formation and could eradicate pre-established bacterial biofilms (CFU/mL, Cristal Violet and CLSM). These findings showed that biofilms and intracellular growth represent a strong protection against antibiotic therapy. However, combined therapy represents an efficient, safer and greener strategy that can be applied during the dry period, which could help to improve antimicrobial treatment against multiresistant bacteria and reduce generation and propagation of them.

Keywords: bovine mastitis, intramammary infection, dry-off therapy

(1728) WEAK AHR SIGNALING RESTRICTS THE DIFFERENTIATION OF CD8+ MEMORY T CELLS DURING *TRYPANOSOMA CRUZI* INFECTION

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The generation and persistence of memory T cells that can provide long-lasting protection against pathogens is depending on signals given by dendritic cells (DC) during the antigen presentation. Thus, changes in any of the factors controlling the activation of T cells during antigen presentation by DC can regulate T effector and memory cell differentiation. The aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor that controls several aspects of immune responses, including the modulation of DC activation and the differentiation of specific T cell subsets. Using B6 WT and B6

mice carrying a mutant AhR protein with reduced affinity for its ligands (AhRd) we demonstrated that AhR activation by endogenous ligands generated during *T. cruzi* infection restricts the differentiation of CD8+ memory T cells. To continue with these studies, we tested the hypothesis that the activation of AhR by weak ligands might contribute to restrict CD8+ memory T cell induction. For that *T. cruzi* infected B6 mice were treated with vehicle or 3-HK (an AhR weak ligand that is generated during the infection) from day 5 to 10 post infection (pi) plus ITE (a weak AhR ligand used in different clinical trials) on days 7, 9 and 11 pi, and the effector phase of memory induction was studied by FACS at day 13 and 21 pi. The treatment of *T. cruzi*-infected B6 mice with 3-HK+ITE induced a significant increase in the percentage of CD8+ T cells specific for the inmunodominant epitope TSKB20 (ANYKFTLV) that showed short lived effector cells phenotype (SLECs: $CD127^{lo}$ $KLRG1^{hi}$) (day 13, $p<0,05$, and day 21 pi, $p<0,01$). In addition, a significant decreased of CD8+ TSKB20+ effector memory cells (EM: $CD44^{hi}$ $CD62L^{lo}$) was observed in 3-HK+ITE vs vehicle-treated mice (day 13, $p<0,05$, and day 21 pi, $p<0,01$). Together, our results confirm that during *T. cruzi* infection AhR signaling restricts the differentiation of CD8+ memory T cells by promoting the development of terminal differentiated SLECs.

Keywords: 3HK-ITE, memory CD8 T cells, *T. cruzi*.

(928) **CHARACTERIZATION OF THE IMMUNE RESPONSE AGAINST *CLOSTRIDIUM DIFFICILE***

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C*lostridium difficile* is a gram-positive, anaerobic, spore-forming bacteria. *C. difficile* infection (CDI) is one of the major causes of nosocomial infections. Transmission of *C. difficile* occurs through the fecal-oral route and the clinic symptoms go from a transitory intestinal colonization to death. The major cause of susceptibility to CDI is the use of antibiotics and the standard method of treatment is the use of antibiotics.

Little is known about the role of T cells during CDI. Therefore, and given the pro-inflammatory character of CDI, we studied the innate and adaptive immune response against this pathogen. We stimulated PBMCs from healthy donors with *C. difficile* extracts, heat inactivated (CD Heat) or inactivated with formaldehyde (CD Formol). First, we evaluated SLAM expression and TNF-α production on monocytes. *C. difficile* stimulation up-regulated SLAM and TNF-α expression on $CD14^+$ cells, greater effects were observed with the heat inactivated extract.

Next, we determined the levels of the pro-inflammatory cytokines, IFN-γ and IL-17, and we also evaluated the expression of the costimulatory molecules SLAM, ICOS and PD-1 on $CD3^+$ cells. We found that, both *C. difficile* extracts, induced IFN-γ production (measured by ELISA). Furthermore, we analyzed the IFN-γ production by flow cytometry and Real Time PCR. IFN-γ expression was higher when PBMCs were stimulated with CD Formol for 5 days. Moreover, more than 50% of IFN-γ^+ cells were also IL-17^+ after 5 days of stimulation with CD Formol. In addition, *C. difficile* stimulation led to an increase of $CD3^+PD\text{-}1^+IFN\text{-}\gamma^+$ and $CD3^+SLAM^+IFN\text{-}\gamma^+$ cells. Our results suggest that *C. difficile* induce a strong po-inflammatory response and that PD-1 and SLAM could be implicated on the regulation of IFN-γ.

The study of T response regulation through costimulatory molecules could be crucial for the discovery of new therapeutic targets and biomarkers that could improve the quality of existing treatments and diagnoses.

Keywords: *Clostridium difficile*, cytokines, costimulatory molecules.

(1346) **IMMUNE RESPONSE IN THE ADVENTITIAL LAYER OF BOVINE *ECHINOCOCCUS GRANULOSUS SENSU STRICTO* HYDATID CYSTS**

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Introduction: Cystic echinococcosis is a major zoonotic disease caused by infection with the metacestode stage (hydatid cyst) of the flatworm *Echinococcus granulosus*, where different genotypes of this flatworm has been described. Helminth infections cause mixed Th1-Th2 immune responses; however, the balance between the Th1 and Th2 responses may differ between the survival strategy of the parasite (parasite protection) and the host animal (host protection).

Material and Methods: Fertile and infertile *E. granulosus* hydatid cysts were obtained from bovine lungs at an abattoir in Santiago, Chile. The strain was identified performing a PCR and posterior BLASTn analysis of the mitochondrial cox1 sequence. Then, formalin-fixed and paraffin-embedded cyst sections were assessed immunohistochemically using antibodies against Interleukin-4 (IL4) and Interferon Gamma (IFN-γ). Finally, positive cells of the adventitial layer were automatically measured with Image-Pro Plus software (ver. 4.5) through the Measure command and then the Count/Size command.

Results: All cyst corresponded to *sensu stricto* strains. In the adventitial layer of the fertile cyst, the IFN-γ mark was 28.2% and the IL-4 mark was 63.9%. In infertile cyst, the IFN-γ mark of the adventitial layer was 79% and the IL-4 mark was 0.2%

Discussion: In the adventitial layer of the fertile cyst, the IL4 mark was higher than the IFN-γ. In the adventitial layer of the infertile cyst, in an opposite way, the IFN-γ mark was higher than IL4. These findings are consistent with a predominant Th2 response in the fertile cyst and a Th1 predominant response in the infertile cyst, supporting the idea that the host immune response plays a role in the *sensu stricto* hydatid cyst fertility.

(1352) **DEVELOPMENT AND PARTIAL EVALUATION OF A RECOMBINANT VACCINE AGAINST *Anaplasma marginale.***

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Anaplasma marginale is an intraerythrocytic bacteria that cause bovine anaplasmosis, disease characterized by high lethality in adults. The disease is in expansion and has become a serious threat to cattle industry. The drawbacks of the currently used *A. centrale* live vaccine, produced in splenectomized calves, boosted the search of safer, more efficient and cost-effective immunogens. The aim of this preliminary work was to evaluate the humoral and cellular immune responses generated by the inoculation of the recombinant form of five subdominant and surface exposed *A. marginale* proteins, members of the type IV secretion system. VirB9.1, VirB9.2, VirB10, VirB11 and Et-Tu proteins were cloned and expressed in *Escherichia coli*. Twenty Holstein steers, two years old, were randomly clustered into four groups (G) of five steers each. GA and GB were immunized with a cocktail of 50 μg of each recombinant protein (rP) in Quil-A® or Montanide® adjuvants, respectively. GC and GD (controls) were inoculated with Quil-A or Montanide adjuvants, respectively. Steers received four inoculations within three-week interval. An indirect ELISA, and a lymphocyte proliferation assay (LPA) was standardized to evaluate the humoral and cellular immune response against the five rP. The production of rVirB9.1/9.2 was higher (25 mg/L) than that produced by the remaining three rP (<12 mg/L). In GA and GB, specific antibody response against the 5 rP was detected 14 days post inoculation while the steers of GC and GD remained sero-negatives. IFN-γ and IL-4 were not detected by LPA after the stimulation with the rP. The cytokines stimulated by Con-A, produced from lymphocytes of all steers, have no significant differences among groups ($p<0.05$). Currently, the animals are being challenged with 10^7 *A. marginale* to evaluate the protection stimulated by the rP. The evaluation of clinical response will allow establishing the type of immune response developed and the degree of

protection achieved.

Keywords: *Anaplasma marginale*, vaccines, immunity, challenge.

(1426) **IMMUNE RESPONSE INDUCED BY *Trypanosoma cruzi* CYTOSOLIC TRYPAREDOXIN PEROXIDASE**

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Chagas disease is a life-threatening illness caused by *Trypanosoma cruzi* and a major problem in Latin America. Currently used drugs exhibit variable efficacy with undesirable side effects. In order to survive and ensure host infection and persistance, *T. cruzi* depends on the effectiveness of its redox antioxidant metabolism and on the immune response evasion mechanisms. The tryparredoxins peroxidases, which belong to the peroxirredoxin family of peroxidases, are very efficient, and constitute virulence factors. Some proteins of this family are able to bind to TLR4 in macrophages and dendritic cells, promoting secretion of proinflammatory cytokines and maturation of dendritic cells.

In the present study, we evaluated the host immunological response to *T. cruzi* cytosolic tryparedoxin peroxidase (c-TXNPx). We used c-TXNPx and a mutated variant (c-TXNPxC52S), which lacks peroxidase activity. BALB/c mice were inoculated with these proteins and the humoral and cellular response was evaluated. Our results indicate that mice inoculated with c-TXNPxC52S have a higher titer of specific antibodies for IgG, IgM, IgG1 and IgG2a isotypes than those immunized with c-TXNPx.

In addition, we demonstrate that animals inoculated with c-TXNPx have a higher percentage of IFNγ-producing T CD4+ in the spleen than those inoculated with c-TXNPxC52S, and controls. Moreover, splenocytes from c-TXNPx inoculated mice (but not cTXNPxC52S) have a higher proliferation rate of T CD4+ and T CD8+ cells and higher production of IFN-γ than those of the control groups, indicating that c-TXNPx produced a specific and polyclonal proliferation in both CD4+ and CD8+ T cells. Our results indicate that the peroxidase activity is important to generate specific humoral and cellular immune responses. Thus, we are currently testing antigen presentation to determine whether peroxidase activity is requiered for this process.

Key words: tryparedoxin peroxidase, immune response

(926) **EPIDEMIOLOGY OF LOWER-LIMB ULCERS: STUDY OF INPATIENT DURING 2013-2014 IN ARGENTINA**

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Lower-limb ulcer (LLU) affects approximately 1% of the population around the world and is a serious problem for patients, professionals and health systems. In Latin America (especially in Argentina) this is an undervalued problem due the lack of epidemiological studies. The aim of this study was to contribute to the knowledge about this problem in the North-West Region of Argentina.

Materials and Methods: A retrospective study of 359 inpatient medical records with ICD-10 codes related to LLU that were hospitalized at A. Padilla Hospital, in Tucumán, Argentina, during the period 2013-2014 was conducted. This study was approved by the SIPROSA Bioethics Committee and the Hospital Teaching and Research Committee. There was collected information about inpatient social status (city, educational status, employment status, social insurance and marital status), hospitalization (inpatient age, sex, days and weather influence) characteristic of ulcer (type, quantity, location, evolution, microbiology, treatments, etc.) and other epidemiological data.

Results: Frequencies, incidence, prevalence, clinical and dermatological associations, and therapeutic effectiveness were determined. This allowed us to evaluate the situation of inpatient with LLU in our region. Statistical analyses were performed using standard statistical programs, which were compared with similar studies published around world. An average of 23 days of hospitalization per patient was observed, mainly caused by diabetic peripheral circulatory complications (59%). 60% of the patients were amputated before or during the study period. A clear association between the level of education of the patients and the hospitalizations was found (84% only with primary school), as with the employment situation (83% do not have job)

Conclusion: This study allows us to find new opportunities for therapeutic improvement, shortening hospitalization time and associated costs, and improving hospital organization.

Keywords: lower-limb ulcer, epidemiology, inpatient

(1128) **INSIGHTS INTO U-OMP19`S STRUCTURE, A Brucella abortus BROAD SPECTRUM PROTEASE INHIBITOR.**

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U-Omp19 is a *Brucella abortus* protease inhibitor with immune adjuvant properties. U-Omp19 inhibits the main gastrointestinal proteases (α-chymotrypsin, trypsin, pancreatic elastase and pepsin) and lysosomal cysteine proteases (cathepsin L, B and C). These activities may play a role in UOmp19`s adjuvant activity by increasing the half-life of co-delivered antigens and in *B. abortus* virulence by protecting it from the action of host proteases during oral infection establishment. The molecular mechanism and U-Omp19´s regions that interact with proteases are still unknown. In this work, we aimed to obtain structural information of U-Omp19 to understand deeply its role in virulence and adjuvanticity.

In silico analysis predicted that U-Omp19 may belong to I38 family, a family of bacterial protease inhibitors characterized by a beta-barrel fold. Based on homology, U-Omp19´s inhibitor activity may be among residues 61-158. Secondary structure prediction suggests that residues 1-60 are disordered and may not present a regular structure.

SLS and DLS studies showed it behaves as a globular monomer of 16,8 kDa and Far-UV CD spectra indicated a high predominance of β-strand secondary structure.

Assignment of protein resonances in NMR studies using uniformly double labeled (^{15}N, ^{13}C) U-Omp19 confirmed that it bears a flexible N-terminal region (residues 1-64) and a C-terminal compact core of eight anti-parallel β-strands (residues 70-158). The lowest energy structure obtained from fold calculations using backbone chemical shifts as restraints is similar to the structures of other inhibitors from I38 family.

To elucidate U-Omp19´s C-terminal function, we obtained a recombinant truncated version (residues 60-158). Far-UV CD spectra confirmed that it retains the beta-barrel folding, however the inhibitor activity against α-chymotrypsin, trypsin and elastase was lost, indicating that U-Omp19 needs at least some part of its N-terminal disordered region for its inhibitory activity.

TOXICOLOGY 2

(1875) **LONG LASTING EFFECTS OF PRENATAL AGROCHEMICAL EXPOSURE ON *CAIMAN LATIROSTRIS* OVIDUCT DEVELOPMENT AND DIFFERENTIATION**

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Humans and wildlife are daily exposed to contaminants which have the potential to interfere with their endocrine system by acting as endocrine disrupting compounds (EDC). *C. latirostris* is a crocodilian species highly sensitive to EDC. Previously we showed that, early postnatal exposure to estrogen decreases luminal epithelial estrogen alpha receptor (ERα) expression and induces both androgen receptor (AR) luminal epithelial expression and histological changes associated to gland development in pre-juvenile caiman oviduct. The aim of this study was to assess the effect of *in ovo* exposure to atrazine (ATZ) and endosulfan (END), agrochemicals classified as EDC, on juvenile caiman oviducts. Eggs were topicated with vehicle (7), ATZ-0.2 ppm (5) or END-20 ppm (6) and incubated at female-producing temperature until hatching. Animals were raised under controlled conditions and euthanized at juvenile (BM≥1kg). The oviducts were dissected, fixed, processed and paraffin embedded. Gland density (expressed as glands/40x field) and percentage of subepithelium occupied by glands were recorded on PAS stained sections. ERα and AR expressions on luminal and glandular epithelium were assessed by immunohistochemistry and expressed as percentage of immunostained nuclei. Results are presented as mean ± SEM. Mann Whitney test was performed and $p< 0.05$ was accepted as significant. Prenatal exposure to END increased gland density (18.28±1.0 *vs* 9.1±3.8 gland/field) and subepithelial area occupied by glands (11.7±2.0 *vs* 2.9±1.1%), suggesting higher gland morphogenesis. Indeed, decreased ERα expression (52.9±5.9 *vs* 76.7±6.8%) was observed in END exposed caimans mimicking estrogen action. Exposure to ATZ reduced luminal epithelial AR expression (58.6±0.5 *vs* 67.1±2.9%) unlike the effect observed after estrogen exposure. Prenatal exposure to ATZ and END induce changes on oviductal development that last until juvenile and could impair *C latirostris* reproductive health.

(285) **COPPER-INDUCED CELL DEATH IS DRIVEN BY IMPAIRED PROTEIN HOMEOSTASIS RATHER THAN OXIDATIVE STRESS**

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Copper (Cu) accumulation is the underlying cause of toxicity in Wilson disease and Cu imbalances have also been reportedly involved in neurologic disorders such as Alzheimer and Parkinson diseases. The reaction between Cu^{1+} and H_2O_2 gives rise to hydroxyl radical which is believed to drive the cytotoxic effects of intracellular Cu accumulation. However, no antioxidant therapy could be so far developed on this basis and the therapeutic strategies in Cu overloaded patients rely fully on Cu chelating agents. Cu ions have been reported to interact directly with proteins, altering their structure and function. Objective: To determine whether Cu-induced cell death is driven by oxidative stress or impaired proteostasis. Methodology: heat shock response (HSR), unfolded protein response (UPR) and mitochondrial UPR (mtUPR), ubiquitin and autophagy related genes upon Cu overload in BEAS-2B cells were assessed with DNA microarrays. Potentiating effects on cell viability of pro-oxidizing (Fe) and pro-aggregating (Zn) stimuli on Cu loaded cells were assessed by flow cytometry. Protein aggregation was assessed by a turbidity assay. Results: A thorough HSR and a milder UPR with no mtUPR was observed in cells exposed to Cu overload. Additionally, an induction of ubiquitin, LC3 and GABARAPL1 was observed. Cu^{2+} was able to directly induce protein aggregation *in vitro* in a time and concentration dependent manner. Compared to other metals, Cu^{2+} was the most efficient in promoting protein aggregation followed by Zn. Interestingly, co-incubation of Cu loaded cells with Fe did not potentiate cell death while co-incubation with Zn drastically enhanced it. Cu exposure induced HSR and UPR due to impaired proteostasis in the cytosol and the *endoplasmic* reticulum respectively. The ability of Cu ions to directly interact with proteins seems to be the force driving cell death as observed by the potentiating effect with Zn which also promotes aggregation; while Fe, did not show any effect.

Key words: Copper, Glutathione, Proteostasis, Protein aggregation

(936) **EFFECT OF N-ACETYL CYSTEINE ON THE BRAIN REDOX STATUS IN A GLUTAMATE EXCITOTOXICITY MODEL**

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Abstract: Glutamate is an excitatory neurotransmitter in central nervous system. Glutamate excitotoxicity is thought to play an important role in neuronal damage. N-acetyl cysteine (NAC) could be useful as a donor of sulfhydryl groups, increasing glutathione levels. The aims were to evaluate changes in redox status in brain of rats subjected to a model of glutamate excitotoxicity and to identify modifications when an antioxidant therapy was given.

The experimental model consisted of four groups: Glutamate Group was injected with 1g/kg weight of monosodium glutamate, Control Group was injected with saline solution, Treated Glutamate Group was supplemented with 150 mg/kg weight of NAC and with 1 g/kg weight of monosodium glutamate and Treated Control Group was supplemented with 150 mg/kg weight of NAC. The supplementation was given every day while the glutamate was injected at days 1, 5 and 9. CICUAL approved every experimental procedure. The following markers were evaluated in brain homogenates: the activities of superoxide dismutase (SOD), glutathione reductase (GR), NADPH oxidase, protein oxidative damage, and glutathione levels.

Glutamate increased SOD (28%,$p<0.05$), GR (15%,$p<0.01$), NADPH oxidase (31%,$p<0.01$), protein oxidation (24%,$p<0.05$), and decreased glutathione (24%, $p<0.05$) compared to control group. NAC decreased SOD (19%,$p<0.05$), GR (25%,$p<0.05$), NADPH oxidase (48%,$p<0.01$), and increased glutathione (28%,$p<0.05$) compared to glutamate group. NAC also increased glutathione (30%,$p<0.05$) respect control group. No changes were found in protein damage.

Decrease in non-enzymatic antioxidants as well as the compensatory up-regulation of antioxidant enzymes activities could be consequence of an increase in oxidative processes in glutamate excitotoxicity model. NAC could be useful to maintain the redox status as it increases glutathione and produces a decrease in the enzymes related to reactive oxygen species production.

Keywords: glutamate, redox status, brain, antioxidant, N-acetyl cysteine

(584) **EFFECT OF NMDA RECEPTOR BLOCKADE ON THE ANXIETY-LIKE BEHAVIOR INDUCED BY POSTNATAL CHRONIC EXPOSURE TO THE ENDOCRINE DISRUPTOR DI-2(ETHYL-HEXYL PHTHALATE) IN MALE RAT**

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Di-2 (ethyl-hexyl phthalate) (DEHP) is an endocrine disruptor with antiandrogenic action, which is used to confer flexibility to products made with PVC. It modifies the neuroendocrine regulation of the testicular axis, increasing the hypothalamic release of glutamate and induces an anxiogenic behavior. The aim of this study was to investigate whether the blockade of NMDA receptors of glutamate

by the non-competitive antagonist dizocilpine (MK-801; 1 mg/kg, ip) could modify the anxiety-like behavior produced by chronic postnatal exposure to DEHP (30 mg/kg in drinking water). Male Wistar rats (n=8-10/group) were used and sacrificed at 60 days of age. The treatment groups were: water+saline (W+S), DEHP+saline (DEHP+S), water +MK-801 (W+MK) and DEHP+MK-801 (DEHP+MK). Anxiety-like behavior was evaluated by the elevated plus maze test and analyzed by Any-Maze video tracking software (Stoelting Co., Wood Dale, Illinois). The parameters measured were: time spent in open (TSO) and closed (TSC) arms, total number of entries (TE) and time of freezing (FT). It is considered that anxiety-like behavior is characterized by a decrease in TSO and an increase in TSC. Also,TE provides a built-in control measure for general hyperactivity or sedation. Statistical analysis was performed using the Kruskal-Wallis test (non-parametric ANOVA, Dunn post-test). As expected, chronic postnatal exposure to DEHP induced an anxiety-like behavior. This effect was reversed by MK-801: TSO (DEHP+MK vs DEHP+S: 47.8 ± 10.2 vs 4.8 ± 1.2; p <0.001) and TSC (DEHP+MK vs DEHP+S: 238.9 ± 5.7 vs 295.2 ± 1.2; p<0.001). Treatment with MK-801 also significantly increased TE (DEHP+MK vs DEHP+S: 12.1±1.8 vs 3.6±0.4; p<0.001) and decreased FT in the closed arms (DEHP+MK vs DEHP+S (22.7 ± 2.2 vs 154.1 ± 18.5; p <0.01). The results suggest: 1) NMDA receptor blockade by MK-801 can reverse anxiety-like behavior induced by DEHP exposure during early life period. 2) The glutamatergic system could be involved in this effect.

DEHP ANXIETY GLUTAMATERGIC SYSTEM BEHAVIOR

(1131) **MIX OF GLYPHOSATE AND CYPERMETHRIN FORMULATIONS INDUCE OXIDATIVE STRESS IN HEp2 CELL LINE**

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During the last few years, in Argentina there has been a strong process of expansion of glyphosate-resistant soybean crops. Along with this exponential growth, the use of agrochemicals has increased. It has been reported that many pesticides (including herbicides) generate intracellular reactive oxygen species (ROS). However, there is still much to be done to understand the underlying mechanisms in the case of pesticides mixtures. In the present study, we aimed to examine the effects on oxidative balance of the glyphosate and cypermethrin mixture (MIX) in HEp2 cell line compared to the effects of the separately formulations. For this purpose, we determined ROS formation, glutathione (GSH) equivalents content, antioxidant and detoxificant enzymes activity (superoxide dismutase (SOD), catalase (CAT), and GSH-S-transferase (GST) respectively), and carbonyl content as a marker of oxidative damage. Our results show that MIX exposure at NOAEC (no observed adverse effect concentration) produced a significant increase (p<0.05) in reactive oxygen species production, SOD and CAT activity, and GSH levels, while no effects were observed for GST activity. Also, MIX exposure at LOAEC (low observed adverse effect concentration) produced a significant increase (p<0.05) in reactive oxygen species production, SOD and CAT activity, while no effects were observed for GST activity and GSH levels. Also, we had shown that both agrochemicals alone not produce significant oxidative effects on HEp2 cells at the same doses present in the MIXs compared to control. Mix exposure at NOAEC and LOAEC produced a significant (p<0.05) increase in carbonyl content compared to control and to pesticides alone. These results confirm that pesticides together causing toxic effects and induce oxidative stress that are not seen with pesticides alone.

Keywords: oxidative stress, pesticides, HEp2, glyphosate, cypermethrin.

(799) **NEONATAL GLYPHOSATE BASED HERBICIDE EXPOSURE ENHANCES THE SENSITIVITY OF THE RAT UTERUS TO ESTRADIOL**

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Endometrial adenocarcinoma is considered to be related with a hyperestrogenic state, often associated with endometrial hyperplasia. In a previous work, we demonstrated that neonatal exposure to a glyphosate based herbicide (GBH) alters uterine development in prepubertal rats causing endometrial hyperplasia together with increased cell proliferation. Our goal in the present study was to determine whether exposure to low-dose of a GBH during postnatal development might enhance the sensitivity of the uterus to an estrogenic treatment. Female Wistar pups were injected subcutaneously with saline solution (control, C) or GBH using the reference dose (2 mg/kg/day, EPA) on postnatal days (PND) 1, 3, 5, and 7. At weaning (PND21), female rats were bilaterally ovariectomized and treated with silastic capsules filled with 17β-estradiol (E2, 1mg/ml) until they were two months of age. On PND60, uterine samples were removed and processed for histology and for mRNA extraction to evaluate: i) morphological changes, ii) cell proliferation by Ki67 immunostaining and, iii) the expression of the estrogen receptors alpha (ESR1) and beta (ESR2) using immunohistochemistry and real time PCR. GBH treatment elicited uterine histological abnormalities after E2 stimulation. The luminal and glandular epithelium in three out of seven GBH-exposed animals were markedly hyperplastic. Moreover, the luminal epithelial cells of the GBH group showed a dramatically increase in cell proliferation (C: 16.36 ± 1.10%; GBH: 29.07 ± 3.89%, p<0.05) in association with a strong induction of ESR1 (C: 2.03 ± 0.36; GBH: 4.05 ± 0.30, p<0.05). In animals treated with GBH, ESR2 expression was upregulated at transcriptional and translational levels, whereas relative expression of ESR1 mRNA was downregulated when compared to control animals. These results suggest that early postnatal exposure to a GBH enhances the sensitivity of the rat uterus to estradiol and these changes could predispose to the development of endometrial cancer.

Key words: glyphosate based herbicide; uterus; estradiol; proliferation; estrogen receptors

(333) **OXIDATIVE STRESS INDICATORS AND BLOOD PARAMETERS IN MAGELLANIC PENGUINS OF THE PATAGONIAN COAST**

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Physiological indicators are studied to measure the environment-induced alterations in organisms. On the Patagonian coast, Magellanic penguins (*Spheniscus magellanicus*) are exposed to pollutants and tourist activities, which can cause impacts on birds. To assess the impact of these stressors, oxidative stress and hematological parameters were evaluated. Parameters were analyzed in penguins under pressure of tourism at San Lorenzo (SL) colony in Península Valdés, Chubut and in penguins exposed to contaminants due to their proximity to the port of Puerto Deseado, Isla Quiroga (IQ), Santa Cruz.

Adults and chicks displayed differences in the analyzed hematological and oxidative stress parameters. In both colonies, chicks showed higher values of glycemia and cholesterol, but lower total protein and hematocrit values than adults (P<0.01). At SL, the OXY-adsorbent test that measures the total plasma antioxidant capacity, showed lower values in chicks than adults, but TBARS and thiols showed an inverse pattern (P<0.01). In contrast, non-significant differences were observed in the oxidative stress parameters between chicks and adults at IQ colony. Both, chicks and adults displayed higher levels of alanine aminotransferase, alkaline phosphatase and an increased plasma lipid peroxidation at IQ (P<0.01)

although their levels of antioxidants were lower in comparison with SL (P<0.01). As expected, results are suggesting that penguins at IQ are exposed to contaminants. When comparing the SL penguins sampled in an area not exposed to tourists than an area with tourism, significant differences were observed just in oxidative stress parameters (P<0.01). Penguins exposed to tourism showed higher levels of oxidative damage biomarker (ROMs) and lower thiol levels than controls. These are the first results of hematological and oxidative stress parameters of Magellanic penguins in both contexts, being essentials for the evaluation of the environmental impact on this specie.

Keywords: Magellanic penguins, oxidative stress, hematological parameters, tourism, pollutants

(1347) **COMPARATIVE STUDY OF THE EFFECTS OF PERINATAL EXPOSURE TO GLYPHOSATE AND ITS COMMERCIAL FORMULATION ON THE FEMALE REPRODUCTIVE PERFORMANCE AND FETAL DEVELOPMENT IN RATS**

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Glyphosate-Based Herbicides (GBHs) have been the most widely used herbicides during the past three decades. Commercial formulations include other chemical, collectively named as co-adjuvants. Although these substances are classified as inert compounds, it has been demonstrated that the formulations of glyphosate are more toxic than the active principle (glyphosate). In the present study, we investigate the effects of perinatal exposure to glyphosate (Gly) or a commercial formulation on female fertility and fetal development. Pregnant rats (F0) were orally exposed to Gly or to a GBH through food, in a dose of 2 mg of glyphosate/kg/day (RfD, EPA), from gestational day (GD) 9 until weaning (lactational day 21). The body weight gain and the vaginal canal-opening of the F1 females were determined. Sexually mature F1 females were submitted to a fertility test to evaluate the pregnancy rates, and on GD19, the number of corpora lutea (CLs) and the implantation and resorption sites. To analyze transgenerational effects on the F2 offspring development, we evaluated the fetal weight, length and morphology, and the placental weight. Gly and GBH exposure neither altered the body weight gain of the F1 females with age, nor induced changes in vaginal opening. Although all Gly- and GBH-treated F1 females resulted pregnant, a decreased number of implantation sites was detected. Moreover, F2 offspring exhibited a delayed growth, evidenced by lower fetal weight and length in both Gly and GBH groups. No differences in placental weight were detected but an increase in placental index was observed in the Gly group. We concluded that perinatal exposure to Gly or its commercial formulation induced female subfertility in rats by decreasing the number of implantation sites. In addition, the normal development of their progeny was affected. These results suggested that the active principle, Gly, produced these deleterious reproductive effects.

Keywords: Glyphosate, Uterus, Subfertility, Feto-placental parameters

(949) **PRELIMINAR STUDY OF REDOX-SENSITIVE TRANSCRIPTION FACTORS IN RAT BRAIN AFTER ACUTE FE-DEXTRAN TREATMENT**

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The aim of this study was to determine the effect of acute Fe administration on the rat brain Nrf2/Keap1 signaling pathway. It was reported that acute Fe-dextran treatment increased rat brain Fe content after 6 h of administration, and modified the NF-κB DNA binding capacity triggering an enhancement in the activity of antioxidant enzymes (such as catalase). Nrf2, constitutively repressed due to its binding to the cytoplasmic Kelch-like ECH associated protein 1 (Keap1) protein, is a redox-sensitive transcription factor related to cell protection against oxidative stress. Nrf2 function is primary controlled by its subcellular distribution and the effect of cellular Fe content is not clearly established yet. The activity of catalase seems as a target for the nuclear factor erythroid 2-related factor 2 (Nrf2) gene products. Fe-dextran treatment was performed as the intraperitoneally administration of a single dose of 500 mg Fe/kg body weight to male Sprague Dawley rats. Brain samples were obtained both from control and treated animals after 4, 6 and 8 h of Fe-dextran injection. Cell expression of both factors was determined by Western Blot. It was observed a significant increase in nuclear Nrf2 expression levels after 6 h of Fe administration in rat brain with respect to control values ($p<0.05$), whereas no significant differences were observed in the cytosolic fraction. The relationship between nuclear and cytosolic Nrf2 levels showed a significant increase at 6 h after treatment ($p<0.05$), suggesting a greater translocation of the factor to the nucleus, and an increased expression level. However, no significant differences in the expression of Keap 1 protein in nuclear or cytosolic fractions of brain extracts were observed. These results suggested that (among other pathways), the oxidative stress produced by the acute Fe-dextran treatment, produced the translocation of Nrf2 to the cell nucleus, and in turn activated target genes involved in the antioxidant response.

Key words: Brain, Fe-dextran, Keap 1, Nrf2, Oxidative stress signalling

(146) **RADIOPROTECTIVE EFFECT OF LIPOIC ACID IN AN EXPERIMENTAL MODEL OF ACUTE RADIATION SYNDROME**

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Amifostine (WR-2721) is effective in reducing the acute toxicity induced by ionizing radiation. However, it has important toxic effects per se that prevent its repeated use. It is our interest to develop less toxic radioprotectors, either by themselves or as adjuvants to amifostine. In this work, using an experimental model of Sprague-Dawley rats (both sexes) exposed to X radiation (2 Gy, whole body), the radioprotective effect of lipoic acid (oxidized form) was evaluated. Groups of 8 rats were exposed at a dose of 2 Gy. At 48 hours post exposure blood samples were obtained by tail puncturing, followed by sampling at 7, 14, 21, 28 and 60 days. The erythrocyte, leukocyte and leukocyte formula were counted. Survival curves up to 60 days were also performed. Genotoxic effects in leukocytes were assessed by the Comet assay (one hour post irradiation). The effects of lipoic acid were tested by the administration as a single dose of 550 mg/kg (p.o.), 3 hours before irradiation. Amifostine was tested for comparison at a dose of 100 mg/kg (i.p., 30 minutes before exposure to X rays). In the irradiated animals erythrocytes were depleted (females, $p<0,01$), and white blood cell count was drastically reduced with respect to the control (both sexes, $p<0.01$), also presenting an altered formula. The effect of lipoic acid on the parameters tested was protective, with a complete recovery of erythrocytes in females ($p>0.05$ compared to control). However, no statistically significant protection was observed in the recovery of the leukocyte level or the leukocyte formula, either with lipoic acid or amifostine (both sexes). Genetic damage revealed in leukocytes from irradiated animals was significantly reduced by treatment with lipoic acid (both sexes, $p<0.01$). However, no significant protective effect was observed for survival. These findings suggest a potential radioprotective effect for lipoic acid as a coadjuvant of amifostine.

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Keywords: ionizing radiation; radioprotection; acute radiation syndrome; oxidative stress

CELL SIGNALING 4

(937) **NITRO-OLEIC ACID (NO_2-OA) ACTS AS A PLANT DEFENSE SIGNAL MOLECULE IN TOMATO CELL SUSPENSIONS.**

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Nitro fatty acids act as signal molecules in mammals, through liberation of nitric oxide (NO) and/or the formation of adducts with cellular thiols (like glutathione or protein cysteine). In plants, its formation was recently reported. In Arabidopsis, nitro-linolenic acid is produced in response to abiotic stress. In addition, exogenous application increases expression of reactive oxygen species (ROS) generation/metabolisms - related genes.

To have a deeper understanding of the role of nitro fatty acids on plant physiology, we analyzed effects of exogenous application of NO_2-OA on tomato cells cultures. Tomato cells cultures were treated with NO_2-OA at different concentrations and as control we used oleic acid (OA). We analysed ROS and NO, plant defense related gene expression, cell death and glutathione content. Lipid extracts from tomato cells incubated with NO_2-OA analyzed by mass spectroscopy showed that NO_2-OA could by internalized and further metabolized. NO_2-OA induced ROS but not NO production in a dose dependent manner. Using pharmacological approach we determined that NO_2-OA activates the NADPH oxidase and that ROS production requires extracellular Ca^{2+} influx and the activation of protein-kinases but not NO. Additionally we showed that NO_2-OA increased expression of plant-defense related genes, induced cell death and reduced total content of glutathione.

These results indicate that NO_2-OA triggers modifications of tomato cell physiology leading to responses associated to plant pathogen interaction, revealing its possible role in plant defense as a signal molecule.

(1792) **MOLECULAR BASES OF NON-SYNONYMOUS POLYMORPHISMS OF PHYTOCHROME B GENE IN LIGHT HYPOSENSITIVE RESPONSES OF PATAGONIA GENOTYPE**

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The plant model, *Arabidopsis thaliana*, has a natural distribution in Eurasia and North Africa. Our research group collected seeds of introduced populations of this species (PAT) in the Patagonia, Argentina. Physiological studies in controlled laboratory conditions showed that PAT is hyposensitive to red light and in response to low red/far-red ratios occurring when plants grow in dense vegetation canopies. We hypothesize that polymorphisms in phytochrome B gene (*PHYB)* is responsible for the PAT phenotype. To gain insight on the molecular causes of light hyposensitive in PAT, we studied the contribution of genetic variation on the promoter and four non-synonymous polymorphisms in the coding region of *PHYB*. We are generating transgenic plants of different versions of *PHYB*-PAT expressed in *phyB* mutant background, and over-expressing the *PHYB*-PAT promoter in PAT and Col-0 backgrounds. The material generated will be characterized under different light conditions to evaluate the contribution of each *PHYB* polymorphism on plant phenotype. Advances in this line of work will be presented.

Keyword: Phytochrome B, polymorphisms, Sahde advoidance

(313) **BIOCHEMICAL CHARACTERIZATION OF TWO GROUP III-MEMBERS OF THE *Solanum tuberosum* CALCIUM DEPENDENT PROTEIN KINASE (CDPK) FAMILY**

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Abstract: As sessile organisms, plants have developed complex signal transduction pathways to cope with the environmental fluctuations to which they are exposed during their life cycle. Calcium is a ubiquitous messenger involved in the signaling of environmental stimuli, whose oscillations are decoded by different sensors. Calcium-dependent protein kinases (CDPKs) are key components of calcium regulated signaling cascades in plants. In this work, isoforms StCDPK22 and StCDPK24 from the CDPK family of *Solanum tuberosum* were characterized. Both isoforms belong to Group III, and contain only three EF-hand sites in their calmodulin-like regulatory domain (CLD) instead of four sites like all other members of the family. StCDPK22 encodes a 59.4 kDa protein (524 aa; pI 7.6) while StCDPK24 encodes a 60 kDa protein (532 aa; pI 6.12), both with an N-terminal variable domain (NTV) which presents myristoylation and palmitoylation consensus sites. *StCDPK22* gene (circa 4 kb) is localized in chromosome 10, while *StCDPK24* gene (circa 5 kb) is localized in chromosome 11. Both isoforms share the eight exons and seven introns structure and have 80% identity and 89% similarity at the protein level. According to RNAseq data from the potato genome StCDPK22 and StCDPK24 expression is ubiquitous, however StCDPK22 transcripts are much more abundant in stolons, leaves, shoots, tubers and roots. The recombinant 6xHis:StCDPK22 and 6xHis:StCDPK24 were obtained and kinase assays were performed. Although both isoforms are active kinases, StCDPK24 activity is ten-fold higher. Kinetic parameters were determined for StCDPK24. We observed that this kinase can use either Mg^{2+} or Mn^{2+} as cofactor and full activity was obtained with 1μM calcium, however kinase activity was also observed in the presence of 10 mM EGTA suggesting that calcium may not be a prerequisite for activity. However, in vitro assays indicate that calcium is required for autophosphorylation.

Keywords: CDPK, Potato, kinase activity

(691) **ROLE OF GASOTRANSMITTER HYDROGEN SULFIDE (H_2S) IN PLANT IMMUNE RESPONSES.**

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Abstract: As a member of the family of gasotransmitters, hydrogen sulfide (H_2S) is endogenously synthesized and has specific molecular targets. In plants, H_2S is produced by the enzyme L-cysteine desulfhydrase 1 (DES1) which degrades L-cysteine into H_2S, piruvate and ammonia. H_2S participates in several plant processes such as stomatal closure. Although the study of H_2S in plants has increased in the last years still much remains to be discovered.

Stomata are pores surrounded by a pair of cells, guard cells, through which plants regulate the gaseous exchange with the environment and the loss of water by evapotranspiration. Moreover, when open, stomatal pores are "access points" for pathogens, therefore the regulation of stomatal closure is considered a first immunity barrier. So the stomatal pore regulation is a key process for carbon and water homeostasis and also for plant defense.

In this work we study the role of H_2S in pathogen-induced stomatal closure. We made use of mutant and silenced *A. thaliana* plants in specific genes as H_2S-source *DES1* and those that participate in immune-responses such as the NADPH oxidase RBOHD and the phosholipase C2 (PLC2). Experiments were performed in Arabidopsis isolated epidermal peels. Stomata were preincubated in opening buffer for 3 hours under light and subsequently incubated with the different treatments on the same buffer. Stomatal aperture assays show that bacterial elicitor flg22-dependent stomatal closure was partially blocked by 200 μM of H_2S scavenger, Hypotaurine (Dunn's;P<0.05). Moreover, preliminary results show that *des1* mutant plants do not close stomata under 5 μM flg22 treatment (Dunn's;P<0.05). On the other hand, plants lacking *rbohD* or *plc2* genes do not close the stomata when epidermal peels are treated with 100 μM of H_2S donors (Dunn's;P<0.05). All together the presented data strongly support H_2S as a novel component of flg22 signaling in guard cells.

Key words: H_2S, flg22, stomata.

(1486) **BIOLOGICAL EVALUATION OF 24-HYDROXY-4-CHOLEN-3-ONE AS A POTENTIAL TOOL FOR CONTROLLING ROOT KNOT NEMATODE INFECTION**

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The root-knot nematode *Meloidogyne incognita* is one of the most damaging parasites because it infects almost all cultivated plants. Second-stage juveniles (J2) penetrate the root and migrate to the vascular cylinder inducing the development of root knots. J2 becomes sedentary and undergoes three molts to become an adult female that lays eggs. After egg eclosion, J2 larvae move in the soil, locate root tips and initiate a new cycle of infection. We have previously demonstrated that the synthetic 24-hydroxy-4-cholen-3-one (C_{24}OH), a dafachronic acid (DA) analogue, is able to control *C. elegans* life cycle by interfering with the DAF-12 regulated endocrine network (C_{24}OH displays an antagonistic activity). Since *C.elegans* and parasitic nematodes share the DA system, we decided to test if C_{24}OH has an effect on *M.incognita* life cycle. Eggs were incubated in presence of increasing concentrations of C_{24}OH and significant differences were observed in the hatching at 96 h ($p< 0,0001$) between 100 and 200 μM C_{24}OH treated ones compared to the control. In addition, a significant dose response effect of C_{24}OH in *M. incognita* J2 mortality was observed. After 48 h incubation 16, 28 and 53% of dead J2 were found in the presence of 50, 100 and 200 μM C_{24}OH respectively, whereas only an 8% of dead J2 were found in the control. The effect of C_{24}OH on *M. incognita* infection was evaluated in tomato plants. Roots were treated for 48 h with increasing concentrations of C_{24}OH and then infected. C_{24}OH treatment decreased significantly the number of galls/root system in a dose response manner (100 μM C_{24}OH produced a 72% reduction). Moreover, plants presented a general improvement in their growth parameters. Specifically, root and aerial part fresh weight from 100 μM C_{24}OH treated plants were a 150% and 215% higher than those of untreated plants respectively. Altogether, our study supports a novel use of C_{24}OH as a nematicide. *Keywords M.incognita*, DAF-12, nematicide.

(1777) **ROLE OF PII PROTEINS IN THE NITROGEN STRESS RESPONSE IN *Bradyrhizobium diazoefficiens* USDA 110**

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The Nitrogen Stress Response (NSR) is a specific mechanism that bacteria have developed to acquire and metabolize nitrogen (N) in an efficient way. The core elements of NSR include an uridylyltransferase/uridylyl-cleavage enzyme, GlnD, and two PII proteins: GlnB and GlnK. In N-starved cells, GlnD uridylylates PII proteins which, in turn, activates the bacterial NSR leading to more efficient ammonium assimilation by increasing glutamine synthetase (GS) activity.

The genome of *Bradyrhizobium diazoefficiens* USDA110, the N_2-fixing symbiont of soybean plants, encodes one copy of *glnB* and two copies of *glnK* (called *glnK1* and *glnK2*). Generally, rhizobia have only one copy of *glnK*, so we decided to investigate whether both copies are functional as well as understand the role of the three PII proteins in the N metabolism.

To achieve our objective we obtained mutants strains lacking *glnK1* (ΔglnK1), *glnK2* (ΔglnK2) or g*lnB* (ΔglnB). The phenotype of the strains was evaluated by monitoring growth kinetics in minimal medium containing 20mM of NH_4Cl as N source. Only Δ*glnB* mutant showed a significant delay in growth rate compared with WT strain. However, when we measured GS activity by □-glutamiltransferase assay, ΔglnK1 and ΔglnK2 showed higher activity than WT meanwhile activity values of ΔglnB were lower.

In view of these results and taking into account that in other bacteria GlnK could interact with AmtB (ammonium transporter) to regulate the NH_4^+ entrance to the bacteria, we decided to evaluate whether the lack of any PII affects the NH_4^+ uptake. Neither ΔglnK1 nor ΔglnK2 showed significant difference compared to the WT. Surprisingly, ΔglnB resulted strongly affected showing a pronounce decrease in the amount of NH_4^+ able to get in the bacteria.

The results suggested that both GlnK are functional in *B. diazoefficiens* and they are involved in GS activation. Moreover, the data obtained showed that GlnB is crucial to sense N status and activate NSR in free-living bacteria.

Keywords: rhizobia, nitrogen metabolism, PII proteins.

(775) **PROLINE TO THE RESCUE: A POINT MUTATION IN THE ESSENTIAL 2-HELIX COLIED COIL REVEALS MECHANISTIC DETAILS OF *BACILLUS SUBTILIS* HISTIDINE KINASE DESK**

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Bacillus subtilis responds to a sudden decrease in temperature by transiently inducing the expression of the *des* gene encoding a lipid desaturase, which introduces a double bond into the acyl chain of preexisting membrane phospholipids. This membrane remodeling is controlled by the cold-sensor DesK, through the reversible formation of a continuous two-helix coiled coil (2-HCC), including the fifth transmembrane (TM) segment and the N-terminus of the cytoplasmic domain.

By random mutagenesis, we isolated a point mutation in the 2-HCC (L174P) that could restore the cold sensing ability of single Pro to Ala replacement mutants in TM1 and TM5. To understand how this modified 2-HCC functions, we constructed a series of mutants by site directed mutagenesis, and expressed them in strain DAK3, engineered to test kinase activity by measuring β-galactosidase activity. As expected, when two Pro residues located in different TM segments were simultaneously replaced by Ala, or the zipper of serines of TM5 was disrupted, the protein showed only phosphatase activity. However, when L174P mutation was incorporated, the thermosensing capacity of all the mutant variants of the sensor was restored. So, we wondered if this modified 2-HCC was indeed sensing temperature, independently of the membrane domain. However, when we incorporated L174P mutation into a non-cold responding DesK allele, composed of TM5 linked to the cytoplasmic domain, we found that this modification was not sufficient to turn this protein into a cold sensor, indicating that structural elements of the sensor domain are necessary for signal detection and transduction.

The present work supports previous analysis suggesting that the input signal must promote the rotation of the 2-HCC to destabilize it. We believe that introduction of Pro174 results in a slight destabilization of the 2-HCC that favors the kinase-competent state, counterbalancing modifications into the sensing domain.

(1358) ***Chlamydia trachomatis* ALTERS PHOSPHOINOSITIDES DISTRIBUTION IN INFECTED HOST CELLS**

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Chlamydia trachomatis (CT) is an obligate intracellular pathogen which resides and multiplies in a vacuole called inclusion. This pathogen establishes complex interactions with host cells to facilitate the acquisition of host-derived molecules that are essential for its survival. Phosphoinositides (PIs) regulate key cellular processes such as vesicular trafficking and cellular signalling, by controlling the

subcellular localization and activation of effector PI-binding proteins. However, little is known about the role of PIs during chlamydial infection. In this study, we proposed that CT targets PIs metabolism to promote the biogenesis of its replicative compartment. First, we analysed the subcellular distribution of different PIs in both, un-infected and CT-infected cells by indirect immunofluorescence. Particularly, we assessed modifications in the level of the different PIs species at the plasma membrane (PM) and the chlamydial inclusion membrane. In addition, we quantified the amount of PI(4)P, PI(4,5)P2, and PI(3,4,5)P3 at the plasma membrane (PM) in CT-infected cells by using a novel micro-pattern technique. Furthermore, by spinning disk microscopy, we observed PIs dynamics at different post-infection times. By these approaches, we demonstrated that this intracellular bacterium alters PIs subcellular distribution and metabolism. Mainly, CT infection produced a decrease on PI(4,5)P2 production at the PM while promoted the recruitment and accumulation of this particular PI at the chlamydial inclusion membrane. Collectively, our results suggest a novel host-pathogen interaction that may be important for understanding CT pathogenesis.

(322) **PHAGOCYTOSIS IS REQUIRED FOR TRANSDUCTION OF CHOLESTEROL SIGNALING IN *TETRAHYMENA THERMOPHILA***

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T. thermophila synthesizes the triterpenoid tetrahymanol instead of sterols. The presence of exogenous cholesterol is sensed by the ciliate, triggering several physiological processes: cell motility increases immediately, cholesterol is taken up and converted to 7,22 *bis*dehydrocholesterol (bDHC), the synthesis of tetrahymanol is repressed and the triterpenoid is replaced by bDHC in the ciliate membranes. Cholesterol uptake appears to proceed exclusively via phagocytosis, as previously determined by using fluorescent analogues. Here we show that, in presence of cholesterol, genes involved in the synthesis of bDHC and putatively in sterol uptake, were transcriptionally up-regulated whereas that of the tetrahymanol synthesis were down-regulated. It was revealed after the analysis of a differential transcriptome and confirmed by RT-qPCR. Using a *T. thermophila* thermosensitive mutant, defective only in phagocytosis (strain II8G), we found a drastic reduction of cholesterol uptake and a negligible bioconversion to bDHC at the restrictive temperature (37°C) when compared to the wild type strain under the same conditions. RT-qPCR carried out on the mutant strain grown at 30°C in the presence or absence of cholesterol, showed similar up- or down-regulation of the genes as described above, but not significant regulation was detected at the restrictive temperature. It indicates that cells need to be competent for phagocytosis, not only to incorporate cholesterol, but also to sense its presence and to transduce the signal to the macronucleus.

Keywords: sterols, signaling, transduction, phagocytosis, ciliates

BIOPHYSICS 4

(1556) **A MOLECULAR DYNAMICS CHARACTERIZATION OF THE STRUCTURE AND DYNAMICS OF THE SPIN LABEL DOXYL CHOLESTANE IN DPPC MODEL MEMBRANE IN FLUID PHASE**

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The sterol 3-beta-**D**oxyl-5-alpha-**C**holestane (**DC**) is used in Electronic Paramagnetic Resonance spectroscopy (EPR) as a spin label to study the dynamics of biological and model membranes. DC merges into the lipid bilayers due to its structural similarity with the cholesterol molecule, becoming a local sensor of the dynamics of its environment. The EPR spectra are interpreted assuming that the sterol moiety of the DC molecules locates at the hydrophobic region of the membrane and its doxyl ring sites around the polar heads. Nevertheless, there are no additional experimental results that confirm these assumptions. We performed long molecular dynamics simulations (~ 0.8μs) of DC in molar concentrations lower than 0.5%, in Dipalmitoyl PhosPhatidyl Choline (DPPC) membranes at 50ºC (fluid phase) to characterize the dynamics of this sterol. The evolution of the systems toward the equilibrium state were followed by analyzing the atomic density depth profiles of the phospholipids and DC atoms in consecutive intervals of simulation time along the trajectory. We observe many events of the DC molecule switching hemi-layer (flip-flop) along the simulated time with a mean time between them of the order of 100ns. This value between flip-flop events is 3 orders of magnitude smaller than those observed for cholesterol in lipid membranes with 30% molar of this sterol. In time intervals between flip-flop events, the doxyl ring moiety of DC distributes mainly at depths among the carbonyl oxygens and the phosphate groups of the phospholipids. From the analysis of the order parameters associated to the orientations of the nitroxide and the sterol rings, we conclude that the orientational dynamics of the normal to the doxyl ring, which determines the EPR spectrum, closely follows that of the sterol rings. The sterol ring moiety locates within the hydrophobic region bellow the deepest carbonyl oxygen of DPPC.

Keywords: Biomembranes, Molecular Dynamics, Spin-labels, Doxyl-cholestane.

(37) **EFFECT OF PSYCHOSINE ON MODEL LIPID MEMBRANES**

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*Abstract:*Psychosine or β-galactosylsphingosine(PSY) is a toxic intermediate in the biosynthesis of sphingolipids occurring in the lysosome lumen. Its accumulationinto the cell has been proposed as responsible for the Krabbedisease.Several evidences propose that this amphiphilic molecule exerts its pathological effect by partitioning into membranes and affecting its function.

In this work, we have studied how this cationic lipid affects electrostatic and rheological properties of the membranes. We explored the interaction of PSY with phospholipid membranes at neutral pH in different phase state,used as classical model systems. Additionally, we explored PSY interaction with a complex lipid mixture of phospholipids, sphingolipidsand cholesterol that mimic the lipid composition of myelin. For this purpose, we combined monolayer techniques,Brewster Angle Microscopy and Fluorescence, and Zeta Potential.

Our results show that PSY at neutral pH exhibit surface activity, being able to lower the surface tension of water from ~72 to ~32 mN/m,and a CMC of 38 ± 3 μM. This value is ~30 times lower than the reported CMC at pH 4 evidencing a more hydrophobic character. PSYshows high sensitivity to the phase state. Partitioning preferentially into expanded phasesin phospholipid membranes. This result suggests that PSYmay stabilize phase separation; affecting the subtle balance that regulates phase separation in myelin. Furthermore, we observed that PSY alters the surface electrostatic of lipid membranes in a complex manner.It adds a positive net charge to the membrane surface, evidenced by more positive values of zeta potential, but induces some structuring of the surface water evidenced by surface potential measurements and Laurdan fluorescent probe.

Being the Krabbe disease a demyelinating process, our results are relevant to the supramolecular interpretation of the mechanism of biomembrane perturbation by PSY.

*Keywords:*Psychosine, Langmuir monolayer, liposomes, lipid mixture mimicking myelin

(1319) **EFFECT OF XANTHONES ON LIPID MEMBRANES**

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Xanthones are heterocyclic compounds with the dibenzo-γ-pyrone structure. In nature xanthones are a class of secondary metabolites that are produced commonly in several plant families. The fused rings that form the xanthonic system and the eight possible types of substitution provide an interesting framework from which different systems can be designed. Interest in their chemical and structural

properties has increased due to many biological activities such as inhibitory activity in tumor cell lines. The interest in this compound is related to its possible integration to biological membrane in order to design lipid particles for drug delivery. For this reason we investigated the insertion of Xanthone (9H-xanthen-9-one) and Hydroxy-xanthone (1-hydroxy-9H-xanthen-9-one) in lipid monolayers of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), di-Oleoylphosphatidylcholine (DOPC), 1,2-di-O-tetradecyl-sn-glycero-3-phosphocholine (etherPC) and phosphatidy lethanolamine (16:0 DPPE) by measuring the changes in the dipole potential, the area per lipid and compressibility properties. In addition, the changes in packing and membrane polarity was studied by measuring Generalized Polarization in gel and liquid crystalline states. The experimental results show that xanthone inserts in DPPC membrane interface orienting the CO group with the oxygen towards the aqueous media normal to membrane surface that explains the dipole potential increase. The aromatic ring locates parallel to the first C atoms of the acyl chains apparently by interaction with the non bound populations of the CO groups of the phospholipid. This produces a modest area increase and a depolarization of the interface. This was confirmed by molecular dynamics analysis. The presence of OH group attenuates the increase in the dipole potential demonstrating that it is essential the full insertion of the non polar moiety in the membrane phase to produce the dipole potential increase.

Keywords: Lipid membranes, Monolayers, Xanthones, Dipole potential, Generalized Polarization

(315) **STUDY OF THE EFFECT OF PERFLUORO DECANOIC ACID ON MEMBRANE MODELS**

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Perfluorinated acids could have harmful effects on living organisms, a possible hypothesis states that they may interact with biological membranes decreasing their stability. Due to the difficulties of carrying out these studies in vivo, a more simplified model of artificial membrane was proposed, based on phospholipids molecules adsorbed at water / air interfaces. In the present work, distearoyl phosphatidic acid (DSPA), dilauryl phosphatidic acid (DLPA) and distearoyl phosphatidyl ethanolamine (DSPE) monolayers were used as membrane models and their interactions with perfluro decanoic acid (PFD) were studied. Langmuir isotherms (surface pressure, π, as a function of molecular area) were performed and the permeability of the perfluorinated acid on these monolayers was evaluated. The results obtained demonstrate that PFD penetrates into all the monolayers studied, producing an expanding effect. Brewster Angle Microscopy (BAM) shows that PFD affects the formation of lipid domains by destabilizing them. Finally, the detergent effect of PFD on DLPA and DSPA large unilamellar vesicles (LUVs) was confirmed and the lysis dose 50 was determinated.

(1907) **LIPID-VASELINE MICROEMULSIONS AT THE AIR/WATER INTERFACE**

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Lipid microemulsions (ME), commonly used to encapsulate molecules of pharmacological interest, are oil in water dispersions stabilized by an interfacial layer of a surfactant. Previously, studies on monomolecular layers (ML) of phospholipids at the Vaseline (VAS)/water interface ($ML_{V/W}$) used as an experimental model of ME, allow us to define a surface pressure (π) vs. composition phase diagram which indicated that $ML_{V/W}$ consists of a EPC/VAS mixture. In turn, EPC/VAS mixed ML at the air-water interface ($ML_{a/w}$) can be used as a model of $ML_{V/W}$ to allow topographic analysis by different microscopies. Thus, in the present work we could evidenced the appearance of surface droplets along compression that did not disappeared upon decompression. Spectral confocal microscopy of VAS/DPPC mixed $ML_{a/w}$ using Nile Red (NR), confirmed that droplets were composed of VAS since this probe partitioned in the droplets exhibiting a fluorescence emission spectra (ES) similar to that in VAS bulk phase. However, NR also partitioned in the liquid expanded PC monolayer phase with an ES similar to that in VAS droplets but different from that reported in bilayers. On the other hand, epifluorescence microscopy revealed that droplets were not marked by NBD-PE probe which appeared as dark points. Brewster Angle Microscopy data (assuming the liquid VAS refractive index) revealed that inspite of the micrometer radii, these lenses have a nanometer (3-6 nm) thickness. Then, by geometrical analyses of these lenses, estimation of contact angles can be performed. Taken together, these experimental results indicate that VAS collapsed structures correspond to isotropic VAS (lenses) and are consistent with the null spreading coefficient (resultant from the interfacial tension (IT) values for VAS/W and VAS/A (37±2 and 33±2 mN/m, respectively) which also predicts that VAS against an A/W interface (IT=72 mN/m) would not form stable monolayers.

Keywords: Microemulsions, Vaseline, monolayers, contact angle

(1184) **MODULATION OF MEMBRANE PHYSICAL PROPERTIES BY PULEGONA AND MENTHONE.**

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The essential oils are natural products extracted from species of aromatic plants and exhibit a variety of biological properties, whose effects are attributed mainly to terpenes, usually the major chemical component. Moreover, some terpenes have proven to possess antimicrobial or insecticidal activity, directly connected with the incorporation of these chemicals into cell membrane. The insecticidal activity of *Mentha* oil and its main components has been tested and established against various insects/pests. In the present work, we analyzed the capacity of the monoterpene ketones most commonly found in *Mentha* species, pulegone and menthone, to interact with membranes. The interaction of both ketones with DPPC membranes was analyzed by using Langmuir monolayers and Brewster angle microscopy (BAM). The presence of these compounds in the subphase modified the interfacial characteristics of DPPC isotherms indicating their capacity to penetrate in the lipidic phase. The changes were reflected mainly as expansion of the isotherms in the LE or LC phases of the film, softening and appearance of phase transition at larger areas and reducing the collapse surface pressure. The experimental data indicate that compounds can incorporate into the membrane and their presence between lipid molecules would induce an increasing intermolecular interaction, decreasing the film elasticity. These results suggests that the insecticidal activity of *Mentha* could involve the interaction of its major components with lipid molecules causing disturbance of the cell membrane as was postulated for several larvicide compounds.

Keywords: insecticidal,membrane, pulegone, menthone,BAM.

(1011) **THERMODYNAMIC AND TOPOLOGIC CHARACTERIZATION OF MAMMAL AND INSECT NATURAL MEMBRANES AT THE AIR-WATER INTERPHASE.**

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The aim of the present work was to characterize natural membranes by studying their ability to form stable monolayers at the air water interface (Langmuir films) and the topology of the formed films.

The natural membranes were synaptosomal membranes obtained from bovine brain cerebral cortex (SM) and ganglion membranes (GM) prepared from the head-thorax portion of *Aedes aegypti* larvae (IV stage) or *Triatoma infestans* nymphs (V stage).

The general composition of the membranes was determined by quantification of protein, phosphoplipids and cholesterol total content.

Mammalian and insect membranes were able to form compressi-

ble monomolecular layers at the air water interface. Different methods were tried to spread the aqueous suspension of the natural membrane at the interface and no isotherm shape differences were observed.

Langmuir films isotherms showed clear differences between mammalian and insect membranes. The SM Langmuir film exhibited a collapse pressure of π_c = 50 ± 0.2 mN/m at a minimal area of A_{min} = 100.4 Å^2 and a typical phase transition at 36 mN/m, usually associated with protein reorganization at the surface. On the other hand, GM showed a π_c between 45-47 ± 0.2 mN/m at A_{min} = 105 Å^2 but the phase transition was not present.

Langmuir films were also observed with an inverted epifluorescence microscope (EFM) to evaluate the topology of the monolayers as well as for checking the absence of vesicles in the subphase. SM and GM were doped with 1mol% of Dil-C_{18} and directly observed from the interface or transferred to a solid support.

In Langmuir films, vesicles were identified as big bright dots in EFM images. The wet bridge spreading method did not prevent the apparition of vesicles, though it reduced the number of vesicles considerably, resulting the most appropriate.

Key Words: Langmuir Films; Synaptosomal membranes; Ganglion membranes; EFM.

(967) **DEVELOPMENT OF AN ELECTRONIC PARAMAGNETIC RESONANCE TECHNIQUE TO QUANTIFY THE PROPORTION OF PHOSPOLIPIDS IN LIQUID DISORDERED PHASE IN AN EXOGENOUS PULMONARY SURFACTANT**

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Pulmonary surfactant (PS) is a mixture of phospholipids (PL), neutral lipids (mainly Cho) and at least four specific proteins. The particular lipid composition of surfactant induces segregation of liquid-ordered phase (Lo) and liquid-disordered phase (Ld) in surfactant membranes and films at physiological temperatures. This coexistence of phases would be crucial for the surfactant activity, and the role of Cho in this organization has been extensively researched. Currently the proportion of phases is determined by qualitative methods. Although the role of the proportion of these phases in the surfactant function is unknown, it would be of great relevance elucidate it, since the essential proteins of the PS are in the Ld phase.

Objective: To design and standardize an electronic spin resonance (ESR) spectroscopic technique that allows the quantification of the proportion of Lo/Ld phases present in an exogenous pulmonary surfactant (EPS).

Methods: EPS with or without extra Cho added was labeled with 5DE and TEMPO for its study by ESR. The original technique developed by McConnell (1972) was adapted to quantify Ld proportion in this EPS. The order parameter of the EPS for each sample in the different experimental conditions was also evaluated. PL were determined by Stewart method (1980) and Cho by enzymatic method.

Results: We found that at 50°C all PL of EPS were in Ld phase. At this temperature, the TEMPO partition coefficient obtained for this EPS system was 0.81±0.03. The order changes in EPS membranes did not affect the TEMPO partition coefficient, nor did the Cho concentration. The results obtained with the different batches of EPS showed no significant difference in the phase proportion values. For this EPS, the reference value of the Ld/Lo ratio, calculated at 25°C is 0.30±0.02.

The relevance of this technique is that allows the quantification of the proportion of Ld/Lo phases evaluating if changes in the lateral structure affects the EPS physiological properties.

Keywords: exogenous pulmonary surfactant, Ld/Lo phases, ESR

(1275) **HEMOLYSIS AND ANTIHEMOLYSIS INDUCED BY ARGININE-BASED SURFACTANTS**

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Two novel arginine-based surfactants, Bz-Arg-NHC_{10} and Bz-Arg-NHC_{12}, were characterized in terms of surface properties and interaction with human red blood cells (HRBC) membranes. *CMC* values, Γ_{max} (maximum surfactant adsorption at the air/liquid interface) and A_{min} (area per molecule) revealed better surface properties for Bz-Arg-NHC_{12}. The observation of cylindrical worm-like aggregates of Bz-Arg-NHC_n through atomic force microscopy (AFM) supported the predictions based on the surfactant packing parameter value (SPP). Erythrocyte membrane solubilization was induced by surfactant aggregates, since cell lysis was only evidenced at surfactant concentrations above *CMC*. Changes in HRBC shape observed at different surfactant concentrations allowed to conclude that a slow mechanism based on the insertion of surfactant monomers into the HRBC membrane, followed by shedding of microvesicles is responsible for the hemolysis produced by both surfactants at the lower concentrations tested. On the other hand, the extraction of membrane lipids upon collisions between HRBC and surfactant aggregates competes and prevents the microvesicles release for the higher concentrations assayed.

Moreover, we study the interaction of Bz-Arg-NHC12 with sheep red blood cells (SRBC) and HRBC due to their different membrane protein/lipid composition. SRBC is a little more resistant than HRBC to the hemolytic effect of surfactant, but in both cases, the micellar form of the surfactant is the entity responsible of the hemolytic effect. As HC_{50}, cAH_{max} value was higher than the CMC value. Thus, a biphasic behavior was observed for the surfactant studied, showing a wide range of protective concentrations when HRBC were tested, while for SRBC, the degree of protection of Bz-Arg-NHC_{12} was about 50% lower than for HBRC. However, only for SRBC treated with Bz-Arg-NHC_{12} a remarkable volume expansion was evidence, although no correlation with the antihemolytic potency was found.

Key words: Arginine based surfactants; hemolysis; antihemolysis; lipid composition; microvesicles

(390) **INTERACTION OF AMPHOTERICIN B WITH A SYNTHETIC GLYCOLIPID IN LANGMUIR MONOLAYERS**

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Amphotericin B (AmB) is an amphipathic polyene antibiotic used to treat systemic fungal infections. The therapeutic action as well as the toxic side effects of AmB depends directly on the molecular organization of the drug. AmB posses very low solubility in aqueous media and new alternatives for its administration are needed. An interesting strategy is the use of surfactants to transport hydrophobic drugs. In previous work we have synthesized a glycolipid formed by β-cyclodextrin and an alkyl chain (βCD_{anf}). The surface behavior at the air-water interface of βCD_{anf}, alone and in the presence of other amphiphiles, is well known.[1]

As this glycolipid could offer an alternative to transport AmB our aim was to get inside about the interactions established among them. For this purpose, Langmuir monolayers of films formed by AmB and AmB/βCD_{anf} were studied at the air-water interface and their topography accessed by Brewster angle microscopy (BAM). Also spectroscopic techniques such as NMR, circular dichroism and FT-IR were performed to elucidate the interactions. Our study indicates that strong interactions take place among AmB/βCD_{anf} mainly through the sugar moieties. Also, the organization acquired by AmB at the interface varies considerable due the presence of βCD_{anf}. The

presence of βCD_{anf} favors the monomeric form of the drug which highly desirably for clinical use.

Keywords: Amphotericn B, synthetic glicolipid, cyclodextrin, monolayer, nuclear magnetic resonance.

STRUCTURAL AND FUNCTIONAL BIOCHEMISTRY 4

(551) DIACYLGLICEROL LIPASE ACTIVITY IN ROD OUTER SEGMENTS DEPENDS ON THE ILLUMINATION STATE OF THE RETINA

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The aim of the present research was to determine how the synthesis of endocannabinoid 2-arachidonoylglycerol (2-AG) for diacylglycerol lipase (DAGL) enzyme is modulated by the illumination state of the retina. DAGL activity was analyzed in purified rod outer segments (ROS) obtained from dark or light adapted retinas. Retinas were dissected from the eyes after dark or light adaptation. Dark-adapted bovine ROS (DROS) and bleached ROS (LROS) were purified by a discontinuous gradient of sucrose from retinas whose optic cup was either maintained under dim red light or exposed to light (3000 luxes) for 30 min. This activity was assayed using [^{3}H] glycerol-DAG as substrate and determined by [^{3}H]-MAG production. MAG was partially metabolized to glycerol by MAGL action. DAGL activity in LROS was higher than in DROS under all conditions assayed. When the enzyme activity was assayed in a range between 100 μg and 200 μg of ROS protein, [^{3}H]-MAG production was increased in LROS ($p<0.01$). However, the light effect on DAGL activity disappeared when 300 μg of protein were used. It was also observed that light increased DAGL activity at 1 and 2 hours of incubation. Interestingly, endocannabinoid production increased at 2 hours in DROS ($p<0.001$) and LROS ($p<0.01$) with respect to 1 hour of incubation. Additionally, it was observed that MAGL associated to DAGL activity was stimulated by light ($p<0.01$). The expression of cannabinoid receptors (CB1 and CB2) was also increased under light conditions. The data was analyzed using Student t-test, two way-ANOVA and Bonferroni test to compare different conditions. These results suggest a light effect on DAGL, the principal enzyme involved in 2-AG synthesis, as well as on the receptors to which this endocannabinoid binds to, thus indicating a potential role of 2-AG in phototransduction processes.

Keywords: Retina, Photoreceptors, 2- arachidonoylglycerol, diacylglycerol lipase

(609) AMYLOIDOGENIC TENDECY OF N-TERMINAL VARIANTS OF HUMAN APOLIPOPROTEIN A-I

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Amyloidosis is a heterogeneous disease involving protein´s misfolding. Among more than 20 described natural variants, some N-terminal mutated apoA-I (as Leu60Arg (L60R) and Trp50Arg (W50R) are involved in renal amyloid protein deposition.

Previous studies with other mutants (Gly26Arg, Lys107-0 and Arg-173Pro) suggested that structural conformational shifts determine the involvement of apoA-I in this pathology. In the present study we extended our knowledge and compared protein stability, aggregation tendency and susceptibility to proteolysis of the protein with the native sequence (Wt), with the N-terminal variants W50R and L60R.

Structural parameters were analyzed under pH 7.4 and 5.0 by fluorescence, following protein chemical denaturalization, Trp arrangement by the quenching with acrilamide and hydrophobic pockets by the binding of BisAns. The aggregation tendency was evaluated measuring Thioflavin T associated fluorescence following incubation at 37°C in the presence and absence of heparin (as a glucosaminoglycan model). Susceptibly to Trypsin-induced proteolysis was determined at pH 7.4.

Our results show that both mutants are less stable than Wt, especially L60R (p 0.05) and showed a higher quenching of Trp residues (p 0.001); this variant evidenced a loss of hydrophobic pockets. While the aggregation tendency of both variants was similar than Wt at pH 5.0, W50R showed higher ThT binding than Wt or L60R under this condition. No aggregation of the variants tested was detected at pH 7.4, either pure or combined with heparin. Susceptibly to proteolysis was increased for L60R (p 0.05) respect Wt and W50R.

We suggest that structural unstability of the variants induce misfolding that could expose binding sites of ligands and cleavage sites for proteases in the protein which could either increase protein catabolism or to favor an aggregation-prone conformation. Maybe induced under a pro inflammatory microenvironment.

Keywords: misfolding, aggregation

(1746) OXIDATIVE STRESS AND PEROXISOMAL BIOGENESIS IN MICROSCLEROTIA PRODUCED BY THE ENTOMOPATHOGENIC FUNGI *BEAUVERIA BASSIANA* AND *METARHIZIUM ROBERTSII*

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Insect pathogenic fungi are able to produce resistance structures called microsclerotia (MS), which are potential candidates for use in biological control programs. Although oxidative stress was reported to be involved in MS differentiation in some plant pathogenic fungi, there is no information available for entomopathogenic fungi.The main goal of this study was to compare the microsclerotial growth in *Beauveria bassiana* strain GHA and *Metarhizium robertsii* strain ARSEF 2575, and to characterize the expression pattern of genes involved in oxidative stress responses and peroxisomal biogenesis. Fungi were cultured in agitated (250 rpm) complete liquid medium with optimal carbon/nitrogen ratio for MS production. Daily aliquots were collected and examined by both optical and transmission electron microscopy (TEM) after staining with the peroxidase activity marker 3,3-diaminobenzidine (DAB). Samples were also used for qPCR analysis to study the expression pattern of superoxide dismutase genes (*sod*), catalase genes (*cat*), and peroxins (*pex*) involved in peroxisome biogenesis. DAB staining showed high peroxidase activity in MS for both strains, with lower staining in hypha close to the borders of the structure. TEM images also showed higher peroxidase activity in mitochondria and peroxisomes. Although *pex* genes were induced in both strains, *Bbpex7* was more induced in *B. bassiana*, whereas *Mrpex19* showed higher expression levels in *M. robertsii*. At least one of each oxidative stress marker family was also induced in both strains. We conclude that an oxidative stress scenario is triggered in MS producing fungi, including proliferation of peroxisome-like organelles and high peroxidase activity. More studies are need to be carried out to elucidate the relationship between MS formation, oxidative stress and peroxisomal biogenesis to better understand the similarities and differences found in microsclerotial metamorphosis of entomopathogenic fungi.

(1234) STRUCTURAL STABILITY STUDIES OF HUMAN GLYCOGENIN-1 MUTANT ALA16PRO ASSOCIATED WITH GLYCOGEN STORAGE DISEASE XV

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Abstract: Glycogenin is a glycosyltransferase that catalyzes the transfer of glucose residues from UDP-glucose to itself, creating a linear polymer of approximately 12 glucose units bound by α-1,4-glycosidic linkages. This oligosaccharide chain serves as the primer for the combined action of glycogen synthase and glycogen branching enzyme that will complete the polysaccharide synthesis. In humans, there are two forms of glycogenin, glycogenin-1 and glycogenin-2. Glycogenin-1 (HGN1) is mainly expressed in skeletal muscle and heart and to a lesser extent in lung, kidney, brain, pancreas, and placenta. Instead, glycogenin-2 is primarily expressed in the liver.

Glycogen storage disease (GSD) XV is a rare metabolic disorder caused by mutations in the GYG1 gene, which encodes HGN1. To date, eight point mutations have been described in GSD XV patients. One of them was homozygous for an N-terminal missense variant (c.46G>C, p.Ala16Pro) of the protein and exhibited skeletal myopathy with storage of polyglucosan in muscle fibers. The mutation was confirmed at the RNA level but the mutant protein was not detected in the skeletal muscle biopsy of the patient.

Since human and rabbit glycogenin amino acid sequences are 93% identical, we have introduced Ala16Pro mutation into rabbit enzyme, the most studied member of the family, and expressed the mutant in *E. coli.* We have previously described that Ala16Pro mutant was inactive for auto- and transglucosylation and has a diminished substrate binding affinity, probably due to a conformational change. In order to explain the absence of the protein in the patient muscle tissue, in this work we have analyzed its stability and oligomerization state by different *in vitro* techniques. Here we show that, in contrast to wild type HGN1, which exists as a dimer, the Ala16Pro variant forms soluble high molecular weight oligomers. Besides, our results suggest that the mutant has a less stable conformation, more prone to proteolytic digestion.

Keywords: glycogen, metabolic disorder, conformational stability

(1242) **S-NITROSYLATION OF HUMAN TRIOSEPHOSHATE ISOMERASE: STRUCTURAL AND BIOCHEMICAL STUDIES**

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Abstract: Nitric oxide (NO) is a diatomic gaseous free radical produced in large amounts by macrophages upon the induction of NO synthetase. One of the mayor consequences of NO exposure is the generation of the protein S-nitrosylation, which could produce alterations in protein function. Even though the S-nitrosylation mechanism *in vivo* is unclear, several reactive nitrogen species were proposed as mediators including NO gas, or non protein S-nitrosothiols such as S-nitrosocysteine (CySNO) and S-nitrosoglutathione (GSNO). Triosephosphate isomerase (TPI) has been frequently identified as a target of S-nitrosylation by proteomic studies. TPI is a dimeric enzyme that catalyzes the isomerization of dihydroxyacetone phosphate (DHAP) to glyceraldehyde-3-phosphate (G3P), and it is found in almost all organisms. However, the effect of S-nitrosylation on activity were only explored in plants and algae. Here, we present results about the S-nitrosylation of human TPI (hTPI), and the effect on its enzymatic parameters. hTPI is S-nitrosylated by S-transnitrosylation mechanism in a time-dependent manner by both, CySNO and GSNO, being CySNO more efficient. Both, X-ray crystal structure and mass spectrometry analysis showed that Cys217 was S-nitrosylated just in one subunit of the dimer. hTPI S-nitrosylation produced a 15% inhibition of the Vmax of the DHAP to G3P conversion. However, we did not observe effect on the Km. This is the first *in vitro* study of the hTPI S-nitrosylation. Further studies will be required to identify the structural basis of the inhibition.

Keywords: triosephosphate isomerase, nitric oxide, S-nitrosylation X-ray crystallography

(1286) **PARKINSON'S ASSOCIATED P5-ATPases. INSIGHTS ON THE REGULATORY PROPERTIES OF SPF1 SEGMENT CTPI**

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The P5-ATPases are membrane transporters that couple the uphill movement of ions with the hydrolysis of ATP. The precise function of the P5-ATPases and the identity of the transported ion are still unknown. Mutations in the genes coding for human P5-ATPases have been found to be associated with early onset Parkinson, Neuronal Ceroid Lipofuscinosis and Hereditary Spastic Paraplegia. The primary sequence of P5-ATPases shows the insertion of a unique stretch of 60-120 amino acids so-called segment ctPi with no homology in other P-ATPases. This insertion is flanked by motifs "h" and "g" which are extremely conserved in other P-ATPases and have been implicated in the coordination of the catalytic Mg^{2+} ion. Previous studies from our group have shonw that the P5A-ATPase SPF1 from *Saccharomyces cerevisiae* is an active ATPase and forms the catalytic phosphoenzyme that characterizes the P-ATPases. With the aim of advancing the knowledge of the structural or functional importance of the ctPi segment we have used site-directed mutagenesis to obtain mutant (ΔctPi)SPF1 in which the ctPi segment has been deleted. (ΔctPi)SPF1 was expressed at levels similar to WT and was purified following the same protocol. The (ΔctPi)SPF1 enzyme was able to hydrolyze ATP at a rate comparable to WT. However, in contrast with WT, the ATPase activity of (ΔctPi)SPF1 was activate by lower concentrations of Mg^{2+} and then was inhibited . These results show that the segment ctPi is not essential for the stability or the function of SPF1 but may be involved in the regulation of the catalytic activity by Mg^{2+}. With grants from CONICET, UBA and ANPCyT.

Keywords: ATPases, Spf1, Parkinson`s Disease, protein structure funtion

(1293) **STRUCTURE AND FUNCTION OF PARKINSON'S ASSOCIATED P5-ATPases.**
LIMITED PROTEOLYSIS OF SPF1.

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The family of active transporters so-called P-ATPases are essential for the cellular homeostasis. The known substrates transported by the P-ATPases are ions or lipids. The subgroup P5 is the not very well characterized and its transported substrate is unknown. Loss of function of P5-ATPases in humans has been associated with early-onset Parkinsonism (Kufor-Rakeb syndrome) and other neurodegenerative diseases. With the aim of advancing the knowledge of the structural organization of P5-ATPases we have performed experiments of limited of the recombinant SPF1 P5-ATPasa of *Saccharomyces cerevisiae* and of its fluorescent version GFP-SPF1. The products were characterized by SDS-PAGE, fluorescence, mass spectrometry and sequencing. We found that chymotrypsin rapidly splits the 135 kDa SPF1 molecule at site CH1 resulting in a larger N-terminal fragment of about 100 kDa and smaller peptides of about 35 kDa containing the C-terminal end of the protein. Site CH1 apparently occurs in a catalytic important region of the phosphorylation domain of SPF1 preceding transmembrane segment M5 which is highly conserved in all P-ATPases. Finally, at longer times the N-terminal end is cleaved. Both GFP-SPF1 and SPF1 are active ATPases and when exposed to proteolysis exhibit a similar fragmentation pattern. Curiously, GFP-SPF1 seemed to be is more rapidly degraded at site CH1. This result may indicate that, as proposed in other P-ATPases, the N-terminal segment of SFP1 interacts with the catalytic central portion of the molecule. In GFP-SPF1 the GFP moiety would disturb this interaction making CH1 more accessible to the protease. With grants from CONICET, UBA and ANPCyT.

Keywords: Parkinson's disease, membrane protein, limited proteolysis, P-ATPase, Protein structure-function.

(1330) BIOCHEMICAL CHARACTERIZATION OF AN APTAMER THAT BINDS TO MYELIN BASIC PROTEIN

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The discovery of new chemical entities for medical therapy, diagnostics or basic research is being obtained by the screening of large chemical libraries. A relatively new type of chemical libraries includes aptamers, which are short single-stranded nucleotidic molecules that interact sterically and through electrostatic interactions with a target molecule. So far, the accumulated data on aptamer functionality have demonstrated that antibodies are no longer the only entities that may be developed to bind to targets. The application of aptamer technology for the detection of myelin basic protein (MBP) has not fully been exploited and a MBP-specific aptamer could lead to a potential therapeutic drug for multiple sclerosis (MS) or a novel reagent

for myelin detection. MBP is the most widely studied myelin protein in MS and the second most abundant myelin protein that constitutes the myelin sheath. MBP has various isoforms. These proteins are highly basic and are considered to be an intrinsically unstructured protein. Much evidence suggests that MBP may be an autoantigen candidate in MS. In particular, MBP-specific T cells have been isolated from MS patients and healthy individuals. Hence, MBP seems to play an important role in MS progression. In the present study we characterized an aptamer that binds to MBP (MBPCl3). Since MBP presents several isoforms, we first established by SDS PAGE and Mass Spectrometry which isoforms were present in our mouse MBP sample. We found 3 isoforms: 14 kDa, 17.2 kDa and 18.5 kDa, being the 14 kDa the most abundant form. We then demonstrated that MBPCl3 was able to detect the three isoforms in Western Blots (WB). The MBPCl3 affinity towards MBP resulted in 137nM and was calculated by capillary electrophoresis. Finally, we performed and compared MBPCl3 detection towards MBP in native and denaturing WB, showing that MBPCl3 recognized different epitopes and/or folded differently in each technique.

(1447) **THE CANNABINOID RECEPTOR CB1 IS EXPRESSED AND FUNCTIONAL IN ISOLATED NUCLEI OF RAT CEREBRAL CORTEX**

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Lipids in nuclei are membrane components as well as signaling molecules with a potential role in regulating gene transcription. Former studies from our lab demonstrated an active nuclear glycerolipid metabolism in the central nervous system. We detected diacylglycerol lipase, monoacylglycerol lipase, and lysophosphate phosphatase enzymatic activities, which are responsible for maintaining the levels of endocannabinoid 2-arachidonoyl-glycerol (2-AG), a bioactive lipid mediator. The endocannabinoid system is a cellular signaling mechanism with a protective role in many pathophysiological processes, especially in the nervous system. 2-AG activates CB1 and CB2 GPCR receptors and triggers different signaling cascades, modulating intracellular Ca^{2+} levels, ERK1/2 phosphorylation, and other second messengers. Different studies have reported that CB1 is expressed not only in plasma membrane but also in intracellular compartments where they also seem to be functional. Therefore, the aim of this work was to study CB1 expression and function in isolated nuclei from rat cerebral cortex (CC). To this end, CC from Wistar rats were dissected, homogenized and highly purified nuclei (CCN) were isolated on a sucrose-density ultracentrifugation. CB1 protein expression was observed in CCN as well as in isolated cerebellum nuclei by Western Blot, which was confirmed by immunocytochemistry. In order to evaluate if CB1 is functional, CCN were incubated at 37 °C with a CB1 agonist (WIN 55-212-2) and ERK1/2 and Akt signaling cascades were studied by Western Blot. Interestingly, it was observed that ERK1/2 phosphorylation increased in nuclei treated with WIN 5 μM for 30 min with respect to controls ($p<0.01$) while no changes were seen in Akt phosphorylation. Taken together, these results demonstrate that CB1 has also a nuclear localization in the cerebral cortex where it could have a potential role in chromatin regulation and gene expression.

Keywords: CB1, nuclei, cerebral cortex

(1487) INSULIN SIGNALING EFFECTS ON 2-ARACHIDONOYLGLYCEROL HYDROLYSIS IN SYNAPTIC TERMINALS EXPOSED TO AMYLOID BETA OLIGOMERS

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2-arachidonoylglycerol (2-AG) behaves as a neuroprotective agent in Alzheimer's disease (AD). Aβ oligomers (OAβ) are responsible for the synaptic dysfunction observed in AD and were shown to disrupt the synaptosomal membrane and to diminish 2-AG availability. Insulin (Ins) is involved in synaptic plasticity and its signaling was shown to be downregulated in AD. OAβ can bind to insulin receptor (IR) and can, therefore, be internalized into neurons, while Ins prevents this binding and thus its neurotoxicity. Here, we aimed to study Ins preincubation effects on 2-AG hydrolysis in cerebral cortex synaptosomes (Syn) exposed to OAβ. To this end, Syn were isolated by differential centrifugation, purified in ficoll gradients, and preincubated with 10 μM LY294002 (phosphatidylinositol-3-kinase -PI3K- inhibitor) or 100 μM genistein (tyrosine kinase -TK- Inhibitor) for 10 min, and subsequently incubated with 0.2 mM vanadate (protein-tyrosine phosphatase inhibitor), 100 nM Ins, or 0.2 mM vanadate plus 100 nM Ins, for 30 min. Syn were then incubated for 10 min with or without 0.1 μM OAβ, and for 20 min with [^{3}H]monoacylglycerol, to assay 2-AG hydrolysis. It was observed that Ins and vanadate -either separately or coincubated- decreased 2-AG hydrolysis ($p<0.01$), and that their effect was not seen if Syn were preincubated with LY ($p>0.05$). On the other hand, in the presence of OAβ, while Ins and vanadate failed to alter 2-AG hydrolysis ($p>0.05$), LY increased this activity ($p<0.001$). However, the presence of Ins plus vanadate after incubation with LY could restore the activity ($p<0.001$) to basal levels in Syn treated with OAβ. Additionally, the presence of genistein previous to OAβ did not change the activity ($p>0.05$). Our results show a regulation of 2-AG hydrolysis by Ins, possibly decreasing its availability via IR and involving PI3K pathway, which is abolished by OAβ. The effect of OAβ appears to be independent of TK receptors and to involve PI3K activity.

Keywords: Insulin, 2-arachidonoylglycerol, Synaptic terminals, Amyloid β oligomers

ONCOLOGY-ONCOIMMUNOLOGY 9

(120) **ABERRANT MIRNAS EXPRESSION PROFILE INDUCED BY METABOLIC SYNDROME IN THE MAMMARY GLAND MIGHT BE CRITICAL FOR BREAST CARCINOGENESIS.**

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Abstract: Breast cancer (BrCa) is the most common malignant neoplasm and the leading cause of cancer female death in the world, excluding skin cancers. Metabolic Syndrome (MeS) is a risk factor for BrCa that and increase its aggressiveness and metastasis. Recently, we generated a MeS experimental model by chronically feeding mice with a high fat diet which induced alterations in the mammary glands such as an increase of postnatal development and prominent duct patterns. These ducts showed high expression of CtBP1, a tumor suppressor gene that is activated by low NAD+/NADH ratio. Moreover, we found that CtBP1 and MeS increased breast tumor growth and progression modulating the expression of 42 miRNAs involved in cell proliferation and tumor progression. The aim of this work was to identify the miRNA expression profile induced by MeS in normal mammary glands.

We selected a panel of miRNAs obtained from the miRNA microarray analysis to determine expression levels in samples of mammary tissue from mice with MeS or control using RT-qPCR stem loop metodology: miR-378a-3p, miR-146a-5p, miR-223-3p, miR-381-5p, miR-433-3p, miR-194-1-5p.We found that MeS significantly repressed the expression of miR-194-1-5p while induced miR-433-3p in mammary tissue. Using the bioinformatics tool ChemiRs, that integrates the information of ten miRNAs databases, we analyzed the molecular pathways modulated by these miRNAs. We found that miR-194-1-5p and miR-433-3p are involved in several molecular pathways including cancer, metabolism, developmental biology, adherent junction and apoptosis. Finally, evaluating microarray datasets from cBioPortal, we demonstrated that miR-194-1-5p presented DNA amplification in 20 % of BrCa patients.

Altogether, these results suggest that MeS induces an aberrant miRNA expression profile that could be critical in breast carcinogenesis.

Keywords: Breast carcinogenesis, metabolic syndrome, miRNAs

(1318) **N-TERMINAL DOMAIN OF cFOS AND FRA1: A NOVEL APPROACH TO INHIBIT BREAST TUMOR PROGRESSION**

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Breast cancer is the most common type of cancer and the leading cause of cancer death in women worldwide. Although early detection has improved survival, in less developed countries most cases are diagnosed at late stages when available therapies are not efficient. Highly proliferating breast tumor cells require high rates of phospholipid (pl) synthesis to support membrane biogenesis for their exacerbated growth. Both Fra-1 and c-Fos are overexpressed in breast tumors, contrasting with their undetectable levels in normal tissue and both promote pl synthesis by activating rate limiting enzymes such as CDP-DAG synthase (CDS) through a physical association with the activated enzyme. We have previously demonstrated that the basic domain of both Fra1 and cFos are involved in the activation of CDS whereas the N-terminal domain of Fra1/cFos physically associates with CDS. Herein we demonstrate using *in culture* experiments, that both N-terminal domains together inhibit the proliferation of the breast tumor cell line MDA-MB-231. *In vivo,* experiments in Balb/c mice bearing tumors generated with the 4T1 breast tumor cell line, revealed that the treatment with both N-terminal deletion mutants significantly decreased tumor growth rate. Moreover, inoculating 4T1 cells in both flanks of each mouse eliminated the possibility of a contribution of systemic effect on the results: One tumor was treated with both N-terminal domains and the other with vehicle. Tumors treated with the N-terminal domains grew to a significantly reduced tumor volume with a decreased tumor growth rate when compared with the contralateral vehicle-treated control present in the same mouse.

(829) **PROTON CHANNEL INHIBITION ALTERS CELL PROLIFERATION AND CELL CYCLE IN BREAST CANCER CELLS**

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Metabolic reprogramming of cancer cells conduces to a high production of acidic substances which must be extruded to maintain cell viability. Here, we studied the effect of 2-(6-chloro-1H-benzimidazol-2-yl)guanidine (ClGBI), an inhibitor of the proton channel Hv1, on proliferation and cell cycle of tumorigenic (MDA-MB-231 and MCF-7) and non-tumorigenic (MCF-10A) human breast cell lines. Concentration-response curve (0-20μM) and time-course (10-48h) were assayed to study cell proliferation (clonogenic assay), cell viability (MTT), cell cycle (flow cytometry) and mitotic cells (immunofluorescence using DAPI and anti-α-tubulin antibody). Results: ClGBI reduced the clonogenicity of MDA-MB-231, MCF-7 and MCF-10A cells in a concentration dependent way (IC50: 3.4±0.2, 2.6±0.7 and 6.0±0.4 μM, respectively), getting the maximal inhibition at 10μM ($p<0.001$ vs. C). This effect was partially reversed after removing the inhibitor only in MCF-10A cells. ClGBI 10μM reduced the viability of MDA-MB-231 (70±10% vs. C, $p<0.001$) and MCF-7 (60±15% vs. C, $p<0.001$) cells after 48h of treatment, and this effect was not reverted by removing the inhibitor. However, MCF-10A viability was not altered. Additionally, ClGBI 10μM increased the percentage of mitotic cells in MDA-MB-231 (3.7±0.7% vs. 0.8±0.2% present in control cells, $p<0.05$) and MCF-7 (4.9±1.2% vs. 0.7±0.1% present in the control cells, $p<0.05$) after 10h of treatment. This parameter was not affected in MCF-10A cells, even with a greater concentration (20μM) or time (48h) analyzed. Finally, 10h of exposure to ClGBI 10μM increased the percentage of cells in G2/M, in MDA-MB-231 (27.8±2.5% vs. 21.0±0.7% observed in the control cells, $p<0.05$) and MCF-7 (35.4±1.6% vs. 31.2±0.7%, p:ns) cells. Cell cycle distribution was not affected in MCF-10A cells. In summary, 10μM ClGBI alters viability, cell proliferation and cell cycle in tumorigenic human breast cells, without affecting these parameters in the non-tumorigenic cell line, MCF-10A.

Keywords: Proton channel, breast cancer, cell proliferation, cell cycle.

(307) **SEARCH OF VASOPRESSIN ANALOGS WITH ENHANCED ANTITUMOR ACTIVITY USING AN AGGRESSIVE LUNG CANCER MODEL: DRUG DESIGN BASED ON RATIONAL AND EVOLUTIONARY APPROACHES**

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The development of safe and effective medicines is a task that requires the interaction of different disciplines. Design of peptides with therapeutics properties based on an evolutionary approach from natural sources, and the rational design of drugs based on studies of structure-activity relationship (SAR) are widely used for the search of new compounds. Several tumors express vasopressin (AVP) receptors with contrasting effects depending on receptor subtype, While V1r are associated with stimulation of cellular proliferation, V2r is related to cytostatic effects. Desmopressin (dDAVP) is an AVP synthetic analog that acts as a selective agonist for the V2r with antitumor properties. The aim of this work was to compare new AVP analogs obtained by two different methodological approaches in a model of human SCLC. Besides confirming V2r expression by immunofluorescence and qRT-PCR, we showed that NCI-H82 cells respond to agonistic intervention of AVP receptors, and that cell growth inhibition or stimulation depends on ligand specificity by MTS assay ($p<0,05$). The evolutionary approach, paying special attention to the specific physiological activity of peptides, led us to design a new analog derivate from the beetle peptide INT, called dDINT. After evaluating all Alanine-substituted dDAVP derivatives, we demonstrated a close relationship between the amino acidic sequence belonging to the cycle of dDAVP and its cytostatic activity. As a result, we evaluated the new selective analog, [V^4Q^5]dDAVP, which shows increased cytostatic effect in comparison to dDAVP as well as newly-designed dDINT ($p<0.01$). Cancer cell growth inhibition induced for [V4Q5]dDAVP was abolished by V2r chemical blockade ($p<0,05$) or gene silencing ($p<0,01$) indicating that reduction of cell proliferation mainly results from V2r activation. Combination of these strategies could provide the basis for future studies for the development of improved compounds with potential therapeutic applications.

Keywords: drug discovery, SAR studies, evolution approach, anticancer, V2r agonists

(391) **SOLUBLE GUANYLYL CYCLASE ALPHA1 SUBUNIT IS INVOLVED IN CELL PROLIFERATION, SURVIVAL AND MIGRATION IN HUMAN ENDOMETRIAL TUMOR CELL LINE ECC-1**

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Soluble guanylyl cyclase is an heterodimeric enzyme catalyzing cGMP formation composed by two subunits, alpha1 (a1) and beta1 (b1). Previously we have shown that a1 increased levels strongly correlate with E2-induced proliferation in E2-depentent tissues and a1 knock-down decreased cell proliferation in endometrial and breast cancer cell lines. The aim of the present study was to investigate the role of a1 in proliferation, survival and migration in the E2-responsive endometrial tumor cell line ECC-1.

a1 expression was silenced through siRNA specific sequences using scramble sequences as control. Cells were incubated with or without 1 nM E2 for 48 h. Protein levels were measured by western blot. Apoptosis and mitosis were assessed by nuclear morphology (Hoechst). Cell cycle was studied by flow cytometry. Migration was determined through scratch motility assay.

a1 knock-down (a1KD) significantly reduced cell proliferation markers' expression both in presence and absence of E2 (PCNA as

% of control (C), E2: 151.6±7.2*, a1KD:55±10*, a1KD+E2:93±11#; cyclin D1, E2:141.2±12.9*, a1KD: 33.3±18.2*, a1KD+E2: 40±15#; cyclin E, E2:127.6±11.6*, a1KD:29.8±14.2*, a1KD+E2:28±17#; *p<0.05 vs C, #p<0.05 vs E2). a1KD produced an arrest of cells in G0/G1 phase and augmented (2-fold increase) the percentage of hipodiploid cells. Nuclear staining confirmed that a1KD decreased mitotic index (C:2.27±0.03, E2:5.96±0.04**, a1KD:0.68±0.16**, a1KD+E2:0.44±0.24##) and increased apoptotic index (C:1.14±0.36, E2: 0.84±0.01, a1KD: 5.62±0.87**, a1KD+E2:3.87±0.75#, **p<0.01 vs C, #p<0.05, ##p<0.01 vs E2). Additionally, a1KD dramatically inhibited cell migration (8-fold decrease vs C).

Our results show that a1 participates in cell proliferation, survival and migration of ECC-1 cells and suggests that a1 not only can mediate E2 procarcinogenic effects but also can be involved in hormone-independent tumor progression.

Keywords: soluble guanylyl cyclase a1 subunit, estrogen, proliferation, migration

(1030) **SPHINGOSINE-1-PHOSPHATE PROMOTES A MIGRATORY PHENOTYPE OF MELANOMA CELLS IN HYPOXIA**

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IMIBIO-SL

Melanoma is the most aggressive type of skin cancer with a high mortality percentage. Half of melanoma patients display the mutation V600E in the BRAF protein kinase. Indeed, FDA and ANMAT have approved the use of Vemurafenib and Dabrafenib (BRAF inhibitors) in melanoma patients. Unfortunately, patients develop resistance after a short period of disease control, indicating that new targets are needed. In that regard, several evidences confirm that tumor microenvironment modulates proliferation, migration and acquired resistance of cancer. Precisely, hypoxia is a remarkable feature of the tumor microenvironment and controls cancer growth and progression. On the other hand, the bioactive sphingolipid sphingosine-1-phosphate (S1P) has been linked to multiple mechanisms leading to chronic inflammation and cancer. Thus, the aim of this study was to elucidate how hypoxia and S1P influence the viability and migration of melanoma cells. To this end, we used two melanoma cell lines: SkMel2 ($BRAF^{WT}$) and Lu1205 ($BRAF^{V600E}$). Viability was studied by MTT and migration analyzed by Wound Healing Assay. Hypoxia augments the viability and reduces apoptosis of melanoma cells cultured in serum-withdrawal conditions but does not affect cell migration. On the other hand, S1P has no effect on cell viability in hypoxia, but stimulates the migration of Lu1205 melanoma cells through engagement of S1PR1 and S1PR3 receptors, suggesting that the sphingolipid promotes a migratory and invasive phenotype when oxygen levels and nutrients availability are low. In addition, inhibition of BRAF with Vemurafenib diminishes S1P-induced migration in hypoxia. Altogether, these results suggest that hypoxia protects melanoma cells from the apoptosis induced by growth factor's shortage. In addition, the presence of S1P in the tumor microenvironment is critical for establishing a migratory phenotype on melanoma cells.

Keywords: melanoma; hypoxia; S1P; BRAFV600E mutation; migratory phenotype

(1440) **TNFα BLOCKADE OVERCOMES TRASTUZUMAB AND PERTUZUMAB RESISTANCE IN HER2 POSITIVE BREAST CANCER**

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HER2 positive (HER2+) is a subtype that affects 13-20% of breast cancer (BC) patients. They receive trastuzumab (T), an anti-HER2 monoclonal antibody, but resistance events hamper its clinical benefit in 40-60% of the cases. We demonstrated that TNFα overexpression turned T-sensitive cells and tumors into resistant ones by upregulating mucin 4 (MUC4) expression. Pertuzumab (P, a monoclonal antibody that disrupts HER2/HER3 dimerization) is another anti-HER2 therapy that is used in combination with T. The aim of this work was to explore whether TNFα and TNFα-induced MUC4 expression play a role in the resistance to the combination therapy T+P. Our approach consisted in blocking TNFα, either with Etanercept (E) or the dominant negative protein XPro™1595 (DN) in JIMT-1, *de novo* T+P resistant cell line which produces TNFα. We established JIMT-1 tumors in female nude mice to explore whether TNFα blockade overcomes T+P resistance. Animals were treated with 5 mg/kg of IgG, P, T+P, 10 mg/kg of DN or P+T+DN i.p. twice a week. The combination of T+P+DN inhibited tumor growth vs. T+P or P+DN (p<0.05). Proliferation of cells treated with IgG, T, P, DN, E (10 μg/ml, 10 μg/ml, 10 μg/ml, 2 μg/ml and 5 μg/ml respectively) as monotherapy and in different combinations was evaluated by cell count or [^{3}H]-thymidine incorporation. The combination of TNFα blockade with T+P inhibited cell proliferation vs. IgG, P+T, P+E, P+DN (p<0.0001). In spite of these results, indirect immunofluorescence and flow cytometry analysis proved that P binding was lower in E and DN treated cells vs. IgG treated cells (p<0.01). Similar results were obtained using MUC4 siRNA, suggesting that MUC4 has a controversial role in T+P resistance, which is being investigated. These results suggest that TNFα blockade could overcome T+P resistance in HER2+ BC. Moreover, patients with expression of TNFα and MUC4 could be eligible for a combination therapy with TNFα-blocking agents to overcome/avoid resistance to therapy.

Keywords. TNFα, breast cancer, trastuzumab, pertuzumab, MUC4

(1482) **POSSIBLE ASSOCIATION OF LOW GLYCOLYTIC PHENOTYPE WITH BRAFV600R MUTATION IN MELANOMA CELLS**

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Abstract: Altered metabolism of cancer cells favors not only proliferation but also therapy resistance. BRAF mutation represent the most prevalent alteration in melanoma patients being V600E mutation more frequent than V600R. Sensitivity to BRAF inhibitors seems to be related to glycolysis dependence. The aim of the present work was to characterize the metabolic phenotype of six BRAF mutated human melanoma cells lines, five V600E subtype (hM1, hM2, hM9, hM10 and A375) and one V600R (hM4). We studied glucose consumption, lactate production, LDH and G6PDH activities (commercial kits), GLUT-1 (WB) and extracellular pH. Interestingly, we found that hM4 cell line presented not only lower glucose consumption and GLUT-1 expression, but also lower LDH and lactate production in parallel with significant higher pH than the other assayed melanoma cells lines. Our data suggest that V600R mutation drives melanoma cells to a lower glycolytic phenotype than V600E mutation.

Keywords: *glucose, lactate, GLUT-1, BRAFV600E, BRAFV600R*

(719) **ANALYSIS OF TUMORAL SPHERES GROWING IN A MULTICHAMBER MICROFLUIDIC DEVICE**

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Introduction: Lab on a Chip (LOC) farming systems have emerged as a powerful tool for single cell studies combined with a non-adherent cell culture substrate and single cell capture chips for the study

of single cell derived tumor spheres. Furthermore this systems have the additional advantage of using low amounts of reagents and cells. Cancer is characterized by its cellular heterogeneity where only a small population of cancer stem cells (CSCs) are responsible for tumor metastases and recurrences. Thus, the *in vitro* strategy to the formation of a single cell-derived sphere is an attractive alternative to identify CSCs. The objective of this study, was to test the effectiveness of microdevices in order to analysis the heterogeneity within CSC populations and its interaction with different components of the extracellular matrix, using MB49-I bladder cancer cell line. The results show the usefulness of LOC as an effective method for quantification of CSC, through the formation of spheres under low adhesion conditions, establishing 32 MB49-I/ul as the optimum number of seeding cells. The device allowed the tracking of single cells derived spheres, observing different patterns of growth, resulting in large, medium, and small spheres, and the further identification of Oct-4 and CD44, CSC markers by immunofluorescence. Furthermore, we observed that spheres were able to grow in collagen IV, but no in matrigel. In conclusion, LOC devices have not only the already known advantages, but they are also a promising tool since they use small amounts of reagents and are under specific culture parameters. LOC devices could be considered as a novel technology to be used as a complement or replacement of traditional studies on culture plates.

Keywords: Lab on a Chip; Cancer Stem Cell; Tumoral sphere; Oct-4; CD44

ONCOLOGY-ONCOIMMUNOLOGY 10

(484) A CELL-BASED SCREENING TARGETING THE HUMAN KINOME REVEALS A NOVEL SYNTHETIC LETHAL INTERACTION WITH BRCA1

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One of the current challenges in the fight against cancer is the development of therapeutic approaches with selective tumor cytotoxicity. A precision strategy of this kind is the induction of synthetic lethality, which takes advantage of tumor mutations to induce cytotoxicity in tumor cells with a reduced impact on normal cells.

Mutations in BRCA1 and BRCA2 genes have been established as drivers of breast and ovarian cancer development, and therefore constitute an interesting niche to search for novel synthetic lethal interactions. Herein, we developed a high-throughput screening platform based on flow cytometry to identify new synthetic lethality inducers in BRCA1 and BRCA2-deficient contexts. This platform involves the co-culture of BRCA1/2-deficient and proficient cells from isogenic origin that are tagged with different fluorescent proteins. The isogenic culture is exposed to the compounds from the libraries and the relative cell survival of each cell population is assessed at the end of the experiment. Using this platform, we screened a library of 684 protein kinases inhibitors and we identified a mitotic kinase that is synthetic lethal with BRCA1. We describe here the screening platform, the early validation phase using different cell lines and commercial inhibitors, and the preliminary study of the mechanism that triggers the synthetic lethality induction in BRCA1-deficient cells.

Keywords: Synthetic Lethality, BRCA1/2, Breast Cancer, cell-based screening, mitotic kinases.

(1523) ANTITUMORAL EFFECTS AND CALCEMIC ACTIVITY OF THE NOVEL ANALOGUE OF CALCITRIOL ML-344.

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The active form of vitamin D_3, calcitriol, has been shown to display antitumoral effects on various types of cancer. However, hypercalcemia is observed when effective antitumoral doses of calcitriol are employed, thus precluding its therapeutic application. In collaboration with the laboratory of Organic Chemistry of the University of Vigo we synthesized an analogue of calcitriol, called ML-344, in order to obtain a compound that retain or even increase the antitumoral activity but prevent the side effects. In this work we aimed to evaluate the biological effects of ML-344 by employing in vitro, in vivo and in silico assays. We demonstrated that the analogue exerts a significant decrease in the viability of different cancer cell lines ($p< 0.001$): head and neck squamous cell carcinoma (HN12), glioblastoma multiforme (GL26 and U251) and breast adenocarcinoma (4T1 and LM3) cells. Importantly, the cellular viability of human primary astrocytes and murine non-malignant mammary epithelial HC11 cell line is not affected after ML-344 treatment. Also, the analogue retards cell migration of 4T1 and LM3 cells (16 h: $p< 0.001$; 21 h: $p< 0.01$) while it does not affect HC11 cell motility. In concordance with the antimigratory effects, ML-344 decreases LM3 invasive capacity ($p< 0.01$) and induces a rearrangement of actin cytoskeleton by a reduction in the amount of cells with stress fibers ($p< 0.001$). In vivo studies showed that the analogue, in contrast to calcitriol, does not cause hypercalcemic effects in CF1 mice administrated daily at 5 μg/Kg of body weight during 96 h. In addition, hematocrit remained within the normal levels and no changes in body weight were found. Finally, computational studies showed that ML-344 is able to bind to VDR with greater affinity than calcitriol (ΔG= -82.5 and -73.5 Kcal/mol, respectively). Altogether, these results suggest the potential use of this analogue as an antitumor drug with a differential effect between tumor and non-malignant cells.

Keywords: Calcitriol, Analogue, Antitumoral, Neoplasms, Therapeutics.

(1479) COMPANION ANIMALS MELANOMA CELLS: A USEFUL MODEL FOR THE *IN VITRO* STUDY OF CHEMO-GENE THERAPY

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Abstract: The aim of the present work was characterize two canine (*Bsk* and *Rk*) and two feline (*Dc*, *Rn*) melanoma cell lines derived from spontaneous tumors, evaluate their sensibility to chemo-gene treatments and compare it with the *in vivo* response. We evaluated the cytotoxicity of bleomycin (BLM) alone, or combined with HSV thymidine kinase/ganciclovir suicide gene (SG) system or interferon-β gene (IFNβ) lipofection on these cell lines cultured as monolayers (mnl) or spheroids (sph). The *in vivo* behavior after SG therapy (final tumor volume: *Dc* 65±5%, *Rn* 110±5%, *Bsk* 46±2%, *Rk* 43±3%) was reflected by the treated spheroids of their derived cell lines (*Dc* 67.5±4.6%; *Rn* 100.5±8.7%; *Bsk* 27.1±2.4%; *Rk* 39.3±3.6%), confirming the behavioral similarity between *in vivo* tumors and their *in vitro* models. BLM enhanced the individual effects of both genes in feline lines (mnl and sph), and the cIFNβ effects in canine lines (mnl) ($p<0.05$). Interestingly, the colony forming capacity (CFC) of *Dc*, *Bsk* and *Rk* survivor cells almost disappeared after 3 days of BLM alone or combined with gene treatments ($p<0.001$), while *Rn* cells

only showed a small decrease ($p<0.05$). Accordingly, this cell line displayed the most resistant phenotype and was enriched in cells with higher CFC, by producing respectively 2-, 2- and 4-fold more colonies in soft agar; and 3-, 3- and 7- fold more adherent colonies than *Dc*, *Bsk* and *Rk* ($p<0.005$). Consistent with the highly aggressive and advanced *in vivo* tumor, *Rn* derived cell line, displayed the greatest proportion of cells with lower basal ROS levels, lower size and higher complexity. All these characteristics have been associated with a pluripotent/stem cell phenotype. These results support companion animals' cells as alternative promising models for optimizing and predicting the *in vivo* response of their respective tumors to therapeutic strategies, and encourage further studies to evaluate the clinic potential of these combined treatments.

Keywords: melanoma, HSV-thymidine kinase, interferon-β, bleomycin, spheroids

(119) **DETECTION OF CIRCULATING TUMOR CELLS IN PATIENTS WITH BREAST CANCER THROUGH EVALUATION OF THE TWIST1 GENE EXPRESSION**

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The mortality in women with breast cancer is mainly due to the development of metastasis. The reason is the spread of tumor cells to distant organs when they are released into the bloodstream, known as circulating tumor cells (CTCs). Some CTCs overexpress the TWIST1 gene, which is associated with the epithelial-mesenchymal transition of tumor cells facilitating metastasis. The objective of this study was to detect CTCs in blood of breast cancer patients by analyzing the expression of TWIST1. The results were correlated with prognosis factors for breast cancer. Blood samples were obtained from breast cancer patients (n=36) and healthy donors (n=14). Nucleated cells were isolated, and RNA was extracted. After a retrotranscription, real time PCR for TWIST1 and glyceraldehyde 3-phosphate dehydrogenase (control) genes was performed. The expression of TWIST1 was analyzed using the method $2^{-(\Delta\Delta Ct)}$, comparing with the expression in healthy controls. Seventeen percent (6/36) of patients presented increased levels of TWIST1 expression up to 6 times respect to controls. The patients who showed the highest levels of TWIST1 expression, in addition, presented local and distant metastasis. Analysis of correlation by the Fisher test between the TWIST1 gene expression and the presence of estrogen and progesterone receptors, the overexpression of HER2/neu, the presence of metastatic nodes, the tumor size, the histological grade and the nuclear grade, did not indicated significant associations ($p > 0.05$) with the different prognosis parameters analyzed. The increase in the TWIST1 expression would indicate the presence of CTCs in epithelial-mesenchymal transition, and could suggest an increased risk of metastasis development. The level of expression of TWIST1 in blood of patients with breast cancer would be an independent parameter of other prognosis factors. Therefore, the detection of TWIST1 expression could provide additional prognostic information.

Keywords: circulating tumor cells, TWIST1, breast cancer.

(1665) **DRUG REPOSITIONING FOR COLON CANCER TREATMENT: EXPLORING THE PUTATIVE USE OF METFORMIN AND PROPRANOLOL**

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Drug repositioning or repurposing in oncology refers to the use of drugs originally formulated for other indications that showed antitumor potential. In this work we made a screening of repurposing drugs that included metformin (M; diabetes treatment), propranolol (P; indicated to treat hypertension), chloroquine (malaria treatment or prevention), DHEA (sexual hormones precursor), orlistat (obesity treatment), atorvastatin (cholesterol treatment) and dichloroacetate (a pyruvate dehydrogenase kinase inhibitor). The antitumor potential of these drugs was evaluated *in vitro* with HCT116 human colon cancer cells through standard viability assays, either individually or in a combined manner. All the tested drugs had the potential to inhibit significantly HCT116 cells proliferation in a dose-dependent manner. From the combinations tested, M+P resulted attractive as it showed a strong growth inhibition even combining low doses of both drugs ($P<0.001$). Also, preliminary data of an *in vivo* experiment with BALB/c mice under a standard azoxymethane (initiating carcinogen)/dextran sulfate (promoting agent) carcinogenesis regimen indicated a potential benefit of M+P combination in the prevention of colon tumors development, with no associated symptoms of toxicity. Altogether, our results suggest that therapy with repositioned drugs might be of interest for colon cancer treatment and, in particular, the combination of M+P could inhibit colon cancer development. These data prompt us to carry out new experiments to validate our hypothesis and corroborate our preliminary results.

Keywords: drug repositioning, colon cancer, metformin, propranolol

(236) **HEME-OXYGENASE 1 DRIVES THE METABOLIC FATE IN PROSTATE CANCER**

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Prostate cancer (PCa) is the second leading cause of cancer-associated death in men. Energetic metabolism alterations have become a new hallmark of cancer, since variations in a single gene can orchestrate changes in metabolic pathways and confer an adaptive advantage. Heme-oxygenase 1 (HO-1) exerts an antitumoral role in PCa inhibiting proliferation, migration, tumor growth and angiogenesis. The aim of this work was to assess the role of HO-1 in the metabolic signature of PCa.

Through RNA-Seq we found a set of metabolic genes deregulated under pharmacological induction (hemin treatment) or genetic induction of HO-1 in PC3 cells. STAR and ATP5L2 were upregulated, while HMGCS2, PRODH and ACOT12 were downregulated. These genes encode for steroid hormone metabolism, ATP synthesis, ketogenesis, proline and lipid metabolism. The analysis of the deregulated genes by Gene Ontology revealed alterations in several metabolic pathways such as steroid, proline and lipid metabolism, and ATP synthesis. Functional analysis highlighted a decrease in oxygen consumtion rate and ATP production under hemin treatment. Furthermore, HO-1 induction led to a decrease in the extracellular lactate levels.

Bone is the only site of PCa progression, and bone cells are able to produce factors that favor progression. However, the molecular nature of this interaction remains elusive. Our results performed on co-cultures of PC3 cells (treated or not with hemin) with Raw264.7 (pre-osteoclastic) or MC3T3 (pre-osteoblastic) cells demonstrate that HO-1 directs the metabolic fate of bone precursor cells due to the deregulation of glycolytic genes. HO-1 induction in PC3 cells downregulated PKM2 and LDHA expression in co-cultured Raw264.7 and MC3T3 cells ($p<0.05$).

Based on our results, we propose HO-1 as a key regulator of the metabolic status of PCa cells and a powerful mediator capable of redefining the metabolic signature of bone precursor cells, favoring the establishment of a less aggressive phenotype.

Keywords: prostate cancer, metabolism, Heme-oxygenase 1

(600) **MIFEPRISTONE AS A MODULATOR OF THE MULTIDRUG RESISTANT PROTEIN 1 ON THE BLOOD BRAIN BARRIER TO INCREASE THE EFFICACY OF DOXORUBICIN ON BRAIN METASTASES**

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The presence of multidrug resistance efflux transporters, such as the P-glycoprotein (P-gp) on the blood brain barrier (BBB), limits the efficacy of chemotherapeutic agents on brain tumors or metastases. The antiprogestin mifepristone (MFP) is known to inhibit P-gp activity on tumor cells. We hypothesize that MFP treatment improves the efficacy of Pegylated doxorubicin liposomes (doxo) on brain tumors. We used the murine mammary carcinoma C4-2-HI whose growth is not inhibited by MFP treatment. Tumor cells ($2x10^{5}$) were injected into the brain of BALB/c-GFP mice using a stereotactic frame. Treatment with MFP (6 mg pellets, sc) and /or two weekly doses of doxo (4,5 mg/kg, iv) was initiated 10 days after cell inoculation. We analyzed the results by fluorescence microscopy, measuring the tumor volume on brain slices after DAPI staining. **Confirming the hypothesis,** a significant decrease in tumor size was only observed in mice treated with MFP and doxo ($p<0,05$). This did not occur when tumors were transplanted sc. In order to confirm the inhibitory effect of MFP on P-gp activity, HepG2 hepatocarcinoma cells, which express high P-gp levels, were treated with doxorubicin (20 µM) with or without MFP (10 µM) for 60 or 120 min. The presence of doxorubicin in cell lysates was quantified measuring fluorescence intensity. We observed an increase in doxorubicin intracellular retention in MFP-treated cells ($p<0,05$). Similar results were obtained in a preliminary experiment using HUVEC cells that are part of the hematoplacentary barrier, thus sharing similar features as BBB endothelial cells.

Our data suggest that MFP may be a promising agent to increase the effectiveness of chemotherapeutic agents on brain tumors, regardless of progesterone receptor expression. The participation of the P-gp mediating this effect needs to be further confirmed.

Keywords: mifepristone, P-glycoprotein, blood-brain-barrier, brain metastases, chemotherapy.

(1303) **NOVEL HSP90-PHARMACOLOGICAL INHIBITORS IN PROSTATE CANCER**

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The heat shock protein of 90 KDa (Hsp90) is known to be associated with many of so named hallmarks of cancer. Hsp90 is the major soluble protein of the cell and interacts with nearly 10% of the eukaryotic genome, acting as a heterocomplex. This molecular chaperone is related to the activity and stability of many oncoproteins, thus tumor cells evade death programs. Tumor cells are thought to be “addicted” to Hsp90 and the inhibition of the chaperone ATPase activity shows strong antitumor effects. Consequently, Hsp90 inhibitors seem to be interesting chemotherapeutic agents. However, side effects are still an important concern. Geldanamicyn, a known Hsp90 ATPase inhibitor, has shown concerning side effects such as nephrotoxicity and hepatotoxicity. Previous studies exhibit that some Schiff bases like imines and azometines showed cytotoxic properties on tumor cells, and some showed only a moderated effect on the Hsp90 ATPase activity. In this work, we studied novel drugs designed and analyzed by *in silico* molecular docking simulations. Then, the effects on the ATPase activity of *in vitro* Hsp90 was studied and with these results viability of prostate cancer cells, and inhibitory action on GR and AR nuclear translocation were assessed. Geldanamycin (GA) was always used as a control of all tests. A total of 20 drugs were tested (named as 4a to 5j) and although the effects on the ATPase activity of the drugs were similar to those observed for GA, as the *in silico* analysis predicted; the effects on cell viability showed no relation with the drug ATPase inhibitory capability. This finding support our previous works that hypothesized that the Hsp90 ATPase activity is not completely correlated with its biological activity. The study here presented provides novel insights to design more active and less toxic drugs with promising future perspectives.

Keywords: Hsp90, ATPase, Synthetic Compounds, Prostatic cancer

(811) **ROLE OF THE ACTIN CYTOSKELETON IN THE FORMATION OF OVARIAN CARCINOMA TUMORAL SPHEROIDS *IN VITRO***

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Epithelial ovarian cancer is highly proliferative and metastatic. During the dissemination, exfoliated ovarian tumor cells are carried via peritoneal fluid to secondary sites in the abdominal cavity where they survive like individual cells or attach themselves forming tumoral spheroids. We propose that the actin cytoskeleton and the activation of the ERM proteins are key to ensuring cell survival, proliferation and tumor formation in the peritoneal fluid.

The *in vitro* model of cell spheroids formation in soft agar was used since is one of the most well characterized models for 3D culture due to its simplicity, reproducibility, and similarity to peritoneal metastasis model. The cell lines SCOV3 (epithelial ovarian cancer) and HeLa were cultured at different densities on 0.5% soft agar. After 5, 10, 15 and 20 DIV (days *in vitro*) the diameter of the spheroids was measured. Survival, proliferation and number of cells per spheroid were analyzed by immunostaining with DAPI, PhosphoHistone3, Tubulin and Phalloidin. WB and IF were used to observe expression levels and distribution patterns of the activated ERMs proteins.

HeLa cells spheroids were significantly large in size and more compacted compared to SCOV3 cells spheroid after 20 DIV. Cell survival and proliferation decreased after 15 DIV. PhosphoERM was distributed in the peripheral layer of the HeLa cells spheroids, whereas in SCOV3 spheroids showed cortical distribution around each cell forming the spheroid.

In this work the experimental conditions were standardized for the formation of cellular spheroids in soft agar for the analysis of peritoneal metastasis. It could be observed that the ERM proteins in SCOV3 cells probably play a structural role, guaranteeing the survival and stability of the spheroid in a different way to HeLa cells.

Keywords: Epithelial ovarian cancer, cells spheroids, actin cytoskeleton, ERM proteins.

(1570) **CHLOROQUINE: REPOSITIONING OF A MALARIA DRUG FOR COLORECTAL CANCER TREATMENT NEW DATA FROM A CHEMICALLY INDUCED INTESTINAL TUMORIGENESIS MODELS**

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Colorectal cancer (CRC) is the second leading cause of cancer-related death in Argentina. The application of mouse models or study of patient disease development is the gold standard to the identification and pre-clinical validation of novel therapeutic targets in colorectal cancer as well for the search for early disease biomarkers. However, it have been suggested that preclinical xenograf rodent-based tumor models are not predictive of human clinical outcomes for this reason we have established an animal tumor models that are induced by exposure to chemical carcinogens (IEC models) like azoxymethane (AOM) and dextran sulfate sodium (DSS) treatment in mice that may offers a powerful model rather xenograf models in the characterization of the initiation of aberrant crypt foci (early lesions) and in the evaluation of CRC chemopreventive strategies.

This study was performed to investigate the anticancer effect and the tumor reverting capacity of Chloroquine on AOM/DSS induced CRC in Balb/c mice. We induced colorectal tumors in male mice and evaluated the effects of Chloroquine

treatment after week 11 of induction on tumor tissue morphology. By using histology, Haematoxylin and Eosin staining and TEM, we confirmed the establishment of tumour and aberrant crypt foci ultra structure by week 10 and 20 from the induction. The administration with Chloroquine from week 11 significantly decreased the incidence of tumor formation and inflammation by week 30 (P<0,01), as well as it results in reverting metabolic status. This was confirmed by microscopy, H&E staining and *Periodic acid–Schiff* (*PAS*) to detect polysaccharides and glycoproteins, glycolipids and mucins in tissue. Our findings suggest that Chloroquine is one of the candidates not only for the prevention of inflammation-associated colon carcinogenesis as other previous results have shown in xenograf model but for treatment in a well established cancer tissue.

Keywords: Chloroquine, Balb/c, colorectal cancer, Induced AOM/DSS model, 5-FU.

GENETICS AND MOLECULAR BIOLOGY 9

(607) DISSECTING THE ROLE OF CRL4^{CDT2} UBIQUITIN LIGASE IN THE MAINTENANCE OF CHROMATIN STABILITY

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The formation and progression of cancer is ultimately regulated by the abundance and activity of both oncogenic and tumor suppressor proteins. The Ubiquitin-Proteasome System (UPS) consists of a complex network of enzymes where the E3 ligases are the final effectors and dictate the specificity of the UPS machinery. CRL4^{Cdt2}ubiquitin ligase is emerging as a master regulator of cellular proliferation involved in multiple DNA repair processes. Different lines of evidence demonstrate that alterations in the expression and activity of CRL4^{Cdt2} induce genomic instability. Therefore, it is not surprising that Cdt2 protein levels are increased in many tumor cells and its expression correlates with tumor grade, metastasis and poor survival. In order to broaden our understanding on how deregulation of CRL4^{Cdt2} might contribute to cancer development, we used an affinity purification and mass spectrometry approach to identify and characterize novel CRL4^{Cdt2} substrates. Hek293t cells were transfected with Cdt2 containing vector (Cdt2) or empty vector (pCDNA) and were treated with different DNA damage agents (Hydroxyurea, Camptothecin and UVC), alone or in combination with the proteasome inhibitor MG132 and an inhibitor of NAE1 (MLN4924). All known CRL4^{Cdt2} substrates are ubiquitinated when they are bound to PCNA, therefore,we focused our attention on chromatin fraction and we identified several novel putative Cdt2 interactors. Among the most abundant putative Cdt2 interactors identified, we centered our work on different members of a multi-protein complex implicated in the maintenance of chromatin structure, gene transcription and DNA replication. These proteins play an important role as barriers of cancer stem cell self-renewal and thus the understanding of their functional interaction with CRL4^{Cdt2} might unveil new potential therapeutic point of intervention in cancer treatment.

Keywords: Ubiquitin-Proteasome System, E3 ligases, CRL4^{Cdt2}.

(136) EFFECT OF RETINOIDS AND LAPATINIB TREATMENTS ON DIFFERENTIATION AND METASTATIC CAPACITY OF CANCER STEM CELLS IN A MURINE MAMMARY TRIPLE NEGATIVE CELL LINE

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Abstract: Cancer stem cells (CSC) are resistant to chemotherapy and radiation and they are also considered as “metastasis seed”.

In order to suggest CSCs as a new therapeutic-intervention target, in this work we propose to study the effect of retinoid ATRA and HER2 inhibitor Lapatinib treatments on:

A) Expression profile of pluripotent genes, retinoid receptors system and E-Caherin levels.

B) *In vitro* invasive capacity and *in vivo* metastatic potential.

For this purpose, the triple negative murine cell line 4T1 (tumorigenic and metastatic in BALB/c mice and expressing HER2 in its CSC component) was used.

Through RT-qPCR, we could observe that ATRA (1μM) and Lapatinib (1μM) treatments, separately or in combination, were able to increase both RARα and RARγ expression and decrease RARβ receptor levels. Moreover, the same treatments induced an increment in E-Cadherin and reduced the expression of the main pluripotential genes: Nanog, OCT4 and SOX2. Regarding parameters associated with malignant progression using Matrigel-coated transwells, we observed that ATRA treatment increased CSC invasive capacity, however in experimental metastases assays with pretreated-CSC, this retinoid significantly decreases lung colonization.

The treatments with both ATRA and Lapatinib were able to induce CSC differentiation and reduced their lung nesting ability, leading to a less malignant phenotype.

Keywords: cancer stem cell, triple negative mammary cancer, metastasis.

(1336) STARD7 DEFICIENCY MODULATES THE EXPRESSION OF EXTRACELLULAR MATRIX ASSOCIATED-PROTEINS IN HTR8/SVNEO CELLS

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StarD7 transcript encodes an intracellular lipid transport protein, a member of the START domain superfamily, which is involved in many physiological processes. It facilitates the delivery of phosphatidylcholine to the mitochondria and previous results indicated that StarD7 knockdown decreases ACBG2 multidrug transporter level, cell migration, proliferation, and phospholipid synthesis. Additionally, we reported that its suppression promotes reticulum endoplasmic stress and ROS production. Here, we examined the effect of StarD7 silencing on several extracellular matrix (ECM) associated-proteins in HTR8/SVneo cells derived from human first-trimester. Data from qPCR, western blot and immunofluorescence analysis demonstrated a significant increase in the mRNA and protein levels of integrin α5 in the HTR8/SVneo cells transfected with StarD7 siRNA compared to control siRNA. Additionally, a clear increase in the transcript level of integrin β1, as well as in the mature β1 and integrin α1 proteins were detected. Furthermore, StarD7 silencing leads to an increase in β-catenin and nidogen-1 at both protein and mRNA levels, as well as in the amount of MMP9 secreted to the culture medium. Collectively, our studies indicate that StarD7 depletion causes a dysregulation in several ECM-associated proteins suggesting that beyond its role in lipid transport, StarD7 contributes to maintain cellular homeostasis. Supported by FONCyT, CONICET, SECyT-UNC

Keywords: ECM, placenta, extravillous trophoblasts, StarD7;

(510) USE OF SHRNA SCREEN TO IDENTIFIY UBIQUITIN RELATED GENES INVOLVED IN THE REGULATION OF TUMOR-CELL MIGRATION AND INVASION.

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The development of metastasis foci in patients suffering from cancer represents a significant reduction in their survival and life quality. The Ubiquitin-Proteasome System (UPS) plays a fundamental role in the maintenance of protein homeostasis both in normal and stressed conditions, thus regulating almost every single cellular process. Since alterations in the ubiquitination cascade have been shown to be associated with malignant transformation, invasive

potential of cells and metastasis, we sought to investigate the role of the UPS in the regulation of tumor-cell migration and invasion. To this end we performed a genetic screen using a shRNA library against UPS associated genes, and Boyden chambers. After the selection process, we characterized the non-migrating cell population and determined the relative abundance of each shRNA by *Illumina* NGS sequencing. We obtained a list of 30 candidate genes, half of which had already been associated with regulation of migration/invasion/tumorigenesis processes or metastasis. Among the candidates, we focused on a specific DUB and demonstrated that its silencing reduces the migratory/invasive potential of different tumor-cell lines using Boyden chamber ($p<0.05$), wound healing ($p<0.05$) and agarose drop invasion assays ($p<0.05$). *In vivo* studies demonstrated that NOD/SCID mice inoculated with silenced tumor cells present a delay in the onset of the tumor formation, compared to the tumors generated by control cells ($p<0.001$). In addition, our results also show a significant impairment in the generation of metastatic foci ($p<0.05$). Altogether, these findings demonstrate that shARN screens using Boyden chambers are useful for finding novel genes that regulate migration and invasion, which might represent novel therapeutic targets for the development or improvement of cancer treatments.

Keywords: screen, migration, UPS, DUB.

(1833) **ALTERED STRESS GRANULES DYNAMIC MODULATES CELL FATE IN BORTEZOMIB-TREATED HUMAN GLIOMA CELLS**

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Among different human glioma cells, we determined a differential susceptibility to Bortezomib treatment (BT) that correlates with variable expression levels and subcellular localization of arginylated calreticulin (R-CRT), together with ER stress induction. Those cells (MO59K) that are resistant to BT showed a small variation of R-CRT, which appears confined to increased stress granules (SGs) formation and sharp ER stress. Whereas those cells (HOG) that are susceptible to BT showed intracellular increased levels and plasma membrane enrichment of R-CRT, where it participates in a caspase 3 dependent pro-apoptotic signaling, after strong ER stress induced by the drug. Co-treatment of HOG cells with BT and Tannic Acid (TA, Ate1 inhibitor) reverts the cytotoxicity shown by BT alone. Since the formation of SGs is associated with a greater resistance to chemotherapy, we evaluate how its dynamism is affected in both HOG and MO59K. Firstly, by immunofluorescence, we determined that HOG exposed to both BT and TA showed an increase of SGs formation in agreement with enhanced resistance of these cells to BT, change that is not shown by co-treated MO59K. However, the later showed the formation of smaller SGs after co-treatment. Secondly, as autophagy is known to degrade SGs, we also evaluate whether it is induced in both cell types during BT. After BT treatment MO59K showed a clear induction of LC3 II. By contrast, a strong activation of autophagy was shown by HOG even in control condition (non-treated cells), what may correspond with a reduced number of SGs in these cells. Hence, the increased susceptibility of HOG cells to BT is associated with a markedly active autophagy and robust induction of ER stress, which strongly promotes arginylation of CRT and R-CRT exposure at plasma membrane. By contrast, in MO59K cells a controlled induction of both autophagy and ER stress maintains reduced levels of R-CRT that mainly associates with SGs, as a hallmark of BT resistance.

Keywords: arginylation, stress granules, bortezomib, ER stress

(1425) **OXIDATIVE AND GENOTOXIC EFFECTS OF MONOTERPENES IN HUMAN NON-SMALL CELL LUNG CANCER A549 CELLS**

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Monoterpenes (Mts) are volatile organic compounds mostly found in the essential oils (EOs) of citrus fruits and aromatic plants, synthesized as secondary metabolites with an antimicrobial effect. *In vitro* physicochemical assays have characterized most of them as antioxidants. However, in eukaryotic cells Mts can modify their redox environment according to their chemical structure, the concentration and time of exposure, as well as the type of cell evaluated. EOs are widely proposed as anticancer natural compounds. We aim to test biomarkers of oxidative stress such as lipids, DNA, and antioxidant enzymes in human non-small cell lung cancer A549 cells incubated in different culture conditions and treated with antiproliferative concentrations of the acyclic unsaturated oxygenated Mt geraniol (G) and mandarin *Citrus reticulata* peel oil (MPO) composed in a 95 % of unsaturated cyclic Mts. Cells were treated with 200 μM of G or 50, 100, and 130 μl/L of MPO. Lipid peroxidation (LP) was measured by TBARS assay, genotoxic activity by comet assay, and superoxide dismutase (SOD), catalase (CAT) and glutathione-S-transferase (GST) activities by spectrophotometric analysis. We observed that G induced LP ($p<0.05$) and DNA damage ($p<0.05$), while MPO decreased their levels ($p<0.05$). No significant changes were observed in CAT and GST activity in either of the cases. However, SOD activity was increased with MPO ($p<0.05$) and slightly decreased with G. These findings suggest that even though all Mts showed an antiproliferative effect, unsaturated cyclic ones have antioxidant potential, while acyclic unsaturated oxygenated Mts act as pro-oxidant. The regulation of SOD activity by Mts may also contribute to cell redox state. Therefore, to elucidate the molecular mechanism of Mts in tumor cells, the relationship among oxidative stress, genotoxicity, and their antiproliferative effect should be deeply analyzed.

Keywords: A549 cells, Monoterpenes, Oxidative stress, Genotoxicity, Antiproliferative effect

(807) **CLB2 PHOSPHORYLATION AND DEPHOSPHORYLATION DYNAMICS SET THE CONDITIONS FOR PROPER ANAPHASE PROGRESSION**

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Mitotic events are orchestrated by oscillations in the activity of cyclin-dependent kinase 1 (Cdk1), which is modulated by the mitotic cyclins (Clb2 in yeast) associating with it. To progress through anaphase and next exit from mitosis, cells must inactivate Cdk1. The latter is achieved mainly via proteolysis of Clb2 by the proteasome. Clb2 degradation is mediated by the ubiquitin ligase APC/C (anaphase-promoting complex) in association with co-factor Cdc20 or Cdh1. Clb2 degradation is bi-phasic; about half of Clb2 molecules is turned over at the metaphase-anaphase transition by the APC/C-Cdc20, while the rest becomes degraded at mitotic exit by the APC/C-Cdh1.

One question that has remained unanswered is why and how APC/C-Cdc20 degrades only half of the Clb2 pool. We propose that Cdc5-mediated phosphorylation of Clb2 represents a signal for APC/C-Cdc20 recognition. Concomitantly with Clb2 turn over, the phosphatase Cdc14 becomes activated. This initial activation of Cdc14 requires the function of Clb2. We show that a non-phosphorylatable Clb2 protein besides being protected from degradation is also impaired in Cdc14 release. Taken together, Clb2 phosporylation by Cdc5 is required for APC/C-Cdc20-mediated Clb2 degradation and for Clb2 function within the FEAR network to promote Cdc14 activation. Active Cdc14 dephosphorylates Clb2 therewith restricting its own activity at early anaphase and protecting the remaining Clb2 molecules from degradation.

Keywords: Cell Cycle, APC/C, CDK, UPS.

(1155) **KRÜPPEL-LIKE FACTOR 6 AND OXIDATIVE STRESS IN HUMAN EXTRAVILLOUS TROPHOBLAST CELLS**

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Oxidative stress implies an imbalance between the generation of reactive oxygen species (ROS) and their clearance by defensive antioxidant molecules and mechanisms. Normal pregnancy requires an active placental antioxidant system to keep ROS level under control for normal trophoblast proliferation, invasion, and angiogenesis. On the other hand, impaired redox homeostasis is associated with miscarriage, preeclampsia, intrauterine growth restriction, and preterm birth. Krüppel-like factor 6 (KLF6) is a ubiquitous transcription factor enriched in the placenta. It has been implicated in angiogenesis, proliferation, apoptosis, and differentiation. However, its function is highly dependent on the cell context. We have previously demonstrated that KLF6 regulates the expression of pregnancy related genes such as *PSG* and β-hCG, it is required for proper trophoblast syncytialization, and hypoxia induces its expression in human term placenta and extravillous trophoblastic cells. Herein, we evaluated whether KLF6 contributes to ROS balance in HTR8/SV-neo cells, a cell line derived from human first trimester extravillous trophoblasts. KLF6 expression was early up-regulated in response to chemical hypoxia, decreased serum concentration, and chlorpyrifos treatment, a pesticide that generates ROS. KLF6 expression was down-regulated with a specific siRNA (siK) or a non-targeting siRNA (siC). Cell viability was not compromised after 48 h of transfection; however, a significant increase in ROS was detected by flow cytometry in siK-cells compared to siC ones. In addition, higher levels of ROS were detected in siK-cells after 3 and 6 h of H_2O_2 treatment. Moreover, upon exposure to H_2O_2 for 24 h viability was reduced in KLF6-silenced cells compared to siC transfected and non-transfected cells. Altogether, these results suggest that KLF6 contributes to extravillous trophoblast survival under oxidative stress conditions. Supported by FONCyT, CONICET, SECyT-UNC.

Keywords: Oxidative stress; placenta; extravillous trophoblasts; KLF6

(1514) **ANALYSIS OF CREB3L1 TRANSCRIPTION FACTOR EXPRESSION IN THYROID CELL LINES IN BRAF OR HRAS ONCOGENIC CONTEXT**

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CREB3L1 is a transcription factor, member of the CREB3 subfamily,that is expressed in a tissue specific manner. In some tissues CREB3L1 participates in cell differentiation processes regulating expression of tissue specific proteins, as well as proteins involved in the secretory pathway (transport factors). Moreover, CREB3L1 was described as a tumor suppressor factor that represses metastasis, migration and angiogenesis in breast cancer cell lines. We have previously described that CREB3L1 regulates the expression of proteins that contribute to the thyroid specific activity. However, the role of CREB3L1 in the differentiation process of thyroid cells is still unknown. Here, we aimed to study the role of CREB3L1 in normal thyroid cells by analyzing the effect of overexpression and inhibition of CREB3L1 on NIS, a thyroid specific protein, and transport factors. Moreover, we analyzed expression levels of CREB3L1, transport factors and NIS, in normal thyroid cells in comparison with BRAF/HRAS expressing cells. Our results show that CREB3L1 expression was able to increase NIS and transport factors. In addition, in cells silenced for CREB3L1 expression, NIS and transport factors levels were reduced relative to control cells. Furthermore, CREB3L1 immunofluorescence levels increase in cells expressing BRAF(V600E) or HRAS (G12V) in a time dependent manner (after 1, 3 and 5 days of induction). In the same conditions and in agreement with previous reports, NIS protein levels decreased until becoming undetectable after 5 days of oncogene induction. These results show a differential expression pattern of CREB3L1 and other proteins involved in the secretory pathway in an oncogenic context when compared to the expression of the same proteins under normal conditions. This could suggest a role of CREB3L1 in the progression of thyroid cancer, but further analyses will be required.

Keywords: CREB3, Thyroid Cells, HRAS, BRAF.

(1601) **USE OF ANTIHER-2 APTAMER FOR SPECIFIC DELIVERY OF MIR-205 IN BREAST CANCER.**

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Epigenetic is the scientific discipline that studies the modifications that affect gene expression without altering the DNA sequence. These changes include DNA methylation, histone modifications, and alterations in the expression of small non-coding microRNAs (miRNA), which are inheritable and reversible. The importance of these miRNAs in cancer and its role in tumor growth, invasion, angiogenesis and immune evasion has been demonstrated. Breast cancer is the leading cause of death from tumors in women, accounting for 17.8% of the total cancer incidence in Argentina. Overexpression of HER-2 is present in approximately 30% of the different types of breast cancer, indicating greater malignancy and invasiveness among the different classifications of this cancer. For this reason HER-2 is an attractive target for a specific delivery. Expression of miR-205 is decreased in breast tumors. Therefore, we decided to use an aptamer against HER-2, the aptamer was developed using the standard SELEX technique. The primary sequence of the HB5 aptamer is: 5'-AACCGCCCAAATCCCTAAGAGTCTGCACTTGT-CATTTTGTATATGTATTTGGTTTTTGGCTCTCACAGACACACTA-CACACGCACA-3'. The aptamer's Kd for the union with the HER-2 peptide was 18.9 nM, At the 3 'end of the aptamer an extension of 23 nucleotides was added in order to bind biotin with mir-205. In this way, we want to increase its concentration in cells with breast cancer, and try to decrease the tumor capacity of these cells. The specific drug delivery system based on aptamers is a very attractive therapeutic strategy, since it can increase the efficiency of the chemotherapeutic treatments and reduce the toxicity improving the quality of life of the patient.

GENETICS AND MOLECULAR BIOLOGY 10

(750) **ROLE OF PII PROTEIN ON THE METABOLISM OF *M. tuberculosis*: MODULATION OF ACYL-COA ACTIVITY**

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PII proteins are a family of signal transduction proteins which play a key role in the regulation of nitrogen metabolism. Recently, it has been suggested that the range of metabolic pathways regulated by them may be greater than described in the literature. In *Azospirillum brasilense*, *Escherichia coli* and chloroplasts of *Arabidopsis thaliana* PII proteins are able to interact with the acetyl-CoA carboxylase complex and therefore to inhibit its activity. Those results led to the proposal that PII-BCCP interaction would be conserved throughout other organisms.

This interaction has not been previously studied in actinomicetes. In general, these bacteria codify for several acyl-CoA carboxylases (ACCase) but only one PII protein. Unlike other organisms their ACCases are able to carboxylate other short chain acyl-CoA substrates apart from acetyl-CoA. From the analysis of the *M. tuberculosis* genome, it can be predicted up to six putative ACCases, some of which are essential for the pathogen.

In this works we evaluated the possible interaction between the PII protein and the AccA3 α subunit of an ACCasa complex from *M. tuberculosis* and analyzed the effect of PII protein in the bacteria physiology. We proved the ability of PII to bind AccA3 by the co-purification of the proteins from a cell extract of *M. Smegmatis* which overexpresses PII. The ACCase activity, studied by measuring the incorporation of radiolabelled bicarbonate, was modulated in pres-

ence of PII. Finally, it has been observed an increase on the *M. Smegmatis* survival after being internalized by macrophages when overexpressing PII. According to these results PII would be also involved in the regulation of carbon metabolism, which could be of relevance in different stages of the infection process.

Keywords: mycobacterium; tuberculosis; PII; Acyl-CoA carboxylases; ACCasa

(978) **CHARACTERIZATION OF PHOSPHATIDIC ACID PHOSPHATASE ENZYMES IN MYCOBACTERIA**

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Abstract: One of the most striking features of the tuberculosis (TB) granuloma is the presence around the lesion of a specific population of macrophages enriched in lipid droplets known as foamy macrophages (FM). Within FM, *Mycobacterium tuberculosis* decreases its multiplication rate and accumulates intracytoplasmic lipid inclusions (ILI) in its own cytoplasm which consist mainly of triacylglycerides (TAG). However, the mechanisms by which *M. tuberculosis* induces the differentiation of these FM and by which ILI accumulates in their cytoplasm within infected cells are not known.

The main biosynthetic pathway for TAG synthesis involves the sequential esterification of glycerol-3-phosphate to produce phosphatidic acid (PA). In *M. tuberculosis*, the PA can be dephosphorylated by a phosphatidic acid phosphatase enzyme (PAP) giving diacylglycerol (DAG), which is the direct precursor of TAG synthesis. Therefore, DAG synthesis is the first reaction specifically dedicated to the synthesis of TAG, suggesting a key role for the PAP enzyme in the regulation of PA flow towards the synthesis of TAG or membrane phospholipids.

The main goal of our project is to elucidate the role of the key enzymatic step that governs the decision of *M. tuberculosis* to synthesize TAG and, therefore, slow its growth and enter dormancy. To accomplish this goal, we overexpressed two candidate PAP proteins in *Mycobacterium smegmatis* and found that both strains showed higher levels of DAG and consequently of TAG. Genetic analyses are being carried out in order to define the physiological role of these proteins, by the construction and further characterization of knock out mutants.

Keywords: tuberculosis, triacylglycerides, phosphatidic acid phosphatase, *Mycobacterium smegmatis*

(183) **CHARACTERIZATION OF THE FACTORS INVOLVED IN THE REGULATION OF FAS I SYSTEM IN MYCOBACTERIA**

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IBR - CONIET

The genera *Mycobacterium*, which includes the causative agent of tuberculosis, unlike other microorganisms has two fatty acid synthase (FAS) systems, FAS I and FAS II, working in concert with the polyketide synthases to produce a vast diversity of complex lipids. Briefly, FAS I performs the biosynthesis of acyl-CoAs *de novo* and FAS II elongates them to synthetize very long-chain meromycolyl-ACPs, the precursors of mycolic acids. These mycolic acids are the main component of mycobacteria cell envelope and play an important role in the reduced cell wall permeability, virulence and acid fastness characteristic. Elucidation of the mechanisms involved in the regulation of fatty acid biosynthesis would shed light on the ability of *M. tuberculosis* to adapt and survive within the infected host.

To study the interaction between the two FAS systems we constructed a conditional mutant of the *fas* gene in order to reduce the levels of this enzyme and consequently *de novo* fatty acid biosynthesis. Although we found that the expression of the *fas* gene was reduced, the production of mycolic acids was not inhibited. These results were obtained both analyzing *de novo* production of mycolic acid using ^{14}C acetate incorporation as well as mycolic acid accumulation by LC-MS experiments.

We also performed a shotgun proteomic analysis in order to study the general protein expression profile responsible for the phenotype observed. We found that many proteins involved in the synthesis and assembly of mycolic acids in the envelope were upregulated when the levels of FAS I activity were reduced.

We also found that the fatty acids necessary to synthesize mycolic acids were taken from TAG, a storage lipid produced by mycobacteria. In summary, we proved that when the *fas* gene expression is reduced mycobacteria uses TAG as an alternative source of fatty acids to synthetize mycolic acids but it is not able to synthesize phospholipids, leading to the arrest of the growth and lysis of the bacterium.

(920) **INHIBITORY MECHANISM OF ACTION FOR *COILED-COIL* PEPTIDES AGAINST TYPE THREE SECRETION SYSTEM FROM ENTEROPATHOGENIC *ESCHERICHIA COLI***

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Human pathogenic Gram negative bacteria, such as enteropathogenic *Escherichia coli* (EPEC), rely on type III secretion systems (T3SS) to translocate virulence factors directly into host cells, interfering and altering their cellular processes. The *coiled-coil* domains, present in the structural proteins of T3SS, are conformed by amphipathic α-helical structures that play an important role in the protein-protein interaction and are essential for their assembly. In order to inhibit the function of EPEC T3SS, peptides between 15 and 34 aa were synthesized based on the *coiled-coil* domains of proteins that make up this secretion system. Initially, a molecular modeling study was carried out using docking-simulations of molecular dynamics-mechano-quantum calculations of the different peptide-protein complexes. These results allowed us to better understand the mechanism of action at the molecular level of these peptides, to try to improve their affinity to the target proteins selected from T3SS. The inhibitory capacity of the peptides on EPEC T3SS was then assessed through *in vitro* hemolysis assays, where a reduction in T3SS-dependent red blood cell lysis was observed. Inhibition of peptides on EPEC T3SS could also be demonstrated by *in vitro* epithelial cells infection assays. Crosslinking assays have determined that the peptides block the formation of recombinant EspA polymers, demonstrating that may affect the EspA-EspA interaction, most likely by their association to the *coiled-coil* domains of this protein, and as a consequence its action is affected. This demonstrates that compounds targeting T3SS from pathogenic bacteria can inhibit bacterial infection by presenting a higher specificity than broad-spectrum antibiotics, avoiding selective pressure and thus reducing the development of resistance to antimicrobial agents. In turn, these peptides could be taken as initial structures to design and synthesize new compounds that mimic their inhibitory pharmacophoric pattern.

Keywords: Type III secretion system, peptides, *coiled-coil*, enteropathogenic *Escherichia coli*.

(1338) **CHARACTERIZATION OF THE EXPRESSION AND PRODUCTION OF BIOLOGICALLY ACTIVE SHIGA TOXIN 2 (STX2) IN EUKARYOTIC CELLS**

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The cardinal element virulence of Shiga toxins (Stx)-producing E. coli (STEC) is the production of Stx, which constitute an AB5 toxin. We previously reported the existence of a putative eukaryotic promoter-like sequence (pr1), located upstream of Stx2-A subunit gene.

The aim of this work was to characterize Stx2 subunits expression by eukaryotic cells after transfection with a prokaryotic plasmid carrying the Stx2 gene under its own promoter (pStx2). To determine the relevance of the putative promoter sequence, pr1, on Stx2 expression, pDpr1Stx2 plasmid was constructed.

293T cells were transfected with pStx2, total RNA was purified and specific mRNA was quantified by RT-qPCR. For both subunits, data revealed similar amounts of mRNA using either oligo (dT) or the corresponding subunit-specific primer (Sub A: C_t specific primer: 22.95, C_t oligo (dT): 21.97, C_t random primer: 27.93; Sub B: C_t specific primer: 28.27, C_t oligo (dT): 25.73 C_t random primer: 28.2). Vero cells, as a representative Stx2-susceptible cell line, were incubated with supernatants from 293T cells transfected with pStx2 (SN-pStx) or pDpr1Stx2 (SN-pDpr1) to evaluate Stx2-cytotoxic activity. SN-pStx showed cytotoxicity on Vero cells. In contrast, no cytotoxicity was observed in SN-pr1 (% Vero viability with 1/32 Dilution of SN, SN-Stx2=50.29□2.12; SN- pDpr1=123.81□6.47 ($p<0.001$)). We also quantified pStx2 and pDpr1Stx2 DNA from transfected cells by qPCR, finding that both plasmids were efficiently transfected.

These results show the putative promoter capacity to drive a biologically active Stx2 in eukaryotic context and suggest RNA polymerase II participation on this transcription. Also, the absence of cytotoxicity in SN-pDpr1, despite its high transfection efficiency, suggest its critical role in the expression and production of the active toxin within eukaryotic environment.

Keywords: SHIGA TOXIN, PROMOTER, EUKARYOTIC TRANSCRIPTION, TOXICITY and RNA POLYMERASE II

(580) **BIOCHEMICAL AND MOLECULAR CHARACTERIZATION OF ENTEROHEMORRHAGIC *ESCHERICHIA COLI* NCTC12900 AS ATOXIGENIC MODEL OF STUDY IN MEAT**

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Escherichia coli O157:H7 is a Gram-negative pathogenic bacterium which is responsible for hemorrhagic colitis and the hemolytic uremic syndrome. This organism is recognized as an important cause of food-borne diseases reported in Argentina and other countries. The highly virulent nature of this bacterium demands a rigorous control of its dissemination in the environment to ensure food safety. *Escherichia coli* O157:H7 is producer of shiga toxins (Stx1 and/or Stx2) and has additional virulence factors. *E. coli* O157: H7 NCTC12900 is a natural atoxigenic strain isolated in 1992 in Austria. It is used as a model of study for biological safety reasons in different laboratory tests. The objective of this study was to characterize *E. coli* NCTC12900 in order to propose it as a model for meat studies. Its ability to grow in a meat model system (MMS), the presence of certain virulence genes (related to biofilm, attaching and effacing lesions) and its adhesion capacity to the meat extracellular matrix proteins (MEC) were evaluated.

Meat growth capacity of NCTC 12900 was evaluated in MMS incubated at 25°C for 120 h. The viability was determined by cell counts on Mac Conkey agar. The presence of virulence and biofilm related genes were analyzed by PCR. The ability to bind to MEC proteins was assessed *in vitro* using microplates coated with collagen IV.

E. coli presented an optimum growth in MMS, with a maximal of 8 log units at 24 h. PCR confirmed that NCTC 12900 possesses several virulence genes as well as the absence of genes related to toxins was confirmed. Adhesion tests suggest the ability to binding collagen IV.

These findings propose *E. coli* NCTC12900, as a suitable pathogen to use in meat studies, since it grows in this niche, does not produce toxins but keeps other virulence genes. Its ability to bind MEC proteins guarantee meat colonization and is a key initial step in biofilm formation.

Keywords: Enterohemorrhagic *E. coli, meat, virulence factors, adhesion.*

(665) **RecA DEPENDENT AND INDEPENDENT RECOMBINATION IN *Pseudomonas aeruginosa* and *Escherichia coli***

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Genetic recombination participates in multiple physiological pathways that are crucial for the maintenance and diversification of the genome. The recombinase RecA is a central factor in this process, mediating the exchange between DNA molecules containing perfect homology (homologous, HO) or very low divergence (homeologous, HE). At present multiple mechanisms are known to mediate genetic recombination, both dependent and independent of RecA. In order to study the recombination process in Gram-negative bacteria, we used a LacZ based system to determine both HO and HE recombination. Using this system, we determined the recombination rates in wild-type (WT) and RecA deficient (ΔrecA) strains of *Pseudomonas aeruginosa* and *Escherichia coli*. Moreover, the recombination process creates a functional copy of the *lacZ* gene and thus, the recombinant clones can be detected by their β galactosidase activity.

In both bacteria, HO recombination rates were approximately 60-200-fold higher than HE recombination rates. In the RecA-deficient *E. coli* strain, both HO and HE recombination rates were 60-fold lower than that obtained for the WT strain. The recA deletion mutant of *P. aeruginosa* showed that HO and HE recombination rates decreased 15 and 3-fold respectively, compared with the WT strain. When the β galactosidase activity of recombined clones was determined, we found that all *E. coli* clones showed enzymatic activity as it was expected. In *P. aeruginosa* however, many clones showed no β galactosidase activity. Furthermore, the molecular analysis of the recombined regions of these clones showed the presence of point mutations and also deletions of as much as 800bp.These results indicate the existence of a significantly mutagenic recombination mechanism, independent of RecA in *P. aeruginosa*, at difference of *E. coli*.

Keywords: *P.aeruginosa*, *E.coli*, Genetic recombination, RecA recombinase

(1507) **MUTATION SPECTRA MODULATION LEADS TO HIGHLY INACTIVATED VARIANTS OF THE MULTIDRUG EFFLUX PUMP REPRESSOR NFXB IN *Pseudomonas aeruginosa***

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Abstract: MutS maintains the DNA replication fidelity by recognizing mispairs and recruiting factors involved in the Mismatch Repair. In a previous work, we described a new mechanism by which MutS also contributes to the replication fidelity: regulation of the access to replication sites of the low fidelity DNA Polymerase (Pol) IV in *Pseudomonas aeruginosa* (PA). We showed that MutS inhibits Pol IV association with **β clamp, which** is absolutely required for Pol IV activity. In addition, MutS limited Pol IV mutagenic activity in exponentially growing cells. Briefly, we compared mutation rates to resistance to ciprofloxacin (Cip^R) and the *nfxB* mutation spectra in the wild type (WT) strain, the $mutS^{\beta}$ strain (harboring a chromosomal $mutS^{\beta}$ allele which encodes a MutS mutant that does not bind to β clamp) and the Pol IV-deficient strains *dinB* and $mutS^{\beta}$ *dinB*. There were no differences in Cip^R mutation rates among these strains. However, inactivation of Pol IV did not change *nfxB* mutation spectra

in the WT background, but it significantly decreased spontaneous base substitutions in the *mutS*$^{-}$ background. Here, we evaluated the phenotypic consequences of PolIV action on *nfxB*. This gene encodes a transcriptional repressor of the MexCD-OprJ multidrug efflux pump, which corresponds to one of the primary antibiotic resistance mechanisms in PA. To test NfxB activity, we integrated on the PA chromosome a reporter fusion of the *mexCD-oprJ* promoter to the *lux* operon. We found that a high proportion of Cip^{R} clones in the *mutS*$^{-}$ strain exhibits lower NfxB activity. These highly inactive variants were not detected in the *mutS*$^{-}$ *dinB* strain, indicating that PolIV is a key factor for generation of these NfxB versions. As expected, PolIV had not effect on the generation of *nfxB* mutants in WT strain since there were no differences with *dinB* strain. Remarkably, these results suggest that the modulation of mutation spectra could be an important way to generate highly inactivated NfxB variants.

Keywords: *nfxB*, mutation spectra, MutS, PolIV.

(1578) **CLONING, HETEROLOGOUS EXPRESSION AND FUNCTIONAL CHARACTERIZATION OF A Δ^{9}-DESATURASE OF *Pseudomonas putida* A (ATCC 12633).**

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Adaptive response of *P. putida* A (ATCC 12633) to tetradecyltrimethylammonium (TTAB) involve the immediate fluidification of cell membranes (Polarization value decreased from 0.12±0.01 to 0.08±0.01) with changes in the content of fatty acid (FA) of the phospholipids membrane: the levels of unsaturated FA (16:1Δ9 and 18:1Δ11) decreased while the amount of saturated FA (15:0 and 16:0) increased. Therefore, the effect of TTAB was an increase in the fluidity of the membrane while *P. putida* cells seemed to respond decreasing the degree of UFA in order to counteract the action of this compound. The decrease in the content of 16:1Δ9 and the concomitant increase in the level of 16:0 could be explained by inhibition of a Δ^{9}desaturase enzyme. In this work, a gene coding for a putative Δ^{9} fatty acid desaturase-like protein was isolated from *P. putida A*(ATCC 12633), cloned and heterologously expressed in *Escherichia coli* BL21, a Δ^{9} desaturasedeficient organism. The gene, named *mf539821,* has an open reading frame of 1185 bp, codes for 394 amino acids with a predicted molecular weight of 45 kDa and showed 85% sequence identity to Δ9 desaturase gene of *P. putida* KT2440, *P. putida* DOT-T1E and *P. fluorescens* PICF7.To determine the functional activity *in vivo*of the recombinant desaturase, *E coli* cells were cultured in LB medium with palmitic acid [1-^{14C}] and induced at 28 ºC, with1% lactose. The cultures were collected and their FA were analysed by TLC. Compared to the negative control, *E coli* cells overexpressing the putative desaturase gene increased by twice the amount of monounsaturated fatty acids formed from exogenous palmitic acid [1-14C].Thus, we conclude that *mf539821* codes for a functional desaturase enzyme of *P. putida* A (ATCC 12633).The successful expression of the enzyme will be used to determine if its inhibition is or not involved in the adaptative response of *P. putida* to cationic surfactants.

(1709) **HYPERMUTATOR STRAINS OF *Pseudomonas aeruginosa* BYPASS GENETIC CONSTRAINTS ON THE PHENOTYPIC SWITCHING TRIGGERED IN BIOFILMS**

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Abstract: *Pseudomonas aeruginosa* (PA) grows as biofilm communities in the airways of cystic fibrosis patients, where small colony variants (SCVs) are frequently observed. These biofilm-adapted SCVs show a great instability when grown outside biofilms, thus revealing the ability to switch between phenotypes. Hypermutator Mismatch Repair System (MRS)-deficient strains of PA show an increased phenotypic diversification, particularly in biofilms. Thus, we performed an evolutionary assay to explore the adaptive potential of PA and the role of hypermutability in this phenotypic switching. We founded five lines with the wt strain and four lines with its isogenic hypermutator *mutS* mutant, and subjected them to alternating conversion and reversion cycles, from which we further isolated SCVs and revertant clones from both strains. We next performed whole genome sequencing using an Illumina platform to carry out a comparative genomic analysis of both parental strains as well as endpoint clones of all wild type lines and intermediate clones of three *mutS* lines. All wt lines of PA showed the accumulation of 1 mutation/round in genes of the *wsp* and *yfi* systems, both being regulators of the second messenger c-di-GMP, which governs the transition between planktonic and biofilm mode of growth. By round 4, SCV conversion was dramatically constrained in the wt strain. On the other hand, conversion/reversion rounds continued steadily in all hypermutator lines, showing an average of 20 mutations/cycle. Interestingly, hypermutators also showed 1 mutation/cycle in the *wsp* and *yfi* systems, indicating that this evolutive parallelism was maintained even in the hypermutator lines. Apart from these, at least other 8 c-di-GMP-related genes were found to be mutated, which is still insufficient to explain the switching process carried out by mutators. Fulfilling this study will reveal new adaptive pathways explaining the ability of hypermutators to bypass the genetic constraints on phenotypic switching.

Keywords: *Pseudomonas aeruginosa*, hypermutability, biofilm, genetic constraints, phenotypic switching.

IMMUNOLOGY (ADAPTATIVE IMMUNITY) 3

(582) **PERIPHERAL BLOOD FOLLICULAR T CELLS (CTFH) IN PATIENTS WITH PRIMARY IMMUNODEFICIENCIES**

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Introduction: T follicular helper (Tfh) cells represent a CD4^{+} T cell subset specialized to provide help to B cells and to induce durable antibody response. Tfh cells are essential for the formation of germinal centers (GCs). Peripheral blood CXCR5^{+} CD4^{+} T cells are currently recognized as a circulating counterpart of Tfh cells (cTfh) and are a heterogeneous group composed of distinct subsets with a characteristic phenotype and function. AIM: describe cTfh cells in peripheral blood of CD4^{+}memory cells from healthy donors (n=14) and patients with PIDs due to GC defect (2 BTK mutation, 1 CD40L mutation, 1 STAT-3 LOF mutation) and patients with dysregulatory syndromes (1 CD25 mutation, 2 STAT-1 GOF mutation, 1 STAT-5b mutation, 2 IDCV patients) were stained with anti-human-CXCR5, CCR6 and CXCR3 by flow cytometry (FC). RESULTS: HD had a cTfh percentage mean of 12,7 ± 3,6 (X±SD). Four subsets of cTfh cells were defined: cTfh1 (CXCR3^{+}), cTfh17 (CCR6^{+}), cTfh1/17(CXCR3^{+}CCR6^{+}), and cTfh2 (CXCR3^{-}CCR6^{-}). Patients with PIDs affecting GC development showed a quantitative reduction in cTfh (12,7 vs 4,8) (p<0,005). Unlike those, patients with disregulatory syndromes showed high levels of cTfh (12,7 vs 25,5) (p<0,002). Patients with disregulatory syndromes have impaired distribution of cTfh subpopulation with severe skew to CXCR3^{+} cTfh cells (28,2 vs 48,1) (p<0,04). DISCUSSION: In summary, specific mutations can differentially affect the quantity and/or the quality of circulating Tfh. These alterations play a role in the pathophysiology of primary immunodeficiencies with B lymphocyte functional impairment.

Studying cTfh and their subpopulation may be a diagnostic tool in patients suspected to have a primary immunodeficiency.

Keywords: Follicular T cells, Primary immunodeficiencies

(1529) **SEROLOGICAL EVIDENCE OF HEPATITIS E VIRUS (HEV) CIRCULATION IN BLOOD DONORS AND PATIENTS WITH LIVER DISEASE IN TUCUMÁN, ARGENTINA.**

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HEV infection is a major cause of human viral disease with clinical and pathological features of acute hepatitis. It affects primarily young adults and is generally mild, except for women in late pregnancy with a high mortality of ~20%. This emergent disease is primarily transmitted via the faecal oral route due to contaminated water and food. Chronic HEV infections in immunocompromised patients and HEV transmission by blood transfusion have been recently reported. There are scarce epidemiological data about HEV prevalence in Argentina and no data for the northern region of the country.

Anti-HEV Ig antibodies were assayed by ELISA (DIAPRO,Italy) in blood donors (BD)of Tucumán and in a group of patients(n=17) with high liver transaminases values consulting a private clinical laboratory.

For cross-sectional study was conducted in 250 BD. Written consent was obtained from donors and the protocol was approved by the Ethics Committee of the UNT and CCT-CONICET. The mean age of the study group was 35.5 years old. Most patients were male (78%). 58.4% lived in the city of San Miguel de Tucumán. 4.4% were reactive to at least one of the disease screening assays (HIV;HBV,HCV,HTLV, Brucellosis, VDRL and Chagas). The overall anti-HEV Ig prevalence in BD was of 4.4% (11/250). Multivariate analysis revealed a clear tendency to increasing HEV seroprevalence with increasing age (Chi-square test, P<0.0256) and with reactivity to blood transmitted diseases (P<0.0001), specially with Chagas. Further analysis is needed to establish if this correlation is due to similar life standards or to possible cross-reactivity. Remarkably, two (11.76%) of the patients with liver inflammation were positive for HEV serology.

This is the first report of HEV circulation in Tucumán, which may be a misdiagnosed agent of hepatitis as evidenced in both studied groups. Thus, there is a need for including HEV testing in routine diagnostic of hepatitis and eventually in blood donation screenings.

(302) **HISTAMINE CONTROL OF APOPTOSIS AND CYTOTOXICITY ACTING ON DENDRITIC CELLS**

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Histamine (HIS) plays a key role in inflammation and allergy, acting through four G-protein coupled receptors (H1R-H4R). Previously, we proved that HIS acting on dendritic cells (DCs) enhances cross-presentation of the soluble antigen ovalbumin (OVA). Here, we analyzed the intracellular mechanisms involved in the modulation of DCs functionality by HIS. We used immature murine DCs obtained from bone-marrow precursors of C57BL/ 6 mice cultured for nine days with GM-CSF. DCs were incubated with HIS (1µM, DC-HIS) or not (DC-Ct) for 20 min at 37°C. We show an activation of PKC protein in DC-HIS (DC-Ct 0.14±0.058 vs DC-HIS 0.24±0.079, p<0.05). We also evaluated *in vivo* the $CD8^+$ cytotoxicity. DC-Ct and DC-HIS were stained with differential concentrations of carboxyfluorescein succinimydil ester (CFSE; 5nM and 0.5 nM) and loaded *in vitro* with OVA. Then we inoculated them in OVA immunized mice. Surprisingly, when $CD8^+$ T cells were incubated with DC-HIS, an increase in lytic activity was observed. This function was independent of PKC activation, as shown in the presence of the PKC inhibitor (2nM; PKCi) (DC-Ct 90.88±8.3, DC-HIS 111.1±7.96, DC-HIS + PKCi 77.73±20.87, n=5; p<0.05). Because HIS has been associated with cell death and is activated during inflammatory processes, we evaluated whether HIS modulates apoptosis. For these assays we induced cell death through heat-shock (42ºC, 20 min) and 24 hours later stained them with Annexin-V-FITC/Propidium Iodine (PI). Apoptosis was assessed by flow cytometry and cells were considered apoptotic when DCs were Annexin-V^+. We found that HIS prevents DCs apoptosis, effect dependent of PKC DC-Ct 62.43±6.3,DC-HIS 29.52±5.347, DC-HIS+PKCi 55.01±9.06, p<0.05). Accordingly, HIS inhibited caspase-3 and procaspase-3 expression in DCs. In conclusion, HIS has the ability to prevent the normal apoptosis program during the perception of damage signals by DCs, and its interaction with DCs favors the activation of specific $CD8^+$ T lymphocytes.

Keywords: histamine, dendritic cells, apoptosis, PKC, $CD8^+$ T cells.

(326) **FUNCTIONAL DIFFERENCES IN REGULATORY T CELLS FROM MICE WITH DIFFERENT SUSCEPTIBILITY TO THE DEVELOPMENT OF AUTOIMMUNE DISEASES**

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NOD mice are characterized by their highly susceptibility to develop spontaneous and induced autoimmune diabetes, thyroiditis, sialiditis and prostatitis. It has been suggested that this increased susceptibility could be related to defects in the number and/or functionality in regulatory T cell (Treg) populations. In a previous work, we have shown that NOD mice exhibited significantly lower frequencies, absolute Treg numbers and mean fluorescence intensity (MFI) for Foxp3, when compared with other mice strains (p<0.05). Herein, we analyze if NOD mice also exhibited functional differences within Treg populations. For that purpose, spleen $CD4^+CD25^{hi}$ (Treg) cells from NOD, C57BL/6 and BALB/c mice were isolated by cell sorting and then in vitro activated with anti-CD3, anti-CD28 and different doses of recombinant IL-2 (0,50 and 200 U/l). After stimulation in the presence of rIL-2, Treg cells from NOD mice showed lower frequencies of $Foxp3^+$ $CD25^+$ when compared with C57BL/6 and BALB/c $mice^+$ (p<0.05). Moreover, Treg cells from NOD mice showed diminished expression for Foxp3 and CD25 even with the addition of high rIL2 doses. Furthermore, a lower frequency and MFI values for Ki67 were observed in Treg NOD cultures even after rIL2 stimulation. To evaluate Treg suppression capabilities, $CD4^+CD25^-$ (T Conventional) cells were isolated by cell sorting and then stimulated with anti-CD3/anti-CD28 at different Tconv/Treg ratios. Results showed that Tregs from all strains suppressed T conventional proliferation. However, Tregs from NOD mice showed the lowest suppressive capacity when compared with the other strains (p<0.05). Our results show that Tregs from NOD mice have defects in their activation and suppression abilities. The defective functionality found could be related to the high susceptibility to develop autoimmune diseases observed in NOD mice.

Keywords: Regulatory T cells, autoimmune diseases, NOD mice

(766) **IL-6 MEDIATES GALECTIN-8 COSTIMULATORY ACTIVITY OF ANTIGEN-SPECIFIC CD4T CELL RESPONSE**

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Galectin-8 (Gal-8) is a mammalian lectin endowed with the ability to co-stimulate the antigen-specific immune response. IL-6 is a pleiotropic cytokine that plays an important role in the regulation of antigen-specific T cell response. In the present study, we aimed to elucidate whether IL-6 was mediating Gal-8 costimulatory effect on antigen-specific CD4T cells. Firstly, we quantified IL-6 by ELISA in supernatants from DO11.10TCR_{OVA} mouse splenocyte cultures, stimulated with the cognate peptide OVA (OVA), Gal-8 or the combination of both. Notably, IL-6 was significantly increased in the conditioned media from Gal-8- or Gal-8 plus OVA-treated cells but not from OVA only-treated cells, where IL-6 was present at the same level as in untreated cells. Next, to assess if IL-6 is involved in Gal-8 costimulatory effect, splenocytes were stimulated, as before, but in the presence of an IL-6-neutralizing monoclonal antibody or a matching isotype control, and T cell proliferation was

determined. Interestingly, IL-6 neutralization specifically precluded Gal-8 costimulatory activity but did not affect antigen-specific T cell response. To identify those cells that produce IL-6 in response to Gal-8, intracellular IL-6 was determined by FACS in Gal-8-stimulated BALB/cJ splenocytes. We found that both $CD11c^+$ and $CD11b^+$ cells produced IL-6 in response to Gal-8. Finally, to confirm that IL-6 from antigen-presenting cells was mediating the Gal-8 costimulatory effect, splenocytes from IL-6-deficient (IL6KO) or C57BL/6J (wild-type) mice pre-treated with Mitomycin-C, were co-cultured with purified CD4T cells from OTII mice in the presence of OVA, Gal-8 or the combination of both. In agreement, Gal-8-induced costimulation of antigen-specific CD4T cell response was significantly impaired when APC from IL-6KO mice were used. Taken together, our results argue in favor of the participation of IL-6 in the immunostimulatory pathway induced by Gal-8.

Keywords: Interleukin-6; Galectin-8, CD4T cells

(1003) **IDENTIFICATION OF $CD8^+$ T CELLS EXPRESSING HLA-DR IN THE COLON MUCOSA OF PATIENTS WITH INFLAMMATORY BOWEL DISEASE**

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BACKGROUND: Inflammatory bowel diseases (IBD) are multifactorial pathologies characterized by a chronic and relapsing intestinal inflammation, probably due to an impairment of regulatory circuits. Peripheral blood $CD8^+$ HLA-DR* were described as regulatory T cells with regulatory function acting through the checkpoints molecules CTLA-4 and PD-1 expressed by these cells.

OBJETIVE: The aim of this work was to characterize $CD8^+$H-LA-DR^+ T cells in the colonic mucosa, and to explore the role of the PD-1-PDL-1 axis in homeostasis and inflammation.

METHOD: $CD8^+HLA\text{-}DR^+$ cells were assessed by flow cytometry at the colonic mucosa of healthy controls (HC) (n= 15) and IBD patients, (n= 10). Cells were also examined for the co-expression of CXCR3, CCR5, TIM-3, CD25, FoxP3, PD-L1, CD45 and PD-1 molecules.

RESULTS: The frequency of $CD8^+HLA\text{-}DR^+$ T cells in the inflamed areas of IBD patients was 36.9 ± 4.6%. In comparison with non-inflamed areas of the same patients (22.8 ± 4.6%) or with HC samples (20.9 ± 1.4%) the difference was significant ($p<0.01$). In addition, the $CD8^+HLA\text{-}DR^+$ lymphocytes of inflamed areas showed a significant increase of PD-1 expression compared with non-inflamed areas and HC samples (72.9 ± 3.5%, 47.7 ± 6.2% and 52.4 ± 4.5% respectively, $p <0.01$). The analysis of PD-L1 cell expression showed that colonic epithelial cells from inflamed areas was significantly reduce compared with cells from non-inflamed areas (27.5 ± 7.3% vs. 59.58 ± 6%, $p <0.01$).

CONCLUSION: $CD8^+HLA\text{-}DR^+$ T cells are present in human colon with higher frequencies in inflamed areas of active IBD patients and with increased expression of PD-1. Strikingly, epithelial cells from inflamed areas showed a down modulation of PD-L1, which could be involved in an impairment of the regulatory properties of $CD8^+$H-LA-DR^+ T cells. This could contribute to the persistence of intestinal inflammation in IBD.

Keywords: Inflammation, Ibd, T Cells.

(1213) **FURTHER ANALISYS OF THE PREVENTION OF ATOPIC DERMATITIS BY *TOXOPLASMA GONDII* CHRONIC INFECTION**

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We previously showed that *T. gondii* infection diminishes the susceptibility to develop experimental asthma. Afterwards we extended these results and showed that the parasite can also modulate an atopic dermatitis. Skin histopathology of mice infected before allergic sensitization was similar to normal mice. This result correlated with diminished IgE and IgG1 levels and reduced OVA specific Th1/Th2 systemic cytokines. The aim of the present work was to further study the mechanisms involved in this immunomodulatory effect. Adult BALB/c mice were orally infected with *T. gondii* cysts and, one month later, epicutaneously sensitized with OVA (TDA). Treatment was repeated twice, with a 2-week resting period between each sensitization. Controls included non-infected mice sensitized with OVA (DA) or PBS (N) and infected mice treated with PBS (T). Local IL-4, IL-5 and IFN-γ secretion was analysed in skin explants stimulated with OVA. *T. gondii* infection before allergic sensitization induced a local reduction in both IL-4 and IL-5 ($p\leq0.05$) and a trend to decrease in IFN-γ levels compared to DA mice. The reduction in both Th1/Th2 cytokines suggested that regulatory cells induced by the parasite may account for the immunomodulatory effect. Hence, IL-10 and TGF-β supernatant levels were evaluated after *ex vivo* stimulation of splenocytes with OVA and changes in regulatory T cells CD4+Foxp3+ in spleen were measured by flow cytometry. No significant differences were observed in either TGF-β or CD4+-Foxp3+ T cells between all experimental groups. On the other hand, while a significant increase in IL-10 levels was obtained for the DA group compared to non-sensitized mice; infection with the parasite restored IL-10 levels in TDA group. Altogether, these results show that *T. gondii* infection reduces both skin and systemic Th1/Th2 responses and suggest that Tregs cells would not be involved in the immunomodulatory effect.

(1239) **IDENTIFICATION OF iNKT FOLLICULAR HELPER CELLS AT EARLY STAGES OF *TRYPANOSOMA CRUZI* INFECTION**

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Invariant natural killer T cells (iNKT) are innate T cells wich express semi-invariant T cell receptors. They recognize both endogenous and exogenous lipid antigens presented by CD1d and they rapidly produce cytokines following activation. It has been described that iNKT cells exert a variety of effector functions developing Th1, Th2 and Th17-like phenotype. Recent data showed that iNKT cells could also acquire a Tfh-like phenotype, migrate into the Germinal Center (GC) and sustain B cell responses. It is interesting to note that an exquisite support of GC may actually represent a fundamental mechanism to fine-tune the type and magnitude of antigen-specific antibody responses and that NKTfh could potentially represent a new player in this landscape. The aim of our work was to evaluate the presence and potential role of iNKT in GC response in *T. cruzi* infection. For that, C57BL/6 mice were infected with 5000 trypomastigotes of *T. cruzi* Tulahuen strain and the kinetic of iNKT and Tfh was evaluated at different days post-infection (dpi) by flow cytometry. iNKT cells were identified by CD3int expression and binding to α-GalCer-loaded CD1d tetramers. Tfh were characterized by the co-expression of CD3, CD4, ICOS, PD-1 and CXCR5. At 4dpi, we observed that a substantial proportion of iNKT undergo Tfh-like phenotype defined by the co-expression of ICOS, PD-1 and CXCR5, compared to uninfected controls ($p<0,05$). Interestingly, this population of NKTfh decreased significantly at 7dpi ($p<0,05$) suggesting a potential role in the early stages of infection. In contrast, Tfh cells, who were almost absent at early stages of the infection, increased in number and frequency at 15dpi ($p<0,05$). Remarkably, iNKT evidenced high expression of CD335 and CD160 (which may be involved in NKT cell activation and function) and produce IL-4 at 4dpi. This work extends our understanding of the potential contribution of iNKT cell in humoral immune response to *T cruzi.*

Keywords: NKTfh; *T cruzi* infection; Germinal Centers, Chagas

disease

(1459) **IL33 RECEPTOR EXPRESSION IS SIGNIFICANTLY UP-REGULATED ON B CELLS DURING PREGNANCY**

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IL33 is an alarmin released during cell injury caused by stress or infection and drives immune cells into regulatory functions to maintain tissue homeostasis.

In the context of pregnancy, IL33 was recently shown to induce the production of anti-inflammatory molecules by decidual B cells, protecting against preterm labor in human and mouse.

Here we aimed to fully characterize the expression of IL33 receptor (IL33R) in B cells during pregnancy.

We began performing a genome-wide transcriptome profiling in pure isolated B cells from the spleen of pregnant (P) and non-pregnant C57BL/6 mice (NP). Interestingly, we observed that expression of Il1rl1 (IL33R) was significantly upregulated (≈ 4,5 fold changes) in B cells from P compared to NP mice using Limma eBayes test ($p<0.0001$). This was confirmed at the protein level by flow cytometry. Further analysis showed that IL33R was predominantly expressed on splenic B220lowCD23$^{low/neg}$ B cells. A kinetic analysis depicted that numbers of splenic B220lowCD23$^{low/neg}$IL33R^{+} B cells were significantly increased at early pregnancy (day 12) compared to NP mice. At mid-pregnancy (day 14), numbers of B220^{low}CD23$^{low/neg}$IL33R^{+} B cells dropped as compared to day 12 but were still significantly higher than in NP mice. At late pregnancy (day 16), numbers of B220^{low}CD23$^{low/neg}$IL33R^{+} B cells were similar to NP mice (one-way ANOVA in randomized blocks, $p<0.0001$). Similarly, peritoneal B1 B cells from P mice displayed significantly higher levels of IL33R expression than NP mice (Unpaired t test $p=0.0005$). Next, we cultured splenocytes from NP virgin C57BL/6 females with estradiol (E2), progesterone (P4) or both and analyzed the expression of IL33R on CD19^{+}B220^{low} B cells after 24h. We observed a modest increase of IL33R expression on CD19^{+}B220^{low} B1 B cells upon E2 treatment.

Our results reinforce the idea of IL33 being a crucial cytokine controlling pregnancy outcome through a mechanism involving B cells.

Keywords: Preterm labor, B cells, IL33R expression, pregnancy outcome.

(1811) **HYPOPHYSITIS IN NON-OBESE DIABETIC MICE AS A NEW TISSUE- SPECIFIC AUTIMMUNE RESPONSE**

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Type 1 diabetes mellitus is a chronic organ-specific autoimmune disease that results from the destruction of beta (β) cells in the pancreatic islets and shows a pronounced leukocyte infiltration in the gland. Non-obese diabetic (NOD) mice develop autoimmune diabetes approximately at 6 months of age in females, providing an experimental model for human Type 1 Diabetes. They also display additional autoreactive immune responses against other glands including the prostate and thyroid. Furthermore, female NOD mice exhibit a lower fertility index compared to wild type mice, that develops approximately at the same time as diabetes. To determine whether the low fertility of NOD mice is caused by an autoimmune response against the hypophysis, we assessed the presence of leukocyte infiltration in the gland. To this end, the presence of CD45+ (panleukocyte) cells in the hypophysis of 3 and 6 months old female BALB/c (control group) and NOD mice was analyzed by flow cytometry. An increase in leukocyte infiltration in the hypophysis from 6 months old NOD mice was observed compared to the controls (0.05±0.04 % BALB/c mice vs 3.2±1.0% NOD mice; $p<0.05$), while young female NOD mice did not show infiltration. Furthermore, we found that the older female NOD mice displayed autoreactive IgGs against hypophyseal total proteins measured by ELISA, as well as prolactin specific IgG, that were not present in control mice. These results suggest that NOD mice may have an autoreactive immune response against the hypophysis that may lead to a decrease in the production of hormones such as FSH, LH and prolactin, resulting in a decrease in fertility. To our knowledge, this is the first report that demonstrates the presence of hypophysitis in NOD mice. These results may help understand the heterogeneity of symptoms present in autoimmune diseases.

Keywords: Type 1 diabetes mellitus, hypophysis, autoimmunity, leukocyte infiltration

IMMUNOLOGY (IMMUNOTHERAPY) 2

(27) **GALECTIN-3 DEFECTIVE VACCINE FOR AN EFFECTIVE IMMUNOTHERAPY AGAINST PROSTATE CANCER DEVELOPMENT.**

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Prostate cancer (PCa) is a major problem of health in Argentina and worldwide. Patients are likely to be cured with early diagnosis, while there is little hope when the cancer is metastatic and resistant to anti-hormonal treatments. Therefore, two alternatives are proposed for castration resistant patients. Depending of the symptomatic or asymptomatic status, chemotherapy based on the use of Taxol derivatives or, more recently, immunotherapy are proposed respectively. The latter has generated encouraging results in clinical trials, but requires a deeper understanding in order to increase its efficiency. The main reason for this low efficiency is that tumors develop a robust immune response control system.

Recent results from our group demonstrate that galectin-3 (Gal-3) is one of the key factors of the immune tolerance in PCa. Using lentiviral transduced murine TC1 cells expressing stable anti-gal-3 shRNA, we demonstrated that tumor cell-expressed Gal-3 would act on CD8 + T lymphocytes selectively, without affecting CD4 + T cells, and that inhibiting activation and proliferation of CD8 + T lymphocytes without inducing their apoptosis. This selective effect is original as compared to the known action of other galectins, such as Gal-1. We also observed that low doses of docetaxel (DTX), used in chemotherapeutic treatments of such cancer, leads to the inhibition of Gal-3 expression in human and murine PCa cells in vitro, but also in vivo without distinction of the degree of resistance of the prostate tumors to such chemotherapy. We finally observed that a vaccine composed of Gal3-deficient allogeneic PCa cell lysates allows the control of the development of the Gal-3-defective PCa cancer in an animal model. Thus, in vivo negative regulation of the expression of Gal-3 in tumors allows us to propose therapeutic alternatives of immunotherapy for all PCa patients, including metastatic and castration and DTX resistant one.

(84) **IMMUNOMONITORING OF ANTI-PD-1 THERAPY IN RENAL AND NON-SMALL CELL LUNG CANCER PATIENTS**

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Programmed cell death 1 (PD-1) is an inhibitory receptor expressed by exhausted T cells in chronic infections and cancer. Ther-

apies that block the PD-1 pathway have shown promising clinical results in a significant number of advanced-stage cancer patients (pts), including non–small cell lung cancer (NSCLC) and renal cell carcinoma (RCC). Pre-existing T-cell infiltration and/or expression of PD-L1 in tumors may be used as indicators of clinical response; however, peripheral blood (PB) analysis directed to understand the mechanisms of PD-1 blockade has not been extensively studied.

Until now, we have analyzed PB samples from 11 advanced stage NSCLC and 4 RCC pts before (PRE) and after 11 weeks (range=8-13) of receiving PD-1–targeted therapies Pembrolizumab or Nivolumab (POST). We use an automated hematology analyzer to study white blood cell counts, and flow cytometry to analyze lymphocyte subpopulations (CD3+, CD8+ and CD4+ T cells, T regulatory and NK cells) and markers of activation/differentiation on T cells (CD69, PD-1, TIM-3, ICOS, CCR7, CD45RO and CD95). Pts were classified according to clinical response in responders (n=4) if presented complete or partial response, stable disease (n=2) or progressive disease (PD, n=9).

We have performed a preliminary analysis on this small cohort of pts. Pts with PD showed an increase neutrophil-to-lymphocyte ratio after treatment (Median [IQR]: PRE 3.2 [2.9-3.6] vs POST 9.1 [4.4-12.6]) while this ratio decreased in responder pts (PRE 6.0 [3.6-18] vs POST 2.5 [2.2-3.0]). The same tendency was observed for leukocyte count, neutrophil absolute and relative number, and percentage of TIM-3+ cells within CD8+ T cells.

Promising early data suggest that there is an association between clinical responses and immunological changes in PB, which might provide a better understanding of patients' responses to PD-1–targeted therapies. Patient recruitment is still ongoing, and circulating levels of inflammatory markers will also be studied.

Keywords: Nivolumab, Pembrolizumab, NCSLC, Renal cell carciona, immunomonitoring

(317) **IFNβ SENSITIZES AND IFNα2b PROTECTS PANCREATIC TUMOR CELLS AGAINST THE PRO-APOPTOTIC EFFECT OF GEMCITABINE BY MODULATING AUTOPHAGY**

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Infiltrating ductal adenocarcinoma of the pancreas is the 4th in prevalence in Argentina, with a median survival of 4–6 months. Type I Interferons (IFNs) have important antitumor properties, but the precise mechanisms by which IFNs generate antineoplastic responses remain incompletely defined.

The aim of this study was to investigate the response of MIAPaCa-2 cell line to IFN-I and analyzed the modulation of autophagy and the capability to induce cell death.

MIAPaCa-2 cells were treated with 1000 IU/ml of IFNα2b or IFNβ for 24h. IFNα2b triggered the accumulation of acidic vesicular, evaluated by AO dye and autolysosomes, observed by MDC stain. By contrast, IFNβ decreased the accumulation of these vesicles. Moreover, IFNα2b increased the number and the size of LC3-positive vesicles respect to untreated cells meanwhile IFNβ decreased the number of autophagosomes per cell in RFP-LC3 transfected cells. Similar results were obtained by western blot. In addition, both IFNs inhibited MIAPaCa-2 cell growth assased by [3H]-thymidine incorporation. IFNα2b inhibited cell growth in a 28.3±4.7 % and 43.3±1.5 % for 24 and 48 h, respectively ($p<0.001$). IFNβ induced an inhibition of 52.0±4.0 % and 63.3±1.5 % for 24 and 48 h, respectively ($p<0.001$). Then, we evaluated cell death. In all cases the percentages of TUNEL-positive cells obtained by gemcitabine treatment after incubation with IFNα were lower to those observed by incubation with gemcitabine. The highest value obtained by treatment with gemcitabine was 42.3±1.5 %. This percentage of cell death was reduced to 30.5±6.0 % by pre-treatment with IFNα2b ($p>0.01$). By contrast, all doses tested by IFNβ sensitized cells to the pro-apoptotic effect of gemcitabine, increasing the percentage of cell death to 69.5±2.5 % ($p>0.0001$).

These results demonstrated that IFNα protected MIAPACa-2 cells to the pro-apoptotic effect of gemcitabine by induction of autophagy mean while IFNβ facilitated it, inhibiting autophagy process.

Keywords: Autophagy, IFN-I, Pancreatic cancer, Gemcitabine.

(380) **IMMUNOLOGICAL RESPONSES PROMOTED BY THERAPEUTIC CSF-470 VACCINE PLUS ADJUVANTS IN CUTANEOUS MELANOMA PATIENTS: CONCLUSIONS OF A PHASE II CLINICAL TRIAL**

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Abstract: Cutaneous melanoma (CM) incidence is increasing very fast and, given its aggressiveness, once it has metastasized treatment options are limited and the prognosis is bleak. We present here the inmune monitoring results of Phase II part of CASVAC-0401 study. 30 post-surgery CM patients (pts) stages II/III were randomized to receive CSF-470 vaccine (n=19) or IFN-α2b (n=11) during 2 years. CSF-470 vaccine is a mini-allograft consisting of 4 lethally-irradiated allogeneic CM cell lines, combined with BCG and GM-CSF as adjuvants. With a mean and a maximum follow-up of 36.1 and 90 months respectively, higher distant metastasis-free survival for CSF-470 treated pts was observed ($p=0.02$, G-B-Wilcoxon Test). Given that all vaccinated pts developed inflammation at the vaccination site and an increment in DTH response, we evaluated the immune changes promoted by CSF-470 vaccine and IFN-α2b in PRE and POST1 (6 months, 5 vaccinations) peripheral blood (PB) samples. Immune monitoring showed an increase in antitumoral cellular and humoral responses. Although vaccinated pts did not increase T $CD4^+$ and $CD8^+$ cells, a significant augmentation in NK cell frequency in the POST1 sample was observed (PRE 16.4±7.6% vs POST1 21±11.8% $p=0.01$, paired T test). Phenotypic and functional assays showed functional competence of these cells. To determine PB T cells reactivity to CM antigens, IFN-γ ELISPOT assay was performed after stimulation with vaccine lysate. A significant increase in specific T cells against CM antigens was detected in POST1 samples of 14/15 pts (PRE 65,4±80.6 spots vs POST1 220.2±97.2 spots, $p=0.0001$, paired T test). Besides, we observed a significant increase in antibody response against CM cells in vaccinated pts sera (ELISA assay, 1/10 dilution, PRE 0.3±0.2 vs POST1 0.8±0.3; $p<0.0001$, paired T test). None of these immune modulations were elicited by IFN-α2b. In conclusion, CSF-470 vaccine modulated the immune system towards an antitumoral response in vaccinated pts.

Keywords: cutaneous melanoma; immunotherapy; vaccine; immune monitoring; antitumoral response.

(381) **IN VITRO ANALYSIS OF THE FIRST STEPS OF IMMUNE STIMULATION ELICITED BY CSF-470 VACCINE PLUS ADJUVANTS**

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Abstract: In a Phase II/III randomized clinical trial (CASVAC-0401, NCT01729663), thirty post-surgery stages II/III cutaneous melanoma (CM) patients (pts) received CSF-470 vaccine or IFN-α2b (2:1). The CSF-470 vaccine is a mini-allograft of four lethally-irradiated allogeneic CM cell lines (CSF-470 cells), with BCG and rhGM-CSF as adjuvants. Since immune monitoring demonstrated an increase in anti-tumor innate and adaptive immunity of vaccinated pts, we attempted to investigate the first steps of the immune response elicited by CSF-470 vaccine plus adjuvants. Using an *in vitro* model of healthy donors' mononuclear peripheral blood cells (HD PBMC) in coculture with CSF-470 cells (1:1 PBMC:CSF-470 cells, $0,5x10^6$ each) plus adjuvants, early release of TNF-α (6 hs, 340.5±191.3 pg/ml) and IL-1β (48 hs, 456.3±195.3 pg/ml) were detected. When HD PBMC were stimulated only with adjuvants, TNF-α and IL-1β

release was higher (TNF-α: 1059±470 pg/ml; IL-1β: 1650±555 pg/ml). CSF-470 cells RNAseq analysis revealed an anti-inflammatory expression pattern, including TGF-β and IL-10 overexpression, that could partially mediate the hampered cytokines production, mainly promoted by BCG. On the other hand, IFN-γ release was also detected, but only in some HD PBMC with CSF-470 cells plus adjuvants cocultures (48 hs, 226,6±150 pg/ml), thus showing a high dependency on HD PBMC response to BCG. In conclusion, adjuvants, mainly BCG, were required to induce local inflammation in the presence of CSF-470 vaccine cells, given the anti-inflammatory pattern of cytokine expression, thus helping to create a favorable context for vaccine antigens to be processed and present.

Keywords: cutaneous melanoma; immunotherapeutic vaccine; adjuvants; cytokines

(518) ***IN VIVO* IMMUNOGENICITY ANALYSIS AND *IN VITRO* STABILITY CHARACTERIZATION OF NEW DE-IMMUNIZED RECOMBINANT HUMAN IFN-ALPHA (RHIFN-α) VERSIONS FOR ANTIVIRAL THERAPY**

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rhIFN-α is widely used for the treatment of viral diseases such as chronic Hepatitis B and C (CHC). Although rhIFN-α is a "self" derived protein therapeutic, anti-IFN-α immune responses have been reported in treated patients.

Previously, we used an *in silico* approach to identify immunodominant T-cell epitopes in a hyperglycosylated IFN-alpha2b (4N-IFN) and to modify them so as to generate functional de-immunized variants. Two functional 4N-IFN variants were successfully produced in CHO cells and designated as 4N-IFN(VAR1) and 4N-IFN(VAR3). These de-immunized 4N-IFN variants had significantly reduced *ex vivo* immunogenicity in human PBMC samples.

In this work we deepen our analysis comparing the immunogenicity of these proteins in transgenic mice. After subcutaneous injection, we isolated blood and serum and quantified binding and neutralizing antibody titers by ELISA and antiviral activity assays. Interestingly, no differences in binding antibody titers were detected in serum from mice inoculated with 4N-IFN, 4N-IFN(VAR1) and 4N-IFN(VAR3) ($p \leq 0.05$). However, marked reductions in neutralizing antibody titers were observed for the de-immunized variants, highlighting the success of the de-immunization process.

Along with immunogenicity, protein stability constitutes a major concern in biotherapeutic manufacturing processes. For this, we characterized the *in vitro* protein stability of the new 4N-IFN variants against a heat treatment ranging from 25 °C to 95 °C. Both de-immunized variants exhibited enhanced stability ($p \leq 0.05$) in comparison with a commercial non-glycosylated protein, highlighting an additional advantage of these muteins.

To summarize, the functional de-immunized 4N-IFN proteins exhibit significantly reduced *in vivo* immunogenicity in transgenic mice and increased *in vitro* thermal stability when compared with a commercial nonglycosylated protein and the original molecule.

Keywords: Interferon alpha, in vivo immunogenicity, thermal stability, hepatitis therapy.

(569) **IMPACT OF IMT504 ON THE IMMUNE SYSTEM OF FEMALE NON-OBESE DIABETIC (NOD/Ltj) MICE**

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Immunomodulatory oligonucleotide IMT504 (IMT) improved glycemia, beta cell function, and reduced leukocyte islet infiltration in spontaneous autoimmune diabetic mice (female NOD/Ltj). We analyzed the effects of IMT on immune parameters. Female diabetic NOD/LtJ mice (two consecutive glycemia levels (Gly) ≥ 250 mg/dl) were treated daily with IMT for five days (20mg/kg/day) or saline as control (DC). The day following the last injection, fasted Gly was measured and mice were sacrificed. Pancreatic leukocytes and splenocytes were obtained, immune cell populations (by flow cytometry) and cytokines expression (by q PCR) were analyzed respectively. DC mice showed 11% (2/19) spontaneous reversion of the diabetic condition whereas IMT treatment improved Gly in 72% of mice (13/18) (χ^2: DC vs IMT20: $p<0.01$). IMT treatment significantly diminished Gly at day 6 [(mg/dl): IMT20: Day 1: 307.92±34.7 vs Day 6: 230.08±97.6, $p<0.001$]. Pancreas CD45+ leukocytes showed a near significant reduction in IMT vs DC [DC CD45+%: 8.4± 5.9 vs IMT CD45+%: 3.83±2.14, $p=0.06$]. CD4+, CD8+, B220+, Cd11c+, F4/80+ and FOXP3+ population did not show significant differences. With IMT treatment, spleen IL12p40 expression showed a significant increase [DC: 1.27±0.18 vs IMT: 3.91±1.26, $p<0.01$]; while TNF-alpha expression showed a near significant decrease [DC: 1.33±0.52 vs IMT: 0.81±0.51, $p=0.07$]. IFN-gamma, IL4, and IL10 did not show significant differences. These results confirm that IMT reduced leukocyte pancreas infiltration shown by a near significant reduction in CD45+ population. Increased IL12p40 subunit is shared by both IL12 and IL23 cytokyne. IL23 promotes IL17 secretion, which could have regulatory effects, considering its inhibition on the apoptosis on suppressor myeloid cells. These results encourage further investigation on these cytokines expression (IL23 and IL17), and confirm that IMT modulates the immune system in NOD/Ltj mice.

(CONICET, UBA, ANPCYT, Fund. Williams, Fund. René Barón).
Keywords: Diabetes, Immune-System, Oligonucleotide

(721) **EXOSOMES ISOLATED FROM ASCITES OF T-CELL LYMPHOMA-BEARING MICE INDUCE A TUMOR-SPECIFIC IMMUNE RESPONSE**

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Exosomes are endosome-derived nanovesicles involved in cell–cell communication. Tumor exosomes can either activate or inhibit the immune system. The neoplastic stage and microenvironment can affect the quantity and composition of EVs determining their biological behavior. In this work, we have evaluated the ability of exosomes isolated from ascites of a murine T-cell lymphoma (ExoA) to modulate a tumor-specific immune response.

ExoA induced *in vitro* proliferation of tumor sensitized splenocytes when compared with unstimulated control ($p<0,001$), detected by CFSE dilution. In addition, ExoA stimulated the specific proliferation of CD4+ cells (15%) and CD8+ (3%) when compared to unstimulated sensitized splenocytes (5% and 2%, respectively). In addition, IFN-γ levels after *in vitro* stimulation with ExoA were significantly higher than unstimulated controls ($p<0,001$).

Immunization with ExoA induced both humoral and cellular immune responses that allowed the rejection of the tumor. Approximately 60% of mice immunized and challenged with LBC cells did not developed the tumor, whereas 100% control mice died 22 days following the challenge. Furthermore, the immunization induced immune memory as it also protected mice against a second challenge with LBC cells. However, it had no effect on a non-related mammary adenocarcinoma, demonstrating that the immune response elicited was specific. Intracellular staining

revealed that IFN-γ secreting CD4+ and CD8+ cells from ExoA-immunized mice were significantly higher in immunized individuals ($p<0,01$), showing that a TH1 response is involved in tumor rejection. Our findings confirm exosomes as promising defined acellular tumor antigens for the development of an antitumor vaccine.

Keywords: exosomes, T-cell lymphoma, immune response, tumor

vaccine, ascites

(1023) **EVALUATION OF THE ADJUVANT EFFECT OF *Minthostachys verticillata* ESSENTIAL OIL FOR ITS POSSIBLE USE IN VACCINES**

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The aim of this study was to determine the effect of essential oil (EO) as adjuvant evaluating the humoral and cellular immune response. Balb/c mice (n=4 per group) were immunized by subcutaneous injection (day 0) and revaccinated (at day 14 and 28) as follows: Group 1:100 µl PBS; Group 2:100 µl ovalbumin (OVA) 0.2 mg/ml; Group 3: OVA + $Al(OH)_3$ 0.5 mg/ml; Group 4-6: OVA + EO (1.25; 2.5; 5 mg/ml); Group 7: OVA + $Al(OH)_3$ + EO (2.5 mg/ml). Seven days after revaccination, serum samples were collected and analyzed for antigen-specific total antibodies (IgG, IgM and IgA) by indirect ELISA. For delayed-type hypersensitivity (DTH) test, mice (n=2 per group) were immunized by subcutaneous injection on days 0 and 14 as described for groups 1, 2, 3, 5 and 7. Seven days after immunization a challenge with OVA in the left footpads were performed by intradermal injection. The thickness of hind footpads was measured before and 24 and 48 h after the OVA injection. Results were expressed as the difference in thickness of footpads after and before the inoculation. Any animal died, no clinical signs were observed in the inoculation area either generalized reactions in any group. A significant increase in antibodies (Ab) levels was observed in the groups 3, 5, 6 and 7 respect to group 2 ($p<0.001$; $p<0.01$; $p<0.01$).However, the Ab levels of groups 5 and 6 were significant lower than those observed in group 3 ($p<0.01$) and the Ab levels of group 7 were similar to group 3. A significant increase in footpads thickness was observed only in the groups 3 and 7 respect to group 2 ($p<0.001$) 24 h after OVA injection by DTH test. A significant increase in footpads thickness of group 7 respect to groups 2 and 3 was observed ($p<0.001$; $p<0.01$) at 48 h. EO acts as adjuvant of humoral immune response with lower power than $Al(OH)_3$. Besides it can activate the cellular immunity combined with $Al(OH)_3$ enhancing the effectiveness. Higher doses of EO are being tested to evaluate the optimal dose as adjuvant.

Keywords: *Minthostachys verticillata*, essential oil, adjuvant, humoral and cellular immunity

(1398) **GLYCOSYLATION SIGNATURE AND SUPPRESSIVE PROFILE OF MYELOID-DERIVED SUPPRESSOR CELLS IN HEALTH AND CANCER**

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Myeloid Derived Suppressor Cells (MDSCs) are a heterogeneous population of immature myeloid cells that give rise to DCs and macrophages; in pathologic conditions, these cells stop their differentiation programs and become suppressive. The glycosylation signature of a cell can bring important information about its selective interaction with endogenous glycan-binding proteins (e.g. Galectin-1), present in inflammatory or tumor microenvironments. Here we examined the glycosylation profile of MDSCs in normal conditions and cancer settings. First, we examined the glycosylation pattern of MDSCs during the differentiation process. We found that $Gr1^{high}CD11b^{+}$ and $Gr1^{low/int}$ $CD11b^{+}$ populations presented differential glycosylation profiles: While $Gr1^{high}CD11b^{+}$ cells showed abundant β1-6 N-glycan branching and poly-LacNAc extension but did not show terminally α2,6-linked sialic acid; $Gr1^{low/int}$ $CD11b^{+}$ cells did show terminally α2,6-linked sialic. We then imitated the tumor microenvironment by adding tumor conditioned media and hypoxia (1% O_2). We found an increase of MHCII, CD11c dendritic cell markers and F4/80 and CD206 macrophage markers, with hypoxic conditions; the latter were further stimulated by combining hypoxia and conditioned media ($p<0,5$). PD-L1 and nitric oxide production were increased in hypoxia, as well as complex N-glycans and α2,6-linked sialic acid ($p<0,5$). Next, we examined the glycosylation status of MDSCs in different hematopoietic organs in normal conditions and whether this phenotype could be influenced by tumor growth. We inoculated C57BL/6 with $5x10^5$ Lewis Lung Carcinoma cells and harvested spleens, draining lymph nodes, bone marrow and tumors. The glycosylation profile was not only specific of the different organs, but was also modified by tumor settings. We present here the first evidence of MDSC glycosylation and suppressive profile, which is the first step for establishing the role of these cells in Galectin-1 mediated tumor escape.

Glycosylation, MDSCs, Immune escape, Galectin-1

PLANT BIOLOGY 1

(359) **A NEW PLASTID TARGETING MECHANISM REVEALED BY THE SYSTEMIC DEFENSE-ASSOCIATED LIPID TRANSFER PROTEIN AZI1**

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Right subcelular localization of defense factors is essential for the plant immune system. The mobile signal azelaic acid (AZA) together with the lipid transfer protein (LTP)-like AZI1 are key components for systemic resistance and the priming or immunological “memory” establishment. AZI1 allows the systemic movement and uptake of AZA in Arabidopsis plants. Localization studies indicate that a pool of AZI1 exists near the site of AZA production, in the chloroplast outer envelope. However, AZI1 does not possess a classical chloroplastic transit peptide that can explain its localization. Here, we show that this LTP-like and several members of the AZI1 family use an undescribed N-terminal signature that allows the chloroplast targeting. We will present evidence that cytoskeleton integrity, protein kinases associated to defense and specific features of the AZI1 N-terminus mediate AZI1-plastid targeting. Interestingly, in Arabidopsis ~0.3 % of the coded proteins display a similar N-terminal signature. The study of some of them corroborate their plastid localization although their organelle targeting is not predicted by standard algorithms. Moreover, because we observed that AZI1 play a role and localizes to root plastids, this signal may also be functioning in underground tissues. Taken together, our results suggest the existence of a novel mode of plastid targeting/trafficking possible related to defense responses.

Keywords: Arabidopsis, Plant Immunity, Plastids, Subcellular Trafficking.

(1512) **ABIOTIC STRESS TOLERANCE IN *Arabidopsis thaliana*: A ROLE FOR ALTERNATIVE SPLICING**

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Because of their sessile nature, plants have adopted varied strategies for growing and reproducing in an ever changing environment. Variations in light quality and intensity, temperature, salinity and soil water content, and also predators have a negative impact on plant’s metabolism and plants need to respond accordingly and efficiently to survive. One of the underlying molecular mechanisms of phenotypic plasticity is alternative splicing (AS) of pre-mRNAs. This adjustment of the transcriptome is particularly prevalent in response to abiotic stress. In this work we study the role of a putative splicing factor (SF) in growth and development of *Arabidopsis thaliana* and

its impact on abiotic stress tolerance. We found that the expression null mutants of this SF exhibit alterations in flowering time, light perception and low tolerance to abiotic stress. Furthermore, here we describe a global transcriptome analysis to assess the role of this SF in the AS control.

Splicing; Arabidopsis; flowering; stress; transcriptomics

(258) **ROLE OF MEDIATOR COMPLEX IN DNA DAMAGE RESPONSES**

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CEFOBI

In Eukaryotes, the multiprotein Mediator complex regulates transcription by participating in RNA polymerase II assembly, RNA processing, as well as by modulating chromatin architecture. Moreover, recent reports have shown that Mediator plays an important role by connecting transcription with DNA repair in humans and yeast. In Arabidopsis thaliana, mediator is composed by 34 subunits and acts as a signal integrator. On the other hand, depletion of the stratospheric ozone layer has increased ultraviolet-B radiation (UV-B) level at the earth's surface, causing DNA damage, primarily cyclobutane pyrimidine dimers. These lesions affect DNA replication and transcription, and as a consequence leaf growth is inhibits. The objective of this work is to investigate the participation of Mediator in DNA damage responses by UV-B radiation in plants, using as a model A. thaliana plants. For this purpose, we analyzed the effect of UV-B in Arabidopsis mutants in the Mediator complex subunit 17, mutations of its orthologues in yeast and humans are impaired in transcription coupled DNA damage repair. Our results show that *med17* mutants show increased DNA damage after UV-B exposure ($p < 0.05$), and are also deficient in different DNA damage responses. Together, our results suggest that MED17 has an important role during DNA damage responses induced by UV-B radiation in Arabidopsis.

(1127) **E2F TRANSCRIPTION FACTORS ROLE IN THE DEVELOPMENT OF *ARABIDOPSIS THALIANA* PLANTS UNDER UV-B RADIATION**

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CEFOBI

Plant development involves the production of new cells that arise at the meristems from divisions of pluripotent stem cells, followed by their successive cell cycle exit and differentiation. In particular, cell division is a tightly regulated process that is influenced by innate genetic cues as well as by external environmental signals. The Retinoblastoma E2F pathway plays an important role in the regulation of the cell cycle and in different differentiation processes. UV-B radiation negatively affects cell proliferation and development; thus, we analyzed the effect of this radiation in different *Arabidopsis thaliana* mutants and transgenic lines with altered expression of genes encoding E2F transcription factors. In this work, we analyzed the role of E2Fb, a transcriptional activator which regulates the E2Fe expression, among many others genes. Previous reports have shown that E2Fe is a transcriptional repressor of the photolyase PHR1, and plants *knocked out* in the *E2Fe* gene are more tolerant to UV-B radiation. Through phenotypic analysis, microscopy observations and molecular biology experiments carried out using plants exposed to UV-B radiation, we show that mutant plants in the *E2Fb* gene are less sensitive to this radiation than wild type plants. For example, these mutants show a lower decrease in the rosette and leaf area than wild type plants. Together, our data demonstrate that E2Fb participates in UV-B responses in Arabidopsis and it may regulate the expression of genes involved in the cell cycle checkpoints.

Keyword: E2F, cell cycle, UV-B radiation

(1689) **DIFFERENTIAL SUBNUCLEAR LOCALIZATION OF THE MBD4L DNA GLYCOSYLASE SPLICING ISOFORMS. POSSIBLE ROLE(S) ON STRESS RESPONSES.**

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DNA glycosylases are key enzymes for organisms' genome stability mediating DNA damage repair. Among them, MBD4L is a nuclear *Arabidopsis* DNA glycosylase acting during stress conditions. Interestingly, *MBD4L* gene can generate two-isoforms through the retention of a protein-coding cryptic intron (exitron). It is believed that exitron splicing events result in protein versions with alternative functional domains and/or post-translational modifications. Taking this into account, we analyzed MBD4L splicing-isoforms transcript levels, subcelular localization and contribution to damaged DNA correction. We found that the *MBD4L* alternative transcripts changed their relative levels depending on the stress applied. Surprisingly, when expressed as GFP fusions each of the MBD4 isoforms located at different subnuclear compartments in both *Arabidopsis* and *Nicotiana benthamiana* plants. Moreover, some stresses induced dynamic changes in enzyme subnuclear localization. On the other hand, although showing distinctive localization the overexpression of both MBD4L isoforms increased resistance to a DNA damaging agent. Altogether, our results indicate that MBD4L location can be driven by exitron alternative splicing mechanisms. However, additional levels of control may determine differential subcellular requirements for MBD4L isoforms under particular circumstances. A putative model for MBD4L nuclear role/localization will be presented.

Keywords: Arabidopsis, DNA glycosylases, Biotic and Abiotic Stresses,Subnuclear Localization.

(534) **EVOLUTIONARY ANALYSES OF INDETERMINATE DOMAIN (IDD) TRANSCRIPTION FACTORS IN ANGIOSPERMS**

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The *indeterminate domain* (*IDD*) transcription factors are a subclass of C2H2 Zinc Finger transcription factor family. This transcription factors plays different roles in diverse aspects of plant metabolism and development like, flowering time, seed maturation, root development, shoot gravitropism and leaf development. The functions of most of these genes are not known and the molecular evolution of the family has not been explored in detailed.

Here we present a phylogenetic tree constructed by Bayesian inference using the IDD genes of 10 representative genomes of flowering plants (Angiosperms) The protein sequences were obtained of the GreenPhylDB and Phytozome databases, they were aligned with MAFFT program, and conserved blocks were extracted and used to make a phylogenetic reconstruction using Mr Bayes v3.2.6. We also performed a conserved motif analyses using MEME v4.11.

Our results suggest that the family may be divided in at least nine groups. These lineages share three conserved motives, which include the four zinc fingers of the IDD domain. Additionally, we found motives that are group specific that needs further characterization. Furthermore, four of the nine groups seem to be monocots specific IDD lineages. Interestingly one of these monocots groups includes *Indeterminate1* that controls transitioning to flowering in Zea mays and is the gene for which the family is named.

This study provides a solid framework of the orthology relationships in the Angiosperm IDD transcription factors, thus increasing the accuracy of ortholog identification in model species and facilitating the identification of agronomically important genes related to plant metabolism and development.

Palabras clave: Indeterminate Domain, IDD, C2H2, Phylogenetics, Angiosperms

(1748) **MUTANTS FOR GENES INVOLVED IN THE 5'-3' mRNA DECAY PATHWAY HAVE ALTER CIRCADIAN PHENOTYPES**

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Circadian rhythms allow organisms to adjust biological processes to occur at the most appropriate time of day. They are controlled by a complex gene network —called the central oscillator—, in which genes interact mainly by transcriptional feedback loops, keeping an approximate 24h rhythm. Recent evidence supports the importance of post-transcriptional regulation as a key step for the correct oscillation of the clock.

Among the different post-transcriptional mechanisms, in this work we focus on RNA stability and decay using the plant model organism, *Arabidopsis thaliana*. We studied the 5'-3' messenger RNA (mRNA) decay pathway. This includes genes encoding proteins involved in different steps of the pathway, from translation arrest, to mRNA degradation, including poly(A) tail shortening and decapping.

We characterize different outputs of the clock in mutants for genes involved in this pathway. All the mutants showed a longer period of leaf movement, a late flowering time, and no appreciable change in the hypocotyl elongation. Also, we observed a deregulation of mRNA levels from some core oscillator genes.

This are the first steps to understand how mRNA degradation contributes to the fine-tuning of circadian rhythms. Further mechanistic studies will be done to achieve a deeper comprehension of this regulatory layer.

Keywords: Arabidopsis; RNA degradation; circadian clock; post-transcriptional.

(802) **RALF PEPTIDES DURING POLLEN TUBE GROWTH IN *ARABIDOPSIS THALIANA*.**

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Polarized cell growth involves the expansion of one of its ends, making cell elongation in a single direction. In plants, this process occurs during pollen tube growth along the style. This polarized growth is highly regulated by cytoskeletal reorganization, vesicular movement, Ca^{2+} signaling and reactive oxygen species (ROS). Because plant cells possess a rigid cell wall, regulation of its integrity is also essential to allow this polarized growth.

Plant receptor-like kinases (RLKs) of the subfamily of *Catharanthus roseus* RLK1-Like (CrRLK1L) are involved in the polarized growth of pollen tubes and root hairs. RALFs (Rapid Alkalinization Factor) are secreted peptides encoded by a large family of 39 members in *Arabidopsis thaliana.* Recently, it was reported that peptides RALF1 and RALFL23 act as ligands of FERONIA, one member of the CrRLK1L subfamily. Our objective is to functionally characterize a subfamily of RALF peptides expressed in pollen of Arabidopsis, potentially involved in the pollen tube growth.

We have analyzed the expression pattern of some pollen RALF members through transcriptional fusions of their promoter regions and the bacterial *uidA* gene encoding β-glucuronidase (GUS). To study the role of RALF peptides in the pollen tube growth, we generated RALF overexpressing plants. In vitro pollen germination of these plants is affected when compared to WT plants. These plants showed an abnormal segregation ratio when transgenic pollen was used. In addition, we have determined, through yeast two hybrid (Y2H), that pollen RALFs would function through interaction with proteins of the pollen Leucin-rich extensin family (LRX).

Future investigations will contribute to understand how the pollen RALFs, the CrRLK1L receptor-like kinases and the Leucin-rich extensin family (LRX) control pollen tube growth.

Keywords: RALF, pollen tube, signal peptide.

(1603) **THE ROL OF DEPRESSED PALEA1 (DP1) IN THE ORIGIN AND EVOLUTION OF THE PALEA IN GRASSES**

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Grasses (Poaceae) with more than 11,000 species are the second largest family of monocots. The diversity of this family is particularly evident in the enormous variety of their reproductive structures, particularly in two exclusive pieces of grasses such as the lemma and palea. There have been postulated different hypotheses about the origin of both, suggesting that they can be modified floral pieces or even extra floral pieces such as glumes. Some efforts has been made in order to understand the molecular mechanisms involved in the development of their flowers, but the information is still very limited and fragmented, preventing elucidate a general mechanism about the origin, development and evolution of lemma and palea. In particular, we study the role of DP1, a transcription factors (AT-hook family) involved in the normal development of the palea in Oryza sativa (rice). The molecular evolution of DP1 is being studied, and also its expression pattern in various representatives of the family. In addition, reverse genetics strategies will be used to understand the function of DP1 in the model grass species Setaria viridis. The information gathered will be very useful to know the origin and function of DP1, and to determine if its function has diversified into grasses.

NEUROSCIENCE 8

(1221) LITHIUM AND BONE MARROW MONONUCLEAR CELLS: COMBINED THERAPY IN THE TREATMENT OF SCIATIC NERVE REVERSIBLE DEMYELINATION

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Lithium salts have been the conventional pharmacological treatment for bipolar and major depressive disorders for over six decades. Additional benefits of lithium include neuroprotective, anti-inflammatory, and anti-apoptotic effects, which have been exploited to treat central nervous system trauma and chronic neurodegenerative diseases. In the peripheral nervous system, lithium administration has been shown to foster functional recovery and remyelination after injury. Our group has studied the bioactivity, specificity, and reversibility of lithium's action on cultured Schwann cell (SC) growth, survival, proliferation and differentiation, in addition to basal lamina and myelin formation in SC-neuron cultures.

On the other hand, our group has demonstrated, in a model of sciatic nerve crush that systemically transplanted bone marrow mononuclear cells (BMMC) spontaneously migrate to and remain in the injured nerve for as long as 60 days post injury (dpi). Once in the ipsilateral nerve, some of the cells colocalized with SC markers. BMMC were also shown to exert a beneficial effect on axon regeneration and remyelination and prevent hyperalgesia.

In the present work, we combine these two potential treatments with the aim of improving nerve recovery. After nerve compression, animals received intravenous transplantation of BMMC and orally administered lithium chloride. Axonal regeneration, remyelination and functional recovery were analyzed 14 dpi. While combined therapy did not exhibit differences regarding BMMC treatment in terms of myelin recovery, analyzed through immunohistochemistry or western blot ($p<0.05$); preliminary electron microscopy studies showed smaller but more highly myelinated axons in the combined therapy group. Further morphological studies and functional assays currently underway will corroborate the advantages of combined therapy over single treatment.

Keywords: Lithium; bone marrow mononuclear

cells; transplantation; Wallerian degeneration.

(1652) **META-ANALYSIS OF AXONAL TRANSCRIPTOME DERIVED FROM ADULT MYELINATED OR CULTURED NEURONS**

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Thanks to the advances made in the last years in RNA amplification techniques and accessibility to the platforms of data sequencing and analysis, it was possible to identify massively the mRNAs localized in axons of neurons. In this work we compare transcriptomes of axons derived from neuron cultures (*in vitro*) with transcriptomes of axons derived from fully differentiated myelinated neurons (*in vivo*) to identify a core of transcripts present in a variety of axons as well as the particularities of each axon coming from different types of neurons. For this purpose we analyzed seven transcriptomes of published datasets of *in vitro* axon obtained by neuron culture of divers types of neurons and species. On the other hand, the *in vivo* axonal transcriptomes were obtained by our group using the technique of micro-dissection of myelinated axons derived from ventral and dorsal roots from adults rats. When comparing the transcriptomes of mature axons with those of axons in culture, we found large differences in the number of transcripts located in this subcellular domain, being a greater number in the axons in culture. This may be because these axons are deprived of their microenvironment, and accessory cells. The core of mRNAs present in axons from different types of neuron both *in vitro* and *in vivo* is about one thousand. The pathways and ontologies enriched in this sets are related with maintenance, cytoskeleton and cell-to-cell union. The trascriptomes of mature axons derived from motor and sensory neurons presents differences in the abundance of some transcripts, focusing our study in mRNAs encoding ribosomal and membrane proteins. This work is the first that analyze all the transcriptome data available derived from cultured axons, and also is the first in obtain transcriptomes of *in vivo* axons, allowing to know the difference between the two methodology to obtain the cytoplasm of axons.

Transcriptome - Meta-analysis - Axon

(168) **NEUROSTEROIDOGENESIS IS RECOVERED DURING SPONTANEOUS REMYELINATION IN THE CUPRIZONE MODEL OF DEMYELINATION**

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Studies of Multiple Sclerosis and murine experimental autoimmune encephalomyelitis (EAE) have shown altered neurosteroidogenesis. We previously demonstrated that Progesterone pre-treatment decreases demyelination, neuroinflammation, improves clinical grade and re-establishes steroidogenic enzymes levels in the spinal cord of EAE mice (Garay 2007,2012,2017). Here, we asses hippocampal steroidogenic pathway in a cuprizone model of demyelination. We also analysed demyelination and neuroinflammation parameters in the hippocampus of control (CTRL), demyelinated (CPZ D), and remyelinated (CPZ R) mice. We found in CPZ D mice hippocampus decreased mRNA expression of the steroidogenic acute regulatory protein (STAR), P450scc (cholesterol side-chain cleavage), 5 α-reductase (CPZ D vs. CTRL;$p<0.05$) whereas mRNA levels of 3β-hydroxysteroid dehydrogenase (3β-HSD) showed a large intergroup variation. Altered expression of neurosteroidogenic enzyme mRNA was accompanied by decreased myelin basic protein (MBP) mRNA and protein levels (CPZ D vs CTRL;$p<0.05$). We also found increased mRNA expression of 18 Kd translocator protein (TSPO), which likely originated on the reactive microgliosis revealed by increased number of iba1+ cells and CD11b and TNFα mRNAs (CPZ vs CTRL,$p<0.001$). Fifteen days after cuprizone removal, spontaneous hippocampal remyelination was demonstrated by the increased MBP expression and attenuation of CD11b, TNFα related-microgliosis and TSPO mRNA. In this cuprizone-free period, steroidogenic enzymes expression recovered the levels of control mice (CPZ R vs CTRL;ns). Our results demonstrated that demyelination and neuroinflammation is associated with reduced neurosteroidogenesis. We hypothesized that restoration of protective steroid products, eg.Progesterone, may have a role in recovery from cuprizone-induced demyelination.

Keywords:Demyelination,Steroidogenesis,RemyelinationProgesterone

(496) **PARTICIPATION OF NUCLEAR RECEPTORS PPARγ AND RXR IN THE REMYELINATION PROCESS**

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Demyelination is a pathological process by which myelin is lost from around axons and, in the CNS; it is usually the consequence of a direct insult on oligodendrocytes (OL). In turn, remyelination is the process by which myelin sheaths are restored to demyelinated axons, a function attributed to adult CNS oligodendroglial precursor cells (OPC). Some of these OPC reside in the adult brain, while others are derived from neural precursor cells (NPC) present in the subventricular zone (SVZ) and help remyelinate neighboring areas. Recent work using demyelination models has proven a significant increase in the transcript of retinoid X receptor γ (RXRγ) during remyelination in the CNS. RXRs are nuclear receptors regulating cell proliferation and differentiation, and form homodimers or heterodimers with other nuclear receptors including peroxisome proliferator activator proteins (PPAR), which also participate in the regulation of OL differentiation and maturation. The aim of the present work is to study joint activation of RXRγ and PPARγ by specific agonists 9 cis retinoic acid (RA) and Pioglitazone (PIO), respectively, in remyelination through *in vivo* experiments using cuprizone (CPZ) induced demyelination in mice. Eighy-week-old mice were fed 0.2% CPZ for 5 weeks, when they were stereotaxically injected vehicle or 1 μl containing 0.1μg RA, PIO or PIO+RA, unilaterally into the corpus callosum (CC). Animals were then sacrificed either 2 or 4 days after injection. Immunohistochemical studies in the CC rendered a higher percentage of APC+ differentiated OL in animals sacrificed 2 days after either PIO or PIO+RA injection, without changes in the proportion of PDGFRα+ OPC. In addition, activated microglia and their phenotypic characterization (M1 or M2) are currently being assessed through both immunohistochemical and Western blot analyses of both the CC and SVZ. Preliminary results indicate a pro-myelinating effect of joint RXRγ and PPARγ activation by specific agonists.

Keywords: Remyelination, Nuclear receptor, oligodendroglial differentiation.

(695) **PROTECTIVE EFFECTS OF TESTOSTERONE ON WR MOTONEURON DEGENERATION**

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Wobbler (WR) mouse is an animal model for human amyotrophic lateral sclerosis (ALS). We have shown neuroprotective effects of progesterone in WRs. Here, we studied if testosterone (T) treatment was neuroprotective. Since low T levels were shown in WR's serum and spinal cord from WRs, two month-old male WRs were treated with T (silastic tube s.c. implant with T crystals) for 60 days. We examined clinical motoneuron disabilities, T serum levels and endocrine parameters: hypophysis, testicles, seminal vesicles and

adrenal glands weight were measured. We also evaluated in cervical spinal cord: 1) the number of microglial Iba1+ cells in ventral horn by immunofluorescence, 2) expression of toll-*like receptor 4* (TLR4) and *transforming growth factor* β (TGFβ) mRNAs, and 3) stero*idogenic* acute regulatory protein (StAR) and the translocator protein (TSPO) mRNAs by qPCR. WRs had higher testicle and adrenal ($p<0.01$) mass and lower seminal vesicles ($p<0.01$ vs controls). T treatment yielded a three-fold increase in its serum levels ($p<0.05$ vs WR). WR's hypophysis, a parameter sensitive to aromatizable androgens, and adrenal gland weights were not modified by T, while T decreased testicles ($p<0.01$) and increased seminal vesicles ($p<0.01$). Except for TGFβ mRNA levels, WR's cervical spinal cord showed high levels of: 1) Iba1+ cells ($p<0.01$), 2) TLR4 mRNA ($p<0.05$), 3) StAR and TSPO mRNAs ($p<0.001$). T treatment significantly reduced Iba1+ cells ($p<0.01$), TRL4 and StAR mRNAs ($p<0.05$), although TSPO was not affected. However, high levels of TGFβ mRNA was found in WR+T ($p<0.05$ vs WR). Clinically, T also delayed clinical abnormalities in WRs ($p<0.01$ vs WR). In summary, pharmacological administration of T to WRs reduced inflammatory and steroidogenic mitochondrial parameters, while delayed motor disability and increased seminal vesicles trophism. To conclude, protective effect of T may involve the modulation of inflammatory and steroidogenic factors in WR's motoneuron degeneration.

Keywords: Testosterone; Wobbler; neuroprotection; amyotrophic lateral sclerosis.

(1225) **SHH SIGNALING INVOLVEMENT IN DEMYELINATION-REMYELINATION PROCESS**

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Multiple sclerosis (MS) is one of the most common neurological disorders affecting young people worldwide. MS is characterized by inflammation, and demyelination; in turn, spontaneous remyelination is driven by oligodendroglial precursor cells (OPCs) but is usually incomplete, as OPCs fail to mature into myelinating oligodencrocytes (OLs), thus leading to neurodegeneration.

Among several signaling pathways taking part in OPC maturation, Sonic hedgehog (Shh) has been recently shown to attenuate myelin damage through an increase in OPC number and maturation in a focal demyelination model. The transduction of hedgehog signaling classically involves transmembrane proteins Patched (Ptc) and Smoothened (Smo). In the absence of Shh ligand, downstream hedgehog signaling is repressed, as Ptc suppresses Smo and inhibits the Gli transcription factors to act on target genes. This repression is relieved when Shh binds Ptc.

In this context, the aim of this work was to study Shh signaling during demyelination and remyelination in a cuprizone (CPZ)-induced demyelination model in rats, and to further investigate Shh protein as a potential biomarker of different stages of MS.

After weaning, Wistar rats were fed a 0.6% CPZ diet for 2 weeks to trigger demyelination, later fed a control diet to allow remyelination, and sacrificed 7d before (-7d), upon (0d), and 7, 14 and 21d (+7d, +14d and +21d) after CPZ withdrawal. Demyelination and remyelination were characterized through myelin basic protein (MBP) immunofluorescence (IF) at different times and the Shh pathway was analyzed by Western blot, q-PCR and IF along the process. Western blot analyses showed an increase in precursor and cleaved Shh in the corpus callosum of CPZ animals at -7d, 0d and +7d. q-PCR assays rendered an increase in Smo and Gli1 during remyelination. These preliminary results suggest stage-specific Shh signaling participation in demyelination and remyelination, and its potential use as a biomarker of MS stages.

(286) **THE SELECTIVE GLUCOCORTICOID RECEPTOR MODULATOR CORT 113176 REDUCES MOTONEURON DEGENERATION AND NEUROINFLAMMATION IN WOBBLER MICE SPINAL CORD**

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Abstract: Wobbler (Wr) mice are experimental models for amyotrophic lateral sclerosis. As such they show motoneuron degeneration, motor deficits, and astrogliosis and microgliosis of the spinal cord. Additionally, Wr mice show increased plasma, spinal cord and brain corticosterone levels and focal adrenocortical hyperplasia, suggesting a pathogenic role for glucocorticoids in this disorder. Considering this endocrine background, we examined whether the glucocorticoid receptor (GR) modulator CORT113176 prevents spinal cord neuropathology of Wobblers. CORT113176 shows high affinity for the GR, with low or null affinity for other steroid receptors. We employed five month old genotyped Wr mice that received s.c. vehicle or 30 mg / kg / day for 4 days of CORT113176 dissolved in sesame oil. The mice were used on the 4th day, 2 hours after the last dose of CORT113176. Vehicle treated Wr mice presented vacuolated motoneurons ($p<0.001$ vs. Control (Ctl)), increased glial fibrillary acidic protein (GFAP)+ astrocytes ($p<0.001$ vs. Ctl) and decreased glutamine synthase (GS)+ cells ($p<0.05$). There was strong neuroinflammation, shown by increased staining for IBA1+ microglia ($p<0.001$ vs. Ctl) and CD11b mRNA ($p<0.01$ vs. Ctl), enhanced expression of tumor necrosis factor-α ($p<0.001$ vs. Ctl), its cognate receptor TNFR1 ($p<0.05$ vs. Ctl), toll-like receptor 4 ($p<0.01$ vs. Ctl), the inducible nitric oxide synthase ($p<0.05$ vs. Ctl), NFkB ($p<0.05$ vs. Ctl) and the high mobility group box 1 protein (HMGB1) ($p<0.001$ vs. Ctl). Treatment of Wr mice with CORT113176 reversed the abnormalities of motoneurons and down-regulated proinflammatory mediators and glial reactivity. Expression of glutamate transporters GLT1 and GLAST mRNAs was significantly enhanced over control and untreated Wobblers ($p<0.01$ Wr vs. Wr CORT113176). In summary, antagonism of GR with CORT113176 prevented neuropathology and showed anti-inflammatory and anti-glutamatergic effects in the spinal cord of Wr mice.

Keywords: Wobbler mice; hyperadrenocorticism; glucocorticoid receptor antagonism, CORT113176; neuroinflammation.

(248) **TREM-2 MODULATES TOLL-LIKE RECEPTOR ACTIVITY IN GLIAL CELLS AND CONVERSION TO THE PRONEURODEGENERATIVE PHENOTYPE**

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Abstract: Activation of the innate immune system can be achieved by pathogen or damage –associated molecules (PAMP or DAMP respectively). In the brain, the innate inmune system response is mainly dependent on microglia, but we and others showed that astrocytes can also behave as facultative innate immune system cells expressing pattern recognition receptors such as Toll-like and RAGE. Once activated, showing the classical reactive gliosis morphological features, astrocytes can further polarize into a TLR4-dependent proinflammatory phenotype A1 that facilitates neurodegeneration. Having in mind that TREM-2 and its adaptor DAP12 down-regulate TLR signaling, participating in the fine-tuning of the inflammatory response; we decided to study the TREM-2 role in reactive gliosis and astroglial conversion to the proinflammatory-neurodegenerative phenotype. First, using an experimental model of brain ischemia in Wistar rats, we detected TREM2 expression in astrocytes, with a peak by 7 days post-ischemia (7DPI) in the periphery of ischemic penumbra. Then, using *in vitro* cell culture approach, we observed that TREM2 expression is induced in astrocytes by OGD, PAMP or DAMP HMGB-1 exposure. Glial TREM2 seems to be fully functional since antibody crosslinking or the overexpression of TREM2 adaptor DAP12 partially blocked LPS-TLR4-induced NFkB activation. Following TREM2 crosslinking, we collected glial-cells conditioned me-

dium and tested the neurodegenerative effects on primary cortical neurons. This approach resulted in an improved neuronal survival that required microglia, since microglial blockage with minocyclin reduced the pro-survival effect. Taking together, our results show that astrocytes express a fully functional TREM-2, however its protective effect inducing neuronal survival, requires the microglial cooperation. In this way, TREM-2 modulation emerges as an interesting new target to reduce astroglial conversion to the proinflammatory phenotype that induces neurodegeneration.

Keywords: TREM-2, glial cells, reactive gliosis

(1567) **ROLE OF C-FOS AS AN ACTIVATOR OF PHOSPHOLIPID SYNTHESIS DURING NEURONAL DIFFERENTIATION**

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Cytoplasmic c-Fos activates phospholipid synthesis by its association with particular lipid synthesizing enzymes at the endoplasmic reticulum (ER) which induces an increase in their catalytic activity. This activity of c-Fos is associated with the molecular mechanisms that allow the higher rate of membrane genesis required for the complex events that take place during neuronal differentiation, such as sprouting and branching. Studying this role, we found c-Fos strongly co-localizing with ER markers in particular unidentified structures located at branching sites of growing neuronal processes. Blocking either c-Fos expression or its activity promotes an impairment in differentiation with no observable development of axonal processes. In addition, the expression of N-terminal deletion mutants of c-Fos that are capable of blocking only its cytoplasmic activity produces a similar effect. CTP:phosphocholine cytidylyltransferase-**β**2 (CCTβ2), the key regulatory enzyme responsible for CDP-choline formation in the brain, associates to the ER membranes and plays an important role in the formation of axon branches. To determine if this enzyme is activated by c-Fos, and taking into consideration that this activation mechanism implies an interaction between both proteins, we initially studied this possibility. We found co-immunoprecipitation of c-Fos with the enzyme and positive FRET values between the tagged proteins both in the soma and in the axon, mainly at the branching points of developing neurons, thus evidencing an interaction between these proteins. These results support the participation of CCTβ2 in the regulation of branching formation and sustain the notion that c-Fos-mediated activation of phospholipid synthesis is of importance during neuronal differentiation.

Keywords: Neuronal differentiation, lipid synthesis, membrane biogenesis

BIOTECHNOLOGY AND BIOINFORMATICS 2

(604) **RECOMBINANT CYCLODEXTRIN GLYCOSYLTRANSFERASE SUITABLE FOR EMPLOYMENT IN FOOD INDUSTRY**

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The enzyme cyclodextrin glycosyltransferase (CGTase) biotransforms the starch into cyclic oligosaccharides with different degrees of polymerization called cyclodextrins (CD), widely used in different industries. These compounds have a hydrophobic internal cavity and a hydrophilic surface, whereby they can form inclusion complexes with many molecules and thus modify their physical and chemical properties. *Escherichia coli* is the main production system for recombinant proteins. However, downstream processing is laborious. In addition, *E. coli* cell envelope contains lipopolysaccharides that act as pyrogenic endotoxins in humans and other mammals thus preventing the use of this bacterium in the obtention of products to the pharmaceutical and food industries. Given the industrial utility of the CGTase and its main products, the objective of this work is the production of CGTase isolated from *Bacillus circulans* in *Pichia pastoris*, a GRAS (generally recognized as safe) system. The CGTase gene was amplified by PCR and cloned into the expression vector pPICZαA, with which *P. pastoris* cells were transformed. Expression was performed by the addition of methanol to the culture medium and the presence of the enzyme in aliquots of the culture supernatant, collected at different post-induction times, was analyzed by SDS-PAGE and zymogram. The recombinant enzyme was purified by affinity to α-CD and its cyclization activity was determined. In this way, a recombinant CGTase was produced in the culture medium, which the subsequent one-step purification allows to have homogeneous preparations with high activity, suitable for use in the food industry.

Keywords: *Pichia pastoris*, Starch, Gluten, Cyclodextrin, CGTase

(649) **EXPRESSION OF A RECOMBINANT PROTEIN FROM *Histoplasma capsulatum* IN THE METHYLOTROPHIC YEAST *Pichia pastoris***

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Histoplasmosis is a systemic and endemic mycosis widely distributed in the Americas caused by the dimorphic fungus *Histoplasma capsulatum*. The infection is usually asymptomatic in immunocompetent individuals. However, immunocompromised patients may contract the disseminated form of the disease, which has a bad prognosis and requires rapid diagnosis and treatment. The definitive diagnosis involves the isolation of *H. capsulatum* by culture from clinical specimens, which may take up to 4 weeks. In addition, molecular methods are expensive and have low sensitivity and immunoassays present many false-positive results. Thus, the aim of this work is to express a specific protein form *H. capsulatum* to develop a novel direct immunoassay and to perform characterization studies of this protein as a first approach for the potential development of novel therapeutic strategies.

The gene that codes for this protein was constructed with a secretory signal and a polyhistidine-tag and was expressed in *Pichia pastoris* X-33 strain. Cell culture supernatants and lysates from different induction times were analyzed by SDS-PAGE, Western blot and mass spectrometry. The expression was also scaled-up to 1L using a stirred tank bioreactor as a proof of concept for the industrial production.

A band of the expected size was observed in the supernatants at 24, 48 and 72h of methanol induction in *Coomasie blue* stained gels and its identity was confirmed by Western blot using anti-histidine antibodies and mass spectrometry. The highest expression level was observed at 24h of induction. Also, a lower molecular weight band was observed at 48 and 72h of induction, probably due to degradation processes.

In conclusion, *P. pastoris* proved to be a valid biotechnological tool for the expression of this specific protein, thus encouraging the national production of novel fungal antigens for the potential development of new rapid diagnostic tests for this clinical relevant form of the histoplasmosis disease.

Keywords: Disseminated histoplasmosis, *Pichia pastoris*, recombinant protein, diagnoses, scaling-up

(729) **NOVEL PURIFICATION METHOD OF VIRUS-LIKE PARTICLES CONTAINING THE HEPATITIS B VIRUS SURFACE ANTIGEN EXPRESSED IN THE METHYLOTROPHIC YEAST *PICHIA PASTORIS***

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Virus-like particles (VLPs) are self-assembled systems composed of antigenic proteins, lacking genetic information, that are frequently used for large-scale vaccine production. For instance, currently used hepatitis B vaccines are based on VLPs derived from different eukaryotic systems, such as *Pichia pastoris*. These spherical empty particles are composed of many molecules of the viral S protein (HBsAg). Attempts to purify such immunogen have shown that its antigenicity is sensitive to ammonium precipitation, acidic pH, high ionic strength and PEG; and requires several chromatographic steps. Here, we describe a novel purification method that reduces costs, time-consuming steps and makes the process more efficient, simpler and quicker than others.

VLPs containing the wild-type HBsAg were intracellularly expressed in *P. pastoris*. Transformed cells were grown in shaker flasks and were then lysed by disruption using glass beads. Purification was performed by a first step of adsorption-desorption on colloidal silica followed by ultracentrifugation on sucrose cushion, dialysis against PBS buffer and concentration with AMICON 10K centrifuge tubes. Each purification step was analyzed by Coomassie and silver-stained SDS-PAGE gels. The presence of HBsAg was determined by mass spectrometry (MS) and its antigenicity by chemiluminescent microparticle immunoassay (CMIA). Finally, the assembly of the subviral particles was evidenced by transmission electron microscopy (TEM).

Results showed high levels of expression and purity of HBsAg. The antigenicity of the VLPs was maintained according to CMIA results and its identity was corroborated by MS. Finally, the typical morphological characteristics of the HBsAg-VLPs were observed by TEM.

In conclusion, the whole purification process described in the present work avoided possible alterations of the morphology and antigenic properties of the HBsAg-VLPs and it was simpler and cheaper than the conventional ones used in industry.

Keywords: HBsAg; *Pichia pastoris*; Virus-like particles; Purification

(730) **EXPRESSION OF HEPATITIS B SURFACE ANTIGEN MAJOR GENOTYPES IN SOUTH AMERICA (F4 AND F1b) IN *PICHIA PASTORIS* AND PURIFICATION FOR *IN VITRO* DIAGNOSIS**

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It has been shown that the immune anti-Hepatitis B virus surface antigen (HBs) response to genotype F could be better detected by homologous genotype antigens. As a consequence, we could assess that the use of different antigen genotypes in diagnosis could support a better detection of anti-HBs antibodies against the major genotypes in South America. In order to achieve this goal, we describe in this study the expression in *Pichia pastoris* and the purification of the HBsAg corresponding to the S region of (sub) genotypes A, F1b and F4.

These immunogens were expressed in the methylotrophic yeast *P. pastoris*, as virus-like particles and cultivated to high cell density in Erlenmeyer flasks as well as in a stirred-tank bioreactor. Expression was induced with methanol pulses and cultures have been set up for 72h. The cells were then lysed by mechanical disruption and the recombinant proteins were purified first by adsorption-desorption on Aerosil-380 followed by ultracentrifugation on discontinuous sucrose gradient, dialysis to remove sucrose and finally concentration with AMICON 10K centrifuge tubes. Purified proteins were analyzed by Coomassie and silver-stained SDS-PAGE gels and its antigenicity was demonstrated by chemiluminescent microparticle immunoassay (CMIA).

All recombinant proteins have been efficiently produced in bioreactor with high yield and purified using a short original process in comparison with the growth obtained in Erlenmeyer flasks. The antigenicity of the HBsAg from the different (sub) genotypes was measured by CMIA. Results demonstrated that the different (sub) genotypes have reacted differentially in the CMIA assays.

The different HBsAg (sub) genotypes expressed in *P. pastoris* are going to be used in a novel immunoassay to assess the usefulness of mixing different genotypes.

Keywords: Genotype F; HBsAg; *Pichia pastoris*; Virus-like particles, South America

(908) **EXPRESION OPTIMIZATION OF A PUTATIVE AMYLO-PULLULANASE FROM *LACTOCOCCUS LACTIS*, USING NICE SYSTEM**

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L. lactis is one of the most commonly used lactic acid bacteria in fermented food production. Because it is considered Generally Recognized As Safe (GRAS), the implementation of this strain in biotechnological processes aims to simplify the downstream processing and to diminish contamination risks with toxins in a wide range of commercial products. The NICE system can be used to produce large amount of recombinant proteins using nisin as inductor enabling its application for I+D and industrial enzymes production. Novel amylo-pullulanase, neo-pullulanase, pullulanase and other enzymes that debranch sugar polymers into simple di- or tri-saccharides are of industrial and agricultural relevance e.g. as a food supplement. The aim of this study was to over-express and characterize a putative amylo-pullulanase (*apu*) induced when *L. lactis* IL1403 was grown in the presence of starch or in *L. lactis* KF147 when growing on plant tissues. To simplify protein detection, purification and further application in several food matrixes, the *L. lactis* IL1403 *apu* gene was fused to usp secretion sequence in the N-terminus and/or Histidine-tag in the C-terminus for Ni_2^+-NTA affinity purification. These sequences were provided by a corresponding expression plasmid derived from pNZ8048 where *apu* gene was cloned under the control of Pnis promoter. Expression was optimized in *L. lactis* NZ9000 and NZ9000 Clp^- $HtrA^-$ strains, the best conditions for protein overexpression were 5ng/ml nisin and 3 hs of induction at 30ºC. Intracellular over-expression could be observed by coomasie staining of SDS-PAGE gels, but no secreted protein was detected in medium supernatant by coomassie staining or western blot using anti-his antibodies. Enzyme activity was measured by the DNS method with 1% pullulan solution as substrate, giving 0.282 U/ml of *L. lactis* crude extract. Suggesting that the overexpressed protein has the predicted function, but further optimization will be required to increase its activity.

Keywords: GRAS, protein expression, lactic acid bacteria

(639) **REVEALING TAG SYNTHESIS PATHWAY IN THE GREEN MICROALGA *Haematococcus pluvialis:* A SOURCE OF TRIACYLGLYCERIDES FOR BIODIESEL AND VALUABLE CO-PRODUCTS**

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Haematococcus pluvialis is an oleaginous microalga known as an important source of the red carotenoid astaxanthin. Recently, we proposed *H. pluvialis* as a potential source of triacylglycerides (TAG) for biodiesel production, and of phytosterols with potential nutraceutical properties. In this work, our purpose was to study the metabolic pathways involved in TAG synthesis induced by high-light stress in *H. pluvialis* UTEX 2505. For this end, the strain was grown in Bold's Basal Medium under high-light intensity or control light for 1, 2 and 3 days. Then, the lipid content and the expression of enzymes known

to be involved in lipid metabolism in plants were analyzed. High-light stress significantly stimulated TAG and phytosterol synthesis and accumulation ($p<0.01$) in all the conditions tested. Red Nile staining analyzed by confocal microscopy confirmed the presence of cytoplasmic lipid droplets after *H. pluvialis* high-light exposure. The use of propranolol, a lipin inhibitor, significantly diminished ($p<0.01$) TAG synthesis induced by high-light intensity. Western Blot analysis revealed that high-light stress significantly induced the expression of the fatty acid synthase (FAS) complex ($p<0.01$), lysophosphatidic acid acyltransferase (LPAAT) ($p<0.01$) and diacylglycerol acyltransferase (DGAT) ($p<0.01$) enzymes. In addition, qPCR also showed increased mRNA levels of the above-mentioned enzymes. On the other hand, high-light also induced the expression of the monoacylglyceride ($p<0.01$) and diacylglyceride ($p<0.01$) lipases. However, no significant changes ($p>0.05$) were observed in the release of glycerol to the medium after high-light stress. Together, these results suggest that TAG synthesis in *H. pluvialis* occurs *de novo* through stimulation of the fatty acid synthesis pathway and induction of the Kennedy pathway under light stress. Our results constitute the starting point to design molecular strategies for increasing the synthesis of valuable products from *H. pluvialis*.

Keywords: TAG synthesis pathway, *Haematococcus pluvialis*, biodiesel

(737) **BUILDING THE MOLECULAR BLOCKS OF MULTI-ENZYMATIC COMPLEXES FOR THE IMPROVEMENT OF BIOFUEL PRODUCTION**

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The enzymatic degradation of lignocellulose generates sugars that upon fermentation produce bioethanol. The enzymes currently used in this process are expensive and have low efficiency. It is important to develop new methods to increase their activity and stability for an economically viable production of bioethanol.

Cellulosomes are multienzymatic complexes that colocalize different cellulolytic enzymes and cellulose binding domains, increasing their degradation activity through enzymatic proximity and substrate targeting effects. However, the industrial production of natural cellulosomes has serious limitations because of the properties of their scaffolding protein. Our goal is to use the structure of an oligomeric protein that is highly stable and highly expressed in bacteria, as a scaffold for the colocalization of a consortium of cellulolytic enzymes and the development of artificial cellulosomes. For the assembly of these multienzymatic particles we use a non-covalent coupling strategy trough high affinity heterodimeric peptides complementary fused to the oligomeric scaffold and the target enzymes. For this purpose we cloned and expressed in *E.Coli* the catalytic domain of a variety of enzymes, including endoglucanases, exoglucanases, xylanases, beta glucosidases and cellulose binding domains from different organisms, fused to the coupling peptide. The amount and solubility of these multienzyme building blocks were compared to the original isolated domains. The coupling peptide doesn't perturb the solubility of the target proteins. At least one member of each functional category is solubly expressed in significant amounts, encouraging further development of the artificial cellulosomes.

It is expected that these complexes will help to increase the enzymatic lignocellulose degradation, reducing the cost of bioethanol production and favoring fossil fuels substitution.

Keywords: Cellulase, Macromolecular complex, Protein engineering, Biotechnology, Bioethanol

(1129) **DEVELOPMENT OF BIOTECHNOLOGICAL PROCESSES FOR THE PRODUCTION OF RECOMBINANT THERAPEUTIC PROTEINS WITH HIGH SOCIO-ECONOMIC IMPACT**

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The aim of this work is to develop the biotechnological processes for the production of three recombinant proteins for therapeutic use. The α-galactosidase A (α-GAL) and the α-Glucosidase (α-GAA) are used for the treatment of Fabry disease and Pompe disease, respectively. These diseases are ultra-orphan pathologies whose treatments are amongst the most expensive on a cost-per-patient basis (U$S 150.000-3.000.000/year). The DNaseI is used for the treatment of cystic fibrosis, one of the most common lethal inherited genetic diseases in Caucasian population (approximately 1/2500 births). Initially, we analyzed the transient expression of these proteins in Chinese hamster ovary (CHO-K1) cells. Plasmid pCI-neo (Promega) was used as the expression vector, which promote constitutive expression of cloned DNA inserts in mammalian cells. The coding sequence of each protein followed by HA-tag and preceded by the signal peptide were synthesized (GenScript, USA) and cloned into pCI-neo downstream of CMV promoter region. For the three proteins, the sequences of coding regions and signal peptides used in the constructions were identical to the corresponding human sequences. An additional plasmid was constructed for the α-GAL, in which the signal peptide sequence was identical to the corresponding CHO-K1 cell sequences and the coding sequence was optimized for the codon usage in this cell line. CHO-K1 cells were transfected with the plasmids and the expression of the proteins was analyzed by Western blot and by immunostaining using anti-HA antibodies, followed by confocal microscopy observation. All three proteins were successfully expressed. However, the optimized sequence seems to work worse than the non-optimized sequence. Treatment of transfected CHO-K1 cells with tunicamycin (N-glycosylation inhibitor) modified the relative migration of the three proteins indicating that the expressed proteins are glycosylated, requirement for the functionality of these proteins.

Keywords: α-galactosidase A, α-Glucosidase, DNaseI, Biosimilars.

(1370) **PROCESS DEVELOPMENT FOR THE PRODUCTION OF A THERMOSTABLE STERYL GLUCOSIDASE IN *ESCHERICHIA COLI***

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Biodiesels are renewable fuels consisting of fatty acid methyl esters synthesized via transesterification of oils and fats with short chain alcohols. They offer several technical advantages over petrodiesel, but some unwanted side products that form insoluble contaminants are also generated. These insoluble precipitates consist mainly on sterile glycosides (SGs). Our working group has described an efficient method for the removal of SGs of biodiesel based on the use of an enzyme with steryl glycosidase activity. The SGase is a thermostable β-glucosidase from *Thermococcus litoralis*, which was expressed in *Escherichia coli*. The production process has been optimized through the use of different promoters, the coexpression of molecular chaperones and development of a fed-batch process, reaching a title 260U/ml and productivity 36 U/ml/hour. In this work we describe the effect of using different carbon sources (glucose, glycerol, crude glycerol, sucrose and molasses) in high cell density fermentations of *E. coli*. Furthermore the effects on the production of the enzyme for different feeding strategies and different C/N ratios at the stage of post-induction were analyzed. Changes in fermentation conditions increased productivity 1.7 times, which directly impacts on the costs of the industrial process for the treatment of biodiesel.

Keywords: Biodiesel, oil, sterile glycosidase, fermentations.

(713) **CORE PROMOTER INFORMATION CONTENT CORRELATES WITH OPTIMAL GROWTH TEMPERATURE.**

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IQUIBICEN.

The subtle mechanisms by which protein-DNA interactions remain functional across a wide range of temperatures are largely unknown. In this work, we manually curated available information relating fully sequenced archaeal genomes with organism growth temperatures. We built a motif that represents the core promoter of each species and calculated its information content. We then studied the relation between optimal growth temperature (OGT) and information content (IC) in the promoter region.We found a positive correlation between G+C content and OGT in tRNA regions and not in overall genome. Furthermore, we found that there is a positive correlation between information content and optimal growth temperatures in Archaea. This can't be explained by an increased C+G composition nor by other obvious mechanisms. These findings suggest that increased information content could produce a positive fitness in organisms living at high temperatures. We suggest that molecular information theory may need to be adapted for hyperthermophiles.

We finally explore the posibility of this fenomena becoming evident at lesser temperature differences, such as those found between mithocondria and nucleus.

Keywords: Protein-DNA, Information content, thermophiles, coevolution

CARDIOVASCULAR AND RESPIRATORY SYSTEMS 5

(235) ANTIOXIDANT ADMINISTRATION SUPPRESSES INCREASED REACTIVITY OF TRACHEAL STRIPS IN FRUCTOSE-FED RATS.

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Consumption of high-fructose diet (HFD) in rats promotes the development of experimental metabolic syndrome (eMS). Hypertriglyceridemia is the earliest metabolic perturbation associated with HFD and may promote lipid deposition in visceral adipose tissue. There is considerable evidence that visceral adiposity is associated with IR. Furthermore, oxidative stress (OS) is related with IR, visceral fat accumulation, and eMS. In previous studies in high-fructose fed rats we showed an increased tracheal reactivity. This effect was amplified by pre-incubation in a high glucose solution, a situation that results in OS mainly through superoxide anion accumulation.

The present work was designed to evaluate the effects of chronic administration of antioxidant substances (AS) on tracheal reactivity in eMS.

Rats were exposed for 5 months to a standard diet –SD- or HFD with or without antioxidants (each subgroup n=8). Metabolic variables were assayed every 6 weeks and at the end of the experiment. Body weight and Systolic blood pressure (SBP) were also measured. After euthanasia, lipid peroxides in plasma were estimated by evaluating thiobarbituric acid reactive substances (TBARS). Tracheas were cut into segments and each segment was cut longitudinally through the cartilage ring to create a strip. Dose–response curves for Ach-induced contraction (Ach-IC) of tracheal strips were conducted in SD and HFD groups. Data were analyzed by two-way ANOVA tests adjusted by Bonferroni correction.

HFD induced an eMS. Significant differences were detected in abdominal white adipose tissue and SBP and in metabolic variables (triglycerides, serum cholesterol levels, insulin, fasting total blood glucose and TBARS). Ach-IC of tracheal strips increased significantly when compared with those obtained from SD ($p<0.01$). Administration of AS in HFD treated rats suppressed this effect.

It is concluded that AS counteracts the effect of HFD probably through the scavenging property of O_2- accumulation.

Keywords: High-Fructose-Diet; Tracheal-reactivity; Experimental Metabolic-Syndrome.

(825) AQUAPORIN 1 AND 4 EXPRESSION BEFORE AND AFTER CARDIOPULMONARY BYPASS DURING AORTIC VALVE REPLACEMENT SURGERY IN ADULTS

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Cardiopulmonary bypass (CPB) is an essential strategy in heart valve replacement surgery. However it may produce postoperative myocardial edema, which has been associated to myocardial dysfunction and worst clinical outcomes. Aquaporin (AQP) 1 and 4 are selective water channels expressed in myocardial tissue. In animal models AQP1 increases its expression after CPB, playing an important role in the formation of myocardial edema; information in humans is still scarce. We designed an observational study to evaluate changes in AQP1 and 4 expression before and after CPB in adults undergoing aortic valve replacement surgery. *Objectives*: 1) To quantify changes in AQP 1 and 4 expression in myocardial tissue before and after CPB using Western blot technique. 2) To correlate changes in AQP expression with clinical characteristics and outcomes. *Methods*: Patients with aortic valve stenosis undergoing valve replacement surgery were invited to participate. Patients with previous myocardial infarction, primary cardiomyopathy, other concomitant severe valve diseases or combined surgery were excluded. Biopsies were taken from the anterior wall of the left ventricle before and after CPB using an automatic transmural fine-needle. Western blot analysis was performed using anti-AQP1 and anti-AQP4 antibodies. Patients were followed until discharge. The protocol was approved by an IRB. *Results*: 14 patients were enrolled between August 2015 and June 2017. Patients were mostly elder (70±9.13 years-old) males (62.5%) with hypertension (75%) and degenerative aortic stenosis (86%). Mean biopsy weight was 14±6.9 mg. Western blot showed a mean increase in the 45 KDa (0.57±1.11), 35 KDa (0.30±0.64) and 28 KDa bands (0.33±0.79) of AQP1, and a decrease in the expression of AQP4 (0.61±0.77). Changes in AQP expression were associated with moderate-severe hypertrophy and on-pump time. *Conclusion*: The expression of AQP1 increases while the expression of AQP4 decreases before and after CPB.

(1759) DOES THE SEVERITY OF THE ISCHEMIC INSULT PROMOTE DIFFERENTIAL ACTIVATION OF THE ENDOPLASMIC RETICULUM STRESS PATHWAYS?

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Abstract: Restoration of coronary flow after an ischemic event rescues the heart from further damage. However, reperfusion can cause reversible contractile dysfunction and arrhythmias if the ischemic period is brief or cell death if ischemia is prolonged. Ischemia and Reperfusion (I/R) challenges the endoplasmic reticulum (ER) protein folding capacity, leading to ER stress. The adaptive response to ER stress is the unfolded protein response (UPR) and comprises three pathways which lead to different effects: 1) ATF6 induces the transcription of XBP1 and GRP78 (the main ER chaperone); 2) IRE1α produces the splicing of XBP1 (sXBP1) which also increases GRP78 expression and 3) PERK attenuates protein synthesis. If ER stress is severe or prolonged, PERK can promote the expression of the pro-apoptotic protein CHOP and IRE1α the activation of caspase 12. The aim of this work was to study the UPR associated to a reversible (I/Rrev) or irreversible (I/Rirrev) I/R damage. Isolated perfused rat hearts were subjected to I/R (20/30min, I/Rrev or 30/60min, I/Rirrev). At the end of R, mRNA expression of GRP78, total XBP1, sXBP1 and CHOP (real time qRT-PCR), expression of caspase 12 (Western blot) and apoptosis (TUNEL assay) were evaluated. mRNA levels of GRP78 and sXBP1 increased in both I/R protocols vs. non-ischemic hearts (Ctrl) (I/Rrev 1.68±0.14, 4.17±0.60; I/Rirrev 1.69±0.12, 2.73±0.22 fold change respectively, n=4-10, $p<0.05$). Conversely, mRNA levels of XBP1 and CHOP only increased in I/Rirrev (1.53±0.08, 1.80±0.12 fold change respectively, n=4-10, $p<0.05$). Under this latter condition the activation of caspase 12 assessed by reduction in pro-caspase 12 levels

(67.2±6.8% of Ctrl, n=10-12, p<0.05) and apoptosis were detected. These results indicate that UPR is effectively triggered in I/R with a pattern response which varies depending on the I/R severity. After a prolonged ischemic insult, the ER stress response may play a deleterious role contributing to apoptotic cell death.

Keywords: endoplasmic reticulum stress, ischemia and reperfusion, myocardium

(1473) ***IN VITRO*** **BUENOS AIRES PARTICULATE MATTER EXPOSURE CAUSES DIRECT AND LUNG-MEDIATED INDIRECT EFFECTS ON CARDIOMYOCYTE**

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Air particulate matter has been associated with adverse effects in the cardiorespiratory system leading to cytotoxic and proinflammatory effects. Particulate matter–associated cardiac effects may be direct or indirect. Direct interactions may occur if inhaled ultrafine particles and/or particle components cross the air–blood barrier while, indirect interactions may occur as the result of pulmonary inflammation and consequent release of inflammatory or oxidative mediators into the blood circulation. Therefore, we considered important to investigate the effect of Urban Air particles from downtown Buenos Aires (UAP-BA) and Residual Oil Fly Ash (ROFA), a surrogate of ambient air pollution, a) directly on cardiomyocytes (HL-1) in culture or b) indirectly by analyzing the biological response of HL-1 to alveolar macrophages (AM) particle-exposed conditioned media (CM). After 24h particle (UAP-BA or ROFA (1-200 ug/mL)) or CM exposure, cultured cells we assessed for: viability by MTT, Reactive oxygen species (ROS) by DHR 123 and NRF-2 expression by immunocytochemestry. We found that a) Cardiomyocytes exposed directly to UAP-BA or ROFA showed a marked increase in ROS generation and NRF2expression, without alterations in cell viability. When b) UAP-BA or ROFA indirect effect was assayed on HL-1 cells, we observed that CM provoke a reduction in cell viability (C_{CM}:1.255±0.086 vs $ROFA1_{CM}$:0.941±0.096, $ROFA10_{CM}$:1.016±0.064, $ROFA100_{CM}$:0.464±0.013, $UAP-BA10_{CM}$:1.016±0.064, $UAP-BA100_{CM}$:0,676±0.034, $UAP-BA200_{CM}$:0.725±0.017 ABS, p<0.001) with increase on ROS generation and slight increase on Nrf2 expression. These results indicate the presence of both direct and indirect effects of PM on cardiomyocyte. In conclusion, our *in vitro* findings employing lung and cardiac miocyte cells provide support to the hypothesis that particle-induced cardiac alteration may possibly involve lung-derived mediators.

Keywords: alveolar macrophages, cardiomyocytes, ambient particle, soluble mediators

(1849) **Na^+/H^+ EXCHANGER LOCALIZATION IN VENTRICULAR MYOCYTES MEMBRANES. INFLUENCE ON ITS FUNCTIONALITY**.

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The Na^+/H^+ exchanger (NHE1), an integral membrane protein, is involved in the maintenance of the intracellular pH (pHi). Its activity is regulated by allosteric site sensitivity for H^+, phosphorylation and by the union of ATP, lipids, growth factors in its cytoplasmic tail. The objective of the present work was to evaluate a putative role of certain membrane domains, such as caveolae, in the modulation of NHE1 activity.

In isolated myocytes from normotensive rats (SP: 118.8 ± 6.2 mmHg) and SHR (SP: 182.5 ± 15.5 mmHg, p <0.01 vs W) we determined:

-the NHE1 membrane localization by the technique of detergent-resistant membranes (DRM) and confocal microscopy.

-the NHE1 activity measuring the velocity recovery after intracellular acidification.

Caveolin3 and flotilin1 colocalized at the level of DRM (fractions 4 and 5) in isolated myocytes from Wistar rats. It was also observed that a minor proportion of NHE1 is located in fraction 4, while it was mainly found in the detergent-soluble membrane fractions (9 to 12). On the other hand, in isolated ventricular myocytes of SHR rats we also observed the colocalization of caveolin3 and flotilin1 in fraction 4 of the gradient and also in fractions 7 to 9. NHE1 is entirely located at the level of detergent-soluble membrane fractions (9 to 12).

Confocal microscopy confirmed results observed by the DRM technique.

Finally NHE1 activity in SHR rats was higher than in Wistar rats (dpHi/dt:0.12±0.01, n=4 and 0.07±0.01, n=3, p<0.05). Caveolae disruption (using β-methylcyclodextrin) did not significantly modify the activity of NHE1 in SHR or Wistar rats (dpHi/dt:0.10±0.01, n=3 and 0.07±0.01, n=3).

The results suggest that the differences in lipid composition between Wistar and SHR rats could justify the greater NHE1 activity in SHR rats. In addition the activity of NHE-1 does not seem to depend on its location in caveola-like domains.

Keywords: myocytes; NHE1; caveolae; hypertension.

(1919) **PROTEIN KINASE G AS AN EMERGING REGULATOR OF CARDIAC RYANODINE RECEPTORS: INSIGHTS FROM THE STUDY OF THE STORE OVERLOAD-INDUCED CALCIUM RELEASE.**

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Cardiac Ryanodine receptors (RyR2) are Ca^{2+} channels located within the sarcoplasmic reticulum (SR) membrane of cardiac myocytes and play a key role in excitation-contraction coupling. RyR2 can also open spontaneously in a process known as Store Overload-Induced Ca^{2+}Release (SOICR) given its dependence on SR Ca^{2+} load. We recently demonstrate PKG-dependent phosphorylation of RyR2 at Ser2808. The objective is to study the effects promoted by PKG-dependent phosphorylation of the RyR2.

Materials and Methods: All the experiments performed in stable inducible HEK293 cells expressing RyR2.

-Immunodetection of PKG expression and RyR2 phosphorylation by western blot.

-siRNA-based knock-down of PKG expression.

-SOICR incidence was studied in cells loaded with 2 μM of the calcium sensor Fluo-4 and superfused with crescent concentrations of calcium.

-Monitoring of intra-SR Ca^{2+} in cells transfected with the FRET-based Ca^{2+} sensor D1ER.

Results: The pharmacological activation of PKG (8-br-cGMP 100 μM) increased the level of RyR2 S2808 phosphorylation and the fraction of Ca^{2+} released in every SOICR event (FR), without changing the incidence of SOICR. The increase FR was a consequence of the reduction in the termination threshold (SOICR events terminate at the 61.8 % of total SR content in control group and at 56.3 % in the cells treated with 8-Br-cGMP. T test for statistical comparison). The knock-down of PKG prevented the effects observed with 8-Br-cGMP. Moreover, the effect promoted by 8-Br-cGMP was tested in two groups of cells expressing mutant versions of RyR2. The observed change in termination threshold was reproduced in S2030A RyR2-expressing cells but not present in S2808A RyR2-expressing cells (compared by ANOVA with Tukey as a post-test)

Conclusion: PKG activity does not increase in the incidence of SOICR but reduce the threshold at which release terminates, suggesting that PKG activity can be a positive modulator of Ca^{2+} release without promoting arrhythmias.

(943) **ROLE OF ITO DEFINING CA^{2+} DYNAMICS ON IN-**

TACT MOUSE HEARTS

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During a physiological cardiac cycle, an influx of Ca^{2+} through L-type Ca^{2+} channels is responsible for triggering Ca^{2+} release from the sarcoplasmic reticulum (SR). Although in most of vertebrates this influx occurs during the plateau phase of the ventricular action potential (ph2) in murine models this happens on the early repolarizing phase 1 (ph1). The aim of this work is to asses if changes in the open probability of K^+ channels defining ph1 (Ito) can modulate both Ca^{2+} currents and Ca^{2+} release from the SR during the ventricular AP. A combination of pulsed local field microscopy (PLFFM), loose patch photolysis (LPP) and mathematical modeling was used to test the hypothesis that a decrease in Ito will enhance Ca^{2+} influx and SR Ca^{2+} release. A mouse myocyte mathematical model used to validate our working hypothesis, predicted that a decrease in the ph1 repolarization rate promote an increase in the amplitude of the L-type Ca^{2+} current, the SR Ca^{2+} load and in the gain of the Ca^{2+} induced Ca^{2+} release process. This theoretical prediction was experimentally evaluated using LPP. Indeed, increasing concentrations of 4 aminopyridine (4-AP) slowed down ph1 repolarization and increased Ca^{2+} influx through L-type Ca^{2+} channels. Furthermore, the activation of Ito with NS-5806 reduced the amplitude of nifedipine sensitive Ca^{2+} currents. In addition, simultaneous recordings of Ca^{2+} transients and APs by PLFFM showed that a reduction in the ph1 repolarization rate produced an increase in the amplitude of Ca^{2+} transients and that an increase in the repolarization rate promoted by NS-5806 led to a reduction of SR Ca^{2+} release. Finally, 4-AP effect on AP ph1 repolarization was significantly smaller when the L-type Ca^{2+} current was partially block with nifedipine. This indicates that not only ph1 repolarization rate regulates cardiac contractility, but also that the rate of repolarization is defined by the competition between an outward K^+ current and an inward Ca^{2+} current.

Calcium Signaling, Excitation-Contraction Coupling, Mathematical Modeling,
Intact Heart Ionic Currents

(1646) **SARCOPLASMIC RETICULUM (SR) CALCIUM RELEASE RESTITUTION VS INCREASED CALCIUM SEQUESTRATION IN THE DETERMINATION OF CALCIUM INDUCED-CARDIAC ARRHYTHMIAS**

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Following the discharge of Ca from the SR, the release mechanism enters a refractory period when it is unresponsive to stimulation by cytosolic Ca. The time elapsed between two consecutive full Ca releases is known as *Ca release restitution* (CRR). Interventions that accelerate or slow SR refilling, accelerate or slow CRR, respectively. CRR is significantly hastened in post-infarction myocytes thereby accounting for increased vulnerability of these myocytes to diastolic spontaneous arrhythmogenic Ca waves and arrhythmias. Hypothesis: Acceleration of CRR is associated with an increase in stress–induced arrhythmias. Methods: Wild-type mice (WT), knock-in mice with constitutive pseudo-phosphorylation of RyR2 at Ser2814 site of CaMKII (S2814D) and increased SR Ca leak, and SDKO mice resultant from crossbreeding phospholamban ablated mice (PLNKO), with increased velocity of SR Ca sequestration, and S2814D were used. We estimated Ca transients and SR Ca load with fluorescent dyes and used confocal microscopy to determine diastolic SR Ca leak. Restitution curves were obtained by introducing an additional stimulation pulse at different time intervals with respect to the regular pacing pulses. Results: Compared to WT, S2814D have accelerated CRR (174±6.2 vs. 235±11.4 msec, $p<0.05$), increased SR Ca leak (0.75±0.04 vs. 0.44±0.02 $\Delta F/F0/100\mu m/sec$, $p<0.05$), and arrhythmogenic Ca wave frequency/100μm/sec (1.08±0.15 vs. 0.43±0.12, $p<0.05$), when submitted to stress. In contrast, SDKO myocytes had significantly increased SR Ca leak and accelerated CRR, but paradoxically they presented diminished Ca wave frequency/100μm/sec: 0.1 ± 0.04, $p<0.05$, which were converted in non-propagated events (mini-waves). A mathematical model successfully reproduced these results. Conclusion: Although CRR hastening is associated with increased SR Ca leak, the enhanced SR Ca uptake counteracts the propensity to arrhythmias typical of the increase in SR Ca leak, by aborting Ca wave propagation.

Keywords: Heart, Arrhythmias ,Calcium

(947) **SARCOPLASMIC RETICULUM STRESS (SRS) MEDIATES CELL DEATH ASSOCIATED TO HYPEROSMOTIC STRESS (HS)**

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Abstract: In certain pathological situations, cardiomyocytes may undergo osmotic stress. This stress is associated with electrical remodeling, contractile dysfunction and cell death. Nitric oxide (NO) is a second messenger recognized for modulating contractile function and cell survival. However, if NO contributes to cell death associated with osmotic stress, has not been studied in adult cardiac myocytes. Our aim was to study, in a hyperosmotic stress model, if osmotic stress promotes NO release, and if NO is involved in cell death associated with this type of stress.

Perfusion of myocytes with a hypertonic solution (HS) increases the fluorescence of the intracellular NO indicator, DAF-FM, compared to myocytes perfused with isotonic solution (IS). When cardiac cells are exposed to HS supplement with L-NAME (NOS inhibitor), NO production is inhibited, suggesting that osmotic stress promotes NO release. In addition, in cultured myocytes we confirmed that HS promotes cell death. However, this death is not prevented by inhibiting NOS, indicating that NO is not involved in osmotic stress-induced cell death ($p<0.05$).

Recent studies in non-cardiac cells indicate that osmotic stress could induce SRS.

Since it is recognized that sustained SRS leads to cell death by apoptosis, we evaluated if osmotic stress promotes SRS in adult myocytes, and if this type of stress is responsible for cell death.

The results indicate that osmotic stress increases the expression of SRS markers, GPR78 and CHOP. Furthermore, the decrease in cell viability associated with osmotic stress was prevented when myocytes were cultured with 3 mM of the SRS inhibitor, 4PBA, suggesting that osmotic stress-induced cell death is mediated by SRS ($p<0.05$).

Our results suggest that in adult cardiac myocytes, osmotic stress promotes cell death and increases NO production. However NO does not mediate cell death. Moreover, osmotic stress promotes SRS which would be responsible for cell death.

Keywords: osmotic stress, cell death, cardiac myocytes, sarcoplasmic reticulum stress

(1658) **THE STAIRCASE PHENOMENON OR BOWDITCH EFFECT IN *Drosophila melanogaster***

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Drosophila melanogaster is a genetic useful model for cardiac studies. However, heart *D. melanogaster* physiology is poorly understood and its characterization is a necessary and mandatory step to extrapolate results of mutagenesis of human conserved genes. The main goal of the present experiments is to characterize in *D. melanogaster* intact heart, a fundamental property of the heart: the staircase phenomenon. Staircase phenomenon establishes the relationship between heart rate (HR) and contractile force: an increase in HR, progressively increases the force of contraction. This physiological response is important during exercise and it is observed in healthy individuals and big mammals. Instead, negative staircase occurs in human heart failure, small mammals and other

vertebrates.

To determine the nature of the staircase phenomenon in *D. melanogaster* we measured mechanical activity and Ca^{2+} transient of adult flies exposed to three 0.5 Hz increments of stimulation frequency (F1 to F3) over the basal spontaneous HR in semi-intact preparation. Although results of movement recordings were somewhat variable, in average, the increment in frequency did not produce significant changes in mechanical activity (Basal: 100% n= 9, F1: 103.8±8.03 n=9, F2: 95.3±9.5 n= 7, F3 105.7±23.9 n=4). In contrast, Ca^{2+} transient exhibits a clear significant negative staircase (basal: 100% n= 5, F1: 73.0 ± 6.4 n=4, F2: 62.2±7.4 n= 5, F3 42.7±8.6 n=5, ANOVA analysis), as it is indicated by the tight relationship between peak Ca^{2+} transient decrease and HR increase (correlation coefficient of Pearson $R^2 = 0.89$). Conclusion: 1. *D. melanogaster* has not a positive staircase phenomenon. 2. The uncoupling between the decrease in Ca^{2+} transient and the absence of significant changes in movement, might suggest an increase in myofilaments Ca^{2+} sensitivity. Further experiments are required to confirm this possibility and to study the possible mechanisms involved.

Keywords: Bowditch effect, Contractility, Calcium, *Drosophila melanogaster*

REPRODUCTION AND FERTILITY 5

(1609) **AN IN VITRO CULTURE MODEL OF BOVINE OVIDUCT EPITHELIAL CELLS ON COLLAGEN HYDROGELS**

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The epithelium lining of the mammalian oviduct has critical roles in the female fertility facilitating the gamete transport, oocyte fertilization, and early embryo development. Ciliated and non-ciliated secretory cells are the two major cell types of the oviduct epithelium. Different in vitro systems for the culture of oviduct epithelial cells (OEC) have been established; however, OEC grown on solid supports dedifferentiate into non-polarized cells; free-floating OEC-vesicles with active cilia on their external surface can be cultured for short terms; polarized OEC can be obtained for long term periods on both, permeable membrane inserts at air-liquid interfaces, and in Matrigel with cells orientated with the apical side into the organoid´s lumen.

The aim of this work was to establish and characterize an in vitro culture of functional polarized bovine oviduct epithelial cells (BOEC) grown at an air-liquid interface on free-floating collagen hydrogels disc (10 mm x 2 mm). Type I collagen was extracted from rat tail tendons and sterile collagen hydrogels (2 mg/mL) were obtained. BOEC sheets free of stromal cells were obtained by gently squeezing of the oviducts. Epithelial sheets were immediately cultured on the top of collagen hydrogels submerged in DMEM supplemented with 10% Fetal Bovine Serum. After 48 h, cells adhered to the hydrogel surface; then, the collagen gels were maintained with the top surface above of the culture medium in an air-liquid interphase. Cultures were controlled under an inverted microscope for the presence of active cilia beating with medium changes at every 48 h. After three weeks, epithelial 3-D cultures were processed for histochemical staining and RT-PCR. Both, the epithelial phenotype mimicking the oviduct epithelium lining, and the expression of the oviduct specific glycoprotein 1 (OVGP1) are indicative of the potential of this culture system for being used as a model for the in vitro study of the oviduct epithelial function.

Keywords: oviduct, epithelial cells, 3-D culture

(105) **MATERNAL OBESITY IMPAIRS EMBRYO DEVELOPMENT AND CAUSES ABERRANT EMBRYO DISTRIBUTION PRIOR TO IMPLANTATION.**

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Diverse signaling pathways, including adrenergic receptor beta 2 (β2AR) and lysophosphatidic acid receptor 3 (LPA3) have critical roles in embryo spacing and implantation. Using cafeteria diet-induced obesity as animal model, we previously found that obesity causes aberrant embryo distribution on gestation day (gd) 18.5 and 5.5 despite the total number of embryos was similar in both groups. However, on gd4.5 the total number of embryos was lower in the obese group compared to controls. Objective: analyze if these embryos were still in the oviduct and examine the potential role of the uterine β2AR and LPA3 signaling in causing the alteration in the embryo distribution on gd4.5. For that purpose uterine and oviductal flushings were performed on gd4.5. In control group all the embryos were in the uterus and the number of embryos was similar in both uterine horns. In obese animals some embryos were still in the oviduct and one horn/oviduct held more embryos then the contralateral ($p<0.05$). Screening of the different isoforms of the AR in the uterus was performed by RT-PCR and showed dominant expression of β2AR in both groups. The uterine gene (qPCR, $p<0.05$) and protein (WB, $p<0.001$) ARβ2 expression was higher in obese animals compared to controls concomitantly with a lower mRNA LPA3 expression ($p<0.05$). Moreover, a higher percentage of poor quality embryos ($p<0.001$) and blastocyst ($p<0.001$) and a lower number of compact morulae ($p<0.001$) were detected in the uterus from obese mothers than controls. Obesity causes alterations in the uterine β2AR and LPA3 expression prior to implantation. This may be one of the mechanisms underlying the alteration in the embryo distribution, the delayed embryo development and/or the poor quality of embryos in obese animals. Treatments that revert these alterations may be useful to prevent implantation problems and the consequent adverse effects on pregnancy outcome described in obesity.

Keywords: Obesity; Embryo spacing; Implantation; Adrenergic receptors.

(1456) **SHIGA TOXIN TYPE 2 SUPPRESSES HUMAN EXTRAVILLOUS TROPHOBLAST MIGRATION AND INVASION**

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During human placentation, the extravillous trophoblast (EVT) invades the maternal decidua and reconstructs maternal spiral arteries. We have previously reported that Shiga toxin producing *Escherichia coli* (STEC) infections during pregnancy may cause maternal or fetal damage mediated by Shiga toxin type 2 (Stx2). We propose that an early STEC infection during pregnancy could damage human trophoblast mediated by Stx2. The aim of this work was to evaluate the effects of Stx2 on cell viability, migration and invasion of human EVT. Swan 71 cell line was used as human first trimester trophoblast model. Firstly, cells were submitted to a lipid extraction for globotriaosylceramide (Gb3, Stx2 receptor) analysis by thin layer chromatography. Cell viability was evaluated by neutral red uptake at 24, 48 and 72h of treatment with Stx2 (0.001-1μg/ml). To assess the rates of apoptosis, cells were fluorescently stained with orange acridine/ethidium-bromide and analyzed under optical microscopy. The effects of Stx2 on migration were studied by the wound-healing assay at 8 and 24h. Proteine activity of matrix metaloproteinase-2 (MMP-2) and invasiveness were analyzed at 24h after treatment by zymography and Matrigel invasion assay, respectively. Gb3 was expressed in Swan 71. Stx2 (1μg/ml) inhibited cell viability and increased the apoptotic rate at 72h respectively ($p<0.05$). Cell migration was significantly decreased by Stx2 in a dose dependent manner at 24h compared to control ($p<0.05$). In addition, Stx2 (0.1 μg/ml) decreased the activity of MMP-2 (16% $p<0.05$) and the cell invasion (45% $p<0.05$). These data indicate that Stx2 impairs migration and invasion at 24h, both essential mechanisms for the correct placentation. Stx2 also inhibited EVT viability and increase apoptosis at 72h, suggesting a Gb3 mediated pathway. Although nowadays there are not reports indicating that Stx2 affect early pregnancy in humans, our data suggest that Stx2 may generate complications

during gestation.

Keywords: Shiga toxin type 2, trophoblast cells, matrigel invasion assay.

(1830) **N6 DIETARY FATTY ACIDS EXCESS DOES NOT AFFECT REPRODUCTIVE SUCCESS; HOWEVER, IT ALTERS FETAL DEVELOPMENT AND ITS VITALITY**

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Parents' feeding has significant impact on the fetus and its postnatal life. Essential fatty acids (EFA) n6 and n3 play a wide variety of roles in reproduction and development. Objective: to evaluate the influence of different dietary n6/n3 ratios on reproductive success (RS), placental and fetal development.

Female adult mice fed 25 days with control diet (C,n6/n3=19.1,n=8), SF (C with 10% sunflower oil, n6/n3=87.2,n=8), F (C with 10% fish oil, n6/n3=1.3,n=8) and B (balanced, C with SF-5%- and F-3%-, n6/n3=5.2,n=8). Body weight and food intake were daily recorded. On day 25 they were placed with 2 males until vaginal plugs were detected (gestational day 0.5=GD0.5). On GD16.5 dams were weighed (g) and the following weights were also registered (g): uterus (UW), ovaries (OW), fetal-placental units (FPUW), fetuses (FW) and placentas (PW). RS was calculated. Number of total fetuses (TFN), normal (NF) and reabsorbed fetuses (RF), fetal length (cm), fetal vitality (FV), placental diameter (PD, mm) and fetal macroscopic characteristics (FMC) were evaluated. Statistics: ANOVA, Chi-square, Kruskall-Wallis.

There were no significant differences in final dams' weight or RS. OW in SF and F were lighter than C (SF=0.013±8.2^{-4} and F=0.013 ± 9.9^{-4} vs. C=0.016±3.7^{-4}; $p<0.05$). No difference in UW, or in TFN. RF were more in SF with significant reduction of NF and FV (FV: SF=47.5% n=120, F=85.3% n=102, B=92.1% n=101, C=85.6% n=104; $p<0.05$). FUPW and FW were significantly lower in SF than the other groups (FW: SF=0.58±0.01 vs. B=0.62±0.02; F=0.66±0.03 and C=0.65±0.0048; $p<0.05$) and tended to be shorter in length. Placentas F were heavier and PD was smaller in all treated groups ($p<0.05$). Normal FMC in all groups.

n6 and n3 excess affected ovarian development. SF, with n6/n3 ratio very distant from the recommended, produces deleterious effects on fetal development evidenced in the greater number of resorbed ones and the smaller number of normal and vital fetuses.

Keywords: essential fatty acids; reproductive success; placenta; fetal development.

(64) **PRENATAL ADMINISTRATION OF DEXAMETHASONE DURING LATE PREGNANCY INDUCES INTRAUTERINE GROWTH RESTRICTION (IUGR) IN MICE**

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Fetal exposure to glucocorticoids excess has been implicated as a causative factor in fetal growth restriction. The objectives of the present study were: a) to develop a murine model of IUGR induced by prenatal exposure to dexamethasone, which mimics maternal stress, and b) to evaluate maternal, feto-placental and neonatal status. Pregnant BALB/c mice were treated with various daily doses of dexamethasone (2, 3, 5 and 8 mg/kg, s.c.) starting from gestational day 14 to 16. The control group was sham-treated with saline. Body weight gain and food intake of pregnant mice were monitored. Also, maternal corticosterone levels were determined 24 h before labor. On gestational day 16, 17 and 18 the placental and fetal weights were recorded. At postnatal day 1, crown: rump length, abdominal and head circumference, and the pups weight were measured. IUGR was diagnosed when the body weight of each fetus/pup was below the 10th percentile of body weight of the control group for each gestational age and for the postnatal day 1. Data were analyzed by one-way ANOVA and comparisons were made by Tukey's test. The dose of 8 mg/kg/day of dexamethasone administered on days 14 and 15 of pregnancy greatly increased IUGR rate from 39% (spontaneous IUGR) to 87% at postnatal day 1 ($p<0.05$). Anthropometric measurements showed a symmetrical IUGR. Also, the average weight of the pups was 28% lower than control ($p<0.05$). On gestational days 16, 17 and 18, the decrease in fetal growth also became evident. On gestational day 18, glucocorticoid overexposure reduced fetal weight and placental mass by 12%. Dexamethasone exposure resulted in a significantly decreased maternal serum corticosterone levels (by 13%, $p<0.05$) and maternal body weight gain (by 48%, $p<0.001$) on gestational day 18. Collectively, our results suggest that short-term exposure to dexamethasone during late pregnancy adversely affects fetal and placental growth trajectory leading to IUGR.

Intrauterine Growth Restriction, Glucocorticoids, Fetal Growth, Placenta

(762) **NOVEL REGULATOR OF TRANSCRIPTION FACTOR AP-1 IN HUMAN TROPHOBLAST CELLS: FKBP52**

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Abstract: The AP-1 family plays an important role en the regulation of fundamental trophoblast processes, including cell proliferation, differentiation and invasion. Alteration in a component of AP-1, particulary c-fos protein, has been detected in placentas from preeclampsia (PE), as well as low protein level of FK506 binding protein 52 (FKBP52). FKBP52 has been known by its role as co-chaperone regulating others transcription factors. The aim of this work was to analyze the effects of FKBP52 on AP-1 signalling in trophoblast cells. BeWo cells were used as trophoblastic in vitro model. Cells were transfected with wild type FKBP52 or its PPIase mutants, and then, stimulated by PMA. AP-1 signalling was evaluated by analyzing endogenous c-fos expression and stability (Western blot:WB), pERK/ERK ratio along time (WB) and c-fos sublocalization (immunofluorescence). AP-1 transcriptional activity was evaluated by luciferase assays and endogenous biological target as IL-6 secretion (ELISA) and MMP-2 proteolytic activity (zymography). In presence of FKBP52, we observed a higher pERK/ ERK level for a longer time. Also was detected greater c-fos nuclear translocation, stability and protein abundance (1.9 ± 0,2 fold). Futhermore, FKBP52 stimulated AP-1 transcriptional activity on a concentration dependent manner ($p<0.01$, n=5). Besides, analyzing the effects of these regulatory events, we observed an increased on IL-6 medium concentration (2,3 ± 0,3 fold) and MMP-2 enzymatic activity (2,5 ± 0,1 fold), abrogated by the FKBP52 PPIase mutants. Conclusions: We demonstrated that FKBP52 modulates different points of AP-1 signalling, regulating the expression, sublocalization and activity of target proteins. All in all, FKBP52 could be considered as a positive new regulator of AP-1 in trophoblast cells. Financial support: UBA, CONICET and PICT 2015-1603.

Keywords: AP-1, c-fos, trophoblast cells, FKBP52

(831) **MATERNAL FAT OVERFEEDING PROGRAMS ALTERATIONS IN PPAR ALPHA PATHWAYS RELATED TO LIPID METABOLISM IN LIVERS FROM RAT FETUSES AND OFFSPRING**

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We previously found liver lipid overaccumulation, decreased mRNA levels of acyl CoA oxidase (AcO) and carnitine palmitoyl transferase-1 (CPT-1) and no response to leptin- induced lipid catabolic actions in fetuses from rats fed a fatty diet (SFD rats). PPA-Ralpha is a positive regulator of liver lipid catabolism through the upregulation of AcO and CPT-1 and lipid trafficking through perilipin 2 (plin2).

Aim: to analyze whether liver from fetuses and offspring from the control and SFD group respond to the lipid catabolic effects of the

PPARalpha activator clofibrate (clof).

Methods: Female rats were fed with standard (controls) or saturated fat diet (27% fat) since they were 6 week-old (SFD rats). After 8 weeks, they were mated with control males. Control and SFD rats were euthanized at gestational day 21 or allowed to deliver and their offspring euthanized at 140 days of age. Offspring's and fetal livers were cultured (3h) with or without clof (20 μM). Lipid levels (Triglycerides (TG), phospholipids (PL), cholesterol (Ch), free fatty acids (FA) and cholesteryl esters (CE)) were assessed by TLC, and Plin2, ACO and CPT1 mRNA levels by qPCR.

Results: Clof decreased liver lipid levels in control fetuses (FA and CE: 25%, $p<0.05$) and (PL, Ch, FA, TG and CE: 30%, $p<0.05$) in control offspring. Also, mRNA levels of AcO (30%, $p<0.05$) were upregulated in control fetal livers. Moreover, AcO, CPT-1 and Plin2 mRNA levels were upregulated (65%, 70% and 40%, $p<0.05$) in livers from control offspring. Differently, fetuses and offspring from SFD rats showed no response to PPARalpha activation in their livers, neither in lipid levels, nor in mRNA levels.

Conclusion: Maternal fat overfeeding induces a resistance to the lipid metabolic effects of PPARalpha, alteration probably involved in the liver lipid overaccumulation in the offspring from SFD rats. These results suggest that use of oral fibrates might not be efficient for the treatment of the fatty liver programmed by maternal obesity.

Keywords: Fatty diet- Programming- Liver lipid metabolism- PPARalpha- Rat

(1842) **UNDIFFERENTIATED SPERMATOGONIA CELL CYCLE ARREST CONRIBUTE TO THE IMPAIRMENT OF SPERMATOGENESIS IN THE TESTIS UNDER A CHRONIC INFLAMMATORY PROCESS**

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Testis inflammation and infections are frequent causes of male infertility and are associated to spermatogenic arrest. We developed an experimental model of autoimmune orquitis (EAO) useful to understand the mechanisms underlying spermatogenesis disruption. EAO is characterized by an interstitial lymphomonocyte cell infiltrate, moderate in the focal phase and abundant in severe EAO, higher levels of nitric oxide (NO) and TNFα, apoptosis of post-meiotic germ cells (GC) and cell cycle arrest of pre-meiotic GC, spermatogonia (EP) and preleptotene spermatocytes. The aim of this study was analyze the cell cycle of undifferentiated EP (CD9+) and evaluate if NO and TNFα are able to regulate cell cycle progression of pre-meiotic GC. Flow cytometric analysis showed that in focal EAO the % of EPCD9+ in each phase of the cell cycle was similar to normal (N) rats (G1 N:73.52±1.24, EAO:72.42±0.97; S N:11.56±0.70, EAO:10.76±0.89; G2 N:14.88±0.79, EAO:16.79±0.58). In severe EAO the % of EPCD9+ significantly decreased in G1 and increased in G2 phase vs N rats (G1 N:72.45±1.78, EAO:32.06±6.69*; S N:11.17±1.83, EAO:10.68±3.15; G2 N:16.43±1.22, EAO:56.84±9.41*, *p<0,001) and the total % of EPCD9+ decreased (N:5.67±0.42, EAO:2.86±0.32 p<0.001).

Intra-testicular injection of DETA-NO, a NO releaser, to N rats significantly reduced the number of seminiferous tubules with BrdU+ pre-meiotic GC vs saline (S) (S:25.17±1.35; DETA-NO 10mM:18.58 ±1.23, p<0,01) while TNFα injection (0.5 and 1μg) did not have effect.

Our results showed that in severe EAO undifferentiated EPCD9+ cell cycle arrest reduces the total % of these cells, event that might be responsible of the less proliferation of pre-meiotic GC. In focal EAO, EPCD9+ cell cycle was unaffected, pointing that other pre-meiotic GC population could be the target of inflammatory microenvironment. NO released by interstitial macrophages might contribute to spermatogenesis impairment through pre-meiotic GC cycle arrest.

Keywords: spermatogonia, cell cycle arrest, nitric oxide, inflammation, infertility

(225) **DEVELOPMENT AND HISTO-FUNCTIONAL CARDIAC ABNORMALITIES IN MURINE FETUSES AFTER PERIGESTATIONAL ALCOHOL CONSUMPTION UP TO EARLY ORGANOGENESIS**

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Maternal alcohol consumption leads to congenital diseases and organ malformation, in which the developing heart is one of main target for teratogenic maternal alcohol effects. The aim of this study was to assess if perigestational alcohol intake up to day 10 of gestation (D10) alters the heart rate and ventricular and atrial myocardial and endocardial arrangement, and modifies the proliferation and apoptotic indexes, in fetal heart at day 13 of gestation (D13). Ethanol 10% in drinking water was administered to murine CF-1 females for 15 days before and up to D10, and gestation continued with water until D13 (treated females (TF)). Control females (CF) were administered with drinking water without ethanol. Both in morphologically abnormal and normal fetuses of TF, the hearts presented thinner trabeculation and discontinuous endocardium (Hematoxylin-eosin), and the ventricular and atrial myocardial wall thickness were reduced ($p<0.001$) compared to CF-fetuses. At 20-40 minutes of uterus extraction, the heart rate of TF-fetuses was reduced respect to CF-heart rate ($p<0.01$). In TF-fetuses, cardiomyocytic myofilaments and mitochondrias were disorganized, while high cellular debris was observed in the ventricular lumen near the endocardium (Transmission electron microscopy). Hearts of TF-fetuses had a reduced myocardial proliferation index (Ki67 immunohistochemistry, $p<0.05$) and increased apoptosis, measured by active Caspase-3-positive cells/myocardial area ($p<0.05$) compared to CF. In conclusion, perigestational alcohol consumption up to early organogenesis induced atrial and ventricular abnormal development, decreased growth, altered functionality and histo-morphology in fetuses at D13 of gestation. Despite the cessation of alcohol intake at D10, probably the fetal cardiac defects, induced during alcohol exposure in early embryo organogenesis, continue at term and yield to the typical congenital cardiopathy observed in the Fetal Alcohol Spectrum Disorder.

Keywords: Cardiogenesis, fetus, proliferation, apoptosis, alcohol.

(432) **THE INFLUENCE OF CABERGOLINE ON THE OFFSPRING PHENOTYPE OF HCG HYPERSECRETING FEMALE MICE**

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An altered secretion of LH/hCG has been related to many pathologies of the hypothalamic-pituitary-gonadal axis. Transgenic female mice expressing hCGβ subunit (hCGβ+) produce elevated levels of hCG, prolactin, progesterone and testosterone, are infertile, obese, and develop pituitary and mammary tumors in adulthood. We have previously demonstrated that a short-term treatment of juvenile hCGβ+ females with the dopamine agonist cabergoline is able to normalize the phenotypic changes of hCGβ+ females and to recover fertility in adulthood, even in the presence of high levels of hCG. The aim of this study was to analyze the possible influence of the maternal environment (hCG and/or the cabergoline treatment) on the hypothalamic-pituitary-gonadal axis of both transgenic and nontransgenic offspring. Three-week-old wild-type (WT) or hCGβ+ females were treated with cabergoline (500 μg/dose) every other day for one week. In adulthood, these females were crossbred with hCGβ+ or WT males, respectively, and the offspring phenotype was analyzed at 3 weeks of age. The phenotype of hCGβ+ offspring derived from cabergoline-treated hCGβ+ females was normalized, in terms of serum hormone profile and ovarian gene expression of *Lhcgr, Cyp11a1, Cyp17a1* and *Cyp19a1.* Furthermore, the estrous cy-

cles were regular and the mice were fertile. No changes were found in nontransgenic littermates. In order to define if these findings were due to hCG or to long-lasting effects of cabergoline during gestation, we crossbred cabergoline-treated WT females x hCGβ+ males. Surprisingly, the phenotype of transgenic hCGβ+ offspring was normalized as well. These results showed that a short-term treatment with cabergoline applied prior to the active reproductive age prevented phenotypic alterations on the offspring. It remains to be investigated if other conditions with altered gonadotropin secretion may also be prevented by cabergoline treatment, as well as the possible epigenetic mechanisms involved.

Keywords: Transgenic mice – hCG – Cabergoline – Offspring – Reproductive axis

ENDOCRINOLOGY 5

(1790) DENDRITIC CELLS (DCs) STIMULATED WITH TRIIODOTHYRONINE (T3) ENHANCES ANTITUMOR IMMUNITY IN A MURINE COLON CANCER MODEL

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We reported that mice DCs express thyroid hormone receptor β1 and that T3 stimulates their maturation and ability to direct Th1 adaptive responses, and T cytotoxic and antitumoral effects in an *in vivo* model of B16-OVA melanoma. Antitumor vaccination based on the own patient DCs, loaded *ex-vivo* with tumor antigens, aims to reduce or eradicate tumor cells. However, protocols deserve optimization since tumor cell cargo and DCs' functional state induced by maturation signals influence their *in vivo* immunogenic potential. Our aim was to analyze the anti-tumor efficacy of tumor antigen-loaded DCs matured by T3 in a murine colon cancer model. MC38 cells were UV-irradiated and apoptotic and necrotic (A/N-MC38) cells were measured by AnnexinV / 7-AAD assay. Immature DCs (iDCs, control) or T3-stimulated DCs (T3-DCs) were co-incubated with A/N-MC38 cells for 18 h. Intracellular and secreted IL-12 production were assayed by flow cytometry and ELISA, respectively. MC38 cells were *s.c.* injected on the flank of C57BL/6 mice (day 0). Control or T3-DCs co-cultured with A/N-MC38 cells were injected *s.c.* at days 1, 3, 5, 7 and 12. Tumor size was measured using calipers (tumor volume = $L \times W^2/2$, L = length, W = width). T3-stimulated DCs cultured with A/N-MC38 cells produced higher amount of IL-12 than iDCs ($p<0.01$). Mice immunized with T3-DCs plus A/N-MC38 cells showed a significant decrease in tumor size (day 22, $p<0.05$). An increased number of infiltrating intratumoral $CD8^+$ T cells in mice receiving T3-DCs cultured with A/N-MC38 cells vs control was revealed (flow cytometry, $p<0.05$). Moreover, the immunotherapy based on T3-DCs decreased the frequency of Treg cells from splenocytes, assessed by a reduction of $CD4^+CD25^+FoxP3^+$ cells ($p<0.05$). These results reinforce the adjuvant properties of T3-conditioned DCs as an alternative approach to potentiate T-cell-mediated tumor immunity reported in other murine tumor model and highlight the profound implications for cancer immunotherapy.

Triiodothyronine, dendritic cells, immunotherapy

(536) EXPRESSION OF CYCLOOXYGENASES (COX) 1 AND 2 IN LIVER OF MICE CHRONICALLY EXPOSED TO ELEVATED LEVELS OF GROWTH HORMONE (GH)

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Abstract: Transgenic mice overexpressing GH exhibit high incidence of liver tumors, including hepatocellular carcinoma, and display upregulation of signaling pathways implicated in the activation of the cyclooxygenases. High levels of these enzymes have been associated with carcinogenesis processes in different tissues. We have previously found that the protein content of COX1 was increased in the liver of transgenic mice overexpressing GH. Therefore, the first objective of the present work was to assess the protein levels of COX2 in hepatic tissue from these animals. Considering that transgenic mice are continuously exposed to high GH levels since birth, as a second objective we pursued to determine if the administration of GH to control mice in lower concentration modifies the hepatic expression of the cyclooxygenases, and if the effect depends on the administration pattern (intermittent vs. continuous). For that purpose, GH was administered (6 mg/Kg/day) to Swiss-Webster mice for 5 weeks (from 3 to 8 weeks of life) by means of two subcutaneous injections per day or by implantation of mini-osmotic pumps for continuous deliver. The protein levels of COX1 and COX2 were determined by immunoblotting and immunohistochemistry assays. As previously observed for COX1, COX2 hepatic levels were higher in transgenic mice than in normal controls, both in males and females ($p<0.01$; n=8-10). No changes in the protein levels of the cyclooxygenases were detected upon intermittent GH treatment for 5 weeks. In contrast, the sustained administration of GH for the same period was associated with higher protein levels of COX1 in males and COX2 in females ($p<0.01$; n=9-11). Therefore, we can conclude that the prolonged exposure to GH is associated with upregulation of cyclooxygenases in liver of mice exposed to continuously elevated levels of the hormone. This effect exhibits sexual dimorphism when the hormone is administered as described.

Keywords: growth hormone, GH, liver, cyclooxygenase, COX, cancer

(240) HEME OXYGENASE-1: A TALE OF INHIBITORY EFFECTS.

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Heme oxygease-1 is an inducible enzyme highly upregulated by oxidative stimuli. It catalyzes the degradation of heme to carbon monoxide (CO), free iron and biliverdin/bilirubin. Previously, we have demonstrated that HO-1 diminishes progesterone (P4) synthesis in MA-10 Leydig cells (LC). We have also shown that NO inhibits steroidogenesis in a similar fashion. Considering that both NO and CO are gasotransmitters capable of binding and inhibiting cytochromes P450 involved in steroidogenesis, we studied CO effects on P4 production using dichloromethane as a donor. Our results indicate that CO inhibits P4 production in basal and 0,5 mM dbAMPc- stimulated conditions and increases HO-1 protein levels. HO-1 antiproliferative effect has been described in a variety of tissues. To assess this, we incubated the cells with 1-25 μM hemin for 24hs and observed that LC proliferation decreased in a concentration-dependent manner. We studied cell cycle progression and found that a 24h incubation with 10 μM hemin resulted in G2/M arrest. Our group has also demonstrated that LC express histamine (HA) receptors type 1, 2 and 4 and that HA affects steroidogenesis and proliferation positively through HRH2 and negatively through HRH1 and HRH4 for steroidogenesis and only HRH4 for proliferation. With the aim of dissecting HO-1/HA interaction, we incubated the cells with 10 μM hemin and FMPH, amthamine or VUF 8430 (1 μM), agonists of HRH1, HRH2 and HRH4, respectively. Hemin reverts amthamine stimulation and potentiates FMPH and VUF steroidogenesis inhibition and VUF proliferation inhibition. In all experiments, if a $P<0.05$ was obtained with ANOVA and *post hoc* Tukey test, results were considered statistically different. In conclusion, steroidogenesis inhibition by HO-1 is exerted, at least partially, by CO. HO-1 stimulates its synthesis through its by-product CO. Not only does HO-1 inhibit LC steroidogenesis, but it also impairs proliferation and acts as a negative modulator of HA effects.

Keywords: heme oxygenase-1, carbon monoxide, histamine, Leydig cells.

(862) **NOTCH PATHWAY ACTIVATION IN MAC-T BOVINE MAMMARY EPITHELIAL CELLS**

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We previously described the expression and activation of the Notch pathway in the pubertal development of the heifers' mammary gland, with a possible role of Notch in cell proliferation and angiogenesis. In this work, we aimed to study basal expression and the prolactin regulation of the Notch pathway components in the MAC-T bovine mammary epithelial cell line. MAC-T cells were grown in 50% DMEM and 50% RPMI medium containing 10% fetal bovine serum (FBS), 5 µg/mL insulin, 5 µg/mL hydrocortisone, 1% glutamine and 1% penicillin/streptomycin at 37°C in an atmosphere of 5% CO_2. They were plated at a density of 45,000 cells/well and cultured in 5% FBS as control. Treated cells were cultured with 1 or 5 µg/mL prolactin during 96 h. Proteins were extracted to perform Western Blot assay and mRNA was isolated for Real Time PCR. Notch receptors in its active (80 KDa) and membrane (110 KDa) forms, target gene Hes1 and p-ERK expression levels were determined by Western blot and Jagged1 ligand and the Hes1 and Hey1 target genes were evaluated by qRT-PCR. We determined that MAC-T cells express several components of the pathway: the four Notch receptor isoforms, the Jagged1 ligand and the Hes1 and Hey1 target genes. We found a dose dependent increase in p-ERK expression (p=0.04) and a trend to increase in Notch2 and Notch4 active domains with prolactin treatment. It was described that ERK1/2 phosphorylation is upstream to the Jagged1 gene induction and that it is increased by prolactin in cancer cells. Our results suggest that prolactin would induce Notch pathway activation in these bovine mammary cells.

Keywords: Notch pathway, MAC-T cells, prolactin

(868) **POTENTIAL MOLECULAR MECHANISMS OF STATINS INVOLVED IN THE PREVENTION OF HEPATOCARCINOGENESIS**

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Hepatocarcinoma (HCC) accounts for 90% of liver tumors. Statins, inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoAR), have been used in treatment of tumors. Antitumor activity could involve growth factor-β1 (TGF-β1) and thyroid hormones (TH). Previously we demonstrated that HCB generates proliferation of preneoplastic foci, alteration of HT metabolism, HMGCoAR and TGF-β1 levels, in vivo. The aim of this study was determine the molecular mechanisms of action of statins involved in the prevention of HCC. Hep-G2 cells were used, we analyze dose-dependent effects of atorvastatin (AT) and simvastatin (SM) on HCB (5 µM) treated cells on PCNA (WB) and TGF-β1 (RT-PCR) levels. We assessed whether the effect of pre-treatment with statins on HCB proliferation-induced depends of TGF-β1 as well as HT. Treatment with HCB (5 µM) increased PCNA levels, which were reduced by 71% with 20 µM AT, and 100% with 30 µM AT. Also, with SM 10 µM were reduced by 80% and 100% with SM 20 µM. The increase of TGF-β1 as well as the decrease in DI levels generated by HCB were not observed when cells were preincubated with maximum doses of AT and SM. Pre-incubation cells with an inhibitor of TGF-β1 (SB431542, 10 µM) and then treated with HCB showed no increase in PCNA or decrease in ID mRNA levels. However, preincubation with AT 30 together with TGF-β1 exogenous and then treated with HCB increased PCNA and decreased the DI mRNA. Also, in Hep-G2 pretreated with different T_3 doses (T_3 10^{-9}, 10^{-7} T3 10^{-5} M) for 24 h and subsequent 5 µM HCB, the stimulatory effect of HCB on PCNA levels was not observed. Statins (AT and SM) prevent the proliferative effect of HCB on the Hep-G2 cell line. TGF-β1 as well as T_3 may be partly responsible for the protective effect of statins on cell proliferation generated by HCB, and may be molecular targets in the treatment of HCC.

Keywords: HCC, Statins, Thyroid hormones, TGF- β1

(1564) **SPHINGOLIPIDS DRIVE AKT-MEDIATED DENDRITIC CELL (DC) FUNCTIONING INDUCED BY TRIIODOTHYRONINE (T3)**

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We reported that mice DCs express thyroid hormone receptor β1 and that physiological levels of T3 promote their maturation and ability to direct Th1 adaptive and T cytotoxic responses which were exploited in an antitumor vaccination protocol. T3 effects involved non-classical Akt activation. Besides, sphingolipids are key elements in signal transduction cascades. The major bioactive sphingolipids include: sphingosine, sphingosine-1-phosphate (S1P), ceramide and ceramide-1-phosphate and they are involved in Akt activation. Our aim was to assess the participation of this pathway in T3-induced effects on DC functioning. Murine bone marrow derived DCs were treated with T3 (5 nM) for different times and chemical inhibitors of the sphingolipids (GW4869: neutral sphingomyelinase -nSMAse-, SKI: Sphingosine kinases -SphK-, Imipramine: acid sphingomyelinase -aSMAse- and NVP: ceramide kinase -CK-). Intracellular and secreted IL-12 production (sensitive marker of T3 action on DCs) were assayed by flow cytometry and ELISA, respectively. The expression of mRNAs coding the enzymes nSMAse, aSMAse, SphK and CK was evaluated by RT-PCR, while changes in the levels of mRNAs coding SphK1, SphK2 and CK mRNAs induced by T3 through RT-qPCR. The involvement of sphingolipids in T3-promoted Akt activation was evaluated by Western Blot. Results showed that DCs express mRNA for all evaluated enzymes and that T3 regulates the expression of SphK1, SphK2 and CK. Furthermore, exposure of DCs to T3 and all inhibitors significantly suppressed their ability to produce IL-12 in response to T3 ($p \leq 0.05$). Besides, GW4869 ($p \leq 0.05$) and Imipramine ($p \leq 0.05$) decreased the level of T3-induced Akt phosphorylation. These findings revealed sphingolipids participation in T3 effects at DC level and suggest C1P and S1P involvement in T3-dependent Akt activation. Considering the therapeutic impact of T3-treated DCs, these results provide initial molecular tools to manipulate the immunogenic potential of DCs.

Keywords: dendritic cells, triiodothyronine, sphingolipids, Akt.

(1616) **MOLECULAR EFFECTS OF GH OVEREXPRESSION OVER BREAST TISSUE**

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Breast cancer is a complex and heterogeneous pathology; therefore, approved therapies for its treatment often result in ineffectiveness or resistance. This highlights the importance of identifying new therapeutic targets and understanding the molecular mechanisms involved in their tumorogenic action. Lately, GH and IGF-I receptors have been associated with mammary tumorogenesis and their inhibitors proposed as plausible therapeutic agents. Thus, it is necessary to know in detail the molecular actions of these hormones in breast tissue. In order to achieve this aim, breast tissue from young adult transgenic mice overexpressing GH (PEPCK-bGH) and their non-transgenic siblings, considered as controls, were studied. The activation and expression of several receptors and signaling mediators that have been implicated in mammary carcinogenesis were evaluated by Western blotting and RT-qPCR assays. The results showed that the expression of IGF-IR, EGFR and ERα, involved in normal development but also in malignant transformation of the

mammary tissue, was increased in transgenic mice. The expression and activation of signaling molecules involved in cell proliferation and survival such as Erk1/2 and Src were similar and Akt activation was lower in mice overexpressing GH. In contrast, the activation of p38 was increased in transgenic animals. Finally, the expression of different proto-oncogenes involved in cell cycle progression was determined. The protein levels of c-fos were higher in PEPCK-bGH mice.

These findings suggest that elevated GH levels induce up-regulation of pro-mitogenic receptors and hyperactivation of the stress responding kinase p38, which might mediate transforming and pro-tumorogenic process over epithelial cells. In this manner, the results obtained contribute to a better understanding of signaling pathways involved in GH promotion of breast cancer.

Keywords: Growth hormone (GH), mammary gland, signal transduction pathways, tumorogenesis

(70) **THE EXON 3 DELETION GROWTH HORMONE RECEPTOR GENE POLYMORPHISM FREQUENCY IN ARGENTINIAN POPULATION AND ITS ASSOCIATION WITH SPONTANEOUS POSTNATAL GROWTH IN SMALL FOR GESTATIONAL AGE CHILDREN**

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In humans, there are two isoforms of the growth hormone receptor gene *(GHR)*; a full-length isoform (*GHRfl*) and an isoform that lacks exon 3 *(GHRd3)*. Associations between *GHRd3* and growth have been reported previously.

The aim of this study was: (a) to determine the frequency of these polymorphisms in Argentinian population and (b) to analyze the association between these polymorphisms and spontaneous postnatal catch-up growth in small for gestational age (SGA) patients.

(a) Ninety four children born with adequate weight and/or body lengh for gestational age (AGA) were evaluated to determine the frequency of the polymorphism in the Argentinian population.The genotype was determined by a multiplex PCR assay previously described by Pantel *et al*.

(b) Sixty one prepubertal and early pubertal children (aged 4-12.41 yr) born SGA were evaluated at Endocrinology Service of Garrahan Hospital. Anthropometric measures were recorded, while age of pubertal onset was assessed by clinical examination and serum gonadotropin, estradiol and testosterone levels. Those patients who have reached height >-2 SDS without treatment, were defined as spontaneous catch-up group.

The frequency of *GHR* polymorphism found in AGA group was *GHRfl/fl* 48%, *GHRfl/d3* 38% and *GHRd3/d3* 14%. The *GHRd3/d3* genotype was significantly lower in the SGA group (2%) than in the control group (14%) p = 0.03. No differences in the frequencies of the *GHRfl/fl* or *GHRfl/d3* genotypes were found between SGA patients and AGA group.

The percentage of SGA patients that achieved spontaneous catch-up growth was greater in those with at least one d3 allele (n = 12/23; 52%) when compared with the *GHRfl/fl* group (n = 6/37; 16%), p = 0.003.

The frequency of *GHR* polymorphism found is similar to those described for other populations. In SGA children, the exon 3 deletion was associated with greater catch up growth. Thus, we propose that this common polymorphism could play a role in postnatal spontaneous growth in SGA children.

Keywords: GHR, SGA, growth, polymorphism.

(934) **WHOLE EXOME SEQUENCING REVEALS *ADAMTS6* AS NOVEL CANDIDATE GENE FOR IDIOPATHIC SHORT STATURE WITH ADVANCED BONE MATURATION AND PREMATURE GROWTH CESSATION**

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Aggrecan (*ACAN*) is the major proteoglycan in the articular cartilage, critical to the structure and function of growth plate cartilage. Recently, heterozygous *ACAN* mutations have been reported in families with idiopathic short stature advanced bone maturation and premature growth cessation.

We present a 5 years old male with advanced bone age associated to a significantly short stature without any abnormalities in pituitary function . He presented open ears, short neck and mammary hypertelorism. Bone X-rays showed normal structure, with a wide first metacarpal bone.

Whole exome sequencing (WES) revealed no deleterious variants in the *ACAN* gene. WES generated more than 83,000 variants, filtering strategy constricted the list to 61 variants. Finally, we ascertained a single-base substitution c.2425C>T (p.Leu809Phe) in the gene *ADAMTS6* as probably causal variant.

The ADAMTS (A Disintegrin and Metalloproteinase with Thrombospondin motifs) enzymes are a kind of matrix metalloproteinase and have 19 members that have diverse roles in tissue morphogenesis and patho-physiological remodeling. Can be sub-grouped according to known substrates: namely the "aggrecanases" (ADAMTS1, 4, 5, 8, 9, 15 and 20), the cartilage oligomeric matrix protein-cleaving enzymes (ADAMTS7 and 12), the von-Willebrand Factor proteinase (ADAMTS13) and a group of "orphan" enzymes (**ADAMTS6**, 10, 16, 17, 18 and 19).

ADAMTS6 is expressed in normal cartilage. Furthermore, a translocation has been reported t(5;6)(q11;q25.3) in which one breakpoint disrupted the *ADAMTS6* gene, in an 8 ys old boy with short stature and clinodactyly. Also *ADAMTS6*- knockout mouse show development alterations like short snout, abnormal vertebrae and hindclimb morphology, unfused sternum an others.

To date, there has been no information about the substrates and the exact role of this enzyme protein. We suggest *ADAMTS6* as a candidate gene for idiopathic short stature and recommend further investigation to confirm this hypothesis.

Keywords: Exome; Growth; Bone; ADAMTS

(1928) **ASSOCIATION OF CORTISOL AND VITAMINE D IN SEMINAL PLASMA WITH SPERM QUALITY**

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Given the inverse association between vitamin D (VD) and cortisol(C) found in some pathologies such as obesity, cancer and cardiovascular disease, we decided to evaluate the possible relationship between VD and C in seminal plasma (SP) and its impact on sperm quality.

Ninety-two sperm samples from patients consulting for fertility were analyzed according to WHO standardization and by the employment of a Computer Aided System, thus being classified as normozospermics (G1, n=43) and not normozoospermics (G2, n=48). VD and C were measured by chemiluminescent methods. C was determined in saliva, serum, and SP while VD in the two latter ones in ten patients.

The results obtained for G1 and G2 in SP for C were 1.48±0.56 and 1.85±0.79 ug/dl and for VD 27.5±10.3 and 28.52±7.74 ng/ml and (mean±SD; p=0.020 and NS respectively). While analyzing G1+G2, a negative correlation was found between C and Progressive Motility(%) (r=-0.241, p=0.03) and sperm count (r=-0.395, p<0.001), being positive for lateral amplitude head displacement (r=0.258, p=0.022). VD did not correlate with any of the studied parameters. In G2, an inverse correlation was found between C in SP and semen volume (r=-0.218, p=0.040) and positive one with the beat crossed frequency (r=0.318, p=0.04), while VD in SP correlated with Total Motility/ejaculate (r=0.350, p=0.025). C in SP represents approximately 10% of the serum concentration, with a higher concentration of VD in SP than in serum. A significant correlation was found between serum C and seminal plasma and saliva C (r=0.90,

p<0.001 and r=0.81, p=0.04, respectively). C in SP correlated with C in saliva (r=0.934, p=0.001). There was no correlation of the VD in serum and SP.

Cortisol would inversely affect sperm count and motility, imprinting a particular kinetics. In the pathological population, VD could have a non-genomic effect on sperm motility. Cortisol in SP as in saliva would be an ultrafiltrate of serum, while there would be local production of VD.

Keywords: semen, cortisol, motility, fertility

METABOLISM AND NUTRITION 1

(1169) **CHARACTERIZATION OF ANTIOXIDANT ACTIVITY OF AQUEOUS EXTRACTS FROM *GUAYUSA***

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Growing interest in natural antioxidants has developed due to a need for more effective, less toxic antioxidants, and medicinal plants appear to have these desired advantages. During the recent past, studies have shown that *Guayusa tea* can lower the risk of cardiovascular disease, as well as showing extraordinary antioxidant, antiaging effects and anticancer properties. Infusion and decoction of 5 % w/v were prepared and brain homogenates were used to determine the effect on lipid peroxidation. Antioxidant activity was assessed by 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), and 2,20-azino-bis(3-ethylbenzothiazoline)-6-sulphonic acid radical (ABTS) scavenging activity. Decoction of tea and leaves displayed significant higher values than infusion in terms of DPPH (*p<0.01*) and ABTS *(p<0.01*). The total polyphenol (TP) and total flavonoids (TF) contents were also evaluated. Decoction of tea showed significant higher values in terms of TP (*p<0.01*) and TF (*p<0.01*). In addition, aqueous extract of tea and leaves showed a strong inhibition of lipid-peroxidation of brain homogenates measured as thiobarbituric acid-reactive products of lipid-peroxidation (TBARS). The concentration required to decrease 50 % of TBARS levels in the absence of additives (IC50) was calculated. Decoction of tea produced a higher percentage of inhibition than infusion (IC50= 134.5 ± 2.3 *μ*g/mL for infusion and 35.8 ± 3.0 *μ*g/mL for decoction, *p<0.05*). The same relation was observed in decoction and infusion of leaves (IC50= 57.9 ± 3.0 *μ*g/mL for infusion and 43.4 ± 3.6 *μ*g/mL for decoction, *p<0.05*).

The results obtained in the present study indicate that aqueous extract of *Guayusa* exhibit antioxidant properties and a protective effect on lipid-peroxidation process. At the same time, decoction of tea is the best extract in terms of antioxidant properties. Therefore, they could be used as a source of natural antioxidants in the treatment of several diseases associated to oxidative stress damage.

Keywords: Guayusa, decoction, infusion, antioxidant, lipid-peroxidation

(1679) **CHIA SEED IMPROVES HEPATIC STEATOSIS AND MODIFIES INTESTINAL MUSCULAR LAYER SIZE AND CRIPT WIDTH**

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Introduction: Metabolic syndrome (MS) may be prevented by a balanced consumption of macro- and micronutrients. It is known that chia (Salvia hispanica L.) seeds contain some valuable nutrients, such as certain lipids as polyunsaturated fatty acids (PUFA) that are associated with risk reduction for MS. Evidence suggests that a greater proportion of omega-3 in relation to the omega-6 in the diet could play a beneficial role against the progression of hepatic steatosis. Also, it is not known if this PUFA may affect the intestinal morphology.

Objective: To assess the effect of chia seeds in liver and in the small intestine in Sprague Dawley rats fed an isocaloric diet for a period of 13 months.

Methods: Based on the AIN-93M, two semi-synthetic isocaloric diets were prepared, one containing 10% of chia seeds. Twenty male rats, divided into 2 groups (chia group and control group), were fed for 13 months ad libitum. Food intake and weight gain were recorded daily throughout the experimental period. Liver and small bowel fragments were obtained for histologic analysis and microphotographs were analyzed using Image-Pro Premier software for hepatocytes and vesicles counting in two hepatic zones and measurement of intestinal morphology. The experimental protocol was approved by the Committee for the Care and Use of Laboratory Animals.

Results and conclusions: The food was prepared with good organoleptic practices, and pellets were similar to commercial rat foods. After 13 months, histological analysis of the liver in the control group evidenced hepatocytes with numerous and large cytoplasmic vesicles in centrolobular zones that significantly alter the sinusoidal and lobular architecture, with respect to the chia group. Intestinal muscular layers size, and crypts width were significantly higher in chia group compare with control (p<0.05). Chia seeds may attenuate lipid accumulation and development of liver steatosis accompanied by intestinal histoarchitecture preservation.

Keys words: Salvia hispánica L., Isocaloric Diets, omega-3, Hepatic steatosis, small intestine structure.

(776) **ADRENOCORTICAL INSUFFICIENCY DUE TO A SUSTAINED INCREASE IN THE AVAILABILITY OF SUCROSE IN THE DIET**

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Obesity and insulin-resistance (IR) have been linked to an increase consumption of sweet beverages. Higher glucocorticoid levels could worsen this condition. In this study we evaluate long term effects of the administration of a sucrose-rich diet (SRD) on the morphology and function of the adrenal gland and assessed the therapeutic effect of hemin treatment. Rats were randomized into 4 groups (n=4): Control, SRD-fed rats (C, SRD) and C and SRD-fed rats receiving 15 mg/kg hemin (ip) (H and SRD+H) during the last two weeks of the experimental period (12 weeks). Statistical significance was analyzed by ANOVA followed by Tukey's post hoc test.

At the end of the experiment, serum metabolites such as glucose and total cholesterol levels (vs. C, p<0.01) were increased in SRD and SRD+H animals. Results from an insulin tolerance test indicate that hemin treatment did not modify insulin sensitivity or the triglyceride/HDL-c index (IR parameter) either in C or in SRD-treated rats. Only SRD-treated rats showed lower basal corticosterone levels and a reduced response to an acute ACTH stimulation (vs. C, p<0.05). Analysis of adrenocortical makers of oxidative stress showed an increase in lipoperoxide (vs. C, p<0.001) production while gluthathione levels were significantly reduced (vs. C, p<0.05). Immunohistochemistry studies showed an increase in the number of Iba1 positive cells in the SRD group that was less evident in hemin treated rats (vs. SRD, p<0.05). Some Iba1 positive cells were also positive for ED1 (p<0.01) suggesting a phagocytic phenotype.

In summary, the administration of a SRD has a significant impact on adrenocortical function, increasing oxidative stress and inflammatory markers in the adrenal cortex. As hemin treatment prevented these effects and also restored adrenocortical function, we propose a role for oxidative stress and inflammation-related effects in the adrenocortical dysfunction observed in IR-rats.

Keywords: insulin-resistance, adrenal gland, Hemin, sucrose-rich diet.

(160) **EFFECT OF *Ligaria cuneifolia* (*Lc*) PROANTHOCYANIDINE ENRICHED FRACTION ON PLASMA LIPIDS LEVELS AND BILE SALTS BILIARY OUTPUT IN WISTAR**

RATS FED WITH A HIGH FAT DIET (HFD).

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Proanthocyanidin enriched fraction from L.cuneifolia was purified (PL.cuneifolia) to analyze the effect on Cholesterol (Cho) and Triglycerides (TG) plasma levels, and also biliary excretion. Adult male Wistar rats (n = 12), 70 days old, were fed for 28 days with a HFD (every 100g of standard diet 40% bovine meat juice). Rats were administered with either physiological solution (controls C, n=6) or P*L.cuneifolia* 3mg /100g body weight (treated T; n=6) i.p. each 24hr during 10 days. On day 11 they were anesthetized and the bile duct was catheterized for bile collection each 15 min for 60 minutes, at the end, the blood was obtained by cardiac puncture. In plasma Cho, ChoHDL, ChoLDL and TG were determined by enzymatic and colorimetric methods. Bile flow (BF) was determined by gravimetry, bile salts biliary output (BSO) was calculated (Concentration of bile salts in bile x BF). Student t test was applied for unpaired data. Results are expressed as mean±SD. Cho(mg%): C: 168.00± 6.63, T: 100.11±4.91*****; ChoHDL: C: 32.20±1.46., T: 28.00 ±2.39 $^{n.s}$; ChoLDL: C: ChoLDL: C: 24.00±0.63,T: 17.78±0.62*;TG: C: 191.80±21.45, T: 133.00±9.68*; BF(µl /min. g liver): C: 2.31±0.049, T: 2.91 ±0.064*****; BSO(nmol / min.g liver): C:34.15±3.66, T:54.50±6.50***** (*p <0.05 and **n.s.**: not significant vs. C). The results showed a lipid-lowering effect of P*L.cuneifolia* in rats fed with a HFD. The decrease in plasma Cho might be due to the increase in the bile salts biliary output (main compound of hepatic metabolism of Cho).

Keywords: Proanthocyanidin, *Ligaria cuneifolia,* Cholesterol, Bile flow, bile salts biliary.

(1720) **FIBRE MICROPARTICLES OBTAINED FROM JAPANESE PLUM (*PRUNUS SALICINA)* HAVE ANTIOXIDANT ACTIVITY IN HUMAN CELLS**

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Japanese plums are largely grown for fresh consumption. Plums discarded by the food industry can be used as an important source of bioactive compounds such as antioxidant polyphenols as well as dietary fibres like pectins. Polyphenols are habitually co-extracted with fibres. Certain polyphenols can exert cytoprotective actions in the gastrointestinal tract interfering with the oxidative stress implicated in inflammatory disorders. Fibre microparticles (MPCs) obtained through ethanolic extraction from plums retained polyphenolic compounds, mainly pentameric proanthocyanidins (170–200mg/100g MPCs) and smaller amounts of anthocyanins and flavonoids. The aim of this work was to biologically evaluate in vitro the polyphenols extracted from plum MPCs in relation to their capability to protect colorectal epithelium (Caco-2) human cells and embryonic kidney (HEK293) cells against the oxidative stress induced by tert-butylhydroperoxide (tBOOH), as well as in their cytotoxicity. Cellular oxidation induced by t-BOOH was evaluated through the dichlorofluorescein (DCF) oxidation. When Caco-2 cells were co-incubated with 0.5 to 10 µg/mL, the tBOOH induced oxidative stress was significantly reduced ($p<0.001$), showing a dependence on the concentration, reaching 100% of protection with 10 µg/mL extract. A similar result was obtained in HEK293 cells where polyphenol extract showed significant protection (100%) against oxidation with 3 µg/mL of extract ($p<0.05$). Cell viability was measured in cells using the MTT assay (dimethyl tetrazole). Both Caco-2 and HEK293 cells treated for 24 h with the extract (3 to 100 µg/ml concentration) presented a CC_{50}>100 µg/mL. Therefore, polyphenol extracts showed a protective effect against oxidative stress coupled with low cytotoxicity. Plum MPCs obtained with co-extracted phenolics can be useful as a functional antioxidant dietary fibre, interfering with the oxidative stress implicated in inflammatory bowell disorders.

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(1644) **EFFECT OF CHÍA OIL IN BODY WEIGHT AND CARBOHYDRATE METABOLISM IN RABBITS**

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Excessive fat intake is associated with increase of cardiovascular risk factors. Data from the literature indicate that high carbohydrate or fat diet increases body weight (BW), fasting glucose, insulin levels and induces glucose intolerance (GI). In previous work we demonstrate that diet supplemented with 10% chia oil has beneficial effects on vascular function in spite of inducing GI. The objective of this work was to analyze the effect of reducing % of chia oil in endothelial function, biochemical and hemodynamic parameters. Rabbits were fed either a control diet (CD), CD enriched with 3% chía oil (CD-Ch3%), or a CD enriched with 10% chia oil (CD-Ch10%) for 6 weeks. Blood pressure (BP), heart rate (HR), glucose tolerance, total cholesterol (CT), HDL, LDL, and triglycerides (TG) parameters were determined. The endothelial function was evaluated in aortic rings contracted with phenylephrine (Phe) 5.10^{-6} M and exposed to acetylcholine (Ach, 10^{-8} M to 5.10^{-6} M). Rabbits fed a CD-Ch 3% showed: 1) decrease of BW (CD: 1876±238g; CD-Ch3%: 1526±25g; CD-Ch10%: 2043±63g; $p<0.00001$) and HDL-c (mg/dl; CD: 51.5±7; CD-Ch3%: 18.32±2.11; CD-Ch10%: 48±7; $p<0.001$) 2) decrease Ach-relaxation (% CD: 65±5.2; CD-Ch 3%: 30±6; CD-Ch10%: 56±12). Rabbits fed a CD-Ch10% showed 1) glucose intolerance (mg/dl 120 min CD: 139.8±19; CD-Ch3%: 142±13; CD-Ch 10%: 158.33±12; $p<0.05$). No changes were observed in CT (mg/dl CD: 59 ± 6; CD-Ch 3%: 68 ± 8; CD-Ch 10%: 53 ± 16); LDL-c (mg/dl CD: 23,8 ± 3,1; CD-Ch 3%: 21,2 ± 2; CD-Ch 10%: 24,6 ± 4) and TG (mg/dl CD: 91.7 ± 14.1; CD-Ch3%: 111 ± 28; CD-Ch10%: 120 ± 29). Conclusion: reduction of BW and the normal glucose metabolism observed in rabbits fed a CD-Ch 3% might imply that not only the type of fat in the diet but the energy balance is important to the beneficial effects of chia oil. Reduction of HDL-cholesterol and the minor relaxation to Ach could be related with by the onset of an oxidative process of the chia oil.

Keywords: *Chía oil, body weight., glucose intolerance., n-3 fatty acid., rabbit*

(563) **HEPCIDIN LEVELS IN A GROUP OF MALE BLOOD DONORS: RELATIONSHIP WITH IRON NUTRITIONAL STATUS**

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Abstract: Hepcidin is an iron (Fe) homeostasis regulator peptide. Limited information is available on this biomarker in the argentine population. In order to quantify serum hepcidin levels and their correlation with Fe nutritional status, 40 male blood donors (18-62y) attending Departmento de Hemoterapia, Hospital de Clínicas (UBA) (2017) were enrolled. Serum hepcidin (sHep) (DRG Hepcidin 25 (bioactive) HS ELISA Kit), serum ferritin (SF) (IMMULITE Ferritin, DPC) and transferrin saturation (TS) (%) (IRON2 and Tina-quant Transferrin, Cobas) were determined in blood samples negative for infectious diseases and C-reactive protein (PCR-latex, Wiener lab).

Daily Fe Intake (FeI), hem Fe intake (hem FeI) and Fe from flour enrichment (Ley 25630) were estimated by a "Food Consumption Frequency" questionnaire (ARGENFOODS and USDA National Nutrient Database on Standard Reference). sHep values (ng/mL) were: mean±SD (range): 33.6±20.9 (7-80); median: 25,0; 2.5th-97th Percentile: 8.85-68.9. Two donors (5%) showed sHep > 81 ng/mL, range assay upper limit. SF (ng/mL) and TS (%) were: mean±SD (range): 213±172 (42-753) and 32.6±12.8 (17.9-90.7), respectively. Criteria of Fe overload (SF>300 ng/mL and TS ≥50%) was observed in 5% of donors. FeI (mg Fe/d) was: mean±DS (range): 24.2±9.0 (10.0-47.2). No participant presented FeI lower than EAR (6 mg Fe/d), and one donor surpassed 45 mg Fe/d (UL) (NAS, 2001). Hem FeI and Fe from flour enrichment were 8.7% and 35% of daily FeI, respectively. A significant correlation was found between sHep and SF (r=0.52; p=0.00097), but not with FeI (r=0.014; p=0.9308), nor with hem FeI (r=0.194, p=0.263). These results show high FeI and a strong correlation between sHep and Fe stores. Therefore, local feeding habits (54.9 Kg meat/per capita/yr, FAO 2011) and mandatory flour fortification with Fe, could enhance adverse effects in individuals unaware of any family history of Fe overload. *Universidad de Buenos Aires, UBACyT 20720150100004BA*

Keywords: iron, hepcidin, biomarkers of iron status, iron intake; food fortification

(378) **BONE VASCULAR ACTIONS OF THE NUTRACEUTICAL GENISTEIN**

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Previously we reported that the phytoestrogen (PE) Genistein (Gen) prevents the atherosclerotic plaque genesis via estrogen receptor (ER) activation and the inhibition of the cellular/molecular events involved in vascular damage. Indeed, vascular muscle cell transdifferentation into osteoblasts (OB) like cells was also impaired. Here we studied the role of Gen on the bone-vascular axis interaction with focus on OB growth and angiogenesis. To that end, aortic rings (AR), primary cultures of aortic endothelial cells (EC) and calvaria OB isolated from female Wistar rats, exposed to Gen (10nM-1uM) were employed. OB proliferation and differentiation are regulated by several factors such as BMP-2, Runx2 and the bioactive compound NO. We showed that short term exposure of OB to Gen enhanced NO production (33-20% above C, 15-30 min treatment, p<0.05, Griess reaction). NO was involved in OB proliferation since in presence of a nitric oxide synthase inhibitor, L-NAME (10uM), OB growth was blunted (p<0.001). In RT-PCR assays we found that Gen significantly increased Runx2 and BMP-2 mRNA levels. The PE genomic action was extended to an up-regulation of alpha and beta ER mRNA expression (p<0.05). Angiogenesis depends on EC proliferation and migration that finally lead to capillary formation. These events were evaluated using conditioned medium (CM) obtained from OB exposed to Gen (72h). CM stimulated EC proliferation (0.47±0.07 vs 0.38±0.07, Gen vs C, p<0.02, MTT technique) and markedly enhanced EC migration (30;187% above C, Gen 10;100nM, p<0.05, wound healing assays). Capillaries formation was studied by seeding AR on a collagen matrix for 15 days in presence or absence of CM and quantified by optical microscopy. A high number of three-dimensional tubular structures around AR were detected. This work provided evidence of OB maturation induced by Gen with beneficial impact on vascular tissue promoting angiogenesis, crucial events involved in bone formation and remodeling.

Keywords: phytoestrogens, genistein, bone-vascular axis, angiogenesis

(964) **CALCIUM ABSORPTION EFFECTIVENESS OF PREBIOTICS IS AFFECTED BY THE NUTRITIONAL STATUS OF VITAMIN D**

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Vitamin D (VD) regulates Ca absorption (Abs) which is positively affected by prebiotics through lowering intestinal pH and increasing colonic cells growth. VD insufficiency could affect prebiotic effectiveness on CaAbs.

Galactooligosaccharides/Fructooligosaccharides (GOS/FOS®) effectiveness to increase CaAbs was evaluated in an experimental model of VD insufficiency and established osteopenia. Ovariectomized Wistar rats fed a VD-free (0 IU%) diet to become VD insufficient (-D) (n=32) or a normal VD diet (100 IU%) (+D) (n=16), during 45 days. Thereafter, for an additionally 45-days period D+ fed: AIN´93 (control diet) (+*D0.5%)*; AIN´93 containing 0.3%Ca or 2.5%GOS/FOS® (9:1) (+*D0.3%P*) while D- fed: VD free-AIN´93 (-*D0.5%)*; VD free-AIN´93 containing 0.3%Ca (-*D0.3%)*; last diet containing 2.5% (-*D0.3%P*) or 5% GOS/FOS (-*D0.3%:2xP)*. Food intake and faeces (F) were collected for CaI and CaF and CaAbs% calculated .

Results CaAbs% (mean±SD):.-D0.5%: 32.71±1.74; -D0.3%: 38.33±2.33; -D0.3%P: 44.71±1.84; -D0.3%x2P: 56.40±1.39; +D0.3%P: 87.45±1.82; +D0.5%: 67.80±2.21.

VD insufficiency reduced CaAbs% (-D0.5% and –D0.3% vs. +D0.5%; p<0.001) while GOS/FOS® effectiveness was negatively affected (-D0.3%P vs. +D0.3%P; p<0.001). CaAbs% of D- diets containing GOS/FOS® was improved by increasing dietary prebiotic % (-D0.3%P vs. -D0.3:2xP; p<0.01).

Effectiveness of prebiotics on Ca Abs was affected by VD nutritional status. Grants: UBACyT 20020130100091BA and PIP (CONICET) 11220130100199CO.

(998) **CONSEQUENCES OF MATERNAL FRUCTOSE INTAKE ON BROWNING POTENTIAL OF RETROPERITONEAL ADIPOSE TISSUE FROM ADULT OFFSPRING**

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Beige adipocytes are highly adapted to convert chemical energy into heat through the action of uncoupling protein-1 (UCP1). Cold exposure or β3 adrenergic agonist treatment stimulates generation of these cells in white adipose tissue (WAT). Our aim was to assess whether maternal fructose intake during pregnancy, affects browning capability of retroperitoneal adipose tissue (RPAT) from adult male offspring. On pregnancy day 1, dams were provided with either tap water alone (CTR, control) or containing fructose (10%w/v; FRD) and fed *ad libitum* with chow up to delivery. Lactating dams and their pups (between 21 and 60 days) received water and chow *ad libitum*. C and F indicate pups born to CTR and FRD dams. On experimental day (age 60 days) RPAT was dissected and stromal vascular fraction (SVF) cells were isolated. mRNA expression levels of beige and white adipogenic markers were assessed in RPAT SVF cells and pads. SVF cells were cultured and differentiation parameters were quantified by qPCR. Previously we found that pre-natal nutritional intervention decreased the adipogenic potential of adult RPAT precursor cells, favoring hypertrophic RPAT expansion and a distorted adipokine secretion pattern. Now, we found that F RPAT expresses lower levels (p<0.05 vs. CTR) of UCP-1, whereas those of Zfp423 (transcription factor involved in maintenance of white adipocyte identity and in inhibition of thermogenic program), leptin and enzymes involved in the lipolytic/lipogenic balance (LPL, HSL, and FAS) were higher (p<0.05 vs. CTR). Analysis of F SVF reveals lower expression levels of Pdgfr1α (a beige lineage marker) and CD34, and higher of Pref-1(p<0.05 vs. CTR). On F differentiated cells, UCP-1 and adiponectin expression was lower (p<0.05 vs. CTR) without changes in leptin or PPARg. Our data suggest that a decreased WAT browning potential could be involved in the adverse metabolic-endocrine dysfunction seen in F adult animals. (PICT2013-0930; PICT2015-2352).

Palabras Clave: WHITE ADIPOSE TISSUE, BEIGE ADIPO-

CYTES, METABOLIC PROGRAMMING.

PHARMACOLOGY 6

(537) **ACTIVATION OF SMOOTH MUSCLE BK CHANNELS BY HYDROCHLOROTIAZIDE REQUIRES CELL INTEGRITY AND PRESENCE OF BK BETA-1 SUBUNITS.**

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Hydrochlorothiazide (HCTZ) is used to treat hypertension, their efficacy is linked to a chronic vasodilatory effect. Previous studies suggest that activation of the large conductance voltage- and Ca^{2+}-dependent K^+ (BK) channel is responsible for HCTZ-induced vasodilatory effect. However, direct electrophysiological evidence supporting this claim is lacking. BK channels can be associated with accessory β-subunits, which confer specific biophysical and pharmacological characteristics. The β_1-subunit is mainly expressed in smooth muscle cells (SMCs). Methods: We evaluated the effect of HCTZ on BK channel activity using patch-clamp technique on SMCs from human umbilical artery (HUASMCs) and in HEK293T cells expressing the BK channel. Paired t-tests were used to compare two groups. Results: Using the whole-cell configuration (WCC) in HEK cells expressing the BK channel with β_1-subunit we observed that HCTZ raised the current amplitude with an EC_{50} of 28.4 μM (pD2=4.5 ± 0.2, n:8). However, 30 μM HCTZ did not change the channel activity when it was evaluated in the same cells in the inside-out configuration (IOC), where cell integrity is lost (%current increase (+90 mV): 12.8 ± 38.1, P>0.05; n:4), suggesting an indirect action. Then, we tested the effect of HCTZ in WCC currents in HEK cells expressing the BK channel without any β-subunit. In this condition, 100 μM HCTZ did not change the BK activity (%current increase (+40 mV): 13.9 ± 15.7, P>0.05; n:4). These experimental results were repeated in HUASMCs. Consistent with the previous results, 10 μM HCTZ caused significant activation of BK current in WCC (528 ± 215 to 1379 ± 132 pA at +40mV, n: 4, p <0.05) while, when it was applied in the IOC, it did not produce any changes in BK channel open probability (NPo (+40mV): 0.0114 ± 0.0015 (control) vs 0.0135 ± 0.0037 (HCTZ), n: 4, P> 0.05). Conclusion: A β_1-subunit-dependent mechanism that requires SMC integrity leads to HCTZ-induced BK channel activation.

Keywords: BK channel, human umbilical artery, hydrochlorothiazide, beta-1 subunit, vascular smooth muscle cells.

(525) **GENISTEIN CARDIOPROTECTION ON HEARTS FROM AGED RATS EXPOSED TO STUNNING DUE TO ISCHEMIA AND REPERFUSION: ENERGETICAL STUDY OF MECHANISMS.**

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The phytoesterol genistein (Gst) was more cardioprotective in rats with estrogenic deficiency such as young males (YM) and aged females (AgF) than in young females (YF). Moreover, in YM Gst was synergistic with estradiol for cardioprotection in severe ischemia/reperfusion (I/Rs). Effect of Gst was not due to higher SR Ca^{2+} store, nor to activation of PI3K-Akt or NO production (SAFE 2016). The aim of this work was to evaluate the underlying mechanisms of Gst cardioprotection on AgF hearts exposed to stunning due to I/Rs without infarct. Hearts from >20 months AgF rats were perfused inside a calorimeter at 37°C to measure left ventricular pressure (LVP, in mmHg) and total heat rate (Ht, in mW) during I/Rs (30 minI/45min R). Effects were compared with those of hearts from YF and YM rats. To evaluate whether the Gst-induced increase in postischemic contractile recovery (PICR) was due to inhibition of tyrosine-kinases (TK), YF and YM were treated with the phosphatases blocker ortho-vanadate (OV) 15 mg/kg i.p. 48 hs before, followed by Gst 5 mg/kg i.p. 24 hs before the I/Rs. OV did not change the effect of Gst, with a PICR of 34.1±5.1% in YF and 50.8±4.9% in YM, with no changes in muscle economy (P/Ht) nor in diastolic contracture (+ΔLVEDP). In order to evaluate whether the activation of mKATP channels underlies Gst cardioprotection, AgF rats were treated with Gst 3 mg/kg i.p. 24 hs before isolating heart, which then was perfused with 100 μM 5-hydroxidecanoate (5-HD) before I/Rs. 5-HD reduced PICR of Gst-ip-treated AgF from 63.9±6.2% to 9.8±2.9% of initial P, which was similar to PICR of non-treated AgF (13.3±3.0%) during R. According to this, 5-HD also reduced the total muscle economy (P/Ht) from 3.2±0.4 to 0.6±0.3 $mmHg.g^{-1}.mW$, which was similar to the non-treated AgF. Results suggest that cardioprotection of Gst-ip was not due to the inhibition of TK, but it is due to activation of mKATP channels, which reduce the mitochondrial Ca^{2+} overload during I/Rs. *UNLP-X-795.*

Keywords: heart, age, genistein, ischemia/reperfusion, mKATP

(1926) **VAPREOTIDE AND CHLORPROMAZINE EFFECT IN AN ANIMAL MODEL OF SCHIZOPHRENIA**.

Maria Graciela Lopez Ordieres

Vapreotide is a somatostatin analogue that it is able to cross the blood brain barrier, being of interest to study its potential effects on the CNS. For this reason, the purpose of this study was to evaluate the vapreotide effects on cortical acethylcholinesterase activity from mice subjected to an epigenetic model of schizophrenia. Then, Swiss Albino mice were injected with a daily dose of methionine 5.2 mmol / kg for 15 days. In this experimental condition, vapreotide addition produced a reduction in the enzyme activity, which was increased by treatment.

Additionally behavioral tests were performed to assess the animals undergoing treatment. Exploratory behavior is fundamental to the rodent nature and the open field task is currently used to test drugs. It was evaluated the performance of the control animals (saline solution injected) versus methionine injected animals, at an initial period, considered when methionine treatment finished, at 90 min and 24 hours later.

Then, the number of crosses (average ± SE; N=5) during the initial period, at 90 min and 24 hours later, it was 34±3; 23±4 and 25±5 for the control animals respectively, whereas in animals treated was 44±8; 45±6 and 44±9 respectively. Number of rearing showed a similar pattern, which would indicate changes in short-term and long-term memories.

Finally, taking into account that schizophrenic patients have increased lipid peroxidation is that it was desirable to examine whether in this model, high levels of lipid peroxidation were produced. Thiobarbituric acid reactive substances are formed as a product of lipid peroxidation. Results showed a significant increase of 127% and 37% in homogenized of cerebral cortex and cerebellum from eight methionine injected mice, respectively. These values were normalized by chlorpromazine administration, a typical antipsychotic agent. Therefore, both vapreotide and chlorpromazine have shown to restore the altered parameters due to the model of schizophrenia.

Vapreotide, antipsychotic, schizophrenia, lipid peroxidation, acetylcholinesterase

(637) **THERAPEUTIC POTENTIAL OF SYNTHETIC PYRAZOLOTRIAZINONES FOR THE HYPERURICEMIA TREATMENT**

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Abstract: Xanthine oxidase (XO) is a widely distributed enzyme involve in the later stages of purine catabolism, catalyzing the oxidation of hypoxanthine to xanthine and of xanthine to uric acid (UA). High levels of UA in the bloodstream produced hyperuricemia, which could develop to gout, nephrolithiasis, cardiovascular disease, hypertension and diabetes, among others. Allopurinol is a well-known

XO inhibitor, a purine analogue, currently used for the treatment of hyperuricemia. However, numerous side effects have been reported due to Allopurinol administration, such as hepatitis, nephropathy, and allergic reactions.

In this study, pyrazolotriazinone compounds structurally related to purine bases were synthesized and evaluated for their ability to inhibit XO *in vitro*. Molecular docking studies were performed to explain the binding mode of XO with the selected compounds.

Pyrazolo[3,4-*d*][1,2,3]triazin-4-ones and ethyl 4-oxo-4,6-dihydro-3*H*-pyrazolo[4,3-*d*]triazine-7-carboxylates with different substituents in the N-7 and N-6, respectively, were synthesized with moderate to very good yields using a simple protocol (Colomer *et. al*). Following, all compounds were evaluated as XO inhibitor as indicated by Schmeda-Hirschmann *et. al.* and IC_{50} values were estimated. As a result, most of the tested compounds presented significant XO inhibition with IC_{50} values in the micromolar-submicromolar range (0,9-8,5 µM).

Considering the experimental results, docking studies were performed to determine the binding mode of both pyrazolotriazinones scaffolds. Main interactions between catalytic residues of XO (PDB ID: 1N5X) and each inhibitor were determined and the differences of inhibition behavior for each compound were stablished.

In summary, two sets of compounds containing the pyrazolotriazinone nucleus were synthesized and identified as potent XO inhibitors. Molecular modeling provided a reasonable explanation for the structure–activity relationships observed in the *in vitro* assays.

Keywords: xanthine oxidase, pyrazolotriazinones, XO inhibitor, docking studies, hyperuricemia.

(714) **INFLUENCE OF DEXAMETHASONE ON MARBOFLOXACIN PHARMACOKINETICS IN CALVES**

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Marbofloxacin is a second generation fluoroquinolone with concentration dependent activity against Gram-negative, some Gram-positive and mycoplasma organisms due to the interaction with DNA gyrase enzyme. The study was conducted to determine the plasma pharmacokinetics of marbofloxacin alone and associated to dexamethasone to assess whether the pharmacokinetics of marbofloxacin is modified by dexamethasone in weaning calves. In a crossover design, each animal group A (n=3) received 6 mg/kg intramuscular marbofloxacin. Group B (n=3) received marbofloxacin 6 mg/kg plus 2.2 mg/kg intramuscular dexamethasone. Treatments were exchanged after 2 weeks. Blood samples from each animal were collected in heparinized tubes at different times up to 24 hours, samples were immediately centrifuged and stored at -20 °C until HPLC analysis. Preparative analyte assay consisted of liquid-liquid extraction using 200 microliters of plasma, 200 microliters of water, 800 microliters of methanol and enrofloxacin as internal standard, stirred 30" in vortex then centrifuged 30´ at 13,500 rpm at 4 °C. Separation and quantification were performed by isocratic elution in reverse phase, at 0.8 ml per minute flow rate, 50 microliter injection volume, pre-column, C-18 octadecylsilane column, fluorescence detector at 295 nm excitation and 490 nm emission and mobile phase composed of water, acetonitrile and triethylamine (79: 19: 1 v/v/v), pH 3. Plasma concentration data were analyzed by PK Solution 2.0 software. Results indicates that intramuscular marbofloxacin has rapid absorption and distribution from the central compartment with moderate retention in body tissues. Statistical non-parametric Mann-Whitney test was used. It can be verified that the coadministration of dexamethasone did not significantly modify ($P < 0.05$), the kinetic parameters of marbofloxacin.

Keywords: dexamethasone, marbofloxacin, pharmacokinetics, calves

(1900) **PRELIMINARY PHARMACOLOGICAL CHARACTERIZATION OF ONE OF THE LESSER-KNOWN ENZYMES FROM SNAKE VENOMS: A PHOSPHODIESTERASE ISOLATED FROM *Crotalus durissus terrificus* VENOM**

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Phosphodiesterases (PDEs) belong to a super-family of enzymes that have multiple roles in the metabolism of nucleotides. Snake venoms contain PDE (e.g. Trimeresurus stejnegeri, Daboia russelli russelli,Bothrops jararaca) but their function is poorly understood at the present. These venom proteins have not received the scrutiny comparable to other venom proteins likely due to their apparent lack of a leading dramatic role in the pathophysiology associated with snake envenoming. In this work a PDE fraction was isolated from C.d.t venom (CDT-PDE) by superdex 75 and ion-exchange chromatographies (HiTrap Q-FF). Homogeneity of the pool was verified by SDS-PAGE. Two bands around 100 KDa were analyzed by mass spectrometry (Q Exactive LC-MS/MS system) and the study of fragment peptides determined the presence of two fosfodiesterase isoforms (96 and 91 KDa). First, the activity of the CDT-PDEs on different substrates was tested: AMP, ADP, ATP and DNA in various reaction conditions and the results showed that PDE fraction hydrolyze AMP > ADP > ATP and DNA. Taken in mind that the generation of adenosine could be involved in smooth muscle relaxation and vasodilatation as well as other effects on cardiovascular function, the open field test (OFT) was used for examining the behavioral effects of PDE fraction. Each mouse was tested in the OFT only once and 15 min before testing received an intraperitoneal injection (1.2µg/30µl/mouse) of the PDE or of the saline solution (control groups). The time of animal in movement was recovered. Also, this enzymes inoculated in mice reduce the movements on 30 or 50 % in the first 5 or last 5 minutes respectively. Probably the decrease in blood pressure is the cause of lower mobility evidenced in mice. So, this study suggests that CDT-PDE may also contribute to the overall pathology/symptomology of snake envenomation. This preliminary study will be expanded in a full characterization of this enzyme that it has a potential biomedical interest

(268) **DEVELOPMENT AND CHARACTERIZATION OF A PRECLINICAL MODEL OF DISSEMINATED RETINOBLASTOMA**

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In the absence of prompt diagnosis and appropriate treatment, retinoblastomadisseminates outside of the ocular globe. In lower-income countries, patients more often present with disseminated disease at the time of diagnosis. These patients receive an empirical and aggressive form of chemotherapy treatment, but still, fatal outcomes are observed. Currently, there is no second line of treatment and so disseminated retinoblastoma is considered an unmet medical need.

There are no previous reports of any type of animal model for disseminated retinoblastoma. In order to study the tumor biology and new or alternative pharmacological treatments we need an adequate preclinical model that still is not available. Thus, ouraimwas to develop an animal model that closely resembles the patient´s condition.

The protocol (#838) was approved by the local IRB. The cell model (396LNP) was derived from a unilateral patient with disseminated retinoblastoma in the cervical lymph node. These cells were injected into the posterior segment of the eye in 6-week old athymic mice. The animals were observed

till the tumor invaded the ocular globe causing proptosis. The mice were then enucleated and sacrificed. Eyes were conserved for histopathological studies. Tissue sampleswere collected to assess for retinoblastoma disseminationquantifying thespecific lineage molecular markerCRXby means of RT-qPCR.
A total of 19 eyes were injected with 396LNP cells with a 79% of engraftment. The eye survival was 23 days. In3 of 15animals cells disseminated into the lymph nodes and in7/15 optic nerves which resembled the patient´s condition. All the bone marrow samples were negative for CRX.
We have established a new disseminated RB animal model for the study of metastatic disease. This animal can be used to evaluate more advanced and efficient alternative treatment options.It will also allow for the development of a pre-clinical research platform which can later be applied for translational studies.
Keywords: Disseminated Retinoblastoma, preclinical model, metastasis.

(50) **EFFECT OF OMEGA-3 FATTY ACIDS ON NEUROPHATIC PAIN IN RATS FOLLOWING CHRONIC INJURY OF THE SCIATIC NERVE**

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Neuropathic pain results from an injury or disease of the nervous system (which may be both peripheral and central), and causes the nociceptive system to behave abnormally, with there being a total lack of causal relationship between tissue injury and pain. It is characterized by pain of a spontaneous type and painful hypersensitivity that we can differentiate between allodynia and hyperalgesia.

The aim of the present study was to evaluate the potential effect of the use of omega-3 fatty acids (O3) for 21 days on a chronic constriction injury (CCI) model in rats (CCI +O3 group) that induced signs and symptoms which mimicked human conditions of neuropathy. Assessment of nerve regeneration was based on neuropathological examination and histomorphometric criteria, and the thermal heat *hyperalgesia* was *evaluated* using the *hot plate test (HPT).*

We found that O3 (720 mg/kg/day) administration increased the response latency significantly compared with the CCI group after 7 days of treatment, with this the effect continuing and gradually increasing toward day 21 in the HPT. **Histological** studies revealed that compared to the CCI group, the O3 *treated* group (CCI + O3 group) *showed* normal axonal density and morphology with an increased number of Schwann cell nuclei according to a process of regeneration after a Wallerian degeneration, as well as a significantly higher axonal Regeneration Index.

Our results demonstrated that O3 treatment alleviated thermal heat hyperalgesia and promoted recovery in the axon number after sciatic nerve injury. Therefore, the present study identified the potential use of O3 in the treatment of neuropathic pain, which merits further preclinical and clinical investigation.

Keywords: neuropathic pain, omega-3 fatty acids, sciatic nerve injury, hyperalgesia.

(924) **METABOLISM OF BENZNIDAZOLE IN HEPATIC MICROSOMES FROM 3 DIFFERENT MAMMALIAN SPECIES**

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Chagas disease constitutes a major public health problem in Latin America and there are more 7 million infected people in the. Benznidazole is an antiparasitic drug with proven efficacy reason why is chosen as first-line treatment, but there is few information about benznidazole pharmacokinetics, particularly its metabolism. The knowledge about biotransformation pathway of this drug is critical to understanding side effects and predicting potential drug-drug and drug-disease interactions.

This work describes a study to analyze a possible metabolism pathway of benznidazole using hepatic microsomes that contains cytochrome P450 (CYPs) enzymes involved in phase I metabolism. Hepatic microsomes were isolated from 3 different species: Pig, Mouse and Rat. After microsomal purification, protein was determined by Bradford technique and activity of two CYPs (CYP1E2, CYP3A4) was detected reading hydroxylation of p-nitrophenol. Experiments were made incubating microsomal protein with necessary cofactors and benznidazole in a final concentration of 10 μg/ml.

Reactions were initiated by adding NADPH and were incubated 60 minutes at 37ºC. After incubation and samples processing it was measure concentration of benznidazole with UHPLC. Data were analyzed by multiple linear regression using R.

A decrease of benznidazole concentration comparing to controls (i.e. microsomes with benznidazole and without NADPH) was observed in the three species (highest in Rat).

These results suggests that biotransformation could be mediated by CYPs as a phase I reaction. Next step is to identify metabolites with mass spectrometry and which isoforms of CYPs were involved in benznidazole metabolism.

Keywords: benznidazole, metabolism, microsomes.

(1118) **DOCKING STUDY OF THE SESQUITERPENE LACTONE CUMANIN AND ITS DERIVATIVES ON TRYPANOTHIONE REDUCTASE FROM *TRYPANOSOMA CRUZI***

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Chagas disease, caused by the protozoan parasite *Trypanosoma cruzi*, is endemic in Latin America. Trypanothione reductase (TcTR) is an enzyme critical in maintaining the thiol redox balance in trypanosomatids. That is why it is one of the most important anticha-gasic drug target.

Based on the experimental evidence of the trypanosomicidal activity of the sesquiterpene lactone cumanin, acquired by this working group and reported in the literature[1], several derivatives were obtained from it.

The use of docking methods to identify potential candidates is a valuable tool extensively used for evaluating compounds and for guiding the synthesis of derivatives. On the basis of the knowledge of the biological target and its crystallography (PDB: 1GXF), and having already validated docking protocols[2], we have developed an hybrid method of DOCKING / QSAR3D, to study cumanin and its derivatives in order to have a theoretical evaluation protocol to estimate their potential as TcTR inhibitors. The best estimate of activity has been determined.

The hybrid method let us to identify cumanin and its derivatives as good inhibitors of TcTR, making them promising lead compounds. Based on these promising results and the estimates for the derivatives, the biological assays will be carried out.

Keywords: Sesquiterpene lactones, derivatives, docking, trypanosoma cruzi.

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INFECTOLOGY 5

(1299) **CHAGAS DISEASE IN AN OBESOGENIC CONTEXT: A HIGHLIGHT ROLE FOR VISCERAL ADIPOSE TISSUE AS A CHRONIC RESERVOIR OF *Trypanosoma cruzi* THAT CONTRIBUTES TO ASSOCIATED CARDIOVASCULAR COMPLICATIONS**

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Chagas disease and obesity are chronic and public health concerns with associated cardiovascular complications (CV). Oxidative stress (OS) and inflammation are common mechanisms that predispose to CV. We hypothesize that in an obesogenic context, the immune-metabolic dysregulation exerted by *T. cruzi* infection could drive CV.

To elucidate this complex interrelation, we evaluate the lipid and lipoprotein systemic changes and the oxidative and immune-inflammatory alterations in visceral adipose tissue (VAT) in uninfected C57BL/6 mice feed with LFD (4% fat diet) or DIO (14% fat/ 5% fructose diet), or infected (LFD+I and DIO+I) groups. Although an improvement on plasma triglycerides (TG) and total cholesterol (TC) levels were seen in DIO+I compared to DIO mice ($p<0.001$; $p<0.001$), apoB100 levels were increased in all groups in relation to LFD ($p< 0.05$); suggesting the presence of atherogenic small and dense LDL particles in infected groups. Concomitant qualitative differences in lipoprotein bands were observed. Significant decreases in the adiponectin levels were seen in both infected groups compared to DIO and LFD ($p<0.001$; $p<0.001$). In VAT, inflammatory cell-infiltration ($p<0.001$) and OS (measured by lipid peroxidation) were exacerbated in DIO+I compared to LFD+I mice ($p<0.05$). Despite a higher number of macrophages observed in VAT (ATM) from DIO+I than in LFD+I mice ($p<0.001$), they presented a M2 phenotype (F4/80+CD11c-CD206+). Furthermore, increased CD36 expression ($p<0.05$) and parasite load ($p<0.05$) was seen in VAT from DIO+I compared to LFD+I. Conversely in heart, a low parasite load was observed independent of the diet, highlighting VAT as a more suitable chronic reservoir.

The strong inflammatory and oxidative response in an obesogenic context is probably counter-balanced by the parasite, which may induce the polarization of ATM to a M2 phenotype to favor its own survival. Thus, parasite persistence would be a key trigger in the progression of CV Chagas disease.

Keywords: OBESITY; *Trypanosoma cruzi*; IMMUNOMETABOLISM; OXIDATIVE STRESS; INFLAMMATION; CARDIOVASCULAR DISEASE.

(1586) **HSP20 PROTEIN FROM *Toxoplasma Gondii* GENERATES PROTECTION IN A MURINE MODEL OF CHRONIC TOXOPLASMOSIS**

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Toxoplasma gondii is an intracellular protozoan with a worldwide prevalence in human and animal populations. No vaccine is currently available for use in humans or food producing animals. The *T. gondii* surface chaperone Hsp20 appears as a possible candidate to be included in the development of a preventive vaccine, since it is recognized by almost 80% of sera from seropositive individuals and also, anti-Hsp20 antibodies are able to block *in vitro* parasite motility and host cell invasion. In the present work, we studied the immune response and protective effect of rHsp20 in C57BL/6 mice, a strain highly susceptible to *Toxoplasma* infection. Mice were immunized 3 times with 15 day-intervals with rHsp20 alone (20µg/dose) by intranasal (rHsp20 IN) or intradermal route (rHsp20 ID), or combined with Alum (rHsp20 ID+Alum) or complete Freund´s adjuvant (rHsp20 ID+ACF). Naive mice were used for the control group (Control). Two weeks after last immunization, mice were orally challenged with a non-lethal dose of tissue cysts of the ME49 strain. One month later, cysts were counted in the brain of infected mice and a significant decrease in parasite load was observed only in the group vaccinated with rHsp20+ACF. All groups vaccinated by the intradermal route elicited an antigen-specific systemic humoral response. However, protection in the rHsp20+ACF group correlated with the induction of both, a strong humoral response with a Th1/Th2 mixed profile and also a cellular response characterized by significant levels of antigen-specific proliferation of and cytokine production (IFN-γ, IL-10 and IL-4) after *in vitro* stimulation with the antigen. No systemic responses could be detected in the intranasal vaccinated mice. Our findings demonstrate that even rHsp20 is a good antigen able to elicit high levels of specific antibodies, protection is achieved only when cellular specific responses are also induced. So we postulate Hsp20 as a good candidate to be used in a vaccine formulation.

Keywords: Toxoplasma gondii, Vaccine, Hsp20

(657) **INACTIVE *TRANS*-SIALIDASE KNOCK-IN INCREMENTS *TRYPANOSOMA CRUZI IN VIVO* VIRULENCE**

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Abstract: *T. cruzi trans*-sialidase (TS) has arised as a relevant parasite virulence factor. *TS* gene family contains alleles encoding for active (*aTS*) and inactive (*iTS*) TS isoforms with a $His_{342}Tyr$ conserved mutation. We have reported that, in contrast to high-virulence strains (DTUs II, V and VI), lower-virulence strains (DTUs I, III and IV) do not contain *iTS* genes. Previous *in vitro* results using recombinant iTS suggest that it could play a different or complementary pathogenic role to that of aTS. We have constructed a recombinant iTS-encoding gene where a 3XFLAG epitope was inserted (T-iTS) and used to transfect iTS^{null}-parasites (K98 DTU I). Flow Citometry, Confocal Microscopy, Western Blot and TS activity evaluation showed its adequate expression and localization. *In vitro* assays showed that T-iTS-transfected clones were able, at low interaction time (1h), to infect significantly more cells (Vero, Cardiocytes and fibroblasts, $p<0.05$). Higher amastigotes number ($p<0.0001$) were observed in T-iTS respect to control parasites (T-aTS, empty vector (T-O) including phagocytic cells. In in vivo assays, infected BALB/cJ mice (n=12 to T-iTS; n=11 to T-O), showed that T-iTS parasites could establish infection. At 60 days pi, mortality was 50% for T-iTS and 25% in T-TO-infected mice. Survivor mice samples (heart and skeletal muscle) were evaluated. Inflammation score in heart (auricle and ventricle) and skeletal muscle was significantly superior ($p<0.05$, both cases) to T-TO-infected mice. Moreover, the presence of amastigote nests was detected only in skeletal muscle from T-iTS-infected mice (4 of 6). iTS expression in trypomastigotes naturally lacking it, shows the increment of invasiveness as well as the ability to induce strong inflammation, that are in turn involved in increased *T. cruzi*-induced pathogenesis. Our *in vivo* assays shed light on iTS isoform pathological role as member of the TS family and support that it must be considered as a virulence factor.

Keywords: pathogenesis; Chagas disease; viulence factor; invasiveness; *trans*-sialidase isoforms

(1252) **PANCREATITIS-ASSOCIATED PROTEIN, A NOVEL BIOMARKER FOR HEART FAILURE, IS INCREASED IN PATIENTS WITH SEVERE CHRONIC CHAGAS CARDIOMYOPATHY**

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Chronic Chagas Cardiomyopathy (CCC) manifests as a dilated cardiomyopathy and includes arrhythmias, atrioventricular and intraventricular conduction disorders, apical aneurysms, cardiomegaly and heart failure. In Chagas disease, it is important to identify patients who still asymptomatic, have risk of progressing to CCC. We aimed to determine the circulating levels of different biomarkers in serum of patients with CCC and to evaluate if their concentrations correlate and/or predict the severity of the disease.

The study was carried in 72 chronic patients and 15 non-infected donors. Patients were classified according to the Consensus on Chagas-Mazza Disease in non-demonstrated (non-DC, n=23)

or demonstrated (DC) pathology groups; the latter group was subclassified in patients with arrhythmias (DC-Arr, n=25) or with congestive heart failure (DC-CHF, n=24). Interleukin (IL) 6, IL10, Tumor Necrosis Factor α (TNFα) and Pancreatitis-Associated Protein 1 (PAP1) were measured using ELISA kits. Our results showed higher levels of IL6 in sera of DC patients compared to non-DC patients (p=0.035). PAP1 level was significantly higher in DC-CHF *vs* DC-Arr or non-DC (p<0.05) showing an important increase with severity of CCC. However, no differences in IL10 and TNFα concentration were observed among groups.

Discriminant Analysis of Principal Components (DAPC) was performed to assess the association between studied biomarkers and the clinical manifestation of Chagas disease. The principal components considered for the group explained the model in 95.3% of the variation in the dataset. DAPC showed a clear grouped for non-DC and DC-CHF patients, but not for DC-Arr group. This result may be due to the fact that these patients could eventually be in an intermediate status of clinical symptoms.

In conclusion, changes in the levels of these serological biomarkers, especially an increase in the concentration of PAP1, could predict the disease progression.

Keywords: Trypanosoma cruzi, CHRONIC CHAGAS CARDIOMYOPATHY, BIOMARKERS

(1496) **ROLE OF GALECTIN-8 IN THE OUTCOME OF *TRYPANOSOMA CRUZI*-INDUCED MYOCARDITIS**

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Galectins are a family of lectins that contain characteristic aminoacid sequences and have affinity for B-galactosides. Galectin-8 (Gal-8), which belongs to the tandem repeat group, it is widely distributed in tissues and secreted to the extracellular media, acting in an autocrine/paracrine manner. It participates in cellular adhesion, migration, and apoptosis, mainly in cancer and autoimmune disorders. Different authors have also proposed Gal-8 to be involved in pro-inflammatory or anti-inflammatory events. Chagasic cardiomyopathy is a chronic disease, whose development is based on the induction of a strong inflammation by *Trypanosoma cruzi* persistence in cardiac tissue. The aim of this work was to analyze the role of Gal-8 in an inflammatory context induced by *T. cruzi.* Gal-8 knock out (KO) and C57/BL6 (WT) mice were infected with *T. cruzi* strain Ac and sacrificed at 4 months post infection for histopathology and phenotyping analysis. The inflammation score in cardiac tissue of KO mice was significantly higher compared with WT mice. No difference was found in fibrosis induction. The cellular population present in infected heart tissues, was characterized by flow-cytometry analysis. There were no differences in the levels of (CD-11b+Ly6C^{high}Ly6G-) and (CD11b+Ly6C^{low}Ly6G-) monocytes, dendritic cells (CD11c+F4/80-CD11b+) ;(CD11c+F4/80-CD11b-) and T lymphocytes (CD3+CD4+ and CD3+CD8+) between KO and WT infected hearts. However, inflammatory cells isolated from KO infected mice showed higher frequency of neutrophils (P=0.0097) and macrophages (P=0.0021) compared to WT mice, with concomitant increase of MCP-1 mRNA expression levels (P=0.0023). In agreement, increased levels of neutrophils (p=0.033) were observed in spleens from KO infected mice compared to those of WT mice.

The reported participation of Gal-8 in preaperesis induction on leukocytes is in agreement with our findings.

Altogether, our results suggest a Gal-8 anti-inflammatory role in *T. cruzi* infection.

(1375) **TRANSMIGRATION OF DIFFERENT STRAINS OF *TRYPANOSOMA CRUZI* ACROSS A 3D-MICROTISSUE MODEL**

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Three-dimensional (3D) cultures are physiologically relevant because mimic the microarchitecture of tissues and provide an environment similar to the encountered in natural infections. Here, we studied the transmigration of *T. cruzi* trypomastigotes of different strains (DTUs I, II, V and VI) into 3D-microtissues (spheroids). Spheroids of HeLa cells expresing RFP (HeLa-RFP) were cultured with CFSE-labelled trypomastigotes; transmigration into spheroids was analyzed at 24 h post infection. 3D-reconstruction from confocal microscopy images showed a broad spectrum of migration among strains, being some strains much more migratory (>40 μm in depth) than others (<10 μm). Also, parasites of "high migrant" strains (e.g. CL Brener strain) were evenly distributed in all the spheroid surface, while "low migrant" parasites (e.g. SylvioX10 strain) presented a focalized "patch-like" distribution pattern. A more detailed analysis carried out with these two opposite strains, showed that the highly migrant CL Brener strain was ~four-fold more infective than the low migrant SylvioX10 strain (qPCR and flow cytometry). However, we could not associate transmigration capacity with the lineages of the strains (i.e. DTU). We also studied the migration into spheroids of two recently isolated strains belonging to the same DTU. The strain isolated from a congenital infected child (173BB) showed a "high migrant" phenotype, while the strain isolated form a chronically-infected mother (that did not transmit the infection to any of her 3 children, 733MM) was significantly less migratory. Altogether results suggest that in a 3D microenvironment, each strain presents a characteristic migration pattern that can be associated to their *in vivo* behavior. Remarkably, the findings presented here cannot be studied with traditional 2D monolayer cultures. Supported by PICT2014-1151 and PIP2015-186.

(32) **Y CHROMOSOME HAPLOGROUPS ASSOCIATED WITH CARDIOVASCULAR MANIFESTATIONS OF CHAGAS DISEASE**

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A sexual dimorphism exists in the incidence and prevalence of the heart disease. In a cohort of seropositive individuals to *T cruzi*, the male patients have at higher risk of worse disease. Recent studies showed an association between I haplogroup of Chromosome Y with an increased risk the carotid disease. These data would highlight the importance of gender and genetic background in the pathophysiology of the chronic chagasic stage. We propose to explore the role of the Y chromosome in men diagnosed with Chagas Disease and assess the relationship between their ancestry and disease status.

In a comparative study, we assessed 300 unrelated Argentinian men to the serological determination of Chagas disease, biochemical parameters, Thoracic X-rays, electrocardiogram, and echocardiography, and DNA to genotype a set of 17 microsatellites from the Y chromosome by AmpFISTR® Y filer kit (Applied Biosystems). The population was divided in Chagasic (CG) and non chagasic groups (nCG). We examined associations between common Y chromosome haplogroups and the clinical parameters of the risk by logistic regression. The most common haplogroups were R1b (43%), G2a and E1b1b (both 9%). The R1b haplogroup was more frequent in nCD (p=0.0421) and G2a (p = 0.0338) among the CG. Men from both group classified as R1b haplogroup show less clinical evidence of disease (p=0.038). But the proportion of symptomatic individuals is higher among CG regardless of whether they carry haplogroup R1b. The Regression Logistic analysis adjusted for the traditional cardiovascular and socioeconomic risk factors shows, that the OR to chance of having a cardio-thorax index more than 0.5% is 3.11

IC 95% 0.11-0.92 and Echocardiograms alterations detect is OR= 2.5 IC 95% 0.16-0.94 in CG patients without R1b haplogroup. Our results showed a high European patrilineal genetic contribution. R1b haplogroup has a potential protective cardiovascular effect, but this is lost when the subject is infected with *T.cruzi*.

Keywords: Chagasic cardiomyopathy, chromosome Y haplogroups, cardio-thorax index

(1874) **INTERLEUKIN-10 AND INTERLEUKIN-6 POLYMORPHISMS AND CHAGAS DISEASE CARDIOMYOPATHY**

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Development of Chagas heart disease (CHD) is often associated with dysregulation of Immune response. During parasite-host interaction, Interleukin-10 plays a key role as a regulatory cytokine in CHD progression, while IL-6 would maintain the inflammatory environment. The G and C allelic variants of the single-nucleotide polymorphisms of IL-10 (SNP-1082 A/G) and IL-6 (SNP -174 G/C) promoter regions are associated with high and low gene expression levels, respectively. This may alter the balance of immune response.

These SNPs were evaluated in individuals with more than 20 years of *Trypanosoma cruzi* infection, divided into two groups: asymptomatic and with CHD. We used High Resolution Melting (HRM) analysis, (Applied Biosystems® HRM Software).

We evaluated 65 samples for IL-10 SNP; the proportion of AA genotype was 45.5% in the asymptomatic and 68.8% in heart disease patients, whereas for the heterozygous AG genotype it was 54.6% and 31.3%, respectively ($p = 0.056$); being similar for the GG genotype (3% and 2.9%, respectively). To date, for IL-6 SNP only 35 individuals were evaluated: 52.9% GG genotype in asymptomatic patients and 72.2% in heart disease patients, with 41.2% and 22.2% GC genotype, respectively ($p = 0.282$). The distribution of CC genotype was similar in both groups.

These results suggest the highest frequency of the A allele of IL-10 SNP could be associated with a protective effect in individuals who remained asymptomatic over 20 years of infection. Similar effect could be generated by the C allele of IL-6 SNP, also present in a higher frequency within this group, although the difference with the CHD did not reach statistical significance. The evaluation of a larger number of individuals and the analysis of the combined effect of these two polymorphisms will allow a better analysis of this trend.

Chagas - polymorphisms - Interleukin-10 - Interleukin-6- cardiomyopathy

(1244) ***T. CRUZI* INFECTED HUMAN PRIMARY CARDIOMYOCYTES IMPROVES MITOCHONDRIAL BIOENERGETICS EARLY AFTER INFECTION.**

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Chagasic cardiomyopathy is one of the most frequent and severe manifestations of American Trypanosomiasis (Chagas Disease, CD) caused by the parasite *Trypanosoma cruzi*. More than 100 years had been passed since the discovery of *T. cruzi* by Carlos Chagas, and to date, the pathogenic and biochemical mechanisms responsible for the cardiac lesions are still not completely understood. In this work, we aimed to evaluate, by transcriptome analysis, using microarrays, the biochemically-altered pathways in infected human cardiomyocytes at early times post infection (2-24h) with *T. cruzi* (Dm28c strain). After 2h of infection, human cardiomyocytes genes belonging to biochemical pathways related to energy metabolism (glycolytic pathway, oxidative phosphorylation and components of the electron transport chain) were up-regulated together with the cytosolic ribosomal proteins. We thus search for improved mitochondrial function in *T. cruzi* infected cardiomyocytes, assayed with the Seahorse XF Analyzer. Results obtained herein show that mitochondrial function were enhance in *T. cruzi* infected human cardiomyocytes (24h post-infection), moreover the mitochondrial biogenesis was also enhance in the infected cells. These results were accompanied by the activation of the mammalian target of rapamycin complex 1, mTORC1. Inhibition of mTORC1 cascade partially restores the control phenotype in the infected cardiomyocytes. The results of the present work, evidence the complexity of the multiple host-parasite interactions that happens early during the infection process identifying, activation of mTORC1, mitochondrial biogenesis and improved in energy metabolism as key biochemical changes that may provide new insights in the host response to parasite infection and pathogenesis.

Keywords: Chagas Cardiomyopathy, energy metabolism.

(1348) **RAB 32 IS PRESENT IN THE *Trypanosoma cruzi* PARASITOPHOROUS VACUOLE AND CONTRIBUTES TO THE INFECTION PROCESS**

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Trypanosoma cruzi, the etiological agent of Chagas´ disease is a protozoan parasite which infects both phagocytic and non-phagocytic mammalian cells. At early stages of infection, trypomastigotes localize in a vesicular compartment called the *T. cruzi* parasitophorous vacuole (TcPV) until the escape to cell cytoplasm to continue their cycle. Rab and SNAREs proteins take part of the molecular machinery of membrane traffic. Rab GTPases have emerged as central regulators of vesicle recognition and transport, whereas SNAREs are mainly involved in the fusion process between membranes. In a previous research, we characterized that the *T. cruzi* infection process in non-phagocytic cells occurs in two stages, the formation and maturation of the TcPV. We also showed that the v-SNARE VAMP7 is required for the TcPV development and for the establishment of infection. The aim of this work was to identify other molecular components of the vesicular transport pathways and their participation in the *T. cruzi* infection. CHO cells were transfected to overexpress GFP-Rab proteins from the endocytic and exocytic pathways and then infected with trypomastigotes for 1 h. After this time, cells were fixed and processed to detect intracellular parasites by indirect immunofluorescence. Similar procedures were performed in CHO cells overexpressing GFP-vector as control. By confocal microscopy, we observed that Rab32 was recruited to the membrane of the TcPV. The frequency of this localization was higher (43 +/- 0.3 %) than control and similar to that observed for VAMP7 at the same time. In addition, we found that the overexpression of Rab32 significantly increased *T. cruzi* infection. Due to Rab32 effectors can regulate binding of VAMP7 and therefore the fusion events triggered by them, these results open a novel way to understand the mechanisms that control the formation and maturation of the *T. cruzi* vacuole.

Keywords: *Trypanosoma cruzi*, host cell infection, Rab 32, VAMP 7

TOXICOLOGY 3

(1719) **CHRONIC EXPOSURE TO PARTICULATE AMBIENT AIR POLLUTION FROM BUENOS AIRES CITY ALTERS OXIDATIVE METABOLISM IN MOUSE BRAIN CORTEX**

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Increasing evidence indicates that the central nervous system is a target of air pollution, causing alterations such as neuroinflammation, and neurodegeneration. However, the mechanisms mediating these effects have not yet been fully elucidated. The aim of this work was to study the effects of chronic exposure to ambient air pollution on mice brain cortex oxidative parameters. C57BL/6J mice were continuously exposed to polluted air from a central area of Buenos Aires city for 7, 15 or 30 days. Mice were housed in chambers specially designed for that purpose. Control mice were subjected to the same protocol but interposing filters to the air flux to the chamber in order to retain particulate matter. TBARS, carbonyl and glutathione levels were measured in brain cortex homogenates as oxidative stress markers. SOD and NOX activities were also assesed in order to evaluate superoxide anion metabolism. Lipid oxidative damage, measured as TBARS, was increased after 7 days of polluted air exposure compared to control mice (36%, $p<0.05$). Carbonyl levels, evaluated as a protein oxidative marker, were significantly increased after 15 and 30 days of exposure to polluted air (42% at day 7, and 49% at day 30 compared to control group, $p<0.05$). Regarding glutathione levels, a decrease in GSH/GSSG ratio was found after 15 days of exposure to ambient air pollution respect to control mice (26%, $p<0.05$). No changes were found for SOD and NADPH oxidase activities for the different times studied.

Taken together, our data demonstrate that chronic exposure to ambient air pollution produces lipid and protein oxidative damage and alters redox status in mice brain cortex. These findings indicate that oxidative stress may play a key role in central nervous system damage mechanisms, triggered by air pollution.

(771) **CHRONIC FUMONISIN INTAKE INDUCES ERYTROCYTE OSMOTIC FRAGILITY**

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La fumonisina B1 (FB_1) es una micotoxina que se presenta como contaminante frecuente del maíz y de los alimentos a base de maíz en Argentina. Tiene un efecto inhibidor de la ceramida sintetasa, enzima clave en la biosíntesis de esfingolípidos. Nuestro *objetivo* fue determinar los efectos de FB_1 "*in vitro*" sobre eritrocitos humanos y evaluar el efecto de una ingesta crónica de un cultivo de fumonisinas sobre la función eritrocitaria en ratas. Se utilizaron ratas Wistar macho de 1 mes, separadas en dos grupos de 5 ratas. Al grupo control se le administró solución salina; el grupo tratado recibió durante 60 días 1mg/kg/día de FB_1 de un cultivo de *Fusarium proliferatum*. Para el estudio de resistencia osmótica eritrocitaria inmediata (ROE i), los eritrocitos de ratas controles o tratadas se mezclan con soluciones de ClNa de 0 a 9 g/l y para el ROE 24 hs se realizó una incubación previa de los eritrocitos durante 24 hs a 37°C y luego tratados con ClNa (4g/l). Para los estudios "*in vitro*", glóbulos rojos humanos se incubaron con cantidades crecientes de FB_1. La diferencia entre medias se calculó por análisis de ANOVA. Los parámetros hematológicos mostraron una disminución significativa de las ratas tratadas *vs* controles, en la concentración de hemoglobina (12,9±0,4 g% *vs* 14,1±1,1 g%) y hematocrito (38,2±0,0 % *vs* 40,7±3,1%). En los estudios de resistencia osmótica, se observan diferencias entre los valores de porcentaje de hemólisis en tratadas *vs* controles 97%*vs* 9,8%, 5g% ClNa y 100 *vs* 74 %, 4g% ClNa; con valores de ROEi de 5.25 g% para los tratados y 4.75 g% para los controles. Los % hemólisis "*in vitro"* no mostraron diferencias significativas frente a cantidades crecientes de FB_1 ($p>0.05$). *Discusión*: La ingesta crónica de fumonisinas en ratas lleva a alteraciones en los eritrocitos que se manifiesta en el aumento de su fragilidad osmótica. Futuros estudios relacionados con una posible senescencia precoz de eritrocitos por efecto de las fumonisinas deben ser abordados

(929) **EFFECT OF ARSENIC IN EPITHELIAL CELLS WITH IMPAIRED ACTIVITY OF THE CFTR**

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Little is known about the effects of environmentally relevant levels of arsenic on ion channels. Cystic Fibrosis, an inherited disease affecting 1:2500 newborn babies, is characterized by mutations in the CFTR, gene encoding a cAMP-regulated chloride channel. The aim of this study was to determine how arsenic affects mammalian cells with a diminished activity of the CFTR; it is known that the metalloid elicits its effect through mitochondrial pathways which, in cystic fibrosis is deeply compromise. IB3-1 (ATCC CRL-2777, a bronchial cell line derived from a cystic fibrosis patient with a DF508/W1282X CFTR genotype) and S9 (ATCC CRL-2778, which are IB3-1 cells transduced with an adeno-associated viral vector to stably express wt-CFTR) were exposed to As III ($NaAsO_2$) (0 and 200 uM) for 2 hrs. Crystal violet assay results in S9 and IB3-1 epithelial cells showed IB3-1 cells to be less viable than S9 to As 10 uM when exposed for 2 hrs, but S9 cells were more prone to apoptosis measured by Annexin-V and Propidium iodide apotosis/necrosis assay and flux cytometry (significant differences were assessed by ANOVA and Fisher's LSD at $p = 0.01$). This might be due to mitochondrial activity impairment and its involvement in apoptosis. Using synchrotron technology at the Synchrotron facility of Campinas in Brazil, we determined speciation in cells exposed for 2 hrs to As (III) 100 uM. This result showed us that As was mainly found as As^{+3} form and that might be binding sulfur, such as As-Glutathione, a well-known detoxifying pathway for As. Taken together, this results partially explains the importance of mitochondrial functionality and GSH detoxifying pathway in cells exposed to As III, even at concentrations of As below permitted levels in drinking water, and for as little as 2 hrs of exposure. Our next step is to study the effects of the metalloid on the expression of CFTR and CFTR-dependent genes.

Keywords: CFTR, Chloride, Arsenic, Synchrotron

(173) **ESTABLISHING A 3D MOUSE PRIMARY CELL CULTURE MODEL TO STUDY THE DIRECT EFFECTS OF ENVIRONMENTAL ESTROGENS ON THE FUNCTIONAL DIFFERENTIATION OF THE MAMMARY GLAND**

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There are several human-made chemical compounds with hormonal action. Among them, Bisphenol A (BPA) and benzophenone-3 (BP3) have been shown to have estrogenic activities. Previously, we had demonstrated that the development and differentiation of the mammary gland are affected by perinatal exposure to xenoestrogens *in vivo*. In the present study, our aim was to develop and establish an *in vitro* culture model to study the direct effects of environmental estrogens during the functional differentiation of the mammary gland. Mammary organoids were obtained and isolated from 8 weeks-old C57BL/6 female mice using enzymatic and mechanical digestion. The organoids were cultured on top of Matrigel with growth medium for 24 hs at 37°C, 5% CO_2. The next day, the medium was changed to a differentiation one containing lactogenic hormones and exposed to: vehicle (1% ethanol), 1 μM of BPA and 1 μM or 1 ηM of BP3 for 72 hs. The mRNA expression of estrogen alpha (ERS1), progesterone (PR) and prolactin receptors (PRLR) and the mRNA levels of the milk protein beta-casein (CSN2) were analyzed by real time RT-PCR. Also, CSN2 and alpha-lactoalbumin (LALBA) protein expression was quantified in histological sections by an immunofluorescence assay. After 72 hs of exposure with 1 μM BPA, ESR1 and PR mRNA levels were like controls, but CSN2 mRNA and protein expression was increased, indicating that this

3D culture model responded to BPA treatment during differentiation. Furthermore, the exposure to both doses of BP3 induced an increase of ERS1, PR and PRLR expression; and 1 μM BP3 augmented CSN2 and 1 ηM BP3 increased LALBA protein expression compared to controls. In conclusion, our results show that a 3D mouse primary cell culture model could be an appropriate tool to study the direct effects of environmental estrogens, and demonstrate that direct exposure to low doses of BPA and BP3 induce alterations on mammary gland functional differentiation and milk protein synthesis.

Keywords: environmental estrogens, 3D cell culture, mammary gland, milk proteins.

(1142) EFFECTS OF ACOUSTIC STRESS ON GLUCOSE HOMEOSTASIS

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Inflammatory bowel diseases (IBD) are frequently associated with diabetes mellitus (DM2). Our previous results showed that acoustic stress (AS) induces long term severe intestinal inflammation in the mouse. In the present study, our aim was to investigate whether AS affects glucose homeostasis. Two-month old CBA/J male mice were subjected to two oral glucose tolerance tests (OGTT) determining glucose levels at 0-30-60-120 min. Then, mice were randomized into AS (n=7) and control (C) (n=6) groups. AS mice were exposed to noise (300Hz-70dB) during 24hs with food and water *ad libitum.* Three AS protocols were assessed: I- 3 month old mice were exposed weekly to 1 AS during 5 weeks, II- At 6 months old, the same animals received weekly 2 AS during 5 weeks and III- Mice were re-exposed to AS twice a week for 5 weeks at 13 mo old. After each AS protocol, two OGTT were assessed. All animals were weighted throughout the whole experiment. Finally, animals were exanguinated for insulinemia and glucagonemia determinations and killed. Results showed: I- AS increased blood glucose and the OGTT area under curve (AUC) by 20% ($p<0.01$). This effect was associated with a higher body weight. II- AUC augmented by 36% ($p<0.01$). III- Interestingly, we detected a high rate of hepatic tumors (4/6) in the AS group while in the C group the proportion was 1/6. AS mice with tumors showed similar AUC values to C group. However, the two AS animals that lacked tumors, showed the highest values of AUC. The AS group showed a ns decrease of plasma insulin levels (C: 1214 ± 752 vs AS: 849 ± 670 pg/ml) and a significant diminishing in glucagon levels (C: 225 ± 60 vs AS: 67 ± 3 pg/ml, $p<0.01$). These data could be due to liver or pancreas disfunction in response to AS. Animals from protocols II and III showed similar body weights. In sum, AS increases glucose levels in a dose-dependent way. Its persistence along life can maintain this effect or, more frequently lead to the generation of tumors.

(1334) EPIGENETIC DISRUPTION OF ESTROGEN RECEPTOR ALPHA FOLLOWING PERINATAL EXPOSURE TO A GLYPHOSATE-BASED HERBICIDE

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Previously, we showed that perinatal (*in utero* and lactational) exposure to a glyphosate based herbicide (GBH) produced subfertility associated with implantation failures in female rats. Implantation process is regulated by endocrine signaling pathways in which ERα is one of the primary mediators. Five promoters (E1, OT, O, ON, and OS) control the ERα transcription initiation, yielding different transcripts with alternative 5´ untranslated regions (5'UTRs). This work investigates whether a low dose of a GBH modifies uterine ERα expression and induces epigenetic modifications in its regulatory regions during the pre-implantation period. Pregnant rats (F0) were orally exposed to 200 mg of glyphosate/kg/day (NOAEL, EPA) through food, from gestational day (GD) 9 until weaning (lactational day 21). When F1 females reached the sexual maturity, they were pregnant and uterine samples were collected on GD5 (pre-implantation period). ERα expression was determined using RT-qPCR. ERα mRNA levels of transcript variants containing alternative 5´UTRs were also evaluated. Then we *in silico* evaluated the presence of CpG islands and specific restriction sites (for *Mae*II and *BstU*I) to analyze the methylation status using Methylation-Sensitive Restriction Enzymes-PCR technique. Post-translational changes of histones were also studied by chromatin immunoprecipitation assay. GBH treatment increased total ERα mRNA expression mediated by an increased expression of ERα-O variant. GBH rats exhibited a decreased DNA methylation in one of the three sites evaluated in the O promoter. In addition, we detected a higher level of histone 4 acetylation and a decreased level of histone 3 methylation at Lys 27. All these epigenetic changes are in accordance with the higher transcriptional activity of ERα in GBH treated rats. We demonstrated that low-dose perinatal GBH exposure induces lasting epigenetic disruption in the uterus possible related with the implantation failures.

Keywords: Glyphosate based herbicide, Uterus, Estrogen receptor alpha, Epigenetic regulation

(329) GLUTATHIONE PREVENTS COPPER-INDUCED CELL DEATH BY BINDING CUPROUS IONS INDEPENDENTLY OF ITS REDUCED THIOL GROUP

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Wilson Disease is a genetic disorder where copper (Cu) builds up in the organism due to defective metal extrusion from the hepatocytes into the bile. Intracellular free Cu ions result highly toxic for cells and the metal-induced oxidative stress is assumed to cause cell death. Thereby, the major antioxidant glutathione (GSH) has been acknowledged as a protective molecule. Nonetheless, the nature of Cu-induced cell death is unclear and besides the antioxidant features of the molecule, GSH may protect the cell independently of the reactive thiol (-SH). Methodology: Cell viability (FACS), –SH content (reaction with DTNB), Cu content (atomic absorption) and DCFH-DA oxidation were assessed after Cu exposure in HCT116, BEAS-2B, HeLa, MEFs and Hepa1-6 cells. Transcriptome profile upon Cu overload in BEAS-2B cells was assessed with DNA microarrays. Protein aggregation was assessed by a turbidity assay. Results: Cu overload enhances intracellular oxidant production. However, no induction of peroxiredoxins, glutathione peroxidases or catalase was observed in the transcriptome profile and -SH homeostasis in the yet living cells is not altered. Intracellular Cu is accumulated as Cu^{1+} either when cells are exposed to Cu^{1+} or Cu^{2+}. GSH protects cells from Cu ions independently of the reduced –SH in the molecule, thus preventing the interaction between the metal and intracellular targets. Interestingly, Cu-induced protein aggregation *in vitro* was fully prevented either by GSH or GSSG, confirming that GSH binding of Cu^{1+} is independent of the reactive –SH group. Conclusion: Cell exposure to lethal concentrations of Cu^{2+} does not

alter –SH groups homeostasis of yet living cells. While the antioxidant GSH is a major protective molecule against the toxicity of these ions, the nature of this function is not based on its redox chemistry but rather on its ability to form an inactive complex with the metal.

Keywords: Copper, Glutathione, Oxidative Stress

(1636) **N-ACETYL CYSTEINE PREVENTS BUENOS AIRES URBAN AIR PARTICLES ADVERSE EFFECTS ON MICE LUNG AND LIVER**

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Particulate matter (PM) is one of the main air environmental pollutants. Epidemiological studies have shown that PM can increase cardiorespiratory morbimortality, being the lungs the main PM body entrance target organ. Ultrafine particles (PM 0.1), because of their size, can translocate from the lung into the circulation affecting other body organs. Among them, the liver plays a key role in xenobiotics metabolism and its malfunction has been associated with increased cardiovascular risk. Previous work from our group demonstrated that Urban Air Particles from Buenos Aires (UAP-BA) induces in lung, inflammation and oxidative stress. Therefore, the aim of this study was to analyze the influence of the antioxidant, N-acetyl cysteine (NAC), on UAP-BA acute impact on mice lung and liver.

BALB/c mice (N=20) were *i.p.* injected with NAC (150 mM/kg BW) or vehicle, and 24h later exposed by intranasal instillation to UAP-BA (1 mg/kg BW) or vehicle. We evaluated 24h post-instillation the total cell number (TCN), and superoxide anion generation (O_2^-) by NBT in bronchoalveolar lavage fluid (BAL) and, superoxide dismutase (SOD) and catalase (CAT) activities spectrophotometrically, reduced glutathione (GSH) by DTNB and lipoperoxide levels by TBARS in liver homogenates.

Exposure to UAP-BA increased TCN and O_2^- production in BAL ($p<0.001$ *vs.* control), while NAC pretreatment prevented this increment ($p<0.01$ *vs.* UAP-BA). Liver homogenates from UAP-BA exposed animals showed altered SOD and CAT activities, and augmented levels of both GSH and lipoperoxides ($p<0,001$ *vs.* control) while NAC pretreatment prevented the increase in lipoperoxide levels ($p<0,001$ *vs.* UAP-BA), but had no effect on SOD and CAT activities or GSH levels induced by UAP-BA.

We demonstrated that UAP-BA induces lung inflammation and liver oxidative stress. Antioxidant NAC pretreatment impairs UAP-BA adverse effects both in lung and liver, suggesting that oxidative stress is a common path for UAP-BA negative impact.

Keywords: Liver, Oxidative Stress, Particulate Matter, N-Acetyl Cysteine, Lung.

(1657) OVEREXPRESSION OF THIOREDOXIN IN HEART HAS A PROTECTIVE EFFECT AFTER EXPOSURE TO AMBIENT AIR PARTICLES

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The exposure to environmental particulate matter (PM) is associated with increased morbidity and mortality rates due to several cardiovascular diseases, including stroke and myocardial infarction. Thioredoxin (Trx) is a key antioxidant system that plays an important role against oxidative stress; it regulates redox homeostasis and has cardioprotective effects reducing infarct size. A byproduct of fossil fuel combustion known as Residual Oil Fly Ashes (ROFA) is frequently used as a PM surrogate to evaluate the biological effects of the exposure to air pollution. Therefore, the aim of this work was to evaluate the role of Trx after an acute exposure to ROFA and clarify the mechanisms by which PM inhalation triggers cardiovascular adverse health effects. Wild type mice (WT) and transgenic mice overexpressing Trx-1 in heart (TRX) were intranasally instilled with a ROFA suspension (1 mg/kg) or saline solution (control group), and hearts were removed after 3 hours. Heart O_2 consumption was significantly increased by 39% in TRX_{ROFA} mice compared to WT_{ROFA} ($WT_{ROFA:}$ 1040 ± 90 ng-at O/min.g tissue, $p<0.05$). No differences were found in GSH levels, but an increase was observed in GSSG levels in TRX_{ROFA} group ($WT_{CONTROL}$: 0.87 ± 0.10 μg GSSG/mg tissue, $p<0.05$), which lead to a decrease in the GSH/GSSG ratio in TRX_{ROFA}. Moreover, SOD activity was decreased in TRX_{ROFA} mice compared to WT_{ROFA} and $TRX_{control}$ animals (WT_{ROFA}: 3.4 ± 0.6 USOD/mg protein, $p<0.05$). Oxidative damage to lipids was evaluated by the TBARS assay. A decrease was observed in TRX_{ROFA} mice when compared with WT_{ROFA} mice (460 ± 10 pmol/mg protein, $p<0.01$). The present findings indicate that overexpression of Trx-1 confers cardioprotection after an acute exposure to environmental PM, and therefore contribute to the understanding of the adverse health effects triggered by PM inhalation.

Keywords: air pollution, thioredoxin, heart, oxidative stress, antioxidant

(145) RADIOPROTECTIVE EFFECT OF SODIUM ALIZARINSULFONATE IN AN EXPERIMENTAL MODEL OF ACUTE RADIATION SYNDROME

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Increased intracellular calcium is a factor known to be involved in the process leading to cell death. Changes in calcium homeostasis are relevant to the late stages of cell injury. Sodium alizarinsulfonate (ASR) has a potent action as calcium chelator, thus interfering with the entrance and action of this element in the cell. It is our interest to develop less toxic radioprotectors, either by themselves or as adjuvants to drugs approved for their use in humans. In this work, using an experimental model of Sprague-Dawley rats (both sexes) exposed to X radiation (2 Gy, whole body), the radioprotective effect of ASR was evaluated. Groups of 8 rats were exposed at a dose of 2 Gy. At 48 hours post exposure blood samples were obtained by tail puncturing, followed by sampling at 7, 14, 21, 28 and 60 days. The erythrocyte, leukocyte and leukocyte formula were counted. Survival curves up to 60 days were also performed. Genotoxic effects in leukocytes were assessed by the Comet assay (one hour post irradiation). The effects of ASR were tested following its administration as a single dose of 100 mg/kg (i.p.), one hour before irradiation. In the irradiated animals erythrocytes were depleted (females, $p<0.01$), and the white blood cell count was drastically reduced with respect to the control (both sexes, $p<0.01$), also presenting an altered formula. Genetic damage revealed by the Comet assay was significantly reduced by treatment with ASR ($p<0.01$). The effect of ASR on blood parameters tested was protective in the recovery of erythrocytes in females ($p<0.01$). No statistically significant protection was observed in the recovery of the leukocyte level or the leukocyte formula (both sexes). However, a significant protective effect was observed for survival ($p<0.05$). These findings suggest a potential radioprotective effect for ASR, either by its known action as calcium chelator or by scavenging free radicals sparked by ionizing radiation.

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Keywords: ionizing radiation; radioprotection; acute radiation syndrome; oxidative stress; calcium homeostasis

CELL SIGNALING 5

(1503) A NOVEL LINK BETWEEN CELLULAR STRESS

AND NEURODEGENERATION

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Cellular stress is a common feature in diverse pathologies, including cancer and neurodegeneration. Several stress factors, including oxidants and unfolded proteins trigger a global translational shut down, while the translation of protective proteins is facilitated. Quite recently, independent work from several labs including ours indicates that mRNAs that aren't translated during the stress response form large cytoplasmic aggregates termed Stress Granules (SGs). SG formation is conserved in mammalian, insects, yeasts, trypanosomes and plants. SGs are transient and highly dynamic and their contribution to the stress-induced translational reprogramming or to cell survival remains unknown. Understanding SG composition and dynamics will help to unveil their regulation and relevance.

To identify the signaling pathways that regulate SG formation and dissolution we performed a loss-of-function screen in Drosophila cells. We developed BUHO, a MATLAB script for image analysis and we identified 21 positive and 16 negative modulators of SG dynamics (Z-Score > 2). We decided to focus on Leucine Rich Repeat Kinase (dLRRK), given that mutations in the human homolog hLRRK2 are causative of Parkinson disease, which has not been linked to SG formation previously. We found that the knock down of dLRRK or hLRRK2 enhances SG formation. Experiments in human cell lines and fly brains indicate that LRRK dysfunction leads to the accumulation of ubiquitinated protein and provokes proteotoxic stress ($p<0.01$, two-way ANOVA). This work provides a novel link between the stress response regulation and Parkinson disease.

Keywords: stress granules, proteasome, proteotoxic stress, neurodegeneration, high-throughput image analysis

(605) **PHOSPHOLIPASE D SIGNALING MODULATES THE INFLAMMATORY RESPONSE DURING 6-OH DOPAMINE (6-OHDA)-INDUCED NEUROTOXICITY**

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Parkinson's disease (PD) is the second most common neurodegenerative disorder related with aging. The death of dopaminergic neurons triggered by oxidative stress and mitochondrial dysfunction is one pathognomonic characteristic of PD patients.

Neuronal exposure to 6-OH dopamine (6-OHDA), a hydroxylated analogue of dopamine, constitutes a very useful strategy for studying the molecular events associated with neuronal death in PD. Our aim in this study was to characterize the role of phospholipase D (PLD) signaling during 6-OHDA-induced neurotoxicity. The exposure to 6-OHDA (50 and 75 μM) induced an increase in reactive oxygen species ($p<0.05$) and lactate dehydrogenase release ($p<0.05$) along with a decrease in mitochondrial viability ($p< 0.001$) in human neuroblastoma IMR-32 cells. We also observed by immunocytochemistry ($p<0.05$) and subcellular fractionation followed by Western blot ($p<0.01$) the nuclear translocation of NF-κB p65 subunit ($p<0.01$) in the presence of 6-OHDA (50 μM). NF-κB nuclear localization was also accompanied by an increase of IkB phosphorylation ($p<0.05$) as well as COX-2 mRNA levels (determined by RT-qPCR, $p<0.001$). On the other hand, pharmacological inhibition of PLD1 (EVJ, 5μM) and PLD2 (APV, 5 μM) was able to prevent the decrease in cell viability triggered by 6-OHDA ($p < 0.01$; $p<0.001$). In line with this, pharmacological inhibition of PLD1 with EVJ inhibited NF-κB p65 subunit nuclear translocation ($p < 0.001$).

Our results indicate that oxidative stress induced by 6-OHDA triggers an inflammatory response that involves NF-κB activation and COX-2 upregulation. Moreover, we also demonstrate here that phosphatidic acid signaling elicited by PLD modulates the inflammatory response associated with neuronal injury.

Keywords: neurotoxicity, phosphatidic acid, oxidative stress, inflammation, 6-OHDA

(1337) **MAPK/JNK PATHWAYS AND THE MOLECULAR CHAPERONE HSP90 ARE INVOLVED IN IMMUNOPHILIN-DEPENDENT NEURODIFFERENTIATION**

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In nervous cells, cellular differentiation occurs through various eliciting agents such as retinoic acid. Our laboratory has previously demonstrated that the immunophilin-ligand FK506 is a good neurotrophic factor. In this study, it was evaluated the possible signaling cascade involved in such process. N2a mouse neuroblastoma cells were stimulated with 1 μM FK506 or 1 μM retinoic acid (for comparative purposes). Using neurite length as a differentiation parameter, FK506 showed as a stronger neurodifferentiation inducer. Then, cells were treated with different inhibitors. It was found that after inhibiting ERK1/2 activation (MAPK pathway) with the MEK inhibitor PD98059, cells showed shorter neurites, suggesting that this pathway is involved in FK506 action. JNK pathway was also involved since the inhibitor SP600125 exhibited similar inhibitory action as the MEK1 inhibitor. Nonetheless, SP600125 also showed an increasing number of neurites per cell. In previous works of our laboratory, where the role of molecular chaperones was analyzed, it was suggested the involvement of Hsp90 ATPase activity in neuronal differentiation, whereas other work in the literature reported inhibitory action. Due to this discrepancy, the requirement of Hsp90 biological activity for the neurodifferentiation process was tested using radicicol, a known Hsp90 ATPase activity inhibitor. Cells showed even longer neurites than those treated with FK506, and also showed more varicosities (usually seen in mature neurons). Taking together, these observations suggest that the MAPK and JNK pathways mediate FK506-dependent differentiation, and that the inhibition of Hsp90 activity is also a positive regulatory mechanism.

Keywords: pathway signaling; neuronal differentiation; FK506; hsp90

(394) **EXPLORING CRHR2α SIGNALING AND TRAFFICKING IN A HIPPOCAMPAL CELLULAR CONTEXT**

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The corticotropin-releasing hormone (CRH) system orchestrates the response and adaptation to stress, acting on the hypothalamic-pituitary-adrenal axis and in different brain regions. A large body of evidence points to dysregulation of CRH system signaling as causally linked to anxiety and depressive disorders. There are two different G-protein-coupled receptors (GPCRs), CRHR1 and CRHR2, encoded by different genes which display different localization and ligand preferences. We are particularly interested in the alpha splicing isoform (CRHR2α) given that it is the most important in the brain. CRHR2α has a pseudo signal peptide which gives this receptor specific trafficking and signaling characteristics. We are exploring the signaling pathways activated by CRHRs in order to identify mechanisms involved in CRH and its related peptides action in the brain. Given that previous results showed specific CRH actions *in vivo* in particular limbic structures such as hippocampus, we perform our studies in HT22 cells, a mouse hippocampal neuronal cell line widely used as a neuronal model. We generated stable clones expressing CRHR1 and CRHR2α in HT22 cells to explore the mechanisms involved in the signaling cascade and trafficking of each re-

ceptor. We used CRH and UCNs as ligands for each receptor to assess whether different signaling pathways are activated depending on the ligand. The stimulation of both CRHR1 and CRHR2α led to an increase of intracellular cAMP measured with FRET biosensors. We compared similarities and differences of the activation of CREB, ERK1/2 and AKT by western blot. We have previously reported that CRH-dependent ERK1/2 activation downstream of CRHR1 is biphasic, being dependent on G protein and receptor endocytosis mechanisms. Remarkably, the same pattern was observed when UCN1 was used as a CRHR1 ligand. However, the kinetics of ERK1/2 activation downstream of CRHR2α were different, either when CRH or UCNs were used for stimulation.

Keywords: CRHRs, UCNs, signal transduction, cAMP.

(579) **CHRONIC ADMINISTRATION OF AN AT2R SYNTHETIC AGONIST INCREASES INSULIN SENSITIVITY IN MICE**

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Abstract:The renin-angiotensin system modulates insulin action. Angiotensin type 1 receptor exerts a deleterious effect, while the angiotensin type 2 receptor (AT2R) appears to have beneficial effects providing protection against insulin resistance and type 2 diabetes. In this study, we explored the role of AT2R in the control of insulin action. To that end, C57Bl/6 mice were administered the non-peptidic AT2R agonist C21 for 12 weeks (1 mg/kg per day; i.p. injection; n=12); vehicle treated animals were used as control (n=12). One week before to the sacrifice, glucose and insulin tolerance tests were performed, which showed increased glucose tolerance and insulin sensitivity in C21 mice (n=8; p <0.05). At the end of the treatment, a supraphysiological dose of insulin via cava vein (10 IU/kg) was administered, to investigate the *in vivo* status of the insulin signaling pathway. Baseline levels were studied in animals that were injected saline solution.The effect of the C21 on visceral adipose tissue was characterized by analysis of adipocyte size on hematoxylin-eosin-stained sections. A statistically significant decrease in adipocyte area was detected in C21-treated mice (n=4; p <0,05). Surprisingly, liver from animals treated with C21 showed increased basal Akt phosphorylation in Threonine 308 and Serine 473 residues and only a slight increase in insulin-induced Akt phosphorylation was observed in C21-treated animals when compared with control mice (n=4; p <0,05). In conclusion, chronic administration of an AT2R agonist to C57BL/6 mice, increased insulin sensitivity and reduce basal glycemia. Such change was associated with decreased adipocyte size in visceral adipose tissue and increased basal activation of hepatic Akt. To understand the effects at the metabolic level of AT2 agonist treatment would be necessary to analyze the status of the insulin pathway in muscle and adipose tissue and the inflammatory state of adipose tissue.

Keywords: Insulin receptor, angiotensin type 2 receptor, type 2 diabetes.

(98) **COX-2 REGULATION BY 1α,25(OH)$_2$D$_3$-VDR LIGAND IN ENDOTHELIAL CELLS EXPRESSING vGPCR**

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The Kaposi's Sarcoma-associated Herpes virus G Protein-Coupled Receptor (vGPCR) is a key molecule in the pathogenesis of Kaposi Sarcoma. 1α,25(OH)$_2$D$_3$ anti-proliferative impact in vGPCR cells occur in part by NF-κB pathway negative modulation, family of conserved transcription factors critical during the inflammatory response. In this work, we studied if COX-2 modulation by 1α,25(OH)$_2$D$_3$ in vGPCR cells contributes to the anti-inflammatory action. As we have previously reported vGPCR cell proliferation is inhibited by 1α,25(OH)$_2$D$_3$ (10 nM, 48 h) due to a reduction on cell number. The blockage of arachidonic acid release by ATK (cPLA2 inhibitor) or the COX-2 inhibition (Celecoxib) decreased vGPCR cell number in a dose dependent manner (10-20 μM), similarly to 1α,25(OH)$_2$D$_3$. These changes were accompanied by morphological modifications observed at the microscope. qRT-PCR analysis of COX-2 gene expression revealed a mRNA increase within 20 min of 1α,25(OH)$_2$D$_3$ treatment and remains increased at least for 72 h. Moreover, ATK (20 μM) could not counteract COX-2 mRNA induced by 1α,25(OH)$_2$D$_3$ (10 nM, 24 h). Finally, COX-2 VDR dependence was evaluated using the stable VDR knock-down cell line vGPCR-shVDR where COX-2 mRNA rise by 1**α**,25(OH)$_2$D$_3$ was found impaired. Significant differences of the data between control (vehicle) and treated conditions were analyzed by one way-ANOVA followed by Bonferroni test (p< 0.05) or t-test (*p< 0.05, **p<0.01). All together, these results suggest that 1α,25(OH)$_2$D$_3$ enhances endothelial inflammation initiation through COX-2 upregulation and contributes to its timely resolution through the antineoplastic action. This shows a possible dual effect as both a promoter and attenuator of inflammation.

Keywords: COX-2, vGPCR cells, Vitamin D, antineoplastic effects

(450) **H1 AND H2 HISTAMINE RECEPTORS CROSS-DESENSITIZATION AFFECTS ANTIHISTAMINE RESPONSE**

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Histamine exerts its effects by binding to four G protein-coupled receptors (H1R-H4R) and modulates numerous physiopathological processes through H1R and H2R, including allergy and inflammation. These receptors are effectively targeted by drugs belonging to the top twenty-used-drug-classes. We have previously described the cross-desensitization (CDS) between both receptors induced by Cetirizine, an H1R antihistamine (AH), on H2R agonist response. The aim of this work is to study the CDS between H1R and H2R caused by others AH and their influence on the regulation of the inflammatory process. In promonocytic U937 cells (endogenously expressing H1R and H2R) and HEK293 (HEK) cells transfected with H1R and H2R, pretreatment with the AHs mepyramine, trans-triprolidine, chlorpheniramine and diphenhydramine significantly decreased the cAMP production following amthamine (AM, an H2R agonist) stimulation, whereas the cAMP response through another Gs-coupled receptor (PGE2) was unaffected. Likewise, the pretreatment with the same AHs in HEK cells transfected only with H2R, did not alter AM response, showing specificity of CDS. On the other hand, we evaluated the AM effect on the inflammatory response of AH. The regulation of COX-2 and IL-8 expression was evaluated by qPCR in *U937 cells treated with PMA*. The pretreatment with AM reverted the inhibitory effects of the AH on the expression of both genes. The same effect was observed for the IL6 promoter activity in HEK cells transfected with both receptors in a reporter gene assay. As expected, the pretreatment with AM did not modify the AH anti-inflammatory response in HEK cells transfected only with H1R. These results show that there is a specific cross-desensitization between H1R and H2R induced by different AHs used in the clinic. Given the wide use of these drugs and the interest in drug repositioning, it is crucial to understand the regulation between the intracellular signaling cascades triggered by them.

Keywords: Histamine Receptors; Antihistamine; Cross-Desensitization

(1800) **MODULATION OF THE SENESCENCE ASSOCIATED SECRETORY PHENOTYPE IN RETINAL PIGMENT EPITHELIUM CELLS**

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Cellular senescence triggers the expression of a wide variety of inflammatory factors named the senescence associated secretory phenotype (SASP). The SASP may contribute to diseases of aging by disrupting tissue structure and function. Our previous findings support the hypothesis that cell senescence of the retinal pigment epithelium (RPE) plays a role in the pathogenesis of Age-related macular degeneration (AMD). Thus, we have reported that oxidative-stress induced senescence in RPE cells dysregulates the expression of factors linked to AMD progression. We now hypothesized that polyphenols can block the senescent secretome by modulating inflammatory signaling pathways. Aims: To evaluate the effect of Caffeic acid (CAF) and Chlorogenic acid (CL) on SASP expression and its paracrine effects. Methods: Human RPE cells (ARPE-19 line) were incubated with 150 **µ**M H_2O_2 for 90 minutes during 3 days (d), and then maintained for 9 d to establish senescent cultures (SEN). These cultures were exposed to 6 **µ**g/ml of CAF or 50 uM CL for the last 6 d. mRNA and protein levels for IL1b, IL8, IL6 were analyzed by qPCR or western blot. Senescence was determined by positive staining for Senescence Associated **β**gal activity and increased expression of p21 and p16. gH2AX was tested by IFI. Results: SEN cultures expressed high levels of IL1b and IL8, which were reduced by CAF and CL treatment ($p \leq 0,01$). SEN cells showed activated phospho - p38 (Tyr182) and phospho-NF-κB p65 (Ser536). Polyphenols inactivated these pathways. CAF and CL diminished p16 expression ($p \leq 0,05$). In contrast, no changes on p21, gH2AX or SA-**β**gal+ staining were observed. Conditioned medium (CM) from SEN cultures promoted increased gH2AX and SA-**β**gal+ staining in control cells. The paracrine effects were suppressed in the CM from CAF and CL- treated cells ($p \leq 0,05$). Conclusions: CAF and CL repressed p38 and NFKB inflammatory pathways, suppressing the SASP and consequently its bystander effects.

Cellular senescence, SASP, aging.

(404) **RGS DOMAIN OF GRK2 MEDIATES BETA3-ADRENERGIC RECEPTOR DESENSITIZATION**

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Beta3-adrenergic receptor (B3AR) mediates some of the effects of adrenaline and noradrenaline on several tissues, mainly the cardiovascular system. B3AR belongs to the superfamily of G protein-coupled receptors (GPCRs) increasing cAMP intracellular levels upon activation. While previous works showed that pre-treatment with isoproterenol leads to receptor desensitization, the mechanism involved remains unidentified since B3AR lacks the PKA- or GRKs-mediated phosphorylation consensus sites known to participate in the desensitization of other GPCRs.

Our aim was to evaluate the mechanism of B3AR desensitization using HEK293T transfected cells. In these cells, the B3 specific ligand BRL37344 significantly increased cAMP levels, and after a 1h pre-treatment, the response was significantly reduced by 40%. When different GRKs were co-expressed, GRK2 and 3, but not GRK5 or 6 potentiated receptor desensitization. Structurally, GRKs possess an N-terminal RGS-homology domain responsible for G-protein activity regulation, a kinase central domain engaged in receptor phosphorylation, and a less conserved C-terminal region responsible for membrane localization. We co-transfected the cells with different dominant negative mutants of kinase and RGS domains. While the expression of kinase inactive mutant K220R, had no effect, receptor desensitization was impeded in the presence of the mutant of the RGS domain, D110A. Since overexpression systems may not be representative for native tissues, we studied B3AR response in cultured rat cardiomyocytes, endogenously expressing B3AR. In this system, transfection with GRK2 dominant negative mutant D110A increased in almost twice the cAMP response to BRL37344 (483 ± 87 versus 879 ± 156; $p<0.05$).

Our results indicate that B3AR desensitization can be mediated by GRK2, in a phosphorylation-independent manner, where the RGS domain plays a crucial role.

Keywords**:** Receptor desensitization; GRK2; Adrenergic receptor.

(364) **ROLE OF THE Gβγ COMPLEX IN CRHR1 SIGNALING MECHANISMS**

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Corticotropin releasing hormone (CRH) plays a key role in the regulation of neuroendocrine, autonomic, and behavioral response to stress. The CRH/CRHR1 system dysregulation is linked to the onset and development of affective disorders, such as anxiety and depression. The aim of our work is to characterize the signaling pathways activated by CRH receptor type 1 (CRHR1). Being a GPCR, the canonical signaling pathway of CRHR1 is linked to the activation of heterotrimeric G proteins, which are composed of α, β and γ subunits. The most studied signal transduction cascade for GPCRs is the one downstream of Gα subunits.

Using as a model the hippocampal neuronal cell line HT22 stably expressing CRHR1 (HT22-CRHR1), we analyzed whether the Gβγ complex was involved in signaling pathways downstream of CRH-activated CRHR1. Cells transfected with a Gβγ scavenger (Gα-transducin) or pretreated with a pharmacological inhibitor (gallein) showed reduced ERK1/2, Akt and CREB activation levels in response to CRH. In HT22-CRHR1 both ERK1/2 and CREB are dependent on cAMP increase. Using FRET-based biosensors we determined that the genetic and pharmacological blockage of the Gβγ complex diminished the CRH-mediated cAMP response. Having established a role for Gβγ, we performed a screening of transmembrane adenylyl cyclases (tmACs) in HT22-CRHR1 cells by RT-PCR finding that tmACs positively and negatively regulated by Gβγ are expressed in these cells. CRHR1 activates G protein–dependent and internalization-dependent signaling mechanisms, being able to signal from endosomal compartments. Our preliminary results suggest that the Gβγ dimer may also play a role in CRHR1 endocytosis, as the blockage of the complex led to a decrease in CRH-induced receptor internalization.

These results indicate that Gβγ participates in CRH/CRHR1 signaling in a hippocampal neuronal context. The specific role of this pathway and the crosstalk between Gα- and Gβγ-activated cascades are currently being explored.

Keywords: CRH, cAMP, Gβγ, GPCR, signal transduction

BIOPHYSICS 5

(1659) **INTERACTION OF ANTIFUNGAL CYCLIC LIPOPEPTIDES WITH FUNGI AND MAMMALIAN MEMBRANE MODEL SYSTEMS**

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Bacillus subtilis is a potent biocontrol agent producing a wide array of lipopeptides for the inhibition of fungal growth. Among them, Bacillomycin D homologues are amphiphilic cyclic peptides composed of seven α-amino acids linked to a β-amino fatty acid that have antifungal activity against human and plant pathogens and also strong hemolytic activity. In order to better understand these phenomena, we explored the interactions of Bacillomycin D analogs having C14 (BC14) and C16 (BC16) β-amino fatty acids with membrane mod-

el systems. The surface activity of BC14 and BC16 was studied in lipid monolayers composed of (phosphatidylcholine (PC)/ergosterol (Erg) *vs* PC/cholesterol (Chol)) and ternary lipid mixtures as simplified models of fungi and mammalian cell membranes (PC/phosphatidylethanolamine (PE)/Erg *vs* PC/sphingomyelin (SM)/Chol). Surface pressure (π) measurements showed similar results for the interaction of BC14 with the different lipid mixtures assayed, with critical surface pressures (πc) of ~30 mN/m. BC16 produced higher π increments than BC14 after its interaction with all the monolayer compositions tested at different initial π, being this feature more notorious in PC/Erg, PC/PE/Erg and also in PC/SM/Chol monolayers for which the calculated πc values were of 44, 38 and 40 mN/m, respectively. In calcein release assays, BC16 showed higher disruptive effects than BC14, being both compounds more active on PC/Erg than PC/Chol vesicles. In summary, results showed that BC14 and BC16 could interact with both fungi and mammalian-like model membranes. In all the systems studied, BC16 produced higher effects than BC14 indicating that the longer hydrocarbon chain in the β-amino fatty acid of BC16 favors the interaction of the lipopeptide with the membrane. This correlates with the higher biological activity of this compound in bioassays.

Keywords: antifungal lipopeptides, model membranes, peptide-lipid interactions.

(1164) INTERACTION OF MELITTIN WITH DIMYRISTOYLPHOSPHATIDYLCHOLINE MEMBRANES

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Melittin (ML) is rich in arginine (Arg) and lysine (Lys). Several studies have been done to understand the mechanism of interaction with neutral and negatively charged lipid membranes. However, it is not known how this interaction depends on electrostatic or hydrophobic forces according to membrane composition. Comparative studies show that ML produces a differential effect on the zeta potential () of DMPC and DMPG liposomes in comparison with Arg-7 and Lys-5.ML and Arg-7 caused a shift of zeta potential () to positive values to reach a final increase of +42.4 mV being this much higher than that observed with Arg-7 (27.63 mV). In contrast, Lys-5 tends to neutralize the surface charges in the lipid membrane, but the final values stabilizes at below zero. These results indicate that not only the electrostatic forces contribute into the interaction of ML to the membrane. This can be explained by a conformational changes produced in the structure of ML during the interaction with the lipid membrane. These changes would expose positive and orient the positive amino acid residues to the interface. The evolution of the effect of ML on the ψ of DMPC membranes during time suggests that ML accommodates in the lipid membrane interphase through different structural conformations as revealed by molecular dynamics. At initial times, ML interacts with the membrane through electrostatic and hydrophobic forces, accompanied by an increase of ψ (=55.73±2.44 mV). Then, ML molecules tend to “cluster”, forming a porous structure, where each molecule of ML penetrates deeper into the membrane with a decrease of to ψ =38.19±2.08 mV. The adsorption isotherms indicate that the process is a Langmuir one i.e. without significant changes in membrane structure. It is concluded that adsorption of ML occurs at different stages rearranging leading to a conformational change that allows to charge liposomes with a net positive ψ.

Keywords: melittin, membrane, zeta potential, molecular dynamics.

(1344) LIPID-PROTEIN INTERACTION OF A NATURAL VARIANT OF APOLIPOPROTEÍN A-I. STUDIES WITH MONOLAYERS AND BILAYERS MODELS

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Human apolipoprotein A-I (ApoA-I) is the major protein component of high density lipoproteins (HDL) serving as transporters for excess of cellular cholesterol (C) through the plasma compartment to the liver. Several single amino acid replacement natural ApoA-I variants have been described in which protein´s function is impaired, resulting in chronic pathologies such as atherosclerosis or amyloidosis. One of this mutant having a deletion of the single residue Lys107 (ΔK107) induces severe atherosclerosis. We have investigated by biophysical approaches the bilayer´s or monolayer´s interaction of this mutant in comparison with the protein with the wild sequence (Wt). In order to compare the ability to interact with both layers, we have measured the leakage induction of POPC and POPC:C (4:1) vesicles loaded with terbium/dipicolinic acid, as well as the interaction with POPC and POPC:C (4:1) monolayers by surface tension measures in a Langmuir balance. Both proteins induce a rapid leakage of the POPC or POPC:C vesicles. Nevertheless, the induced leakage for both proteins is higher with POPC:C liposomes than with POPC vesicles. In monolayers, the differences of surface tension were higher for the Wt (ΔΠ~14,0mN/m) than for the ΔK107 (ΔΠ~8,5mN/m) protein with POPC monolayers at Π_0=10mN/m. However, this effect is not observed in POPC:C monolayers (for both Wt and ΔK107; ΔΠ~9,0mN/m) at Π_0=10mN/m. These results suggest that the interaction of the protein with POPC or POPC:C bilayers are not affected by the deletion. With low Π_0 monolayers, however, Wt protein is most efficient than ΔK107 to increase the surface tension in POPC monolayers.

Keywords: apolipoprotein A-I; deletion mutant; leakage; vesicles; monolayer

(790) MEMBRANE CHOLESTEROL CONTENT IS A MAJOR FACTOR THAT AFFECTS THE ABILITY OF EPIGALLOCATECHIN GALLATE TO INHIBIT ACHE

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Polyphenols are plant secondary metabolites able to interact not only with proteins but also with membrane lipids, what may be related with some of the beneficial properties attributable to these compounds.

We have already demonstrated that epigallocatechin gallate (EGCG) was efficient as acetylcholinesterase (AChE) inhibitor when the enzyme was bound to membranes. Even though EGCG was still able to inhibit AChE in its soluble form, the effect was less pronounced. In fact, the erythrocyte AChE isoform has proved to be a good model for studying the enzymatic activity of membrane-bound AChE isoforms, hence this variant was chosen to carry out the present work.

The activity of AChE, as well as others membrane-bound enzymes, has been shown to depend on membrane lipid composition and order. That is to say, membrane lipid alterations may induce changes in the activities of these enzymes. Therefore, we studied cholesterol-levels influence in erythrocyte membranes on the AChE inhibition by EGCG. In other words, we aimed to know if the interaction of EGCG with membrane varies and if it influences on AChE inhibition degree.

EGCG-mediated inhibition of enzymatic activity was enhanced when AChE was bound to membranes with low cholesterol level. The EGCG localization was studied by biophysical techniques. On one hand, an enhanced quenching of rhodamine R-18 fluorescence by EGCG, was observed when cholesterol content of membranes was reduced, leading us to hypothesize that EGCG might interact with interfacial portion of membranes. On the other, possible deeper localization of EGCG in membrane was assessed by IR spectros-

copy.

It can be concluded that interactions among EGCG and lipid components of membranes are noteworthy since they can determine the inhibitory effect of this polyphenol on membrane bound enzymes.

Keywords: acetylcholinesterase, cholesterol, polyphenols.

(1741) ROLE OF CHARGED RESIDUES IN THE PROTEIN-MEMBRANE AND PROTEIN-PROTEIN INTERACTION DURING ACTIVATION OF THE MITOCHONDRIAL PATHWAY OF APOPTOSIS.

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The intrinsic pathway of apoptosis is activated by signals of cellular stress and regulated at the mitochondrial level. Such regulation is carried out by members of the Bcl-2 protein family (B leukemia cell lymphoma 2). Protein-protein and membrane-protein interactions allow the exposure of hidden domains in the initial conformation of pro-apoptotic members. Bax and Bid are members of the Bcl-2 family of proteins that promote apoptosis by an external mitochondrial membrane permeabilization mechanism (MOMP). The interaction between Bax, Bid and membrane, triggers a series of events that include; opening of a pore in the external mitochondrial membrane, release of apoptogenic factors from the intermembrane space and activation of caspases, to finally culminate in a process of cell death program by apoptosis. Although there is a broad knowledge at the structural level of proteins belonging to the Bcl-2 family, the mechanisms involved in MOMP require a better understanding of the conformational changes and specific contacts required to trigger apoptosis.

In a previous work we demonstrated the influence of non-specific electrostatic interactions in the first approach between Bax-membrane and between Bax-Bid. In the present work, we computationally model the interaction between Bid and membrane, determine the amino acids important for the interaction between Bax and membrane by in silico mutagenesis, and try to elucidate the mechanism involved in the formation of the apoptogenic pore in the external mitochondrial membrane. Free Electrostatic Energy of Binding, was computed using Finite Difference Poisson Boltzmann Equation (FDPB) method as implemented in software APBS (Adaptive Poisson Boltzmann Solver). This type of calculations provided a starting point for further computational analysis through molecular dynamics simulations (MD). To this end, we used GROMACS simulation package.

Keywords: apoptosis, Bcl-2, molecular dynamics.

(1057) STUDIES ON THE STRUCTURE OF FQS SPECIES PRESENT AT DIFFERENT pH´s AND THEIR INTERACTIONS WITH LYSOZYME PROTEIN

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Fluoroquinolones (FQs) are synthetic antimicrobial agents with a broad spectrum of antibiotic activity against Gram-positive and Gram-negative bacteria. They play an important role in the treatment and prevention of disease in both humans and animals. On the other hand, patients often take antibiotics with foods or dairy products such as milk to help swallow easier and to decrease their gastrointestinal side effects. Therefore FQs may be in contact with proteins present in milk as lysozyme (Lyz). It is known, that food–drug interactions may occur by many mechanisms, and they can result in changes both in the rate and the extent of absorption. The pH of the gastric milieu may also be an important determinant of the magnitude of the interaction.

In this context the purpose of this work is to study the structure of Ciprofloxacin (Cpx) and Levofloxacin (Lev) species present at basic, acid and neutral pH´s and their interactions with Lyz structure by Fourier transform infrared spectroscopy (FTIR) and Theoretical Calculations.

Theoretical calculations using DFT/B3LYP/Lanld2z methodology with gaussian09 program calculate that the more stable structure in solution is the neutral ones for both FQs. Docking Molecular analysis predict that the main forces involved in the interaction between FQs species and Lyz protein are Van der Waals forces and hydrogen bond. These results are in agreement with FTIR in solid phase and buffered solution. The spectra show significant changes in the quinolone carboxylic group and the piperazine amine group. When Lyz is present, it is observed that the FQs modify the secondary structure of Lyz. These theoretical and spectroscopic studies give a deeper understanding of the structural changes occurring between Cpx and Lev molecules with Lyz protein.

Keywords: Fluoroquinolones, Lysozyme, DFT calculation, FTIR, Molecular Docking.

(882) FIBRIL LIKE STRUCTURES CAN BE INDUCED IN LIPID/PEPTIDE (BETA-AMYLOID AND MELITTIN) LANGMUIR MONOLAYERS

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Langmuir monolayers at air-water interface is a proper technique to study the interfacial properties of film forming peptides mixed with lipids. It allows manipulating the lipid/peptide mole ratio, the amount the **P**eptide **C**overed **A**rea (**PCA)**, the physical state of the lipid and the degree of lateral compactness in a confined environment, mimicking a biological membrane interface. Using lipid/peptide monolayers we studied the surface properties of AB1-40 **A**myloid **P**eptide (**AP**) mixed with different proportions of lipid that differs in their physical state. As AP form beta-sheet conformation, we also include the amphiphilic alfa-helix Melittin peptide (**Mel**) for comparison. Both AP and Mel form homogeneous monolayers with reproducible Pi-Area isotherms and maximal stability in between 15-25 mN/m. Their rheological properties were related to their secondary structures.

In a liquid-condensed environment (DSPC/peptide mixed systems), we observed immiscible behavior at all proportions with a first collapsing point close to that detected for pure peptide. In a liquid expanded lipid environment (POPC/peptide systems) both miscibility and stability of the film depend on the peptide used. For POPC/Mel at low PCA a mixed film exhibit composition-dependent stability, whereas at high PCA the lateral stability corresponds to that of pure peptide, which together with BAM images indicates lateral segregation induced by compression of the film. For AP/POPC, lateral segregation was observed (BAM images) at all proportions. However we found an unexpectedly complex stability behavior, corresponding to pure peptide at high PCA but composition-dependent at low PCA; interestingly at PCA 5-10% fibrils like structures are clearly observed and the film exhibit a pronounced compression hysteresis. Surprisingly, fibrils could also be observed for DSPC/Mel at certain proportions. We discuss on the conditions and kinetic aspects affecting fibrils formation.

Keywords: beta amyloid, melittin, lipid-peptide interaction, Langmuir films, peptide monolayers.

(65) EFFECT OF AMYLOID OLIGOMERIZATION ON ALPHA-SYNUCLEIN CURVATURE-MEMBRANE SENSITIVITY

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Abstract: The protein α-synuclein (AS) is as a critical regulator of synaptic vesicle dynamics in dopaminergic neurons. The amy-

loid aggregation of AS is pathognomonic of Parkinson's disease, a movement disorder associated with axon degeneration of dopaminergic nigral neurons. In this context, prefibrillar oligomeric species are pointed as highly neurotoxic. AS has a greater affinity for highly curved vesicles, such as that of synaptic vesicles. Here, we investigated the loss-of-function that might be associated to the conversion of AS from its monomeric functional state to its pathological oligomeric form by evaluating the impact of AS oligomerization on its membrane-curvature sensitivity. We used Fluorescence Correlation Spectroscopy to determine AS binding affinity to membranes varying in sizes and compositions. We found that oligomerization abolishes AS binding to physiological-like membranes and changes curvature-sensitivity towards neutral membranes. Our findings provide insight into how amyloid oligomerization can modulate AS physiopathology.

Keyword: synuclein, protein-lipid interaction, Parkinson, amyloid

(163) **STRUCTURAL ORGANIZATON OF AMYLOID OLIGOMERS OF THE PARKINSON'S DISEASE RELATED PROTEIN α-SYNUCLEIN**

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Abstract: The accumulation of intraneuronal amyloid fibrils mainly formed by the 140-aa presynaptic protein α-synuclein (AS) is a hallmark of Parkinson's disease. Neurotoxicity is largely attributed to prefibrillar oligomeric species rather than to the amyloid fibrils. Structural details of the supramolecular organization of AS oligomers (oAS) are needed for understanding the structure-toxicity relationship underlying their pathogenicity. We previously showed that oligomers formed by the wild-type protein (oWT) adopt a distinct antiparallel β-sheet structure and identified a number of intermolecular contacts, many of those involving the N-terminal region. Bioinformatic analysis led us to hypothesize that the N-terminal region adopts a coiled-coil conformation in the oligomer. Therefore, we designed AS mutants that would increase (X3) or decrease (X2) the coiled-coil propensity. To get structural information at the atomic level, all-atom molecular dynamic simulations using the implicit solvent model were carried out for monomeric WT, X2 and X3 variants. Our results indicate that the first 25 residues acquire α-helix structure, in agreement with the helical propensity of this region. In line with our hypothesis, additional simulations of the N-terminal 25-mer peptides of WT and X3 variants demonstrate the tendency of coiled-coil interactions. Infrared experiments indicate that oWT and oX3 have similar secondary structural features whereas oX2 have a reduced content of β-sheet structure demonstrating the importance of N-terminal interactions in oligomerization. We are carrying out complementary fluorescence experiments using pyrene-labeled variants to sense changes in intermolecular proximities within the oligomers in response to the introduced mutations. Our results will provide valuable information on the role of helix-helix interactions on amyloid intermediates formation as well as for the development of molecular models of oAS.

Keyword: synuclein, Parkinson, amyloid, molecular simulation, protein structure

(1883) **CHEMICALLY MODIFIED TETRACYCLINE INHIBITS α-SYNUCLEIN AMYLOID FIBERS FORMATION AND NEUROINFLAMMATION**

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Parkinson's disease related death of dopaminergic neurons has been linked to pathological aggregation of α-synuclein (AS) protein and its transcellular traffic through the dopaminergic system. Tetracyclines, such as minocycline and doxycycline have been shown to be neuroprotective in Parkinson's disease animal models. Neuroprotective activities of minocycline have been attributed to its inhibitory effects on microglia activation and doxycycline has been shown to protect cells by inhibiting the formation of toxic alpha synuclein species. Chemically modified tetracycline (CMT) has been proven to protect neurons through residual anti-inflammatory activity but their effects on AS are unknown. In the present work, we explore the ability of an specific CMT to inhibit the formation of amyloid aggregates of AS using different biophysical techniques such as fluorescence techniques, SAXS and advanced microscopies. In addition, we evaluate the ability of CMT to modulate the inflammatory response of microglial cultures mediated by different inflammogenic compounds. This study represents a milestone in the assessment of CMT as a therapeutic agent for the treatment of neurodegenerative disease.

Keywords: α-synuclein, amyloid fibers, CMT, tetracyclines and microglial cells.

STRUCTURAL AND FUNCTIONAL BIOCHEMISTRY 5

(535) **EFFECT OF ALUMINIUM ON PLASMA MEMBRANE CALCIUM PUMP AT CELLULAR LEVEL**

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Aluminium (Al^{3+}) is a metal widely distributed in the environment. It is involved with the pathophysiology of neurodegenerative disorders, such as Parkinsonism dementia and Alzheimer's disease. The mechanisms that have been proposed to explain the toxicity of Al^{3+} are linked to changes in the cellular calcium homeostasis. PMCA is a P-ATPase involved in the regulation of the calcium homeostasis. Its function is to transport Ca^{2+} from cytoplasm towards the extracellular medium against the electrochemical gradient modulating the cytoplasmic Ca^{2+} concentration. In previous works, we already showed that Al^{3+} irreversibly inhibits Ca^{2+}-ATPase activity of PMCA by preventing the dephosphorylation of the pump.

The aim of this work is study the effect of Al^{3+} on PMCA at cellular level. To characterize this effect, we measured the changes on intracellular calcium in HEK293T cells using a fluorescent probe (Fluo4-AM) in the presence of different concentrations of $AlCl_3$. Subsequently, we used lumogallion as a direct fluorescence molecular probe to follow the presence of aluminium. With this probe we measured by fluorometry the uptake of aluminium and lumogallion to HEK293T cells over time. Further, we studied the location of aluminium in the cells as a function of time using confocal fluorescence microscopy.

Our results show that $AlCl_3$ inhibits calcium efflux mediated by PMCA in HEK293T cells ($p \leq 0.05$). The aluminium entrance to the cell as a function of both incubation time and concentration present a saturable behavior. The lumogallion probe gets into the cell through diffusion and its entrance also depends on the incubation time and concentration. Confocal fluorescence microscopy shows that Al^{3+} first interacts with the membrane and afterwards it migrates near the nucleus of the cell.

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Keywords: calcium pump, inhibition, lumogallion, calcium homeostasis

(774) **A METABOLIC CONTROL ANALYSIS APPROACH TO STUDY OF SYSTEMS: THE RED BLOOD CELL GLYCOLYTIC FLUX UNDER INHIBITION BY ALKYLATING REAGENTS**

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Metabolic Control Analysis (MCA) is a system-level methodology to the study of metabolism in a quantitative fashion. Red blood cells (RBCs) are interesting systems to develop non-reductionist biological studies of metabolic pathways. Moreover, they are relatively simple in structure and do not contain organelles, thus becoming a simple model without internal compartments. This work provides a simple experimental setup to measure the glycolytic flux and the flux response (sensitivity) to external modulators. Here we assayed two different inhibitors of the enzyme glyceraldehyde-3-phosphate-deshydrogenase (GAPDH) often used as text book example for an irreversible enzyme inhibition: iodoacetic acid (IA) and iodoacetamide (IAA). These compounds are widely used as alkylating reagents to modify thiol groups in proteins by S-carboxymethylation or S-cabamidomethylation. IAA and IA inactivate to GAPDH by blocking an essential Cys residue in the active center of the enzyme and, consequently, glycolysis flux is inhibited.

Glucose was *the source* and lactate was *the sink* of the metabolic pathway. The glycolytic flux was determined by measuring the extracellular lactate concentration through time, using a colorimetric low-cost setup based on two coupled enzymatic reactions and photographic analysis using the ImageJ program and a plugin developed to this end. The system reached steady state and, under these conditions, MCA tools were applied to evaluate the sensitivity of the system's flux to the enzyme inhibitors. The response coefficient was calculated by fitting a derivable empirical equation to the experimental data. A lower IA concentration was required to produce the highest flux inhibition compared with IAA. Moreover, the systems showed to be very robust and of easy implementation opening the possibility of RBCs ageing or viability measurements. Besides, testing response to hormones like insulin, among other modulators is also feasible.

All authors contributed equally

Keywords: Metabolic control analysis, red blood cells, glycolytic flux, system

(835) **EFFECT OF CALCIUM ON THE DISTRIBUTION OF TUBULIN IN HUMAN ERYTHROCYTES**

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In previous work we demonstrated that tubulin is present in human erythrocytes and changes its distribution within the cell during arterial hypertension. In addition, we observe that erythrocyte deformability depends on the tubulin content in the membrane. On the other hand, tubulin regulates pmca activity depending on the lipid composition where the enzyme is immersed. Dag and pa activate pmca (decrease in intracellular calcium), causing a redistribution of tubulin in human erythrocytes, increasing the acetylated isotype content in the membrane. In this work we have proposed to study if variations in calcium concentration affect the distribution of tubulin in human erythrocytes and its relation to pmca activity. For this, the erythrocytes were incubated with different concentrations of calcium, in the presence and absence of a calcium ionophore (a23187) and also in the presence of caloxin different concentrations (specific inhibitor of pmca). Our immunofluorescence and western blot experiments demonstrate that intracellular calcium variation causes redistribution of tubulin in erythrocytes. At high concentrations of calcium the tubulin migrates to the membrane and this causes a decrease in its deformability, reaching values similar to those found during arterial hypertension. Therefore, we conclude that the inhibition of pmca and the consequent increase of calcium concentration during hypertension are implicated in the migration of tubulin to the membrane, causing a decrease in erythrocyte deformability that contributes to the increase of blood pressure.

Keywords: calcium, tubulin, hypertension, deformability, PMCA

(955) **β-XYLOSIDASE ECXyl43: 3D STRUCTURE PREDICTION AND KINETIC PARAMETERS DETERMINATION FOR XOS HYDROLYSIS**

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β-xylosidase enzymes hydrolyze short xylooligosaccharides (XOS) to xylose units and catalyze the last step of hemicellulose degradation during biomass bioconversion into added value products.

In the current work, the 3D structure of the recombinant β-xylosidase EcXyl43 from *Enterobacter* sp. was studied *in silico* and its activity on different XOS was characterized.

Phylogenetic analysis of EcXyl43 suggested a common ancestor with previously crystallized bacterial β-xylosidases from GH family 43. Those known structures were employed to model EcXyl43 with good accuracy. EcXyl43 was predicted to have a five-bladed β-propeller catalytic domain connected by a loop with a β-sandwich auxiliary domain of undefined function. Multiple sequence alignment revealed the presence of a conserved active site and residues D14-E186-D127 were predicted to be the catalytic triad. The involvement of residues D14 and W73 in the enzymatic activity was confirmed by site-directed mutagenesis. The separation of the two modules, which were expressed independently, caused the complete loss of enzymatic activity.

The activity of EcXyl43 on xylotetraose (X4), xylotriose (X3) and xylobiose (X2) as substrates was also evaluated. Kinetic studies revealed that EcXyl43 presented activity on all XOS with higher efficiency on X4 than X3 and than X2. Moreover, no transglycosylation activity was demonstrated by TLC, indicating an inverting mechanism of hydrolysis.

Some divalent cations, like Cu^{+2}, Mg^{+2}, Ni^{+2} and Co^{+2}, as well as ethanol above 5% and monomeric sugars affected negatively the enzyme activity, but the presence of Ca^{+2} improved its thermal stability.EcXyl43 has a 3D structure similar to other inverting GH 43 xylosidases, but its higher catalytic efficiency for longer XOS is a differential characteristic that makes it interesting for combining with xylanases in biomass deconstruction.

Keywords: β-xylosidase, xylooligosaccharides hydrolysis, biomass bioconversion

(1357) **EFFECT OF EPIGALLOCATECHIN-3-GALLATE ON CALCIUM HOMEOSTASIS THROUGH INHIBITION OF THE PLASMA MEMBRANE CA2+-ATPASE**

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Catechins are polyphenols that exhibit beneficial properties for health, such as, antioxidant, anticancer, cardioprotective, neuroprotective and vasorelaxant properties. Epigallocatechin-3-gallate (EGCG), which is the major polyphenol component of green tea, is believed to be the main responsible for many of such properties. Different studies have shown that EGCG produces alterations in intracellular Ca^{2+} homeostasis, which could be linked to alterations in the function of the mitochondria, different types of Ca^{2+} channels and calcium pumps, including the Plasma Membrane Ca^{2+}-ATPase (PMCA). PMCA transports Ca^{2+} actively to the extracellular medium coupled to the ATP hydrolysis maintaining a very low concentration of intracellular Ca^{2+}.

The purpose of this work is to investigate the effect of EGCG on the PMCA and its relationship with the calcium homeostasis.

We evaluated the effect of EGCG on the ATPase activity of PMCA purified from human red blood cells. EGCG showed a strong inhibition with an apparent affinity of 30 nM by increasing the phosphorylated intermediate which was found to be ADP sensitive. This suggests that EGCG stabilizes the *E*1P intermediate on the reaction cycle of hydrolysis of ATP by PMCA. In addition, while EGCG did not modify significantly the value of $K_{0.5}$ for Ca^{2+}, it increased the apparent affinity for Mg^{2+}.

In order to assess whether this inhibition may be of physiological relevance, we characterized this effect in the context of a living cell by monitoring in real time the changes in the cytosolic calcium levels. We tested the influence of EGCG on the activity of the PMCA transiently expressed in human embryonic kidney (HEK293) cells. We found that EGCG produced an increase of the cytoplasmic basal Ca^{2+} concentration and decreased the rate of removal of Ca^{2+} suggesting that PMCA activity was inhibited.

These results suggest that inhibition of the PMCA by EGCG can explain the observed effects on intracellular Ca^{2+} levels.

Keywords: Calcium, Epigallocatechin-3- gallate, inhibition, plasma membrane Ca^{2+}-ATPase

(1632) **THE UNIQUE REDUCING STEP OF ORGANIC HYDROPEROXIDE RESISTANCE PROTEINS: THE CRUCIAL ROLE OF THE CATALYTIC ARGININE**

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Organic Hydroperoxide Resistance proteins (Ohr) are Cys-based, lypoil-dependent peroxidases with extraordinary reactivity towards organic hydroperoxides (10^6–10^8 $M^{-1}s^{-1}$). These proteins are present in pathogenic microorganisms, exhibiting a unique barrel shaped fold and are absent in mammals. Such characteristics make Ohr promising drug targets. In the oxidative part of the catalytic mechanism, the peroxide is reduced via the oxidation of the reactive Cys at the active site yielding a sulfenic acid derivative; then a second Cys residue resolves, forming an intramolecular disulfide bridge. The reducing part of the cycle depends on a series of thiol/disulfide exchange reactions, which regenerates the reduced form of the enzyme. The catalytic triad of Ohr comprises a reactive Cys, an Arg and a Glu interacting in a closed state. In an open state, the loop containing the catalytic Arg moves away from the active site.

Here, we describe six new crystallographic structures (including the complex between Ohr and its biological reductant, dihydrolipoamide) that enable us to gain new insights on biochemical properties of Ohr. Furthermore, molecular dynamics simulations indicated that the most flexible regions of Ohr enzymes are located at the periphery of the active site, which might play a role in accommodating the different substrates. Moreover, when dihydrolipoamide was present at the active pocket, it provided additional stability to the close state of Ohr. Our results suggested that even upon disulfide bond formation, Ohr would have low probability of reaching the open state, represented in many Ohr structures available in PDB. In order to evaluate if the catalytic Arg is required to activate dihydrolipoamide, hybrid Quantum/Classical simulations are being performed, aiming to determine the free energy profiles regarding the reducing step, both in the open and close conformations. Taken these data together, an updated scheme for Ohr enzymatic mechanism is presented.

Keywords: Cys-based peroxidases, conformational changes, catalytic mechanisms, QM/MM.

(1905) **ISOLATION OF A TRYPSIN-LIKE ENZYME FROM *Piaractus mesopotamicus* (PACÚ)**

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Piaractus mesopotamicus (pacú) is an omnivorous fish endemic of Paraguay-Paraná river basin, incorporated to the aquaculture production system in the northern region of Argentina. Fish viscera are a potential source of digestive enzymes, especially proteases. An interesting alternative to convert the fish processing wastes into useful products of higher value is the isolation of trypsin, one of the major digestive enzymes with potential industrial applications. This enzyme is currently marketed by its applications in food industry, as additive in laundry detergents, in cell culture technique and in the pharmacology area where is prescribed for several treatments (inflammatory edema associated with post-surgical wounds, traumatic injuries, rhinitis, sinusitis, etcetera). In this work we applied affinity chromatography on Benzamidine Sepharose column to isolate a tripsin-like protein from pacú processing waste. Pyloric caeca was disaggregated and homogenized in saline buffer (pH 7.8). After centrifugation, supernatant was applied to the column equilibrated in Tris buffer (pH 7.8). The adsorbed material was eluted with low pH buffers, 4.5 and 3.2. The eluents were monitored at 280 nm for proteins. Trypsin activity was measured by the change in absorbance at 410 nm using BApNA as substrate. Fractions collected at pH 3.2 which exhibited trypsin activity were pooled, dialyzed and lyophilized. SDS-PAGE showed a single band compatible with fish trypsins. Isolated protease was active in a wide range of temperatures (0–75°C) and the highest activity was found at 45°C. At 75°C, about 50% of maximum activity was retained, this value being 20% higher than that observed for commercial porcine trypsin. This remarkable property makes this enzyme an eligible ingredient in detergent manufacturing. Consequently, the single step method described above can be considered an useful tool to recover a trypsin-like enzyme from piloryc caeca of pacú for industrial purposes.

Keywords: pyloric caeca, serinoprotease, digestive enzyme, detergents

(676) **BIOCHEMICAL CHARACTERIZATION OF AN EUCARIOTIC POLYKETIDE SYNTHASE**

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Methyl-branched fatty acids (FA) are compounds which have numerous industrial applications. They show improved physicochemical properties in comparison to linear carbon chain FA, the most common FA found in natural sources. However, this kind of molecules is extremely rare in nature. Thus, with the aim to develop new compounds with optimized properties for industrial uses, in this work we explored a new strategy to generate structural diversity in FA.

Based on the mycoserosic acid synthase (MAS), a polyketide synthase (PKS) from *Mycobacterium tuberculosis* that synthesizes long chain methyl-branched fatty acids, we searched for a new PKS capable of using methylmalonyl-CoA (MMC) as extender unit. Using MAS as query for a BLASTp search, we identified a new family of un-characterized and highly conserved PKS in birds. The uropygial gland, in birds, is involved in the secretion of methyl-branched fatty acids, alcohols and esters that are used for cleaning and impermeabilization of the plumage. We hypothesized that these compounds would be produced by this new family of PKS. In particular, we started the characterization of a PKS enzyme, that we named ApMAS, from the duck *Anas platyrhynchos;* in this specie the uropygial secretion has a high proportion of methyl-branched- C6 FA.

PKS are enzymatic complexes that condense simple short chain acyl-CoA into larger molecules with diverse biological activities. In an initial biochemical analysis of ApMAS, we determined the ability of this enzyme to covalently bind to the substrates by using radiolabeled precursors, acetyl-CoA/propionyl-CoA as starter units and malonyl-CoA/MMC as extender units. Using this strategy we could also evidence the substrate transfer from an acyl-CoA to the ApMAS acyl carrier protein domain (ACP). We then measured the kinetics of this transfer reaction catalized by the ApMAS acyltransferase domain. Finally, we could demonstrate the *in vitro* condensing ability of the ApMAS ketosynthase domain.

Palabras clave: methyl-branched fatty acids, polyketide synthase

(869) **BIOCHEMICAL CHARACTERIZATION OF THIOREDOXIN DEPENDENT CELLULAR PATHWAYS IN *Trypanosoma cruzi***

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The *S*-nitrosylation is a protein post-translational modification forming part of the cellular signaling mechanisms. In this modification, the cysteine thiol is converted to nitrosothiol by the addition of a nitrosyl group. Several works have shown that thioredoxin (TRX) catalyzes the *S*-nitrosylation and denitrosylation of a large number of proteins. In *Trypanosoma cruzi* (the etiologic agent of Chagas disease), the modification of thiol-proteins by *S*-nitrosylation and its potential relation to regulatory or signaling functions have not been explored yet. Herein we present the functional properties of a TRX of *T. cruzi* (*Tc*TRX), a redox protein with disulfide reductase activity, which have potential functionality to control redox intracellular homeostasis. Our *in vitro* data suggest that *Tc*TRX operates as a redox mediator in trypanothione system. The nitrosylated *Tc*TRX, presented a single SNO group per *Tc*TRX and it not brought about changes in its disulfide reductase activity. *In vivo* assays, *Tc*TRX overexpressing epimastigotes presented a slight increase in its tolerance to exogenous H_2O_2. In contrast, the overexpressing cells were more sensitive to exogenous GSNO. Complementarily, we evaluated the *S*-nitrosylated protein profiles by the biotinswitch technique. *Tc*TRX overexpressing epimastigotes showed higher number of *S*nitrosylated proteins than the non-overexpressing cells. These results suggest that *Tc*TRX participate in protein nitrosylation pathways *in vivo*. In parallel, *Tc*TRX overexpressing cells showed increased metacyclogenic ability (of recombinant epimastigotes) and a rise in the mammalian cells infection capacity (of recombinant trypomastigotes). These results indicate that *Tc*TRX participates in processes related to the infective capacity of this parasite. The influence of *Tc*TRX in several parasite physiological processes suggests novel insights not only in redox metabolism but also in redox signaling pathways in *T. cruzi*. Granted by ANPCyT (PICT2014-2103).

Keywords: *S*-nitrosylation, thioredoxin, *Trypanosoma cruzi,* redox metabolism.

ONCOLOGY-ONCOIMMUNOLOGY 11

(1240) **INTEGRAL ANALYSIS OF GENES RELATED TO TUMOR BIOLOGY AND IMMUNE STATUS FROM A CUTANEOUS MELANOMA PATIENT IMMUNIZED WITH THE CSF-470 VACCINE**

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The therapeutic vaccine CSF-470 is a mixture of four lethally-irradiated melanoma cell lines co-administered with BCG and GM-CSF; it has demonstrated a significant benefit in the distant metastasis-free survival for stages IIB, IIC and III melanoma patients in a phase II Clinical Trial as compared to IFN-α2b (CASVAC-0401,NCT01729663). We present here the genomic and transcriptomic analysis of a subcutaneous metastasis highly infiltrated by immune cells, which developed at the end of the 2-year protocol in a vaccinated patient (#006). *Methods:* DNA and RNA were isolated from a frozen resected cutaneous metastasis and peripheral blood cells. Paired whole-exome sequencing was performed, tumor SNPs and INDELs were analyzed with MUTEC2. RNA-seq from the metastasis was performed; gene-expression profile patterns were analyzed by Gene Set Enrichment Analysis (GSEA). *Results:* somatic variants corresponded to a total number of 4,754 SNPs plus INDELs; 1464 were non-synonymous variants and 302 were missense mutations. Tumor mutational load was high (94.3 variants/Mb), this tumor might have generated multiple immunogenic neoantigens. Among somatic mutations, $BRAF^{V600E}$ (het) was confirmed as an oncogenic driver; also frameshift insertions in NF1 and GRIN2A tumor suppressor genes were found. Regarding RNASeq, genes related to angiogenesis were significantly expressed. Several melanoma associated Ags as well as cancer testis Ags were expressed; HLA transcripts were abundant. Inflammatory response genes were abundant. CD4, CD8, granzymes and perforins, indicative of tumor infiltrating lymphocytes with cytolytic activity, were also highly expressed. Inhibitory immune checkpoints axes and immunosuppressive molecules were also expressed. *Conclusions:* integral analysis of a non-microdissected metastatic melanoma tissue allowed us to determine tumor mutations and expression of genes related to tumor biology and immune status, with impact on patient´s outcome.

(1405) **KLRG-1+CD57+ CD4+ SENESCENT T CELLS ARE INCREASED IN PERIPHERAL BLOOD FROM BREAST CANCER PATIENTS AND DISPLAY DIFFERENTIAL GENES EXPRESSION COMPARED TO CD4+ SENESCENT T CELLS FROM HEALTHY DONORS.**

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Cell senescence is a phenotype characterized by cell cycle arrest, usually due to DNA damage, and alterations in cells functionality. Senescence in CD8+ T cells has been widely described, while senescent CD4+ T cells have been less studied, particularly in cancer patients. In this work we explored the phenotype of CD4+ T cells in peripheral blood of breast cancer patients (BCPs). Using FACS we identified senescent T cells using the classic markers KLRG-1 and CD57. KLRG-1+CD57+CD4+ T cells (DP cells) were increased in BCPs compared to age-matched healthy donors (HDs; $p \leq 0.05$). Comparing with KLRG-1-CD57-CD4+T cells (DN) from BCPs, these DP cells showed lower expression of CD28 and CD27 ($p \leq 0.05$), cell cycle arrest and lower IL-2 production ($p \leq 0.01$), all features of senescent T cells. To identify biological functions and pathways associated with CD4+ T cell senescence, we sorted DN and DP cells from 4 BCPs and also DP cells from 3 HDs and performed a transcriptome study by Microarrays (Affymetrix). We found 1115 differential-expressed genes (DEGs) between DP cells from BCPs and HDs and 1170 between DN and DP cells from BCPs (Welch *t*-test applied, $p \leq 0.05$ for all DEGs). Gene Ontology analysis (DAVID databases) comparing DP cells from BCPs and HDs showed upregulation in the former of genes related to DNA repair and protein glycosylation and downregulation of genes related to apoptosis, proliferation and RNA splicing. Comparing DP cells vs. DN cells from BCPs, many DEGs were related to immune response. Among them, genes related to TCR signaling and co-stimulation were downregulated in DP cells. Our results show that even though DP cells from HDs and BCPs were sorted using same markers, the transcriptome study revealed differences, particularly in genes related to biological processes as DNA repair, proliferation and survival. Further studies will be conducted to identify markers of cancer-promoted senescence on CD4+ T cells and the role of these cells in tumor progression.

Keywords: CD4+ T cells, cancer, senescence, DNA damage.

(81) **L-NAME IMPROVES THE ANTI-TUMOR IMMUNE RESPONSE INDUCED BY BCG IN INOS-EXPRESSING BLADDER TUMORS**

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Bladder cancer (BC) is the second most common malignancy of urological tract in men and is classified in non-muscle invasive (NMI) and invasive tumors (MI). Bacillus Calmette-Guerin (BCG) is the standard treatment for patients with NMI BC. Nitric oxide (NO) is produced by nitric oxide synthases (NOS). We previously described that the inducible NOS (iNOS) is not expressed in normal bladder urothelium, but it is expressed in the 50% of BC patients, associated with early recurrence and invasion. Using a murine NMI BC model (MB49), which expresses iNOS, we observed that inhibition of NO production with L-NAME potentiated the tumor growth inhibition induced by BCG immunotherapy.

Objective: To evaluate immune cells profile in tumor-bearing mice treated with +/- BCG and +/- L-NAME in spleen and tumor draining lymph nodes.

Results: MB49 subcutaneous tumor-bearing mice (TBM) presented a systemic immunosuppressive profile characterized by a reduction of $CD8^+$, NK and $CD4^+CD25^-$ cells and an increase of Treg and MDSC in spleen and draining lymph nodes compared to normal mice ($p<0.05$). L-NAME (0.5g/l in drinking water) treatment showed no significant changes in all these populations. BCG (2mg/ml intratumoral) was able to increase the number of effectors cells such as $CD8^+$, NK and $CD4^+CD25^-$ ($p<0.05$), but was not able to reduce Treg and MDSC. The combined treatment of BCG+L-NAME completely restores the immune profile of $CD8^+$, NK, $CD4^+CD25^-$, Treg and MDSC to those observed in normal mice. In a specific cytotoxic assay against MB49 cells we showed that splenocytes from BCG or BCG+L-NAME treated TBM presented an increased cytotoxicity activity against MB49 cells compared to splenocytes from the untrated TBM ($p<0.001$).

Conclusion: Our results indicated that L-NAME improves the anti-tumor immune response induced by BCG in iNOS-expressing bladder tumors.

Keywords: Bacillus Calmette-Guerin (BCG), Bladder Cancer, Nitric Oxide, Immunotherapy.

(1170) **ME-ALA BASED PHOTODYNAMIC THERAPY AS A NOVEL PUTATIVE IMMUNOGENIC CELL DEATH INDUCER AGAINST MELANOMA**

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The immunogenicity of tumor cells has recently been acknowledged as a critical determinant of efficacy in cancer therapy. It is now clear that cancer cells can succumb to some anticancer therapies by undergoing a peculiar form of cell death that is characterized by the emission of “damage/associated molecular patterns” (DAMPs). The release of DAMPs and other immunostimulatory factors by cells dying through immunogenic cell death (ICD) favors the establishment of a productive interface with the immune system. The purpose of the present study was to evaluate the effectiveness of the PS protoporphyrin IX (PpIX) in promoting the exposure of pivotal DAMPs that characterize ICD, such as calreticulin (CRT), on melanoma cells. B16-OVA cells were sensitized with Me-ALA-induced PpIX during 4 h. Interestingly, confocal microscopy images showed that PpIX was preferentially localized in the endoplasmic reticulum (ER) prior to irradiation (Pearson’s r: 0.934). Subsequent photoactivation of PpIX with lethal dose significantly promoted oxidative stress (100% DCF + cells). ROS-scavenger N-acetylcysteine (NAC) inhibited cytotoxic effect of PDT by 33.3% ($p<0.001$). Moreover, chelation of cytosolic Ca^{2+} with BAPTA-AM suppressed photodynamic-induced cell death by 10.62% ($p<0.05$), partially linking ER-stress to photodamage. In this context, through CRT-GFP translocation analysis, CRT surface exposure was detectable as early as 30 min after photosensitization. Taken together, in agreement with previous reports, our data suggest that Me-ALA-based PDT could be proposed as a novel putative ICD inducer, given that PpIX, an ER-localizing photosensitizer, caused massive production of ROS, ER-stress and CRT mobilization when excited by light of a specific wavelength. Further studies will be done in order to explore whether this selectively target of the ER would be able to promote ICD and potentially activate antitumor adaptive immune response against melanoma.

Keywords: Photodynamic Therapy; DAMPs; Immunogenic cell death; Calreticulin; Melanoma

(475) **MELANOMA MICROENVIRONMENT REPROGRAMMING BY INTRALESIONAL *Mycobacterium bovis* BACILLUS CALMETTE-GUÉRIN (BCG) THERAPY FOSTERS ANTITUMOR T CELL RESPONSES**

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Inoperable cutaneous metastatic melanoma (CMM) has been treated for decades with intralesional *Mycobacterium bovis* bacillus Calmette-Guérin (BCG), a relatively inexpensive therapy. However, skin mechanisms of intralesional BCG used in this manner persist understudied. Tumor-associated macrophages (M2) infiltrate melanomas and impair antitumor immunity. Since macrophages play a pivotal role against both cancer and mycobacterial infections, we hypothesized BCG regulates macrophages to promote antitumor immunity. The analysis of intralesional BCG-treated CMM lesions, in combination with *in vitro* studies, allowed us to further investigate BCG-altered pathways. BCG-treated, *in vitro*-polarized M2 (M2-BCG) showed transcriptional changes enriched for inflammation, immune cell recruitment, cross talk, and activation pathways. Mechanistic network analysis revealed M2-BCG potential to improve interferon gamma (IFN-γ) responses. Consistent with this finding, frequency of IFN-γ-producing CD4+ T cells responding to M2-BCG vs. mock-treated M2 increased ($p < 0.05$). Moreover, conditioned media from M2-BCG vs. M2 elevated the frequency of granzyme B-producing CD8+ tumor-infiltrating lymphocytes (TILs) challenged with autologous melanoma cell lines ($p < 0.01$). These enhancements were also observed for cells or conditioned media from BCG-treated, *in vitro*-polarized M1 macrophage cultures (M1-BCG, $p < 0.05$). When comparing transcriptomes of intralesional BCG-injected CMM with uninjected lesions we found enrichment for immune functions, with the most prevalent pathways representing T cell activation mechanisms. *In vitro*-infected, MM-derived cell lines induced higher frequency of IFN-γ-producing TIL from the same melanoma ($p < 0.05$). Our data hints toward BCG favoring antitumor responses in CMM through direct/indirect effects on tumor microenvironment components including macrophages, T cells, and tumor cells.

Keywords: cutaneous metastatic melanoma, intralesional BCG, melanoma microenvironment, antitumor immunity mechanisms, T

cell response

(99) **ONCODRIVER INHIBITION AND TH1 CYTOKINES COOPERATE TO INDUCE TUMOR SENESCENCE AND APOPTOSIS IN HER2 AND TRIPLE NEGATIVE BREAST CANCER (TNBC)**

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Immune infiltration of breast cancer predicts favorable outcomes. We have described the loss of anti-oncodriver Th1 immunity during breast tumorigenesis. We studied the role that Th1 cytokines may play in the induction of tumor senescence and apoptosis of breast cancer cells.

HER2^{positive} cells were sensitive to senescence induction when cultured with IFN-γ and TNF-α in a dose dependent manner measured by β-galactosidase activity ($p<0.05$) and increased p15 and p16 expression by western blot. Apoptosis was also observed by western blot of active caspase-3 and flow cytometry of Anexin V-PI staining ($p<0.01$). Th1 cytokines synergize with HER2/HER3 blockade by either RNAi or trastuzumab and pertuzumab (TP) addition to increase senescence ($p<0.05$) and apoptosis ($p<0.01$). Anti-HER2 CD4 Th1 co-cultured with DC1 pulsed with HER2 peptides in a transwell culture system separated from HER2^{positive} breast cancer cells treated with TP led to synergistic increases in both senescence and apoptosis ($p<0.001$) which was inhibited by neutralizing anti- IFN-γ and TNF-α. T-resistant cells were sensitive to Th1 cytokine immune senescence that restored sensitivity to oncodriver inhibition ($p<0.001$). IFN-γ and TNF-α led to phosphorylation of STAT1 on tyrosine and serine respectively and a compensatory decrease in STAT3 phosphorylation. Also 4 TNBC cell lines were relatively resistant to Th1 cytokine induced senescence and apoptosis despite expressing IFN-γ and TNF-α receptors. TNBC cells were sensitive to oncodriver inhibition by EGFR RNAi and when combined with Th1 cytokines resulted in highly increased senescence and apoptosis.

Th1 cytokines and oncodriver inhibition cooperate to induce senescence and apoptosis of HER2 expressing and TNBC through activation of STAT1 and inhibition of STAT3. These results suggest that restoring Th1 cytokines and oncodriver inhibition offers a promising approach to the elimination of disseminated cancer cells to reduce risk of recurrence in breast cancer.

Keywords: CD4 Th1 cytokines, oncodriver, breast cancer

(1324) **PHENOTYPIC AND FUNCTIONAL CHARACTERIZATION OF CD39+CD4+ CONVENTIONAL T LYMPHOCYTES FROM B16F10-OVA TUMOR-BEARING MICE.**

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The enzymatic activities of CD39 and CD73 play key roles in the modulation of signals delivered in the tumor microenvironment (TME) through the conversion of ADP/ATP to adenosine. We aimed to study the expression of CD39 on conventional Foxp3- CD4+ T cells (Tconv) from tumor-bearing mice by flow cytometry. C57BL/6 mice were injected with B16F10-OVA cancer cells. Tumors, spleens and draining lymph nodes (dLN) were extracted on day 17. We observed increased frequency of CD39CD73-expressing Tconv in tumor respect to spleen ($p\leq0.001$) and dLN ($p\leq0.001$). This population, together with CD39-CD73+ Tconv showed higher expression of CD26 compared to CD73- Tconv. In tumors, we detected that 18,5±2,6% of CD39+ Tconv co-express PD-1 and LAG-3, and 15,8±6,6% express TIGIT. In addition, PD-L1 expression was increased on this population compared to CD39- Tconv ($p\leq0.01$). We observed that 89,4±2,7% of tumor-infiltrating CD39+ Tconv exhibited effector memory phenotype. Around 50% of this cells were CD49d+ CD11a+, a phenotype associated with antigenic stimulation. The effector function of these cells was assessed analyzing their ability to produce cytokines upon activation. Within CD39+ Tconv we detected decreased frequency of TNF-producing cells, but higher frequency of IFNγ-producing cells compared to CD39- Tconv ($p\leq0.01$ for all). In concordance, CD39+ Tconv showed high expression of T-bet. Also, CD39+ Tconv exhibited higher frequency of CD107a+ and Granzyme B+ cells than their CD39- counterparts. CD39+Tconv did not produce IL-2. Using Hypoxyprobe-1 reagent, we found a higher frequency of hypoxic cells within the subset CD39+ Tconv compared to CD39- Tconv ($p\leq0.01$). Together these results suggest that TME drives the acquisition of immunoregulatory molecules on Tconv and postulate CD39 as a relevant target to be deeply studied in order to understand how this molecule expressed on Tconv may influence the anti-tumor immune response.

Keywords: CD4, cancer, CD39

(1881) **POST-TRASPLANT LYMPHOPROLIFERATIVE DISORDERS IN PATIENTS RECEIVING SOLID ORGANS. CASE SERIES AND INCIDENCE OF EPSTEIN-BARR VIRUS.**

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Post-transplant lymphoproliferative disorders (PTLD) comprises a heterogeneous group of lymphoid proliferations that present after a transplant in 1-2% of organ recipients.

The objective of this research is to describe the cases of PTLD diagnosed in our institution (Hospital Italiano de Buenos Aires) between 2002 and 2017, in terms of morphological, immunophenotypic characterization according to the WHO criteria of 2016, and to study the tissue prevalence of Epstein-Barr Virus (EBV).

Data of all cases diagnosed as PTLD in our hospital was obtained from the institutional data center. Histological material was obtained from archive. Immunohistochemical techniques were performed to reclassify the cases according to the WHO criteria (2016). In situ hybridization was performed to study the presence of EBV in the tissue.

The study population consisted of 40 cases, of whom 20 were female and 20 were male. 55% of the patients were pediatric and 45% were adults. The age range was 1 to 73 years (mean 28.48 +/- 24.42 years). 52.5% of the transplants were hepatic and 47.5% were renal. The localization of the tumors was heterogeneous, mainly involving lymph node (37.5%). Regarding to the histological pattern, 2.5% corresponded to Non-Destructive PTLD, 60% were Monomorphic PTLD, 27.5% were Polymorphic PTLD and 10% were Hodgkin-like PTLD. The prevalence of EBV was 62.5% (25 positive cases).

In conclusion, the data in our series is coincident with data reported in international series regarding morphological classification and EBV status. The presentation of this series of cases is the starting point of a larger investigation, to study prognostic and predictive factors, to achieve a risk-stratification at the diagnosis to improve the management of this entity.

Keywords: PTLD, TRANSPLANT, LYMPHOMA.

(827) **THE ACTIVATION OF THE IMMUNE SYSTEM AS A CONSEQUENCE OF TISSUE REMODELING AFTER ENDOCRINE TREATMENT MAY PARTICIPATE PREVENTING TUMOR RELAPSE**

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The role of the immune system during endocrine treatment of mammary carcinomas (MC) has been poorly investigated. We have shown that mifepristone (MFP, antiprogestin) may induce the regression of murine MC that express hormone receptors. Our aim

was to characterize the infiltrating cells in MFP-treated MC and evaluate the role of the immune system in antiprogestin-induced tumor regression.

Bone marrow (BM) cells from BALB/c-GFP+ mice were *iv* inoculated into NSG mice to establish the NSG/BM-GFP+ mouse model. 59-2-HI tumors, originated in BALB/c mice, were inoculated into NSG or NSG/BM-GFP+ female mice. MFP pellets were implanted sc. when the tumors reached 50 mm^2. Tumors were excised after 6 days and cells analyzed by flow cytometry. An increase in T lymphocytes (CD8+; $p<0.01$), macrophages (CD11b+ F480+; $p<0.01$), NKs cells ($p<0.05$), central memory (CD62L+; CCR7+) CD8+ ($p<0.05$) and CD4+ ($p<0.001$), and effector memory (CD62L-; CCR7- cells) CD8+ ($p<0.05$) and CD4+ ($p<0.05$) T cells was detected. Conversely, a decrease in the T_{reg} subpopulation (CD4+ CD25+ Foxp3+; $p<0.05$), was observed in MFP-treated tumors as compared with control tumors.

We also carried out re-challenge assays. MFP pellets were removed after 6 days and tumors excised. Animals were re-inoculated with the same tumor in the opposite flank 5 days after surgery. Sham operated animals were used as controls. Re-challenged NSG/BM-GFP+ mice had a significant lower tumor take: sham vs. MFP ($p<0.001$); control vs MFP ($p<0.01$).

These data are in agreement with an adaptive immune response elicited during endocrine tumor regression. Regressing tumors may expose intracellular antigens that could be generating a protective immune memory response that might be related with the long free relapse survival induced by endocrine therapy. The characterization of the type of cell death involved, DAMPs exposure and cytokines present in the tumor microenvironment after MFP treatment is being investigated.

(342) **DIFFERENT PHENOTYPE OF PERIPHERAL BLOOD AND INTRATUMORAL NK CELLS AND CD8⁺ T CELLS IN RENAL CELL CARCINOMA PATIENTS MAY UNDERLAY DISTINCT FUNCTIONAL ABNORMALITIES**

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Renal cell carcinoma (RCC) is an aggressive neoplasm with metastatic potential and is considered one of the most common types of cancer in older adults. Partial or radical nephrectomy constitutes the gold-standard treatment for RCC. However this type of cancer remains silent during early stages, becoming evident when metastasis may have already occurred, representing a therapeutic challenge due to patient's poor prognosis. Different tumors express a diverse array of ligands of the NK cell activating receptor NKG2D (NKG2DLs), but their expression pattern and relevance in RCC remains unknown. NKG2DLs comprise MICA, MICB and 6 members of the ULBP family (ULBP-1 to -6). As they may constitute attractive targets for immunotherapy and potential prognostic and therapeutic biomarkers, the aim of this study was: a) to characterize the expression pattern of these NKG2DLs and b) to analyze the expression of NKG2D on NK cells and CD8⁺ T cells in peripheral blood mononuclear cells (PBMC), tumor infiltrating lymphoid cells (TIL) and tumor cells from RCC patients as well as in PBMC from healthy donors (HD) using multicolor flow cytometry. In RCC patients (n=6), high expression of MICA was observed on tumor cells, while on TIL, not only MICA, but also ULBP-3 and ULBP-4 were highly expressed. Conversely, PBMC from RCC patients and HD (n=5) did not express NKG2DLs. Also, NK cells and CD8⁺ T cells in PBMC from RCC patients expressed decreased amounts of NKG2D compared to HD ($p<0.05$), while NK cells and CD8⁺ T cells in TIL displayed increased expression of NKG2D but only in patients that simultaneously displayed increased expression of NKG2DLs on TIL. Our results suggest that NK cells and CD8⁺ T cells from PBMC and TIL exhibit a different phenotype in terms of NKG2D and NKG2DLs expression, which might underlay or be associated with different tumor immune evasion mechanisms in PBMC and TIL.

Keywords: RCC, NKG2D, NK cells, CD8⁺ T cells

ONCOLOGY-ONCOIMMUNOLOGY 12

(1569) **CELL TYPE SPECIFIC PR AND ERA GENE REGULATION IN ENDOMETRIAL CANCER CELLS**

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Estrogen (E2) and Progesterone (Pg) and their specific receptors ER and PR respectively are major determinants in the development and progression of breast and endometrial malignancies. We have studied the cell specificity of E2 and synthetic progestin R5020 action in human Ishikawa endometrial cancer cell line and compared it to similar genomic strategies in human T47D breast cancer cell line. Both hormones regulate pathways linked to cell proliferation in the breast and endometrial cancer cells, through different gene networks and signaling pathways. Using ChIPseq, we identified ~1,400 binding sites for PR (PRbs) and ~1,900 for ERalfa (ERbs) which are largely specific for Ishikawa and distal (>50kb) to TSS (FDR cutoff for peak calling q = 0.05; FDR cutoff for downstream analysis $q<10^{-5}$). Long-range interactions in chromatin structured genome analyzed by HiC showed that Ishikawa and T47D cells exhibit distinct open and closed chromatin compartments, which correlate with different hormone receptor binding distribution and gene regulation as indicated in Pgr and Alpp genes. Among Ishikawa specific sites, the most representative motives found in PRbs were PRE, SOX and PAX, whereas in ERbs they were ERE and PAX. PR and ER bind mostly to non-common sites that exhibit their corresponding consensus sequences, and are adjacent to Pax2 binding. Increased levels of PR by E2 treatment resulted in enhanced R5020-dependent PR binding to chromatin with a stronger association to ER and Pax2 binding, while overexpression of PR by introducing one copy of *pgr* gene produced different PRbs. In conclusion, the endometrial specific hormone response results in part from specific chromatin compartments, unique receptor binding sites and selective TFs binding partners.

Keywords: Ishikawa, progesterone receptor, ChIPseq, chromatin compartments.

(101) **CTBP1 PROTEIN AND METABOLIC SYNDROME IMPACT ON MIRNAS EXPRESSION PROFILE ASSOCIATED TO BREAST CANCER.**

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Abstract: Breast cancer (BrCa) is the main cause of cancer death in women and metabolic syndrome (MeS) increases the incidence and aggressiveness of this disease. C-terminal Binding Protein (CtBP1) is a corepressor of tumor suppressors activated by the low NAD+/NADH ratio. Previously, we generated a murine MeS model by chronically feeding animals with high fat diet. We found that CtBP1 and MeS induced BrCa growth and progression. Using microarray technology we showed that CtBP1 hyperactivation by MeS regulated the expression of multiple miRNAs in BrCa xenografts. Bioinformatic analysis using miRSystem resource allowed us to identify one cluster of miRNAs involved in cell proliferation (miR-378a-3p, miR-146a-5p and miR-381) and tumor progression (miR-378a-3p, miR-146a-5p, miR-381, miR-223-3p, miR-494-3p, miR-940, miR-433, miR-522 and miR-637). The goal of this work was to elucidate CtBP1 and MeS effect on miRNAs expression and to

investigate its function in BrCa.

We studied miRNA expression levels by RT-qPCR stem loop methodology of selected miRNAs from our previous results, in CtBP1-depleted or control MDA-MB-231 xenografts generated in mice with MeS or control .We found that CtBP1 modulated miR-381-5p, miR-378a-3p, miR-146a-5p, let-7e-3p and miR-194-5p in BrCa xenografts while MeS induced miR-381-5p and miR-194-5p. By gene ontology analysis based in the bioinformatic tool ChemiRs, we determined that CtBP1/MeS associated-miRNAs are involved in cancer, apoptosis, focal adhesion and mesenchymal cell proliferation. Finally, we assessed genetic alterations in BrCa patients obtained from cBioPortal datasets. Interestingly, 1.5% of patients presented DNA amplifications in let-7e and 20% in miR-194.

Thus, let-7e-3p and miR-194-5p emerge as CtBP1 controlled miRNAs that might be relevant in a subset of BrCa patients with MeS.

Keywords: Breast cancer, metabolic syndrome, miRNAs, CtBP1.

(108) **DOWN REGULATION OF RAC3 IN COLORECTAL CANCER SENSITIZES TO THE TREATMENT WITH 5-FLUOROURACIL AND OXALIPLATIN THROUGH AN INCREASE IN THE LEVELS OF AUTOPHAGY**

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It has been reported that some chemotherapeutics induce immunogenic cell death (ICD). ICD is characterized by alterations in the plasmatic membrane and the microenvironment of the dying cell. Autophagy is a process that precedes ICD as it allows such changes like ATP exocytosis and avoid the up regulation of the immunosuppresssive ecto-ATPase CD39.

Our group has previously shown that the down regulation of RAC3 expression in HCT 116 colon adenocarcinoma cell line sensitizes to the treatment with 5-fluorouracil 3.5 μM (5Fu) and oxaliplatin 0.4 μM (oxa), chemotherapeutics for colorectal cancer patients. In view, that RAC3 over expression inhibits autophagy; we analyzed if these drugs stimulate autophagy in HCT 116 and in this cell line transfected with a short hairpin RNA against RAC3 (shRAC3); and if this mechanism could lead to ICD.

Autophagy was assayed by Western blot against LC3. After 24 h post treatments, both drugs increase the ratio of LC3II/I on shRAC3 (HCT control, 5Fu: 1.1, oxa: 1.0; shRAC3, basal: 1.0 5Fu: 1.3, oxa: 1.4-fold respect HCT control basal). Furthermore, to study if induced autophagy could lead to ICD, the cell lines were pretreated with TSA (0.4 μM) and then stimulated with the drugs to performed an immunofluorescence against acetylated proteins. Compared with HCT control (87±11), the acetylation percentage decreased in shRAC3 cell line (37±8 p<0,001) and cells stimulated with drugs (HCT control, F+T 41±5; o+T 56±7 p<0,01; shRAC3, F+T 26±5; o+T 14±11 p<0,05). Then, we measured CD39 levels by qPCR. Only oxa treatment but not with 5Fu, induced a decrease in CD39 expression (HCT control 1.0±0,1, 5Fu 0.7±0.09 and oxa 0.7±0.1; shRAC3 basal 0.5±0.08, 5Fu 0.7±0.1 and oxa 0.3±0.08 fold respect HCT control basal; p<0.01).

Altogether, both drugs induce autophagy, a mechanism by which RAC3 over expression could attenuate chemotherapeutics' effect. On the other hand, ICD could be propitiated by acetyl lysine reduction and also by CD39 expression decrease.

Keywords: RAC3, autophagy, chemoresistance, immunogenic cell death

(987) **FOXA1 AND FOXM1 ARE INVOLVED IN MIGRATION AND CELL CYCLE PROGRESSION IN PROSTATE CANCER CELLS**

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The Forkhead Box factors (FOX) belong to a family of over 40 proteins that regulate genes related to: cell growth, proliferation, differentiation, and migration. FOXA1 and FOXM1 have been reported to be cofactors of the androgen and glucocorticoid receptors (AR and GR), which are involved in prostate carcinogenesis. We aim to study the role of FOXA1 and FOXM1 in prostate cancer (PCa). We down-regulated *FOXA1* and *FOXM1* expression through transfection with shRNA in PC3 (AR-, GR+) and C4-2B (AR+, GR+) PCa cell lines. We previously showed that *FOXA1* and *FOXM1* down-regulation reduced cell proliferation (by MTS assay), altered cell morphology and modulated the transcriptional activity of GR. In this work, we further evaluated the role of these factors in PCa. Using a wound healing assay, we observed that the down-regulation of *FOXA1* reduced cell migration in both cell lines (remaining wound area at 24 h >40% compared to control; p=0.02). Similar results were observed for the down-regulation of *FOXM1*; however, it was only significant for C4-2B cells (35%, p=0.02). We found a significant reduction in cell migration when both genes were down-regulated simultaneously (45%, p=0.001 for PC3 and 50%, p=0.003 for C4-2B). Analysis of cell cycle using propidium iodide staining and flow cytometry revealed that the down-regulation of *FOXA1* produced a 10% increased of cells in S-phase in both cell lines, and a decreased in G1-phase (7% for PC3 and 15% for C4-2B). Similar results were observe when *FOXM1* was down-regulated alone or together with *FOXA1* (S-phase increase 5% for both cell lines). Interestingly, the double knocked-down cells showed a 5% increase in sub-G1 (apoptotic) cells. In conclusion, we demonstrated that FOXA1 and FOXM1 are involved in PCa cell migration and cell cycle progression. These results give new insights in the PCa biology and provide new potential therapeutic targets.

Keywords: Foxa1, Foxm1, Prostate Cancer, Cell Migration, Cell Cycle

(227) **INVASIVE MICROPAPILLARY CARCINOMA OF THE BREAST OVEREXPRESSES MUC4 AND IS ASSOCIATED WITH POOR RESPONSE TO TRASTUZUMAB IN HER2-POSITIVE BREAST CANCER**

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Invasive micropapillary carcinoma of the breast (IMPC) is a low-frequent tumor variant (~6% of all breast cancers) characterized by an inside-out formation of tumor clusters surrounded by stroma with a pseudopapillary arrangement and it has a poor clinical outcome that does not correlate with the number of micropapillary features. Pathologists underreport IMPC because it is difficult to identify and there are no biomarkers available. HER2-positive breast cancers (HER2+ BC) have an aggressive behavior. This subtype is treated with trastuzumab (TZ) but 40-60% of the patients do not respond to therapy due to resistance. We recently demonstrated that TNFα-induced mucin 4 (MUC4, membrane glycoprotein that promotes metastasis dissemination) expression in HER2+ BC is a biomarker of poor prognosis to adjuvant TZ.

Here we studied the clinical significance of IMPC and MUC4 expression in HER2+ BC patients.We retrospectively studied 86 HER2+ BC patients treated with TZ in the adjuvant setting. We explored the association between IMPC and clinicopathological parameters at diagnosis and its prognostic value.

IMPC, either as a pure entity or associated with invasive ductal carcinoma (IDC), was present in 18.6% of HER2+ cases. It was positively correlated with estrogen receptor expression and tumor size, but inversely correlated with patient's age. Disease-free survival was significantly lower in patients with IMPC (hazard ratio= 2.6; 95%, confidence interval 1.1-6.1, P = 0.0340). MUC4 was strongly expressed in all IMPC cases tested. IMPC appeared as the histological breast cancer subtype with the highest MUC4 expression with respect to IDC, lobular and mucinous carcinoma (P<0.05).

These results show that IMPC presence in HER2+ BC should be carefully examined and we therefore propose MUC4 expression as

a useful biomarker to determine its presence. Patients with MUC4+ tumors with IMPC should be more frequently monitored and receive more aggressive therapies.

(411) **MUSCARINIC RECEPTORS IN NON-TUMORIGENIC MCF-10A BREAST CELLS. EFFECT OF CARBACHOL ON THREE-DIMENSIONAL GROWTH AND VEGF-A EXPRESSION**

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CEFYBO

It was reported that muscarinic acetylcholine receptors (mAChRs) are constitutively expressed in different types of tumors. In our laboratory we demonstrated that the activation of mAChRs with the cholinergic agonist carbachol (CARB) promotes malignant progression in murine and human breast tumors. In line with these results, we developed a new model to confirm the contribution of mAChRs to malignant transformation, transfecting the non-tumorigenic human mammary cell line, MCF-10A with mAChRs. Considering that bidimensional culturing is the most used technique but not the best model to mimic the first stages of tumor growth *in vivo*, we developed three-dimensional (3D) cultures (spheroids) with mAChRs-transfected MCF-10A cells and analyzed the effect of mAChRs activation on growth (μm^2) and the expression of vascular endothelial growth factor-A (VEGF-A) by Western blot (%). The *de novo* expression of mAChRs in MCF-10A cells promoted the formation of spheroids an VEGF-A expression in 3D cultures. The addition of CARB increased spheroids size ($mAChR_3$:4745±502; $mAChR_4$: 5427±357; $mAChR_3R_4$: 6055±475) in comparison to untreated cells ($mAChR_3$:3350±467; $mAChR_4$: 2903±462; $mAChR_3R_4$: 4330±362)($p<0.05$; n=20). The preincubation of cells with muscarinic antagonists or with specific interference RNA (iRNA) reduced this effect. VEGF-A is an important mediator of tumor angiogenesis. Its expression in spheroids was upregulated by the addition of CARB ($mAChR_3$:62±1.17; $mAChR_4$: 40,8±17,9; $mAChR_3R_4$: 46.9±9.13) ($p<0.05$; n=3) in comparison to control, and reduced by the presence of selective muscarinic antagonists. In conclusion, our results indicate that mAChRs over-expression on mammary normal cells induced the formation of 3D structures in a similar manner than tumor cells and receptors activation increased cell growth the expression of VEGF-A which may promote tumor progression.

Keywords: Muscarinic acetylcholine receptors, breast cancer, VEGF-A, 3D culture.

(1305) **ROLE OF HUMANIN IN THE RESPONSE OF CANCER CELLS TO CHEMOTHERAPY**

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Humanin (HN) is a mitochondrial-derived peptide with potent cytoprotective action in many cell types. Although HN can protect normal cells against the toxic effects of chemotherapy, the role of this peptide in tumor pathogenesis is not well understood. We have previously observed that recombinant HN inhibits the response of triple negative breast cancer (TNBC) cells to cytotoxic stimuli, facilitating tumor progression and chemoresistance *in vivo*. Here we aimed to evaluate whether chemotherapy modulates the expression of HN in cancer cell types that are characterized by their chemoresistance: TNBC and glioblastoma (GBM) cells. We evaluated the expression of HN in murine 4T1 TNBC cells and in human U251 GBM cells by flow cytometry following serum deprivation and chemotherapy with Doxorubicin (DOXO), Cisplatin (CP) or Temozolomide (TMZ). Expression of HN in 4T1 TNBC cells was upregulated by DOXO (500 nM) and CP (1 μM, $p<0.05$), but not by serum deprivation. In U251 GBM cells, the expression of HN was increased by CP (5 μM) and TMZ (15 μM), and at a lesser extent by serum deprivation ($p<0.05$). We next evaluated the role of endogenous HN in the apoptotic response of TNBC cells, using a plasmid encoding a short hairpin RNA to block HN (pUC.shHN). To readily assess transduction efficiency, the plasmid also encodes the red fluorescent protein dTomato as a reporter gene. Transfection of TNBC 4T1 tumor cells with pUC.shHN increased the percentage of apoptotic cells when compared to cells transfected with control plasmid, as assessed by flow cytometry after staining with propidium iodide ($p<0.05$). When transfected 4T1 cells were incubated with different concentrations of DOXO, pUC.shHN increased their chemosensitivity, reducing their clonogenic capacity when incubated with DOXO ($p<0.05$). Our findings indicate that blockade of HN expression could constitute a therapeutic strategy to improve the efficacy of chemotherapy in chemoresistant cancer cells.

Keywords: Humanin, breast cancer, glioblastoma, chemoresistance.

(552) **STUDY OF THE MOST FREQUENT GENETIC ALTERATIONS AND CLINICAL EVOLUTION OF HUMAN CUTANEOUS MELANOMA PATIENTS TREATED WITH CSF-470 VACCINE OR INTERFERON ALPHA 2B**

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Human cutaneous melanoma (HCM) is a skin cancer whose incidence and mortality rates have been increasing over the last 30 years. HCM is relatively resistant to both chemotherapy and radiotherapy; however, it is an immunogenic tumor. A therapeutic vaccine anti-HCM, CSF-470, has been developed in our laboratory. CSF-470 has been tested against interferon alpha 2B in phase II clinical study. It has had better results than interferon alpha 2B.

HCM is the highest mutated cancer. Recent advances in sequencing techniques have greatly increased the knowledge of key molecular events for its genesis and development. It has become evident that molecular alterations may have different effects on the tumor microenvironment.

The aim of this work was to evaluate if there is a relationship between genetic alterations of each patient´s tumor and disease progression; and if a genetic marker may be identified, that correlates with the observed clinical response and may be used to predict whether patients (pts) will respond to CSF-470 vaccine.

The most frequently mutated genes and copy number variation in HCM were studied by Nested-PCR and fluorescence *in situ* hybridization (FISH) respectively, in 12 pts of the phase II CSF-470 clinical trial. It was found that 67% of the tumors harboured BRAF mutations; all of them being V600E. Only one of the tumors had $NRAS^{Q61}$ and $NRAS^{G12}$ mutations, and it also was $BRAF^{WT}$ as expected. PTEN alterations were observed in 3 pts, one of them had a missense mutation and the others had a partial deletion. Point mutations or a small copy number of CDKN2A were observed in 42% of the tumors. 33% of pts had a missense mutation in the DNA-binding domain of P53. No mutations were found in RAC1.

No significant correlation could be established between alterations found in the tumors and metastases-free-survival-period of the pts ($p>0.05$). This suggests that further genomic or transcriptional alterations of tumor cells must be analyzed to predict clinical behavior.

Keywords: human cutaneous melanoma, CSF-470 vaccine, mutations

(1159) **ROLE OF PROTEIN KINASES IN THE DEVELOPMENT OF TAMOXIFEN AND PALBOCICLIB RESISTANCE IN BREAST CANCER**

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Abstract: It is estimated that approximately 70% of breast tumors express hormone receptors for estrogen (ER) and progesterone (PR). Although ER antagonist tamoxifen has been successful for ER+ breast cancer treatment, a significant amount of these tumors eventually develop resistance. In order to study this phenomenon, we generated a tamoxifen-resistant cell line derived from the human breast cancer cell line T47D (T47D-TR), by long-term cell exposure to progressively increasing concentrations of tamoxifen. This resistant cell line showed an increase in basal activation of ER despite reduction in its expression levels (p<0,05), higher expression and activation of PI3K/Akt/mTOR pathway proteins (AKT and S6) (p<0,05), along with greater levels of PKCα (p<0,05) compared to *wild type* T47D-wt line. Furthermore, T47D-TR cell line was able to generate tamoxifen resistant tumors growing as xenografts in immunosuppressed mice. Interestingly, T47D-TR tumors showed more invasive characteristics than those generated from the T47D-wt cell line.

To further understand the mechanisms of resistance, we evaluated novel inhibitor combinations used in breast cancer patients in the tamoxifen-resistant cell line. T47D-TR cells exhibited a lower inhibitory response to palbociclib (a CDK4/6 inhibitor) and to rapamycin (a mTOR inhibitor) than the parental T47D-wt line, while it did not show differences in the expression levels of cyclin D1, cyclin E, CDK4 and CDK2. Furthermore, we developed two new cell line models resistant to both tamoxifen and palbociclib, or palbociclib, fulvestrant and rapamycin. To accomplish this, we treated T47D-TR cell line with increasing concentrations of palbociclib (T47D-TPR) or palbociclib+fulvestrant+rapamycin (T47D-TPFRR). These new resistant cell lines will be useful to understand, both *in vitro* and *in vivo*, specific and common features of tamoxifen, palbociclib and PI3K/Akt/mTOR inhibitors resistance.

Keywords: endocrine resistance, PI3K/Akt/mTOR pathway, palbociclib, breast cancer

(1288) **ELECTRIC CELL–SUBSTRATE IMPEDANCE SENSING (ECIS) FOR THE STUDY OF ADRENOMEDULLIN ON HUMAN RENAL CLEAR CAKI-2 CELLS IN HYPOXIC CONDITIONS**

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Angiogenesis is a crucial process in tumor development and metastasis. The hypoxic feature of solid tumors contributes locally and systemically to tumor progression. Clear cell renal carcinoma (CRCC) is the most common histological subtype of renal cancer (80-90% of cases). It is characterized by an intense vascularization implying a close relationship among angiogenesis, cellular response to hypoxic stress and tumor progression. There is limited evidence about the use of electric cell–substrate impedance sensing (ECIS) assay to evaluate angiogenesis and / or metastatic potential in CRCC. In addition, the function of adrenomedullin (AM) and the effects of its inhibition on solid tumors (such as CRCC) are not fully known. The aim of this study was to assess the effects of AM, a pro-angiogenic molecule, and its inhibition in CRCC using the ECIS assay. Briefly, Caki-2 cells were cultured on ECIS 8W10E electrode plates (Applied Biophysics, Troy, NY). Cellular impedance variations were measured against different concentrations of cobalt chloride (1-100 μM) for simulating hypoxic conditions and with/ without the inhibitory agents: 16311(10 to 1000 nM) and 22-52 (0,01 to 1 μM). Monolayers were summited to 1400µA current at 60Hz for 20 sec for migration assay.

Results showed that 100 μM CoCl2 was the most effective dose for mimicking hypoxia in Caki-2 cultures. Expression of AM was evaluated by Western Blotting (WB), RT-PCR and qPCR at 0,12, 24 and 36 h post cobalt chloride. AM expression increased significatively between 12 and 24 h in hypoxic conditions by WB and RT-PCR (p≤0.001). qPCR revealed a significant increment of AM RNAm at 24 h of hypoxia (p≤0.01). Finally, AM inhibitors caused the decrease in cell growth and migration by ECIS assays. This preliminary study contributes to a better understanding of CCRC tumor biology and encourages us to propose AM as a potential anti-tumor target in relation to angiogenesis.

Keywords: Hypoxia, renal cancer, angiogenesis, adrenomedullin, ECIS.

GENETICS AND MOLECULAR BIOLOGY 11

(181) **RHBDD2 GENE EXPRESSION PROFILE IN LOW AND HIGH-GRADE BRAIN TUMORS REVEALS ITS UP-REGULATION IN THE MALIGNANT SUBTYPES.**

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Abstract: The Rhomboid family is a heterogeneous group of membrane proteins involved in various functions such as cell signaling, development, apoptosis or stress response to endoplasmic reticulum (ER). Several members have been associated to neurodegenerative diseases and cancer. In previous studies, we determined that the expression of the gene RHBDD2, a member of the family, is increased in the advanced stages of breast and colorectal cancers and it is induced by the chemotherapeutic agent 5-fluorouracil (5-Fu) in colon cancer cells. We also showed that RHBDD2 is associated to the ER stress pathway known as the unfolded protein response (UPR). In this sense, UPR is highly active in glioma tumors and its induction drives chemoresistance in glioma cells. We hypothesized that the overexpression of RHBDD2 in the advanced stages of cancer and its induction against 5-Fu provides the tumor cells with a stress-resistant phenotype. In an exploratory study, using human brain tumor samples, cell lines, gene expression databases and bioinformatic analysis we determined the expression profile of RHBDD2 and UPR genes in different subtypes of brain tumors (Oligodendrogliomas, Astrocitomas and Glioblastomas). Using IHC, RTPCR and phenotypic assays we observed that RHBDD2 expression varied across the different types of tumors, being higher in glioblastomas (p<0.01). We also evidenced that silencing of RHBDD2 expression in the cell line SHS5Y, widely used as a cell model of DAergic neurons, contributed with a decrease in cell migration and proliferation (wound healing assay, p<0.05) and also affected the expression of UPR related genes. Survival analysis in TCGA datasets indicated a significant association (p<0.01) between high RHBDD2 expression and short-term survival factors such as grade, age and tumor localization. Although more studies are being carried out, the perspectives of these results position RHBDD2 as an interesting target to be considered for malignant glioma therapeutics.

Keywords: RHBDD2, Gliomas, UPR, chemoresistance.

(500) **INFLUENCE OF RHBDD2 EXPRESSION ON THE RESPONSE TO 5-FLUOROURACIL IN COLORECTAL CANCER CELLS.**

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Abstract: The current standard of care for locally advanced rectal cancer is neoadjuvant radio-chemotherapy (NRC) with 5-fluorouracil (5-Fu) as the main drug, followed by surgery and then ad-

juvant chemotherapy. This has greatly improved patient survival and limited local recurrence; however, tumors do not respond in the same way to NRC. At present, there are no molecular markers that allow monitoring tumor response prior to surgery. In previous studies we demonstrated that RHBDD2 gene is overexpressed in the advanced stages of colorectal cancer and that its expression is induced against 5-Fu. Hence, RHBDD2 could constitute a predictive / prognostic marker of response to 5-Fu therapy. In a pilot study on samples of patients with rectal cancer treated with NRC, we observed a decreased of RHBDD2 protein in most post-treated samples. However, there was a group of samples from patients with worse prognosis, in which RHBDD2 expression remained high after the treatment. With the aim of complementing this clinical approach, in the present study we developed two contrasting *in vitro* models. We overexpressed RHBDD2 in the Caco2 colon cancer cells (low endogenous RHBDD2) and silenced its expression in the HCT116 cells (high endogenous RHBDD2). We evaluated the consequences on the processes of proliferation and sensitivity to 5-Fu. Models were validated by RTPCR, immunocytochemistry and western blot. By wound healing assay and flow cytometry, we found that silencing RHBDD2 decreased proliferative rate of HCT116 ($p<0.01$), whereas the opposite occurred with RHBDD2 overexpressed in Caco2 ($p<0.05$). In addition, sensitivity to 5Fu showed a significant increase when RHBDD2 was silenced ($p<0.01$). These data along with the preliminary data in tumors indicate that RHBDD2 expression obtained from biopsies would be an interesting tool to stratify patients with advanced rectal cancer into responders and not responders to the 5-Fu regimen. Further studies are underway to strengthen this hypothesis.

Keywords: RHBDD2, 5-Fluorouracil, Colorectal cancer, Neoadjuvant chemotherapy.

(1147) **ROLE OF GPAT2 IN THE LANDSCAPE OF SMALL NON-CODING RNAs IN BREAST CANCER CELLS**

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Abstract GPAT2 is expressed in the testis and ectopically overexpressed in cancer cells, where it contributes to tumoral phenotype. Although GPAT2 was initially linked to lipid metabolism, it was demonstrated that it is essential for primary piRNA biogenesis. In this work, we analyzed the impact of GPAT2 silencing in the landscape of small noncoding RNAs of MDAMB231 cells. From the differentially expressed (DE) piRNAs, we found that most downregulated (down) piRNAs (32/39, 82%) are single copy in the genome being mainly intragenic (27/32, 84%), whereas upregulated (up) piRNAs are single (18/38, 47%) and multiple (20/38, 52%) copies in a similar way. Interestingly, snoRNAs constituted the host gene of 81% of the intragenic single copy down piRNAs, which means 56% of total down piRNAs. Furthermore, many of down piRNAs were previously found upregulated in breast cancer growing cells. From the DE tRFs we identified 147 down and 128 up. Down tRFs would be derived from “differentiation tRNAs” whereas up tRFs would be from “proliferation tRNAs”. Moreover, we obtained a protein profile from the amino acids carrying by DE tRFs and identified proteins associated to phosphatidic acid biosynthesis, phospholipid acyl chain remodeling and regulation of cell death. We also found a significant difference ($p<0.05$) in length distribution of piRNAs and tRNAs reads among DE members. From DE expressed miRNA, we identified 109 up and 104 down. Functional enrichment of putative targets revealed specific terms associated to mitochondrial biogenesis, IGF1R signaling, and oxidative metabolism of lipids and lipoproteins. GPAT2 silencing affect the length distribution of piRNAs and tRFs, and their specific abundance and features would define a less tumorigenic phenotype, as was previously observed in the silenced cells. This finding could be indicative of a role of GPAT2 in sncRNA processing, supported by the fact that other mitochondrial proteins have also been involved in at least piRNAs processing.

Keywords: GPAT2, piRNAs, miRNAs, tRFs, cancer cells, small noncoding RNAs.

(1084) **CELL-INTERNALIZATION SELEX METHOD FOR SPECIFIC RNA APTAMER SELECTION AGAINST ACUTE LYMPHOBLASTIC LEUKEMIA CELLS.**

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Acute lymphoblastic leukemia (ALL) is a malignant disorder characterized by clonal proliferation of early B- and T- lymphocyte progenitors. ALL is the most frequent cancer in pediatric oncology. Aptamers are single stranded DNA or RNA oligonucleotides that could target cancer cells with high affinity and specificity. Even more, they could internalize into the target cell. Unlike antibodies, aptamers have low immunogenicity and they are easily synthesized and modified. Furthermore, the smaller size of aptamers facilitates better tissue penetration what improves their clinical applicability. B-cell leukemia represents 80% of ALL cases, however, there is still no aptamer against B-ALL cells in the literature. Therefore, we aimed to select the first aptamer that specifically target and internalize into B-ALL cells, without binding normal blood cells. To achieve this goal, we adapted the cell-internalization SELEX method for suspension cell lines. In this new protocol, the starting RNA aptamer library was incubated with nontarget cells (Ea.Hy926 (human endothelial cells), JURKAT (T-ALL) and peripheral white blood cells of healthy patients). Those RNAs that do not bind or internalize into the nontarget cells were then transferred to the target cells: B-ALL cells (KOPN-8 and NALM-16). Unbound and surface-bound RNAs were washed off and only internalized sequences were recovered from the cancer cells. The recovered RNAs are amplified using RT-PCR and the resulting amplified dsDNA in vitro transcribed for the subsequent round of selection. After a total of 8 selection rounds, we include NGS (Next Generation Sequencing) technology for the bioinformatic analysis of the aptamers obtained. Subsequently, the aptamers found were tested individually for internalization to find the best candidate to be used as a delivery tool. Thus, these novel results could be useful for aptamer-mediated drug delivery that could provide a new therapeutic tool for this disease.

Keywords: Acute lymphoblastic leukemia, cell-SELEX, aptamers.

(856) **CO-EXPRESSED GENES PROFILE IN HUMAN METASTATIC MELANOMA CELLS ASSOCIATED WITH RADIORESISTANCE AND MALIGNANCY**

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Melanomas are highly metastatic and though immunotherapy and targeted therapies have led to improvements in patient outcomes, the advanced disease has still a 5 year overall survival of less than 10%. Although melanoma is resistant, radiotherapy is usually used with systemic therapy for symptomatic metastases and as adjuvant therapy. In order to explore gene profiles associated with radioresistance and malignancy in melanoma, the aim of this study was evaluate gene sets involved in the intrinsic radiation response of melanoma cells with different degrees of malignancy but with the same genetic background (A375 and PCDNA3, non-invasive controls; G10, invasive and metastatic and A7, less aggressive). Bioin-

formatic analyses included expression microarrays data (Affymetrix) from these cells and a priori defined gene sets associated with DNA damage response (DDR) and invasion collected from KEGG and bibliography, to evaluate significant differences (FDR<25%) in gene co-expression among cells by GSEA. G10, unlike A7 and controls, down-regulated 44 co-expressed genes associated with DDR while up-regulated genes associated with invasive melanomas. Thus, radiosensitivity was determined by clonogenic assay after irradiating the cells with a ^{137}Cs gamma source. Curiously, G10 was more radioresistant ($p<0.01$) than A7 and controls, demonstrated by surviving fraction at 2 Gy and α parameter of survival curves fitted to the linear-quadratic model. Double-strand breaks signaling and repair were assessed by the detection of phosphorylated H2AX histone (γ-H2AX) and real time PCR of MRE11A gene. Induction of γ-H2AX foci ($p<0.001$) and expression of MRE11A ($p<0.01$) were lower in G10 vs A7 and control, both at basal level and post-irradiation. In conclusion, the G10 gene expression profile observed suggests that these cells could escape from DNA damage-induced apoptosis with the consequent progression in the cell cycle resulting in genomic instability and increase of malignancy.

Keywords: malignant melanoma, bioinformatics, ioinizing radiation response, resistance

(809) **GENOTYPING OF 22 GENES USING TARGETED NEXT-GENERATION SEQUENCING (NGS) IN NON-SMALL CELL LUNG CANCER (NSCLC)**

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Background: Lung cancer is the leading cause of cancer death worldwide. Understanding the molecular characteristic of NSCLC has allowed for the development of targeted therapy, which nowadays has become standard treatment. However, the prevalence of driver gene mutations remains unknown in Argentine population, except for EGFR, ALK, and KRAS.

Objective: -To assess the prevalence of mutations in 22 genes in NSCLC of Argentine population.

Material and Methods: Prospective observational study, approved by CEMIC's Ethics Committee.

Materials consisted of formalin-fixed paraffin-embedded (FFPE) tissue from 45 patients with NSCLC examined between 2011-2016. Patients belonged to all cancer stages, and had appropriate clinical follow-up.

Genotyping: Tumor was microdissected and DNA was extracted applying the columns method (QIAmp®-DNA-FFPE-Tissue (Quiagen #55404)). DNA quality and quantity were checked with spectrophotometry. Twenty-two genes were sequenced using targeted NGS, with Colon and Lung Cancer Research Panel v2 (Ion Torrent™ AmpliSeq™ technology) on Ion S5 Next Generation Sequencing System. The obtained sequences were analyzed on Ion Reporter™ Software 5.2.1.

Results: Ninety-two amplicons were obtained; depth of the minimum reading was 500x on 95% amplicons. Two samples were inadequate for testing.

Patients' age, gender, smoking status, histological type and disease stage were recorded.

Mutations found: TP53 was the most frequent mutation found on 21 cases (49%), followed by KRAS (47%), EGFR (21%), BRAF (12%), NOTCH1 (7%), MET (5%), ERBB2 (5%), ALK (5%), PIK3CA (5%), and STK11 (2%). Conclusions Our results are concordant to published data. Interestingly, EGFR mutations were more frequent than on previous studies carried out in our institutions. BRAF mutations were more common in comparison to published data. Of all 43 patients, 10 harbored driver mutations, which have target therapies on ongoing clinical trials (cMET, BRAF, ERBB2 y PIK3CA).

Keywords: non-small cell lung carcinoma, next generation sequencing, driver genes, mutations.

(1016) **IDENTIFICATION OF GERMLINE VARIANTS IN CANCER SUSCEPTIBILITY GENES IN PATIENTS WITH HEREDITARY BREAST CANCER SYNDROME BY MASSIVE PARALLEL SEQUENCING IN ARGENTINEAN POPULATION**

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Breast cancer is the most frequent cancer and ovarian cancer the seventh most frequent cancer among females worldwide, representing approximately 25% and 4% of all cancers, respectively. In Argentina, breast cancer and Ovarian cancer cause 6163 and 1332 deaths per year, respectively. The two major susceptibility genes for both diseases are *BRCA1* and *BRCA2*, and several other susceptibility genes have been identified. However, in the majority of high-risk breast and/or ovarian cancer (HBOC) families, the genetic predisposition factors remain unidentified, making the genetic counseling of these families challenging. The aim of the current study is to utilize an NGS approach to identify genetic factors that predispose individuals to hereditary breast and/or ovarian cancer (HBOC) in the high-risk Argentinian *BRCA1/2* mutation-negative HBOC patients. 14 patients were analyzed, the DNA was extracted from a blood samples and was subjected to a targeted massively parallel sequencing platform comprising 141 cancer predisposition genes. We detected in these patient 6 pathogenic variants in *SBDS*, *FANCA*, *MC1R*, *WRN*, *MSR1* and *SDHD* genes. The effect of these variants are of type: non-synonymous (67%) and stop codon gains (33%). We also found 6 variants of uncertain significance (VUS) in *APC*, *RAD50*, *SDHB*, *FANCD2*, *MLH1* and *CDH1* genes. In conclusion, the identification of new variants could help to discover new genes associated to HBOC in the population of Argentina. These findings provide an excellent premise for further studies and could be utilized in the design of more efficient clinical management strategies for HBOC.

Keywords: NGS, breast, cancer, genes, susceptibility.

(620) **LINE1 METHYLATION AND VARIATION IN MITOCHONDRIAL GENOME IN A SAMPLE OF CANCER PATIENTS FROM ARGENTINA**

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Abstract: DNA methylation is a crucial epigenetic element involved in gene regulation and genomic stability. LINE-1 methylation is widely accepted as surrogate marker for global genomic DNA methylation, being hypomethylation a frequent event in cancer. Furthermore, nowadays it is suspected that mitochondrial DNA copy number variation (mtDNA-CN) is related to methylation changes on nuclear gene promoters. As a preliminary approach, we conducted a case-control study on a set of DNA samples derived from 171 controls and 212 cancer patients, all from Argentina: 75 patients with onco-hematological diseases (Hem), 65 with breast cancer (BrCa) and 72 with colorectal carcinomas (CRC). Blood samples were available in all cases, and for solid tumors paired tumoral/non-tumoral adjacent tissues (T/N), were available too. LINE-1 methylation level was analyzed by MS-MLPA method using a custom-made kit. mtDNA-CN were determined by qPCR. In BrCa, we

found a significant mtDNA-CN decrease in 63% of the samples by comparing Tvs.N tissue (average decrease=24%, p<0.01). However, no differences were seen between blood samples in controls vs. Hem, BrCa or CRC cases (p=0.134, 0.142 and 0.139, respectively). For LINE-1 marker, the results did not reach the level of statistical significance when comparing tissues from BrCa patients. However, we found a statistically significant difference (p<0.001) between N and T tissues for CRC samples (mean 0.88 and 0.81, respectively). Furthermore, when grouping CRC samples by age, cut-off 50 years, LINE-1 methylation levels still reveal significant differences for mean values (<50y: N=0.869, T=0.798; and >50y: N=0.882, T=0.827). In this cohort of samples, no correlation was seen between global methylation levels and mtDNA-CN, neither in controls nor in tumor samples; more studies are necessary to confirm this result. Overall, our results confirm that LINE-1 hypomethylation is a characteristic feature in CRC but not very common in other tumors.

Keywords: DNA methylation; mtDNA copy number; cancer; Argentina; epigenetic.

(1433) **GENOTYPE - PHENOTYPE CORRELATION IN TWO CASES OF UNILATERAL RETINOBLASTOMA**

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Retinoblastoma (RB) is a hereditary cancer of childhood caused by mutations in *RB1* tumor suppressor gene. An early diagnosis is critical for survival and eye preservation, thus identification of mutations is important for unequivocal diagnosis and risk assessment in relatives. Unilateral RB is non-hereditary in 80% of cases, while approximately 20% of unilateral RB is hereditary. These patients have several risks: bilateralization of retinoblastoma, development of other tumors in adulthood and transmission of the RB predisposition to their offspring. The purpose of this work was to identify the causative mutations in two unilateral RB patients with different clinical presentations. One of them (#686) was diagnosed at 1 month and the other (#689) at 4 years, both were enucleated. DNA was extracted from blood and tumor and analyzed by sequence and MLPA assays. Analysis of tumor DNA identified mutations in both *RB1* copies in the two patients, which were thereafter sought in blood. Tumor DNA of patient #686 displayed a T duplication in exon 14, which was also present in blood DNA; thus, this was the first *RB1* mutation and it was germline. The second mutation in tumor (the somatic one) was a deletion of *RB1* exons 1 and 2 plus a centromeric gene (*ITM2B5*). Both were frameshift novel mutations Tumor DNA of patient #689 showed an A duplication in exon 2 as a first frameshift *RB1* mutation and a loss of a wild type chromosome 13 (without reduplication of the mutant chromosome) as a second mutation. None of the two mutations were found in blood, thus, they were somatic. These results reveal a hereditary nature of mutations in the patient #686, with an early diagnosis, and a nonhereditary nature of mutations in patient #689, with a late diagnosis. These data are significant for genetic counseling and support the relevance of carrying out complete genetic screening for *RB1* mutations in both constitutional and tumor tissues.

Keywords: Retinoblastoma, *RB1* tumor suppressor gene, *RB1* mutations, Genotype-Phenotype correlation.

GENETICS AND MOLECULAR BIOLOGY 12

(1596) **EXPRESSION OF INTERLEUKIN 1 BETA (IL-1B), IN PATIENTS WITH TYPE 2 DIABETES (DM2) SUBJECT TO PROTOCOLS OF METABOLIC COMPENSATION.**

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We studied the expression of IL-1β, the genotype of a promoter SNP, and the relationship with biochemical-clinical variables; in individuals with DM2 undergoing metabolic compensation treatment. We studied 30 patients with metabolically decompensated DM2 (HbA1c> 8%), and after 6 and 12 months of compensatory treatment (HbA1c <7%). Protein expression of IL-1β was determined in serum by chemiluminescence. Relative quantification of IL-1β mRNA was performed by qPCR by the $2^{-\Delta\Delta Ct}$ method with GADPH as the internal control. The SNP rs16944 of IL-1β (-511C/T) was genotyped by the KASP method. Statistical analysis was performed by ANOVA for paired data and linear regression corrected for age and sex, in SPSS v20.0, with a significance level of 0.05.

Prior to treatment, individuals had higher IL-1β protein expression at higher blood glucose ranges (p = 0.015). The CC genotype at rs16944 was associated with increased IL-1β mRNA expression (p = 0.006).

All Individuals achieved metabolic compensation after treatment, demonstrated by a significant decrease in HbA1c (p <0.001). Those individuals who showed an increase in IL-1β protein expression after treatment, showed the highest decreases in HbA1c (6 months: p = 0.021; 12 months: p = 0.004) and glycemia (12 months: p = 0.040).

Expression of IL-1β depends on various factors such as SNPs and glycemia levels. We demonstrate that IL-1β expression is higher at higher glycemia levels, even in hyperglycemia states. Higher decreases in HbA1c and glycemia were associated with increased IL-1β expression, which could be related to an improvement in translation efficiency at the endoplasmic reticulum level.

Keyworlds: Interleukin-1 Beta, Diabetes mellitus type 2, Gene expression, Polymorphisms.

(893) **COMPLEXIN 2 IS NECESSARY FOR EFFICIENT GLUT4 EXOCYTOSIS IN L6 CELLS.**

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Abstract: The glucose transporter type 4 (GLUT4) is the insulin-regulated glucose transport expressed mainly in striated muscle and adipose tissues. It is well known that insulin mediates the glucose uptake by GLUT4 traffic from intracellular storage (GSVs/IRVs) to the PM, a process also known as "GLUT4 translocation" or "GLUT4 exocytosis". Similarly, muscle contraction also increases the GLUT4-exocytosis. Glucose transport is a rate limiting process for glucose utilization in adipose and muscle tissues, and it is deficient in type 2 diabetes. Intracellular membrane fusions are carried out by canonical SNAREs proteins, which are constitutively active and require a large set of regulatory proteins. In this regard, the calcium sensor synaptotagmin and complexin play a key role in calcium regulated exocytosis in neuroendocrine cells and spermatozoa. Specifically, the t-SNAREs, sintaxin-4 and SNAP23, and the v-SNARE Vamp2 are the main SNAREs in GLUT4-exocytosis. It is well established that this process requires calcium and calcium sensors (ESYT1 and Doc2B), however, there are non-evidences about the presences and participation of complexin during this process in muscle cells. Therefore, the purpose of this work was to study the presences of complexin in muscle cells and its participation in GLUT4 exocytosis. Using approaches like western-blot, immunofluorescence and qPCR we were able to show the presence of complexin in L6-muscle cells and skeletal muscle tissue, being complexin II the most abundant isoform. Moreover, using specific complexins siRNA, we knocked-down the protein expression in L6 cells expressing GLUT4 with a myc tag. Using these cells, we setted up a GLUT4 translocation assay and we observed a deficient GLUT4-exocytosis in complexin-KD cells, compared with control cells. Therefore, this is the first study which evidence of complexin II expression in tissue and cells muscle, showing, furthermore, that complexin II is required for GLUT4 exocytosis in L6 cells.

Keywords: GLUT4 translocation, membrane fusion, SNAREs, muscle.

(901) **AGGREGATED LDL AFFECTS THE REGULATORY ACTION OF LRP1 ON THE INSULIN-DEPENDENT INTRACELLULAR SIGNALING AND GLUT4 TRAFFICKING IN HL-1 CARDIOMYOCYTES**

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Diabetes mellitus (DM) is a metabolic disorder mainly characterized by hyperglycemia, dyslipidemia and hyperinsulinemia, and cardiovascular disease (CVD) is the main complication of DM. DM patients usually have increased levels of atherogenic subfractions of low-density lipoproteins (LDL), such as aggregated LDL (agLDL). This latter lipoprotein is responsible to induce anomalous intracellular lipid accumulation in cardiomyocytes in a low-density lipoprotein receptor-related protein-1 (LRP1)-dependent manner. LRP1 is an endocytic and signaling receptor, belonging to the LDL receptor family. Moreover, LRP1 regulates the intracellular trafficking of membrane proteins and vesicles, including insulin receptor (IR), GLUT4 and GSVs (GLUT4 storage vesicles). Thus, it has been suggested that a functional deficiency of LRP1 would be directly associated with insulin resistance disorders. In the present work, we evaluate whether agLDL via its interaction with LRP1 may affect the insulin-induced intracellular signaling and GLUT4 trafficking to the plasma membrane (PM) in HL-1 cardiomyocytes. By quantitative PCR and Western blot analysis we determined that LRP1 and GLUT4 expression were unaffected after agLDL treatment for 8 h at 37 °C. By confocal microscopy we found a substantial modification of the intracellular localization of LRP1 and GLUT4 in agLDL-stimulated cells. Also, after insulin stimulus (100 nM) in agLDL-preincubated cells, a decreased level of Akt and AS160 phosphorylation was observed compared to unpreincubated cells. By immunoprecipitation we found that agLDL-preincubated cells showed a weakly molecular association between IR and LRP1 with respect to control conditions. Finally, by biotin-labeling protein assay we demonstrated that agLDL reduced the GLUT4 translocation to the plasma membrane in insulin-stimulated cells. Thus, we conclude that LRP1 is a key regulator of insulin response and glucose metabolism in cardiomyocytes, being its action affected by agLDL.

Palabras Clave: endocytosis, atherosclerosis, metabolism, heart.

(1036) **ALPHA-1-ANTI-TRYPSIN INCREASED NA^+/K^+-ATPASE AND CX43 EXPRESSION IN AN *IN VITRO* DIABETIC RETINOPATHY MODEL**

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Purpose: The ophthalmic therapy for diabetic retinopathy is focused on severe stages of the disease. Previous results obtained in our group show that Alpha-1-Anti-Trypsin (A1AT) acts like an anti-inflammatory agent that could play a role on diabetic retinopathy treatment. It is important to evaluate A1AT impact on cellular components that are essential to retina function like Na+/K+-ATPase (NKA). This protein is involved in synaptic activity and action potentials in this tissue. It is known that NKA activity and expression is diminished in diabetic retinopathy. Cx43, part of gap junction channels, play an essential role for maintenance of retinal homeostasis. High glucose or diabetes has been shown to reduce Cx43 expression in retinal cells.

A1AT may stimulate NKA and Cx43 expression through different cellular mechanisms. For this reason, we aimed at evaluating NKA and Cx43 with A1AT treatment in an in vitro diabetic retinopathy cell model.

Methods: Mouse retinas were obtained from freshly enucleated eyes incubated with collagenase I and Trypsin. Retinas were desegregated and incubated with DMEM for 5 days to allow the enrichment of Müller cells population. Müller cells obtained, were incubated 24h with DMEM 30mM glucose (Control), DMEM 30mM glucose + 4.5mg/ml A1AT (Control + A1AT), DMEM 100mM glucose (Diabetic), DMEM 100mM glucose + 4.5mg/ml A1AT (Diabetic + A1AT). Cells were harvested with RIPA buffer for Western Blot Assay or Fixed for Immunohistochemistry.

Results: Alpha subunit of Na^+/K^+-ATPase and Cx43 expression were increased in A1AT treated cells. NKA: Diabetic (46.0±4.3)%, Diabetic+A1AT(69.9±17.7)%, $P<0.05$; Cx43: Diabetic (75.3±3.7)%, Diabetic+A1AT(98.6±1.2)%, $P<0.01$.

Conclusions: Results support the hypothesis that A1AT promotes Na+/K+-ATPase and Cx43 expression. This is a novel aspect about Na+/K+-ATPase and Cx43 expression modulation. Although molecular mechanisms involved remained unknown, A1AT might play a new role in diabetic retinopathy treatment.

Keywords: Alpha-1-Anti-Trypsin, Na+/K+-ATPase, Cx43, Müller cells, diabetic retinopathy

(1085) **RESVERATROL, LIPID METABOLISM AND CELL SURVIVAL**

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Resveratrol (trans-3,4′,5-trihydroxystilbene, Rsv) is a small polyphenol molecule present in a large variety of plant species such as mulberries, peanuts and grapes. Rsv was largely utilized by Asian medicine for treatment of fungal, inflammatory, hypertensive, allergic, cancer and lipid diseases. Nowadays it is used in “natural medicine” and widely consumed by public as dietary supplementation due to its antioxidant properties. Despite various molecular targets have been proposed, AMP-activated protein kinase (AMPK) and NAD^+-dependent histone deacetylase sirtuin 1 (SIRT1) are considered as the main effectors for Rsv actions. Rsv-induced SIRT1 activation would mediate anti-inflammatory by downregulation of NF-kB and COX2 expression and activity. Rsv-induced AMPK would decrease lipogenic genes expression. We have previously shown that both, COX2 expression and activity and remodeling of cell membranes are key factors for adaptation and survival of renal cells subjected to abrupt changes of environmental osmolality. In the present work, we evaluated whether Rsv affects such survival pathways. To do this, lipid profile and synthesis, determined by incorporation of [U-^{14}C]-glycerol to lipid molecules, and COX2 expression were evaluated in cultures of the renal epithelial cell line MDCK incubated in NaCl-hyperosmotic media (~550 mOsm) without or with 100 μM Rsv for 0, 6, 12, 24 48 and 72 h. After treatment, cells were collected, counted and viability determined. Lipids were extracted by Bligh-Dyer and separated by TLC. COX2 expression was assessed by western blot. Both lipid synthesis and COX2 expression were induced after 24, 48 and 72 h of hyperosmolar treatment, and both processes were harmed by Rsv from early time of treatment. Cell number recovered after Rsv was significant lower. These preliminary results suggest that in renal cells Rsv abolished protective mechanism against osmotic stress.

Keywords: resveratrol; osmoprotection; renal cells.

(1408) **ALTERING MICROTUBULE POLYMERIZATION IN SERTOLI CELLS STRONGLY AFFECTS THEIR LIPID METABOLISM.**

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The aim of this work was to study the biological consequences of altering microtubule polymerization in Sertoli cells, with a special focus on their lipid metabolism. For this purpose, the effect of long-term exposures to nocodazole (NCZ) (0.01 μM) was studied on TM4 cells in culture. In addition to the expected effect of NCZ on the depolymerization of tubulin microtubules, a remarkable reorganization was observed in the intermediate filament network (vimentin), which

showed a more peripheral distribution than in untreated cells. As compared to cells cultured in control conditions, those exposed to NCZ displayed an increased mitochondrial membrane depolarization ($p<0.001$). Their plasma membrane integrity was concomitantly altered, as shown by a considerable increase in the leakage of lactate dehydrogenase ($p<0.01$) released into the culture medium. In addition, the cell proliferation was significantly reduced ($p<0.003$) in NCZ-exposed cells. That the drug induced a major alteration on lipid biosynthesis was clearly apparent from a significant increase in triacylglycerol (TAG) levels ($p<0.0002$) and a massive accumulation of lipid droplets in the cytoplasm of cells. This observation was sustained by following the incorporation and distribution of labeled arachidonic acid ([^{3}H]AA). After 1 hour of incubation, the most highly [^{3}H]-labeled lipid class were TAG in control cells, but choline glycerophospholipids in drug-treated cells. Interestingly 72 hours later, the percentages of label in TAG and cholesterol esters were about twice higher in NCZ-treated than in control cells. We conclude that the cytoskeletal alterations that Sertoli cells undergo after being exposed to NCZ, through their impact on mitochondrial functions and cytoplasmic architecture, are closely involved in the deranged lipid metabolism induced by the drug.

Keywords: microtubules; nocodazole; lipid droplets

(1430) **COORDINATION BETWEEN SPHINGOLIPIDS AND PROTEINS REQUIRED FOR PRIMARY CILIOGENESIS**

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The final stage in the process of epithelial cell differentiation is the outgrowth of a primary cilium from the apical surface. We have previously described that extracellular hypertonicity induces the differentiation of MDCK cells by the regulation of the sphingolipid metabolism. The aim of this work was to investigate the ciliogenesis induced by hypertonicity in MDCK cells and the role of glycosphingolipids in the process. Confluent MDCK cells were cultured under isotonicity (150 mM NaCl, control) or hypertonicity (300 mM NaCl) for 48 h in the presence or absence of D-PDMP, a glucosylceramide synthase inhibitor. The presence of primary cilium was detected in MDCK cells stable expressing α-tubulin–GFP subjected to hypertonicity. For the immunofluorescence assays, cells were stained with acetylated tubulin to visualize primary cilium, and co-stained with IFT20 or Rab8, proteins required for vesicle-mediated trafficking pathway from the Golgi to the base of the cilium. Isotonicity-cultured cells did not show cilium formation and IFT20 and Rab8 appeared diffusely distributed. Cells cultured under hypertonicity displayed primary cilium and the number of ciliated cells significantly decreased when the cells were subjected to hypertonicity in the presence of D-PDMP. Cells cultured under hypertonicity showed IFT20 with a Golgi – like distribution and perinuclear Rab8, but when cells were treated with D-PDMP the distribution of these proteins was diffuse. A proteomic analysis performed by mass spectrometry of IFT20+ vesicles immune-isolated from cells cultured under hypertonicity revealed the presence of galectin 3, a protein with an important role in apical sorting that associates with the primary cilium. In conjunction, these results suggest that hypertonicity induces ciliogenesis by displaying a protein machinery that is correctly assembled in the presence of glycosphingolipids.

Keywords: primary cilium, cell differentiation, renal epithelial cells, sphingolipids

(1246) **14-3-3γ AND β HAVE OPPOSITE ROLES IN ADIPOGENIC DIFFERENTIATION**

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The 14-3-3 protein family regulates several cellular processes including differentiation. Regarding adipogenic differentiation, this family binds to regulatory proteins involved in adipogenesis as TAZ, PPARγ2, Lipin-1 and Seipin. 14-3-3 proteins bind their targets through specific phospho-Serine/Threonine residues. Among the seven highly conserved mammalian family members, specifically 14-3-3γ and β mRNA and protein levels increase during adipogenic differentiation of 3T3-L1 preadipocytes. To investigate the role of these paralogs in adipogenesis, we decreased their levels by using lentiviral transduction particles containing short hairpin RNAs (shRNAs) specific to silence the expression of both of these paralogs, and the ZsGreen protein as a marker of transduced cells. 3T3-L1 cells were infected with these vectors, and then induced to the adipogenic linage during 7 days. Adipogenic differentiation was evaluated by Oil Red O staining of lipid droplets followed by confocal microscopy analysis. The results revealed an increment of number and size of lipids droplets after 14-3-3γ silencing compared to neighboring cells that were not infected. The opposite effect was observed upon 14-3-3β silencing, where a pronounced decrease in lipid droplets number and sizes was evident in transduced cells. Taken together our findings suggest that 14-3-3γ and β differentially regulate 3T3-L1 adipogenesis.

Keywords: 14-3-3, 3T3-L1, adipogenesis, differentiation.

(1175) **IDENTIFICATION OF PROTEIN TYROSINE PHOSPHATASE 1B (PTP1B) INTERACTORS INVOLVED IN CELL MOTILITY**

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PTP1B is an ER-bound protein tyrosine phosphatase implicated in the dynamics of cell-matrix adhesions. This regulation relates to a positive role of PTP1B in directional cell migration and attenuation of cell contractility. The aim of the current study is to identify and characterize potential substrates and interactors of PTP1B involved in cell motility. To this end we used a Bimolecular Fluorescence Complementation (BiFC) approach for direct visualization and analysis of PTP1B interactors in intact cells. BiFC is based on complementation and restoration of fluorescence when two non-fluorescent fragments of a native fluorescent protein are a few nanometers apart. These fragments were fused to PTP1B and the potential substrates/interactors, chosen on the basis of previous phosphoproteomic data and their relevance in cell motility. We observed BiFC between PTP1B and epidermal growth factor receptor (EGFR), the scaffold protein Mena and the focal adhesion tyrosine kinase FAK. PTP1B/EGFR interaction was revealed as puncta with increasing size and density in internal locations compared to the cell periphery. The colocalization with cell-matrix markers was not evident. In contrast, PTP1B/FAK and PTP1B/Mena interactions were prominent at peripheral adhesions. FAK BiFC fluorescent signal was attenuated when using a FAK mutant (Y4-9F-FAK) in which tyrosine phosphorylation sites Tyr 407, 576, 577, 861, and 925 were mutated to phenylalanine. The role of individual mutations on FAK, as well as on putative interacting motifs on EGFR and Mena are currently investigated. Our results suggest a dual role of PTP1B on integrin and EGFR signaling driving the cell motility machinery. Supported by CONICET and ANPCyT.

Keywords: Adhesion, Motility; Ptp1b; Bifc

IMMUNOLOGY (INNATE IMMUNITY) 4

(48) **HISTOLOGICAL STUDY OF THE ADVENTITIAL LAYER OF FERTILE, INFERTILE AND SMALL HYDATID CYSTS REVEALS HALLMARK INFLAMMATORY FEATURES**

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Introduction: Cystic Echinococosis (CE), a worldwide-distributed zoonosis caused by the cestode Echinococcus granulosus, is an endemic disease in Chile in both humans and animals, with cattle being the most affected of the intermediate hosts. CE can manifest as either fertile or infertile cysts, the latter unable to continue the biological cycle of the parasite. Small hydatid cysts (smaller than 1 cm in diameter) usually don't fall in neither category, because they are still developing. The following work studies the adventitial layer of bovine hydatid cysts, to better understand the host-parasite interplay.

Material and Methods: Through routine abattoir visits, fertile, infertile and small hydatid cysts samples were collected, all of which were paraffin embedded, cut in 5 µm thick sections and stained with hematoxylin-eosin. The adventitial layer was examined by a seasoned pathologist

Results: Fertile hydatid cysts present an adventitial layer consisting mainly of collagen fibers and fibroblast whereas infertile and small hydatid cysts have hallmark inflammatory features: palisading foamy macrophages in direct contact with parasite tissue, lymphocyte germinal centers and giant multinucleated cells.

Discussion: Small cysts presented similar inflammatory features with infertile cysts, and no small cysts presented histological features of the adventitial layer of fertile cysts. These findings support the idea that both small and infertile hydatid cysts may be the result of the bovine immune system attack on the metacestode.

Keywords: Echinococcus granulosus, immunohistochemistry, hydatid cyst fertility

(90) **HEME-OXYGENASE-1 EXPRESSION CONTRIBUTES TO THE IMMUNOREGULATION INDUCED BY *Fasciola hepatica* AND PROMOTES INFECTION**

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Fasciola hepatica is a trematode that infects livestock and humans causing fasciolosis, a zoonotic disease of increasing importance due to its worldwide distribution and high economic losses. This parasite immunoregulates the host immune system by inducing a strong Th2 and regulatory T immune response by immunomodulating dendritic cell (DC) maturation and alternative activation of macrophages. The objective of this work is to show that *F. hepatica* infection in mice induces the upregulation of heme-oxygenase-1 (HO-1), the rate-limiting enzyme in the catabolism of free heme that regulates the host inflammatory response. We show and characterize two different populations of antigen presenting cells that express HO-1 during infection in the peritoneum of mice infected orally with 10 *F. hepatica* metacercariae. Cells that expressed high levels of HO-1 expressed intermediate levels of F4/80 but high expression of CD11c, CD38, TGFβ and IL-10 suggesting that they correspond to regulatory DCs. On the other hand, cells expressing intermediate levels of HO-1 expressed high levels of F4/80, CD68, Ly6C and FIZZ-1 indicating that they might correspond to alternatively activated macrophages. Furthermore, the pharmacological induction of HO-1 with the synthetic metalloporphyrin CoPP promoted *F. hepatica* infection increasing the clinical signs associated with the disease. In contrast, treatment with the HO-1 activity inhibitor SnPP protected mice from parasite infection, indicating that HO-1 plays an essential role during *F. hepatica* infection. Finally, HO-1 expression during *F. hepatica* infection was associated with TGFβ and IL-10 levels in liver and peritoneum, suggesting that HO-1 controls the expression of these immunoregulatory cytokines during infection favoring parasite survival in the host. These results contribute to the elucidation of the immunoregulatory mechanisms induced by *F. hepatica* in the host and provide alternative checkpoints to control fasciolosis.

Keywords: Helminth, heme-oxigenase-1, immune regulation, dendritic cell, macrophage

(208) **IMMUNOREGULATORY ROLE OF NLRP3 INFLAMMASOME IN A MODEL OF CORONAVIRUS INFECTION**

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Inflamasomes are multiprotein complexes that induce IL1b release. They play an important role in antiviral immunity, since IL1b is crucial for establish an appropriate CD8 T cells immune response. However, the role of NLRP3 activation in coronavirus infection has not been elucidated.

Objective: To characterize the relation between inflammasome activity and antiviral immunity in a model of viral hepatitis.

We performed in vitro and in vivo inflammasome activation assays. We developed survival analysis, WB, ELISA and flow cytometry (FC) assays to determine immunological, biochemical and survival parameters of the infected WT and Casp-1 KO mice. In addition, our group has characterized a novel modulator of NLRP3 activity, the cationic channel Tmem176b, therefore we have also studied infection on Tmem176b KO mice.

Our results have shown that infected BMDCs secrete IL1b in a Casp-1 and NLRP3-dependent manner ($p \leq 0,01$). As well, infected Tmem176b KO BMDCs produce more IL1b ($p \leq 0,01$) and Casp-1 than WT cells ($p \leq 0,001$). In vivo studies have shown that infected Tmem176b KO mice are vulnerable to MHV, as they have lower survival rates ($p \leq 0,01$), higher expression of IL-1b and Casp-1 ($p \leq 0,05$), and elevated viral load ($p \leq 0,05$) than WT mice. Tmem176b KO animals expand their survival ($p \leq 0,05$), after IL1b blockade, in a CD8 T cells dependent manner. On the other hand, Casp-1 KO mice are resistant to infection and present lower levels of viral RNA ($p \leq 0,05$). These differences were also characterized as T CD8 cells-dependent by in vivo depletion assays. Finally, by FC analysis we determined that MHV-specific CD8 T cells from susceptible mice (Tmem176b KO and Casp-1 WT) have high expression of PD-1.

Our results suggest that, in coronavirus infection, IL1b antagonizes the antiviral immune response by the functional inactivation of CD8 T cells through PD-1. Understanding this interaction is an important step in the development of new therapeutic strategies.

Keywords: inflammasome-coronavirus-IL1beta-PD-1

(383) **IMMUNOMODULATORY MECHANISMS EXERTED BY PROBIOTIC BACTERIA IN THE INTESTINAL MUCOSA IMMUNE SYSTEM**

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Probiotics bacteria can interact with mucosal immune cells or epithelial cells lining the mucosa to modulate specific functions of the mucosal immune system. Here, we aimed to study the differences in the mechanisms exerted by probiotic bacteria and its cell wall in the gut mucosa, compared with an enteropathogenic bacteria. Six-

wk-old BALB/c mice received in the drinking water a suspension of viable *Lactobacillus casei* CRL431 (Lc431) (10^8 CFU/mL) or by gavage its cell wall (CW431) (100 mL/day) for 7 days, d8 animals were sacrificed. One dose of *Salmonella* Typhimurium (10^8 CFU/mL) were performed on 6-wk-old BALB/c mice, after 72 h animals were sacrificed. Relative fold change gene expression (a20, irak-m, mkp-1, and tollip) were determined on intestinal epithelial cells (IEC) and on peritoneal macrophages (pMf) by RT-qPCR. The percentages of Treg and Th17 cells were analyzed on the lamina propria of the gut by flow cytometry. Lc431 and its CW431 were able to increase significantly the regulatory genes expression of NF-kB and MAPK pathways on IEC and pMf compared to *Salmonella* ($p<0.05$). At the same time, Lc431 and its CW431 increased the percentages of CD4+ CD25+ Foxp3+ IL-10+ cells in the lamina propria of the gut compared to untreated and *Salmonella* mice ($p<0.05$). However, there were not differences on CD4+ IL-17+ cells percentages, in all conditions evaluated ($p<0.05$). Previously, we demonstrated that probiotics active the NF-kB pathway, however probiotics are not an inflammatory stimulus to the gut mucosa. In this sense, the present results suggest that probiotic up-regulates the regulatory proteins of NF-kB and MAPK pathways modulating the immune response on IEC and pMf, in contrast to *Salmonella* infection. In this scenario, IEC could create the cytokine environment that favor the balance to Treg profile in the gut lamina propria. These results would demonstrate the way by which probiotics are able to modulate the gut mucosal immune system.

Keywords: probiotic, probiotic cell wall, regulatory proteins, immunomodulation, gut homeostasis

(443) IL-10 DEFICIENCY MODULATES INFLAMMATORY RESPONSE AND ANTI-INFLAMMATORY MEDIATORS IN A MURINE MODEL OF HEMOLYTIC UREMIC SYNDROME (HUS)

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Hemolytic Uremic Syndrome is a disease triggered by Shiga toxin (Stx) characterized by hemolytic anemia, thrombocytopenia and renal failure. The concomitant inflammatory response mediated mainly by neutrophils (PMN) is essential to HUS.

Previously we demonstrated that mice lacking IL-10 (IL-$10^{-/-}$) had a higher survival after Stx2 and a delayed neutrophilia compared to control mice (wt). The aim of this work was to determine the mechanisms involved in IL-$10^{-/-}$ protection against Stx2.

Before and 3h, 24h, 48h and 72h after administration of $1LD_{100}$ of Stx2 e.v, IL-$10^{-/-}$ and wt mice were sacrificed. Plasma was collected to evaluate creatinine as an indicator of renal damage and corticosterone since IL-10 regulates glucocorticoid synthesis at the level of the adrenal gland. Peripheral PMN were detected as $Ly6G^+CD11b^+$ cells and CXCR2 and CD62L expression was analyzed by FACS.

Even though creatinine levels (mg/dl) were increased at 72h in both strains, IL-$10^{-/-}$ showed reduced levels comparted to wt suggesting reduced renal damage (Wt_{0h}: 0.5±0.1; Wt_{72h}: 1,4±0,1*; IL-$10^{-/-}_{0h}$: 0.4±0.1; IL-$10^{-/-}_{72h}$: 0.9±0.1*#, *<p0.05 vs 0h, #between strains, n=10 per group). On the other hand, corticosterone levels (ng/ml) were only increased in IL-$10^{-/-}$ mice 3h after Stx2 (IL-$10^{-/-}_{0h}$: 24,1±10,0; IL-$10^{-/-}_{3h}$: 337,9±56,0*# , *<p0.05 vs 0h, #between strains, n=5 per group).

Although there is a delayed neutrophilia in IL-$10^{-/-}$ mice (PMNx10^5/ml: Wt_{0h}: 11.5±0.1; Wt_{48h}: 28.2±0.2*; Wt_{72h}: 28.9±0.2*; IL-$10^{-/-}_{0h}$: 14.7±0.1; IL-$10^{-/-}_{48h}$: 20.8±0.3#, IL-$10^{-/-}_{72h}$: 25.1±0.2*, *<p0.05 vs 0h, #between strains, n=20 per group), there were no differences in CD62L and CXCR2 expression between strains after Stx2 treatment.

This work show that Stx2 protection in IL-$10^{-/-}$ mice is associated with reduced of renal damage and endogenous glucocorticoid circulating levels, the most renowned anti-inflammatory factors.

Keywords: HEMOLYTIC UREMIC SYNDROME, IL-10 DEFICIENCY, INFLAMMATION, RENAL DAMAGE, GLUCOCORTICOIDS.

(495) IMMUNE CHARACTERIZATION OF A NOVEL BIFUNCTIONAL PLATFORM BASED ON BLS

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Brucella lumazine synthase (BLS) is a stable protein that folds into a dimer of homopentamers. BLS activates dendritic cells (DC) via TLR4 and generates a strong long-lasting humoral immune response. In order to generate a bifunctional and polarized platform, we rationally designed two different mutant pentamers, BLS_{DR} and BLS_{KE}. In these mutants the association between identical pentamers is impaired but they are able to generate heterodecamers, BLS_{DRKE}, when mixed.

In order to evaluate if the pentamers are able to activate DC, bone marrow derived DC (BMDC) were stimulated with each pentamer or with decameric wild type BLS and expression of costimulatory molecules and cytokine secretion were analyzed. BMDC were stimulated with 90µg of BLS_{DR}, BLS_{KE} or BLS. After 18h the level of expression of CD80 was analyzed by FACS. The amount of IL-12p70 in culture supernatant was analyzed by ELISA. Results show that both pentamers and BLS induce a similar upregulation of CD80 in BMDC surface. Surprisingly, the secretion of IL12p70 induced by each pentamer is significantly higher than the amount secreted by BLS-stimulated BMDC. To assess the humoral response induced by each protein, BALB/c mice were sc immunized with 10µg of BLS, BLS_{DR}, BLS_{KE} or BLS_{DRKE}. Serum was obtained every 14 days and anti-BLS humoral response was assesed by ELISA. After 14 days post-immunization specific antibodies can only be detected in mice immunized with BLS. After 42 days, mice immunized with BLS_{KE} or BLS_{DRKE} show a significant level of anti-BLS IgG but lower of that induced by BLS; BLS_{DR} does not generate specific antibodies.

In conclusion, these mutant pentamers are capable of activating DC and induce a TH1 response in a similar way than BLS. However, the ability to induce a humoral response is diminished in the heterodecamer and BLS_{KE} pentamer and is impaired in BLS_{DR} pentamer. These results are promising for future biotechnological applications in which humoral response is not desired.

(621) INHIBITION OF THE TYPE I INTERFERON ANTIVIRAL RESPONSE BY ZIKA VIRUS NS4B PROTEIN

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Zika virus (ZIKV) illustrates the importance of flaviviruses as emerging vector-borne human pathogens. The major concern is the association between ZIKV infection and neurological disorders, such as congenital microcephaly and Guillain-Barré syndrome. According to the WHO, there are currently 54 countries with ongoing transmission of the virus, including Argentina. Type I interferons (IFN I) play an important role in innate host defense against viruses. When a cell is infected with a virus, microbial components can be sensed by intracellular receptors and promote IFN I production. However, recent studies have shown that non-structural proteins of dengue virus and other flaviviruses have developed ways to hinder this response. In the present work we aimed to study whether one non-structural protein of ZIKV, NS4b, is able to inhibit IFN I response. For this purpose,

we conducted transfection assays using RAW-Lucia ISG cells, an IFN reporter cell-line that secrete Lucia luciferase under ISRE promoter. Results showed that cells transfected with plasmid encoding NS4b were able to inhibit luciferase signals in a dose dependent manner compared to empty vectors (two-way ANOVA, $p<0.05$). This inhibition was also significant after treatment with TLR ligand, LPS, and STING agonist, c-diAMP (two-way ANOVA+Tukey´s, $p<0.05$). Furthermore, NS4b(residues 25-127) was successfully cloned in the pet21a vector and produced in *E. coli* BL21(DE3)cells. The recombinant protein was used to obtain a specific antisera that could recognize NS4b in transfected cells. Although further analysis should be carried out to elucidate the mechanism by which this protein is able to inhibit IFN I production, this results support the idea that flaviviruses are able to escape early host innate response. A better understanding of the flavivirus-host interactions during the different events of the viral life cycle may be essential not only for vaccine development but also for developing novel antiviral drugs.

Keywords: NS4b, Zika virus, Type I Interferons, Innate immunity

(945) **IMPACT OF THE VIRULENCE FACTOR YopP ON THE DEVELOPMENT OF IMMUNE RESPONSE AGAINST *YERSINIA ENTEROCOLITICA***

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IMIBIO-SL

Yersinia enterocolitica (*Ye*) are Gram-negative enteropathogenic bacteria. *Ye* inject the effector proteins called Yersinia outer proteins (Yops) into host target cells. YopP induces apoptosis in macrophages and dendritic cells (DC). Here, we aimed to compare the invasion properties and immune response induced by YopP-deficient (Ye ΔYopP) and wild type (Ye wt) strains. Therefore, C57BL/6 mice were oragastrically infected with 1-5 x 10^8 or 5 x 10^{10} Ye ΔYopP, Ye wt or Ye wt-GFP. After 30, 60, 120 min and 5 days after infection, Peyer's patches (PP), mesenteric lymph node (MLN) and spleen (S) were obtained. Colony forming units (CFU) were evaluated in the organs at 60 min and 5 days after infection. Cytokines and NF-κβ were measured by ELISA, and cell infiltration by flow cytometry. Mice infected with Ye ΔYopP showed increased CFU in PP at 60 min ($p\leq 0.05$), and in MLN and S after 5 days post-infection ($p\leq 0.01$). Besides, higher amount of pro-inflammatory cytokines (IL-1β, IFN-γ, TNF, IL-6, and IL-17) ($p\leq 0.05$), and increased translocation of NF-κβ ($p\leq 0.05$) was detected in PP of mice infected with Ye ΔYopP compared to those infected with Ye wt. However, IL-10 decreased after Ye ΔYopP infection ($p\leq 0.01$). At 5 days after Ye ΔYopP infection, it was found increased frequency and absolute number of macrophage ($CD11b^+$ $F4/80^+$) in PP and MLN ($p\leq 0.001$), and of neutrophils ($CD11b^+$, $Ly6G^+$) in PP ($p\leq 0.01$). However, we did not observe differences in the absolute number of macrophages, dendritic cells ($CD11c^+$) and $CD11c^{int}$ $CD11b^+$ inflammatory cells in S at 60 min post-infection. Because we did not found early differences in cell recruitment, we evaluated cells involving on immediate dissemination of Ye. We observed an increased number of $CD11c^+$ $CD11b^+$ $CD103^+$ GFP^+ at 60 and 120 min post-infection in S from Ye wt-GFP infected mice ($p\leq 0.05$). We concluded that YopP could be playing a critical role in Ye infection, controlling the immune response at mucosal entry sites.

Keywords: *Yersinia enterocolitica*, YopP, cytokines, oral infection, mucosal immune response

(1038) **IMMUNE-MODULATORY EFFECTS OF THE SERINE PROTEASE INHIBITOR-1 OF *Toxoplasma gondii* ON INNATE IMMUNE RESPONSE**

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CESyMA - ECyT - UNSAM

Toxoplasma gondii infects approximately one-third of the world population, making it one of the most successful parasitic organisms. *T. gondii* serine protease inhibitor-1 (TgPI-1) is a potent inhibitor of neutrophil elastase, trypsin and chymotrypsin but its role during natural infection is not already known. It has been reported that other serine protease inhibitors are able to modulate the immune system. Previously we showed that TgPI-1 was able to down-modulate both a Th2 and Th1 type immune responses already established. Those results led us to explore the ability of TgPI-1 to modulate the innate immune response. In this work we used the J774-A1 murine macrophage cell line to study this possible modulatory role. Cells were stimulated with lipopolysaccharide (LPS) or exposed to residual oil fly ash (ROFA), in the presence of a recombinant form of TgPI-1 (rTgPI-1). We observed that rTgPI-1 was able to decrease nitric oxide production induced by LPS stimulation when compared to LPS alone ($p<0.05$). No variation was detected in cell viability as measured by MTT assay or in production of pro-inflammatory cytokines like TNF-α and IL-6. On the other hand, exposure of cells to ROFA showed detrimental effects on cell viability compared to unexposed control cells ($p<0.05$). Surprisingly, the presence of rTgPI-1 during ROFA treatment restored cell viability to untreated cell levels, resulting in a significant increment of TNF-α and IL6 secretion compared to ROFA stimulated cells ($p<0.05$ and $p<0.005$ respectively). The exposure of cells to rTgPI-1 alone showed similar responses to non-stimulated cells. These results showed the ability of TgPI-1 to modulate the first steps of the development of immune responses. Our results led us postulate a possible role of TgPI-1 during *T. gondii* infection, either diminishing the activation of innate cells that attack the parasite itself or avoiding cell death during infection, contributing to the establishment of chronic toxoplasmosis.

(1087) **GPAT3 AND GPAT4 DIRECT GLYCEROLIPID DE NOVO SYNTHESIS DURING MACROPHAGE TO FOAM CELL TRANSITION AND THEIR DOWN-REGULATION ALTERS CYTOKINE RELEASE.**

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Glycerol-3-phosphate acyltransferase (GPAT) initiates *de novo* glycerolipid synthesis. To study the role of GPAT isoforms during the macrophage to foam cell transition, a model of lipid accumulation during atherosclerosis, we analyzed the expression of the four mammalian GPAT isoforms by qRT-PCR in murine RAW264.7 macrophages and primary bone marrow derived macrophages (BMDM) after oxidized LDL (oxLDL) exposure. The endoplasmic reticulum isoform GPAT3 expression increased 32 and 8-fold after 8 and 24h of treatment, respectively. These data were consistent with an increment in GPAT3/4 activity in both models. The lipid droplet area, as well as triacylglycerol (TAG) and phospholipid (PL) contents also increased after oxLDL treatment. To establish the role of GPAT3 and 4 during macrophage to foam cell transition, we silenced GPAT3 in RAW 264.7 cells (shGpat3 cell line) and obtained BMDM form *Gpat3*$^{-/-}$ and *Gpat4*$^{-/-}$ mice. GPAT3 and GPAT4 deficient foam cells accumulate ~30-40 % less LDs, ~25-40% less TAG and ~20% less PL compared to *wt* or SCR control foam cells. We also analyzed the incorporation of [^{14}C]-acetate and [^{14}C]-oleic acid into lipids during sh*Gpat3* cells, *Gpat3*$^{-/-}$ and *Gpat4*$^{-/-}$ BMDM transition to foam cells. We detected fewer counts in total lipids, mainly in the TAG fraction when GPAT3 and 4 were absent or silenced. To investigate the effect of an impaired lipid synthesis due to GPAT3 or GPAT4 deficiency on the inflammatory response, we analyzed the cytokine release in *Gpat3*$^{-/-}$ and *Gpat4*$^{-/-}$ BMDM. Cytokine secretion after foam cell transition increased when GPAT4 was absent; while no clear effect was observed in GPAT3 deficient cells. Taken together, these results prove that GPAT3 and 4 contribute to the increase in total glycerolipid content due to an increase in de novo synthesis during macrophage to foam cell transition and that GPAT4 is required to regulate cytokine release during this process.

IMMUNOLOGY (CLINICAL IMMUNOLOGY) 3

(93) **RECENT THYMIC EMIGRANTS NAÏVE CD4+ T CELLS (CD45RA+CD31+) IN PAEDRIATRIC PATIENTS INFECTED WITH HIV AND DIFFERENT ADHERENCE TO ANTIRETROVIRAL THERAPY: A LONGITUDINAL STUDY**

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Introduction: CD31 (PECAM-1) is expressed in several cellular types, mediates T-cells adhesion, inhibits its activation and the co-expression with CD45RA defines recent thymic emigrants (RTEs) naïve(N) CD4 +T cells, which have not yet undergone significant homeostatic proliferation.

Objective: to monitor RTEsN CD4+T cells in paedriatic patients infected with HIV with different levels of adherence to antiretroviral therapy (ART).

Material and Methods: RTEsN CD4+T cells percentage levels as marker of immunological reconstitution, were studied in 23 patients with HIV infection by vertical transmission, in ART, at two different time-points of follow-up (i,f). Flow cytometry and specific monoclonal antibodies were used. Plasma HIV RNA viral load (VL) (limit for the assay log<1.70 IU/ml), and clinical evaluation of patients were also determined.

Results: after follow-up period (12 ± 5 months), children were divided according to degree of adherence and virological response: Adherent (A, n: 14) and non-Adherent (NA, n: 9).Group A were in turn divided in children who were already in ART(A1, n: 7), and patients who started ART (A2, n: 7). Children of group A1 maintained CD4+(i: 33±15, f:33±13) and VL (i:<1.70, f:<1.70) levels, while RTEsN CD4+T cells not shown significant differences during follow-up (i:46 ± 15, f: 47 ± 11). CD4+T cells increased and VL diminished levels (i: 16 ± 10, f: 29 ± 10, and i: 4.9 ± 1.43, f :< 1.70) respectively, were recorded in group A2. RTEsN CD4+T cells increased significantly (p≤0.05) (i: 32 ± 11, f: 58 ± 12) in this group of patients. Decreased CD4+ (i: 16 ± 7, f: 19 ± 7), increased VL

(i: 3.90 ± 1.5, f: 3.36 ± 0.8), and decreased but constant, RTEsN CD4+T cells levels, (i: 39 ± 13, f: 38 ± 14) were observed in NA children.

Conclusion: the increase in levels of RTEsN CD4+T cells would reflect the improvement in thymic functionalism, associated with progressive immune-virological stability, induced by efficacy and correct adherence to ART.

Keywords: recent thymic emigrants, CD31, HIV, paedriatic patients.

(446) **SERUM CYTOKINE PROFILE IN TYPE 1 GAUCHER DISEASE PATIENTS CORRELATES WITH BONE PATHOLOGY**

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Gaucher disease (GD) is caused by mutations on the gene encoding for the lysosomal enzyme glucocerebrosidase. Type I GD (GD1) patients present anemia, hepatosplenomegaly and bone alterations. In spite of treatment, bone alterations in GD patients persist, including poor bone mineral density (BMD). Mechanisms leading to bone damage are not completely understood, but previous reports suggest that osteoclasts are involve. It is known that bone remodeling is regulated by the immune system, in part, through cytokines like TNFα (inductor of osteoclastogenesis), TGFβ, IL-10 and INF (inhibitors of osteoclasts differentiation). On the other hand, it has been demonstrated that the accumulation of glucocerebrosidase in GD can induce macrophage activation and secretion of cytokines such as Il-6, IL-1β and TNFα.

The aim of this work was to evaluate cytokines profile (TNFα, IL-6, TGFβ, IL-10 and INF) and bone biomarkers (CTX and BAP) in treated patient´s serum and evaluate its relation with BMD and the pro-osteoclastogenic potential.

The number of osteoclasts was determined by cultured PBMCs with M-CSF and stained for tartrate-resistant acid phosphatase (TRAP). CTX and BAP were assessed by electrochemiluminescence and enzyme immunoassay, respectively; and cytokines were measured by ELISA.

Serum levels of CTX in adult and pediatric GD1 patients were increased (p<0,01; p<0,05, respectively) as well as BAP in adult GCD1 (p=0,05). We also observed that pediatric group had a positive correlation with the pro-osteoclastogenic potential (number of osteoclasts) (r=0,8338 p=0,0052). Serum cytokines were also altered showing a pro-osteoclastogenic status and a positive correlation between IL-10/ INF- with BMD (express as Z-score) was observed (r= 0,5134 p=0,0420; r=0,5751 p=0,0198, respectively).

Our results suggest the involvement of osteoclasts in the bone pathology of GD1, and an altered immune response which may play an important role in bone damage.

Keywords: Gaucher disease, bone pathology, osteoclastogenesis, cytokines, biomarkers.

(1171) **THE EXPRESSION AND FUNCTIONALITY OF THE IL-27 RECEPTOR PATHWAY IS ASSOCIATED WITH THE LOSS OF *Trypanosoma cruzi*-SPECIFIC T CELLS IN PATIENTS WITH CHRONIC CHAGAS DISEASE**

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We have previously shown that chronically *T. cruzi*-infected subjects bear several features of a process of immune exhaustion including an inverse correlation between disease severity and the magnitude of T cell responses, and alterations in the signaling pathway of the IL-7 receptor (IL-7R). Since IL-27 has a wide range of effects on T cells and like IL-7, IL-27 also exerts its function through STAT5, we evaluated the functionality of the IL-27 receptor (IL-27R) and its association with *T. cruzi*-specific T cells responses. Polychromatic flow cytometry was used to analyze the expression of IL-27R components (CD130 and WSX1 chains) on $CD4^+$ and $CD8^+$ T cells. IL-27-dependent signaling events in T cells were also evaluated by polychromatic flow cytometry following stimulation of PBMC of chronic Chagas disease patients with recombinant human IL-27. Subjects with no signs of cardiac disease showed a significant decrease in $CD130^+WSX1^+CD4^+$ T cells and an increase in $CD130^+WSX1^+CD8^+$ T cells compared with, either patients exhibiting heart disease or uninfected controls. *T. cruzi* infection, in vitro, was able to stimulate the downregulation of the IL-27R in $CD4^+$ T cells and the upregulation in $CD8^+$ T cells. IL-27-induced phosphorylation of STAT1, STAT3 and STAT5 was lower in patients with heart disease compared with asymptomatic patients and inversely associated with the frequency of *T. cruzi*-specific IFN-gamma-producing T cells measured by ELISPOT assay. STAT5-downstream gene expression of *tbx21*, *eomes*, *gzmb* and *cxcl9* assessed by q-RTPCR was also reduced in patients with cardiomyopathy. These findings support a possible role of the IL-27R signaling pathway in promoting *T. cruzi*-specific T cells responses.

Keywords: Chagas disease, T cells, IL27, immune exhaustion, STAT

(1193) **PD-L1 REGULATORY B CELLS ARE SIGNIFICANT DECREASED IN RHEUMATOID ARTHRITIS AND INCREASE IN GOOD RESPONDERS TREATED PATIENTS**

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Rheumatoid arthritis (RA) is the most frequent autoimmune disease. Immune tolerance and prevention of autoimmunity can be exerted by different subsets of regulatory B cells (Bregs). PD-L1 functions as a regulatory protein to maintain T cell self-tolerance, and could play a role in determining Breg activity in RA. We aimed

to evaluate PD-L1 expression on B cell subsets in RA patients and healthy individuals and analyze whether PD-L1 expression on B cells had an influence on T cell activity and response to therapy. Peripheral blood samples were obtained from healthy controls (HC, n=25), untreated RA patients (untreated, n=24), RA patients treated with Metotrexate (MTX, n=20), with TNF inhibitors (anti-TNF, n=19) or with JAK inhibitor Tofacitinib (TOFA, n=6). In some patients, samples were obtained at baseline and after 3 months of Tx (n=18). RA activity was evaluated by 28-joint count Disease Activity Score (DAS-28) and the Tx response by the EULAR response criteria. We observed that among B cell subsets, the % of CD19+CD24hiCD38- correlated negative with DAS-28 (r= -0.4, p=0.0315) and that the % of CD19+CD24hiCD38-PDL1+ was not modified by different Tx, but a significant decrease in the % of CD19+CD24hiCD38-PDL1+ and CD19 PDL1+ B cells was observed in untreated RA patients (p<0.01). Moreover, we observed that the expression of PD-L1 strongly increased in HC (p<0.01) and untreated RA patients (p<0.01) when purified CD19+ B cells were cultured with CpG+IL-2. These PD-L1+ expressing B cells, suppressed CD8+ T cell proliferation and cytokine secretion in a PD-L1 dependent manner. Interestingly, the % of CD19+CD24hiCD38-PDL1+ significantly increased in good responders RA patients (p<0.01). Our findings suggest a regulatory role for the CD19+CD24hiCD38- that could be used as predictive biomarker of response to therapy. Furthermore, PDL1 induction on CD19+ B cells from RA patients, could provide news perspectives for future Tx strategies.

Autoimmunity, Rheumatoid arthritis ,PD-L1 regulatory B cells

(1328) **VIP CONTRIBUTION TO THE SELECTIVE RECRUITMENT OF TREGS DURING THE PERI-IMPLANTATION PERIOD**

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Decidualization process involves phenotypic and functional changes on endometrial cells and the modulation of different mediators such as the secretion of the vasoactive intestinal peptide (VIP). This neuropeptide is a key regulator of the immunetolerance capable of inducing Tregs towards alloantigens which are crucial for a successful implantation and the pregnancy maintenance. VIP deficient(+/-) pregnant mice have reduced litter size and increased inter-gestations period.

Here, we evaluated VIP contribution to the Tregs' recruitment during the peri-implantation period. We used two approaches: VIP(+/-) and Foxp3-knock-in-GFP females either pregnant or in estrous. Foxp3 expression by RT-PCR was not detectable in VIP(+/-) mice uterus, in contrast to Foxp3-GFP mice, confirmed by FACS analysis. This reduction was accompanied with a higher expression of RORγt and a decrease in IL-10 (p<0.05 Wilcoxon). When Foxp3-GFP females were mated with WT males we found a peak in Foxp3 expression in uterus and mesenteric lymph nodes at the implantation day 4.5. Therefore, to study Tregs effects and trafficking in VIP(+/-), we performed adoptive transfer of Treg cells (sorted from Foxp3-GFP females) to VIP(+/-) that haven't got pregnant during 6 months (n=4). Vaginal plug was observed during the first 3 days in all of them after mating with WT males. Foxp3-GFP cells were mainly recruited into the uterus in relation to all other tested tissues accompanied with an increase in IL-10 expression. Finally, we performed *ex vivo* migration assays using CD4+ sorted cells from Foxp3-GFP mice towards conditioned media from WT-explants at day 5.5 cultured in the absence/presence of VIP for 24hs. VIP induced an enrichment of CD4+Foxp3+ and a decrease in CD4+ cells.

In conclusion, VIP may contribute to the selective recruitment of maternal Tregs to the uterus and might prevent the development of a hostile uterine microenvironment for implantation.

Keywords: VIP-IMMUNETOLERANCE-TREGS-IMPLANTATION

(1506) **LEUKOCYTE AS KEY PLAYERS IN OPTIC NERVE DAMAGE INDUCED BY OPTIC NEURITIS**

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Optic neuritis (ON) is a condition involving primary inflammation, demyelination, and axonal injury in the optic nerve which leads to retinal ganglion cell (RGC) loss, and visual dysfunction characterized by a decrease in pupil light reflex (PLR) and visual evoked potentials (VEPs).We have developed an experimental model of primary ON in rats through a single microinjection of bacterial lipopolysaccharide (LPS) into the optic nerve.Neuroinflammatory diseases are characterized by disruption of the blood-brain barrier (BBB) and increased leukocyte infiltration. The aim of the present work was to analyze the involvement of cell infiltration on visual damage induced by experimental ON. LPS or vehicle were injected into the optic nerve from adult male Wistar rats. BBB integrity was analyzed through Evans blue perfusion and by using WT-GFPþ/WT chimeric rats. At 6 h post-LPS injection an increase in albumin-Evan's blue leakage and an increase in optic nerve cellularity was observed. At 24 h post-injection, e-GFP(+) cells (likely macrophages and neutrophils) were identify in LPS-injected optic nerves. Experimental ON induced an increase in the chemokine CCL2-immunoreactivity (p< 0.01). The injection of Bindarit (a CCL2 inhibitor),as well as the irradiation of animals with a leadshield in their heads,significantly prevented the effect of ON on the PLR(p< 0.01),VEP amplitude (p< 0.01),and RGC number(p< 0.01).These results indicate that leukocyte recruitment plays a key role in the visual damage induced by experimental ON.

(1608) **NO AND INFLAMMATION AS EFFECTORS OF CARDIAC MITOCHONDRIAL DYSFUNCTION AND ROS PRODUCTION IN LOW-GRADE AND SEVERE ENDOTOXEMIA.**

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Myocardial dysfunction is a complication of severe sepsis and endotoxemia. Previously, we described cardiac mitochondrial dysfunction in this syndrome. Mechanisms of how systemic inflammation could damage heart function remains still unknown. Cytokines, NO and bioenergetics derangements are thought to be involved. The aim was to analyse systemic NO and ROS in a model of low-grade and severe endotoxemia. Female Sprague-Dawley rats (55 days) were injected *i.p.* with a single dose of LPS 0.5 mg/kg, 8 mg/kg or vehicle (control), 6h later animals were sacrificed. White blood cells (WBC) and differential count were analysed. DFC-DA and DAF-2-DA were used to estimate intracellular ROS and NO concentration by polymorphonuclear cells (PMN), respectively. Nitrite/nitrate content and proinflammatory cytokines levels (TNFα and IL-6) were analysed in plasma samples. Mitochondrial H_2O_2 production was measured using Amplex Red probe. WBC count showed difference between groups: control 2638 cells/mm^3 (N:17%/L:77%/M:5%/E:1%/B:0%), LPS 0.5: 6771 cells/mm^3 (N:61%/L:28%/M:7%/E:0%/B:4%) and LPS 8: 1021 cells/mm^3 (N:58%/L:40%/M:2%/E:0%/B:0%). PMN cells from LPS-treated animals increased intracellular NO by 147% (LPS 0.5) and 164% (LPS 8) compared with control (p<0.05). ROS production showed a partial increase at LPS 0.5 and LPS 8 treated animals. Nitrite/nitrate content in plasma increased 4-fold (LPS 0.5) and 7-fold (LPS 8) compared to control group (p<0.05). TNF-α and IL-6 levels increase was related to the severity of endotoxemia. Car-

diac mitochondrial H_2O_2 production (control: 0.128±0.01) increased 140% in LPS 0.5 and 160% in LPS 8 treatment compared with control group ($p<0.05$). Our results show that the severity of the endotoxemic process is correlated to ROS and NO levels in blood, and to cardiac mitochondrial dysfunction previously reported. These results strongly suggest the importance of restoring NO and ROS levels, and cardiac mitochondrial function in inflammatory pathologies.

Keywords: endotoxemia, nitric oxide, ROS, pro-inflammatory cytokines, mitochondria dysfunction.

(1688) **THE INFLAMMATORY RESPONSE DURING THE IMPLANTATION PERIOD: ASSOCIATION WITH THE RETICULAR STRESS AND THE UNFOLDED PROTEIN RESPONSE**

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Embryo implantation in humans involves the generation of a sterile inflammatory response associated with blastocyst invasion; however, it is still unclear how it could be induced. During decidualization, endometrial stromal cells undergo reticular stress (RS) and unfolded protein response (UPR) which allow them to expand their endoplasmic reticulum and change their secretome producing proimplantatory mediators. Here we focus on the impact of the RS/UPR on decidual cells and how could be induced IL-1b production needed for implantation. We used two approaches: an *in vitro* decidualization model of Human endometrial stromal cell line (Dec) differentiated with MPA (1uM) and dbcAMP (2,5mM); and endometrial biopsies obtained from fertile women, with recurrent spontaneous abortions (RSA) or with in vitro fertilization failures (IF).

The decidualization in Dec cells increased the RS-sensors, ATF6, PERK and IRE1a in comparison with non-Dec cells (Thapsigargin was used as ctrol+ $p<0.05$). Then, we evaluated the RS/UPR downstream pathway, by the expression of TXNIP, a kinase/RNAse able to activate the NLRP3 inflammasome and sXBP1, an UPR-transcription factor. TXNIP and sXPB1 expression increased in Dec cells and was accompanied by an increase in NLRP3 expression ($p<0.05$). In fact, we observed an increase in caspase-1 activity tested by FLICA-probe, and in IL-1b production tested by FACS ($p<0.05$) and confirmed by W.blot. Finally we evaluated the RS/UPR pathway on endometrial biopsies by qPCR. We found an increase expression of IRE1a, TXNIP, sXBP1, NLRP3 and IL-1b on RSA-samples in comparison with fertile women; while biopsies from IF patients showed similar expression as fertile women. The present results suggest that human decidualization process is accompanied by a physiological RS/UPR associated with an increase of IL-1b production generating a sterile inflammatory response and this response is differentially affected in RSA and IF pathologies.

Keywords: Decidualization; Reticular Stress; Unfolded Protein Response; Immunomodulation; Inflammatory Response.

(1789) **INTERLEUKIN (IL)-17 MODULATES THE PRO-INFLAMMATORY RESPONSE OF COLONIC MYOFIBROBLASTS FROM PATIENTS WITH INFLAMMATORY BOWEL DISEASE**

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Inflammatory bowel disease (**IBD**), mainly Crohn's disease (**CD**) and ulcerative colitis (**UC**), are chronic intestinal disorders in which pro-inflammatory cytokines are involved. Th17 cells and IL-17 are abundantly found in the inflamed intestinal mucosa. Although IL-17A has been reported as a pathogenic factor in IBD, the exact role for IL-17 in IBD remains controversial. We have previously shown that IL-17 dimmers (IL-17AA, FF and AF) are differentially produced in UC and CD lamina propria, and the anti-inflammatory properties of IL-17AA. In order to identify target cells for IL-17 in IBD intestinal mucosa, ***we aimed to study the effect of IL-17A, IL-17F and IL-17A/F on human colonic myofibroblasts*****,** which may be pro- or anti-inflammatory cells**.**

Myofibroblasts were isolated from intestinal biopsies or surgical samples from IBD adult patients (UC n=3, CD n=4) and healthy donors (HC n=1). Cells were stimulated with recombinant human IL-17A, IL-17F or IL-17AF (1ng/ml), in combination with TNF (1ng/ml) or medium as control. Secreted IL-6 and IL-8 were quantified by ELISA, and IL-33 and ST2 gene expression was quantified by qPCR as pro-inflammatory markers.

We found that IL-17 dimmers did not induce IL-6 nor IL-8 secretion, however only IL-17A combined with TNF significantly diminished IL-6 and IL-8 production by UC and CD myofibroblasts compared with TNF alone ($p<0.1$). Besides, IL-17 dimmers induced a two- to eight-fold increase in IL-33 and ST2 expression on UC and CD fibroblasts.

In conclusion, we found that intestinal myofibroblasts from IBD patients are target cells for IL-17 and IL-17 dimmers differentially modulated the pro-inflammatory response. Moreover, CD fibroblasts expressed IL-33 and ST2, which has been described so far as exclusively produced by UC fibroblasts. These findings suggest that IL-17 may play different roles in the pathogenesis of IBD, depending on the cell subset affected.

KEYWORDS: Inflammatory Bowel Disease, human myofibroblasts, IL-17, cytokines, IL-33

(1110) **POTENTIAL IMMUNOMODULATORY EFFECT OF HUMAN CHORIONIC GONADOTROPHIN IN PATIENTS WITH MULTIPLE SCLEROSIS**

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Multiple sclerosis (MS) is an invalidating, neurodegenerative disease, 2-3 times more common in women than men. It is well known that pregnancy has a strong influence on MS disease activity and, a reduced relapse frequency (70%) is seen during pregnancy. Human chorionic gonadotropin (hCG) is synthetized by the placenta during pregnancy and the presence of its receptor has been described in immune cells, such as B and T cells. The aim of our study was to investigate the capability of hCG to reduce activation and later production of pro-inflammatory cytokines from immune cells in MS patients. We recruited women suffering MS during their reproductive life. PBMC were isolated from whole blood samples and then cultivated *in vitro* with/without (control, C) hCG: urinary hCG (u, 100 IU/ml) or recombinant hCG (r, 5 ug/ml) for 24 h. PMA, Ionomycin and Brefeldin A were added, in some wells, for the last 5h of culture. Flow cytometry was used for the phenotypical characterization of different immune cell subsets: CD19, CD80, CD86 (B cells), CD4 and Foxp3 cells and to evaluate intracellular cytokines levels: TNF-α and IL-10.

We observed that treatment with hCGu significantly reduced the relative numbers of $CD19^+CD86^+$ B cells as compared to control

group. No changes were observed concerning $CD80^+$ expressing B-lymphocytes. Interestingly, addition of either hCGu or hCGr induced a significant reduction in the total numbers of $CD19^+TNF\text{-}\alpha^+$ B cells as well as $CD4^+TNF\text{-}\alpha^+$ cells as compared to controls. Besides a slight increase, no significant differences were observed on IL-10 production by $CD4^+$ or $CD19^+$ B cells upon either hCG stimulation (data were analyzed by One-way ANOVA).

Overall, we demonstrated here that treatment with hCG *in vitro,* lowers the expression of the costimulatory molecule CD86 in B cells and also decreases the production of TNF-α, in both $CD4^+$ and $CD19^+$ B cells. These results highlight the potential use of this hormone for the treatment of MS.

Keywords: Multiple Sclerosis, hCG, immune suppression.

PLANT BIOLOGY 2

(828) **ADP-GLUCOSE PYROPHOSPHORYLASE IS POST-TRANSLATIONAL REGULATED BY PHOSPHORYLATION IN WHEAT SEEDS**

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ADPglucose pyrophosphorylase (ADPGlcPPase, EC 2.7.7.27), a heterotetramer comprised of two small (S) and large (L) subunits, catalyzes the first and rate-limited step of starch biosynthesis pathway. In plants, the activity of ADPGlcPPases is regulated allosterically by metabolites, by redox mechanisms and recently, phosphoproteomic and bioinformatic studies suggest its regulation by phosphorylation. In comparative studies along wheat (storing starch) and castor (storing triacylglycerides) seeds development, we found that ADPGlcPPase is phosphorylated only in wheat seeds at stages with increased ADPGlcPPase activity and higher starch accumulation. To study the effect of phosphorylation in wheat ADPGlcPPase, we used recombinant co-expression strategies in *Escherichia coli* to produce the subunits of wheat endosperm ADPGlcPPase, separately (*Tae*S, *Tae*L) or together (*Tae*SL). Also, we designed and produced different *Tae*S and *Tae*L Ser mutants located in putative phosphorylation sites. *In vitro* phosphorylation assays with ^{32}P-γ-ATP, wheat seed extracts (as kinases resource) or specific recombinant kinases (SnRK1, SOS2 and CDPK) showed that *Tae*ES, *Tae*EL and *Tae*ESL were phosphorylated. As well, all mutants were phosphorylated too, suggesting that *Tae*EADPGlcPPase is phosphorylated in other Ser/Thr residue or the occurrence of many phosphorylation sites. When we evaluated the effect of phosphorylation, the result showed a 4-fold increase of *Tae*SL activity. Altogether, the results point out that posttranslational regulation of *Tae*EADPGlcPPase by phosphorylation would increase the enzyme activity at specific stages of wheat seed development. This work describes a new posttranslational mechanism in the regulation of starch metabolism at the level of ADPGlcPPase, that will required further study to deeply comprehend the interplay with other regulation mechanism and explore possible biotechnological developments.

Keywords: starch, phosphorylation, seeds, wheat, castor oil seed

(840) **ARABIDOPSIS POLLEN EXTENSINS LRX ARE REQUIRED FOR CELL WALL INTEGRITY DURING POLLEN TUBE GROWTH**

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Abstract: Polarized growth of pollen tubes is an oscillatory mechanism which involves high trafficking of vesicles to the tip where a constant remodeling of the cell wall occurs. To maintain the proper assembly of the cell wall is crucial the role of glycoproteins and homogalacturonans in the crosslinking. Here, we studied the function of four pollen specific LRX(8-11) (Leucine-Rich repeat Extensin-like) chimeric proteins which belong to the HRGP (Hydroproline-Rich Glycoprotein) family in *Arabidopsis thaliana*. These LRX proteins have an LRR (Leucine rich repeat) N-terminal domain followed by a cysteine rich region and a C-terminal domain extensin (EXT)-like with Ser-Pro repetitions. LRR domain might be involved in protein-protein interactions and the role of EXT domain might be to establish the crosslink on the cell wall allowing its integrity. Because we did not observe any obvious phenotype for the single mutants and to overcome a possible functional redundancy, we obtained multiple homozygous loss of function mutants. The triple *lrx9 lrx10 lrx11* mutant displayed the most severe pollen tube phenotype with an important reduction (60.7%) in pollen germination and a lower seed set (33% less compared to the WT). Moreover *in vitro*, semi *in vivo* and *in vivo* analyses revealed the presence of abnormal pollen tubes with widen tips and the emergence of bulges mostly near to the tube tip. To study the composition of the primary cell wall in the triple *lrx9 lrx10 lrx11* mutant, we analyzed the distribution of pectins, callose and cellulose using different dyes. Confocal and bright-field fluorescence microscopy revealed an altered deposition of pectin, callose and cellulose and thicker cell walls in the triple mutant. All together these results suggest that LRX8-11 have a structural role and are required for a proper cell wall assembly during polarized growth of pollen tubes.

Keywords: pollen tube, cell wall, extensin, polarized growth, LRX

(1253) ***Arabidopsis thaliana* ASPARTIC PROTEASES AND THEIR ROLE IN DROUGHT STRESS.**

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Plant aspartic proteases have been implicated in protein processing as well as in plant senescence, stress responses, programmed cell death and reproduction. In last years, it has been reported that they might have a role in the adaptation of plants to an environment with less water availability. In our laboratory we determined that an *A. thaliana* gene that encodes for an aspartic protease (*At1g11910*) that is expressed in guard cell, is induced under deficit water conditions.

The aim of this work was to study the role of *At1g11910* during the plant response to drought. We performed assays to compare the response of Col-0 and *At1g11910* mutant plants in a mild water deficit (MWD) condition. Seedlings of 14 days were putted in pots with an equal substrate quantity and were watered until saturation during 10 days. Then, treated plants were watered until reached a 26% of the maximum substrate capacity (0.13 g of water/g substrate) during the next 20 days. We evaluated the phenotype of each plant under stress conditions and quantified different hydric parameters as water content, consumption and loss.

Our results indicated that mutant plants were more susceptible than Col-0 to a MWD, showing a reduction in the total area leaf and in the apical length in a 60% and 40% respectively. On the other hand, we did not observed significant differences in chlorophyll content. We determine that mutant plants had a higher water loss (60%) and consumption (25%) but they presented lower water content (28%). These results allow us to suggest that *At1g11910 A. thaliana* gene would participate in the tolerance to drought. Currently we are completing the characterization of this gene and generating *At1g11910* overexpressing *A. thaliana* plants as a new biotechnological tool to face water deficit conditions.

Keywords: Aspartic Proteases, Drought, *A. thaliana*.

(1059) **CHARACTERIZATION OF A MAIZE FLAVONE SYNTHASE II**

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Flavones, a subtype of flavonoid, are widespread among the higher plants and have diverse physiological functions. There are two classes of flavone synthase (FNS) enzymes that catalyze the conversion of flavanones into flavones. The flavone synthase II comprises oxygen- and NADPH-dependent cytochrome P450 membrane-bound monooxygenases. We identified a gene encod-

ing a putative FNSII in maize named *Zm*CYP93G7. In order to examine whether this enzyme participates in flavone biosynthesis, we cloned the open reading frame in a yeast expression vector, transformed the WATT11 yeast strain and cultures of transformed yeast cells were fed with the flavanones substrates. LC-MS/MS analyses showed that *Zm*CYP93G7 is able to catalyze the conversion of flavanones (naringenin and eriodictyol) into the corresponding flavones (apigenin and luteolin, respectively). The coding sequence was expressed from the constitutive 35S promoter in Arabidopsis plants. Total flavonoid extracts of Arabidopsis transgenic plants expressing *ZmCYP93G7* in *dmr6* mutant background (lacking a FNSI enzyme) analyzed by LC-MS showed apigenin accumulation demonstrating FNS functionality *in planta*. Moreover, when transgenic Arabidopsis lines ($35S_{pro}$::*ZmCYP93G7*) were infected with the pathogen *Pseudomonas syringae,* we found that maize CYP93G7 restores pathogen susceptibility compared to *dmr6* mutant plants ($p<0.001$), which are more resistant to these bacteria. In this way, we were able to show that *Zm*CYP93G7 can complement *Arabidopsis thaliana* mutant plants lacking endogenous FNS DMR6, as we previously demonstrated for a FNSI-type, *Zm*FNSI-1. Additionally, we are evaluating if flavones could play a role in the protection of plants against UV-B radiation. Under that assumption, dot blot assays are being performed to analyze cyclobutane pyrimidine dimer (CPD) accumulation, and preliminary results indicate that transgenic plants show less DNA damage after UV-B exposure than wild type plants.

Keywords: flavones, flavone synthase, maize

(768) **BIOCHEMICAL CHARACTERIZATION OF PLANT PROTEIN KINASES USING A NOVEL COLORIMETRIC METHOD**

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Abstract: Protein kinases (PKs, EC 2.7.11.-) constitute one of the largest protein families and are important components of signaling cascades in eukaryotes. By modifying protein activity, they regulate nearly every aspect of cellular function. Due to their sessile lifestyle, plants developed new signaling systems to cope with the challenges imposed by an always-changing environment. Therefore, plants have expanded PKs families, developing many that are unique to their kingdom. Besides the major role that reversible phosphorylation plays in the regulation of carbon metabolism in plants, information about the biochemical properties of plant PKs is scarce. This is mainly due to the difficulties to assay their activity using radiolabeled ATP and because the non-radioactive methods available to date lack the proper sensitivity and cannot be adapted to every kinase. In this work, we designed a straightforward protocol based on a cyclic enzymatic assay to measure PK activity using a 96-well microplate reader. Released ADP is used to oxidize NADH via pyruvate kinase and lactate dehydrogenase. Afterwards, NAD^+ is recycled using alcohol dehydrogenase/ethanol and phenazine ethosulfate/Thiazolyl Blue Tetrazolium Bromide. The method measures released ADP after the phosphoryl transfer (linear range from 0 to 4 nmol); thus, it can be used to analyze virtually all PKs. To validate the protocol, we characterized recombinant PKs with different catalytic mechanisms and from different plant species, including *Arabidopsis thaliana* SnRK1, *Malus domestica* SOS2 (a member of the SnRK3 subfamily), and *Solanum tuberosum* CDPK1. Assays run in parallel using the colorimetric method and radiolabeled ATP showed a correlation close to 1. Overall, this tool represents a step forward in the functional characterization of PKs, which is critical to expand our knowledge about signaling networks in plants.

Keywords: signaling networks, protein kinase, phosphorylation, non-radioactive assay, biochemical characterization.

(654) **CHARACTERIZATION OF *CITRUS SINENSIS* PYROPHOSPHATE-DEPENDENT PHOSPHOFRUCTOKINASE GENE FAMILY AND ITS RESPONSES TO COLD STRESS**

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Abstract: Phosphofructokinase catalyzes the glycolytic conversion of fructose-6-phosphate to fructose-1,6-bisphosphate, a central step in sugar metabolism. In plants, two forms of the enzyme are known: PFK (EC 2.7.1.11), which is ATP-dependent, and PFP (EC 2.7.1.90), which is pyrophosphate-dependent. Most PFPs are heterotetramers composed of two subunits of 66 (PFPa) or 60 kDa (PFPb), encoded by a varying number of genes. PFP contributes to glycolysis in a number of conditions that lead to a drop in nucleotide levels, such as anoxia, Pi deprivation, and cold stress. Previous work from our group evidenced higher PFP protein levels after frost events in *C. sinensis* juice sacs, thus indicating a role in the enhancement of glycolysis in these tissues.

To gain further insight, we searched *C. sinensis* genome for putative PFP subunits and bioinformatically characterized their sequences and structures. Sequences were compared to those known for other species both by alignment and phylogentically. Expression levels of PFPs were studied in vegetative and reproductive tissues of *C. sinensis* var. Valencia Late obtained from field-grown plants. To further characterize PFP responses to cold in vegetative tissues, PFP gene expression was studied in frost-exposed leaves, and also in leaves from cold-treated plantlets.

We report the identification of four sequences coding for three catalytic and one regulatory subunit in the *C. sinensis* genome. Expression studies showed that PFPa and PFPb1 were expressed at different levels in all tissues assayed and, notably, with different a/b subunit ratios. PFPb2 was almost exclusively detected in flowers, while PFPb3 was found in mature leaves, roots, and fruit tissues. Cold treatments changed the expression pattern of these proteins. This work provides cues to understand the role of PFP during normal conditions and through the energetic rescue of the cell under stressful conditions in citrus.

Keywords: citrus, metabolism, phosphofructokinases, cold, stress.

(1266) **ROLE OF *ARABIDOPSIS* ASPARTIC PROTEASE (AT1G11910) IN RESPONSE TO *BOTRYTIS CINEREA* ATTACK**

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Aspartic proteases (APs) are a family of proteolytic enzymes widely distributed among living organisms. Plant APs are involved in various processes as senescence, programmed cell death, reproduction and stress responses. Purpose roles to these APs are to mediate defense responses either by directly processing pathogen related proteins or to generate elicitors. In our laboratory we identified, purified and characterized two typical APs from potato (*St*AP1 and *St*AP3). These APs have are bifunctional enzymes with antimicrobial and proteolytic activities.

Through the search for sequence homology, we detected the presence of three *St*AP1 highly homologous genes in *Arabidopsis*. These genes are At1g11910 (80% id) (named as *APA1*), At1g62290 (82% id) and At4g04460 (83% id). According with data described in Arabidopsis eFP browser APA1 is induced in leaves after *B. cinerea* and others pathogens infection. However, there are not results that correlate the induction of this gene with the increase or decrease of the defense response of *A. thaliana* to *B. cinerea*. In the current study, we have evaluated the susceptibility degree of *APA1* mutant plants to *B. cinerea* infection. We analysed the defense response of plants Col-0 and homozygous *APA1* mutant lines infected with an inoculum of *B.* cinerea (1 X 10 5 spores/ml). Results obtained shown that *APA1* mutant plants developed more severe disease symptoms at 3 days after infection, than wild type plants. These symptoms include extensive necrosis and chlorosis with the increased in 94% of lesion area than wild-type plants. In this work, we show that mutation of the *Arabidopsis* APA1 gene causes enhanced susceptibility to the necrotrophic fungal pathogen *B. cinerea*. Therefore, we suggest that this typical AP (APA1) would participate in the *Arabidopsis* defense response against to *B. cinerea* attack.

Keywords: *A. thaliana*, *B. cinerea*, Aspartic proteases.

(416) **PHOSPHORYLATION OF THR257 INHIBITS ALDOSE-6-PHOSPHATE REDUCTASE FROM PEACH LEAVES**

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Abstract. In addition to sucrose and starch, glucitol (a sugar alcohol) is a primary photosynthate in a considerable number of agronomically important plant species. Glucitol is produced in mature leaves from glucose 6-phosphate by the combined action of the NADPH-dependent aldose-6-phosphate reductase (Ald6PRase, EC 1.1.1.200) and a specific phosphatase. We recently found that activity of Ald6PRase from peach (*Prunus persica*) leaves (*Ppe*Ald-6PRase) is inhibited by hexose-phosphates, Pi and oxidants. In this work, we show that *Ppe*Ald6PRase from mature leaves is phosphorylated *in vivo*. Treatment of the phosphorylated enzyme with alkaline phosphatase increased the activity 3-fold. Recombinant *Ppe*Ald6PRase was phosphorylated *in vitro* by a partially purified extract from peach leaves, but also by recombinant Mg^{2+}- and Ca^{2+}-dependent protein kinases. Using *in silico* approaches we identified a highly conserved Thr residue as a putative phosphorylation site. The phosphomimetic T257D mutant had negligible catalytic activity and was less phosphorylated than the WT. Because Thr257 is located in the predicted NADPH-binding loop and presence of NADPH prevents phosphorylation of the WT enzyme, we hypothesized that phosphorylation of this residue prevents cofactor binding. Dye-binding coupled to temperature-dependent denaturing assays showed impaired NADPH-binding to the T257D mutant. Our results strongly suggest that phosphorylation regulates Ald6PRase activity and, as a consequence, glucitol levels in peach leaves.

Keywords: carbon metabolism, enzymes, peach, phosphorylation, *Prunus persica*.

NEUROSCIENCE 9

(1073) **REPRODUCTIVE EXPERIENCE ALTERS BRAIN PLASTICITY AND SPATIAL MEMORY IN RESPONSE TO MOTHER-PUP SEPARATION STRESS DURING THE POSTPARTUM PERIOD**

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In mammals, substantial behavioral and neurobiological changes occur throughout the female brain because of reproductive experience (RE: pregnancy, parturition, and mothering). This structural and functional modification remodels the neural circuits of the hippocampus and have a significant impact in learning and memory aspects. Since levels of hippocampal neurogenesis vary in response to a wide range of intrinsic and extrinsic factors, we considered the possibility that neurogenesis is deviated during RE, thus providing potential explanation for the changes in maternal cognitive performance during that period. Female Wistar rats were divided in the following experimental groups: Nulliparous (NP: none RE), Primiparous (PRI: one RE) and Multiparous (MULT: two RE). The PRI and MULT group were subjected to either animal facility rearing (C) or daily 4,5h of separation from pups (S) from postpartum day (PPD) 1 to 21. Spatial memory was evaluated in the Barnes Maze (PPD 21-26). To quantified cell survival dams were injected with bromodeoxyuridine (BrdU) on PPD1 and euthanized 21 days later. To evaluate cell proliferation were injected PPD21 and euthanized 24h later. Neurogenesis were also analyzed in Age-matched NP females. The results revealed that MULT and PRI-C dams show a better performance in spatial memory than PRI-S and NP females demonstrated by less errors in their search during the probe trial ($p \leq 0.02$), PRI-C and MULT-C spent more time in the goal sector than NP and S dams ($p<0.04$), NP and PRI-S has less pokes on goal holes ($P \leq 0.008$), C dams showed a better progression from a random to serial strategy. Maternally experienced show no increases in cell survival in the Dentate Gyrus (DG) of the hippocampus, but cell proliferation was increased in both MULT-S and MULT-C ($p \leq 0.04$). These data demonstrate that multiparity dampened the consequences of disrupting the natural dam-pup interaction in memory aspects and increased cell proliferation in DG.

Keywords: Maternal separation, reproductive experience, spatial memory, bromodeoxyuridine, dentate gyrus.

(1683) **EXPERIENCE TRIGGERED BY ENRICHED ENVIRONMENT PROTECTS THE VISUAL PATHWAY ALTERATIONS INDUCED BY EXPERIMENTAL GLAUCOMA IN ADULT RATS**

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Glaucoma is a leading cause of blindness, characterized by retinal ganglion cell (RGC) loss and optic nerve damage. Increased intraocular pressure (IOP) is the most accepted risk factor for developing glaucomatous neuropathy, however many patients with successful IOP control continue to lose vision. Enriched environment (EE) is a paradigm that involves sensory, cognitive, motor, and social stimulation. The aim of this work was to analyze whether the exposure to EE prevents glaucomatous alterations. Adult male Wistar rats received 30% hyaluronic acid (ZHYALCOAT®) in the anterior chamber of one eye and vehicle in the contralateral eye, once a week, and were housed in standard environment (SE) or EE for 10 weeks. Animals were subjected to functional (electroretinogram (ERG) and flash visual evoked potentials (VEPs) recording with skull-implanted electrodes), and histological analysis. The number of RGCs was assessed by Brn3aimmunoreactivity. An immunohistochemical analysis of Iba1 (microglia and macrophage marker), and glial fibrillary acidic protein (GFAP) levels in the retina and optic nerve was performed. EE housing which did not affect IOP (p

(125) **STRESS-RELATED BEHAVIOR AND HIPPOCAMPAL GENE EXPRESSION CHANGES IN MALE RATS EXPOSED TO PRENATAL STRESS**

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Prenatal stress (PS) is associated with a dysregulation of the offspring Stress System response and higher rates of mood disorders. We have previously demonstrated that PS induces persistent changes in the expression of plasticity-related genes in the offspring hippocampus, which is a brain structure that has a major role in stress regulation. In this work, we tested how the alteration in the gene-environment dialog induced by PS could induce long lasting consequences on offspring Stress System by epigenetic changes in the hippocampus. Pregnant Wistar rats received restraint stress during the third week of gestation (PS group) or left undisturbed (control group, C). Elevated plus maze, forced swimming test and stress reactivity to an acute stress were used to evaluate *stress-related behaviors* on male offspring. The test batteries began on postnatal day 28. After last behavioral test, hippocampi were dissected to assess the expression of candidate genes related to Stress System regulation and to DNA methylation pathways. We found that PS rats had decreased anxiety- ($P=0.002$) and depression-like behaviors ($P=0.016$) in comparison with C group. Gene expression of corticosteroid receptors (*nr3c1* and *nr3c2*) and of chaperones that modulate their activity (*fkbp4, fkbp5, bag1* and *ppid*) did not vary significantly between experimental groups. However, PS increased mRNA levels of chromatin remodeler genes ($P=0.036$ for *dnmt3a*; $P=0.005$ for *mecp2*; $P=0.025$ for *tet1*). Our findings highlight that

the DNA methylation patterns, established by early-life experiences, may induce behavioral phenotypes which then dictate an individual's reaction to new stressors encountered later in life. Because we found no relationship between offspring behavior and the expression of stress-related genes in the hippocampus, on-going studies are being directed to extend the analysis of the expression of these genes in further brain structures related to stress response.

Keywords: Prenatal Stress, Epigenetic Regulation, Male Offspring, Hippocampus

(921) **ENVIRONMENTAL ENRICHMENT PREVENTS BEHAVIORAL IMPAIRMENTS AND MYELIN ALTERATIONS IN THE VPA EXPERIMENTAL MODEL OF AUTISM SPECTRUM DISORDERS**

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Autism Spectrum Disorders (ASD) are characterized by behavioural and connectivity alterations accompanied with neuroinflammation. We previously reported evidences supporting the short-distance hyperconnectivity hypothesis in the valproic acid (VPA) rat model of ASD. Experiences as environmental enrichment (EE) are known to alleviate behavioural impairments and influence brain processes. The aim of this work was to study long-distance connectivity in the corpus callosum (CC) of VPA animals and evaluate behavioural and structural effects of EE. VPA or control male rats were weaned on postnatal day (PND)21 and housed in two conditions: standard or EE. Behavioural evaluation was performed at PND7-16 (maturation parameters) and PND30-35 (exploratory activity and social test). On PND36, CC expression of myelin basic protein (MBP) and markers for microglia (Tomato lectin labelling), astrocytes (GFAP) and mature (CC1) and immature (PDGFαR) oligodendrocytes (OL) were evaluated. Myelin structure was studied in all groups (CS/VS/CEE/VEE) by electron microscopy (EM). At PND7-16, VS and VEE animals showed similar growth and maturation, delay in comparison with the CS group. At PND30-35, while hole pocking number and social exploration index were decreased in the VS group, exploratory and social behaviours in the VEE group did not differ from CS. VPA rats evidenced lower MBP expression, decreased number of $CC1^{+}OL$ and increased $PDGF\alpha R^{+}OL$ but no changes in tomato lectin or GFAP levels. EM of CC from VPA animals revealed lower percentage of myelinated axons and aberrant myelin; both myelin alterations were ameliorated in VEE animals. Our results indicate that defective OL maturation could contribute to myelin defects seen in VPA animals. EE can prevent the behavioural impairment seen in pre-pubertal stage of VPA animals as well as the structural myelin alterations observed in the CC of VPA group.

Keywords: Environmental Enrichment, Myelin, VPA model, Autism Spectrum Disorders, Corpus Callosum

(872) **EVALUATION OF SLEEP QUALITY IN MEDICAL STUDENTS AND ASSOCIATED FACTORS**

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Sleep is a neurobehavioral state related to cognitive functions such as learning, memory consolidation, and mood. A good sleep quality is associated with improved health status, less daytime sleepiness, better overall physical fitness and reduced risk of disease development. The prevalence of sleep disturbances in general population is 35%. Insomnia is the most frequent sleep disorder. The objective was to evaluate the sleep quality in medical students and several factors associated. An evaluation of sleep quality was carried out in 88 medical students who took subjects in the afternoon using the Pittsburgh Sleep Quality Questionnaire (PSQS). In addition, we consulted on certain factors that possibly affect the sleep quality: territorial uprooting, couple living together, child rearing, work activity, medical illness. The average age of students was 24.4 years and 60.2% were women. The majority of the students lived with their parents, 15.9% alone and 11.3% as a couple. The 15.9% belonged to provinces of the interior of Argentina. Nearly a third of the students worked (an average of 5.6 hours daily). Only 3.4% of the students were raising children. Of the total sample, 14.7% of the students had a chronic medical illness (more than one third were endocrinological pathologies), all treated. The mean total score of the PSQS was 6.82 points, using the cut-off point (5 points), 83% of students could be considered as "bad sleepers" compared to 17% classified as "good sleepers". Conversely, 72.7% of the students had a subjective good and very good sleep quality and only 31.8% of students slept more than 7 hours. The majority of the students (87.5%) had a daytime dysfunction as a result of poor sleep. Only 11.3% of students took sleeping medication (benzodiazepines were the most common). In conclusion, most of the students evaluated had poor sleep quality, with daytime dysfunction associated with poor sleep as opposed to the positive subjective quality perceived by them.

Keywords: sleep quality, medical students, bad sleepers

(356) **HIPPOCAMPAL HYPOCONNECTIVITY IMPLIES REDUCED NUMBER AND SIZE OF GLUTAMATERGIC SYNAPSES IN THE VPA RAT MODEL OF AUTISM**

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Autism spectrum disorders (ASD) are characterized by impairments in social interaction and repetitive-stereotyped behaviours. Strong genetic evidence regarding alterations in adhesion molecules and other synaptic proteins redefines ASD as developmental synaptophathies. A well validated ASD animal model, based on prenatal exposure to valproic acid (VPA), mimics the main behavioural and neuroanatomical alterations found in these disorders. We reported a decrease in synaptic protein synaptophysin (SYN) and the polysialylated form of neural cell adhesion molecule (PSA-NCAM) in the hippocampus of juvenile VPA rats. The aim of this study was to evaluate neuronal differentiation, synaptic formation and their temporal correlation with changes in hippocampal PSA-NCAM levels. PSA-NCAM expression was studied in the hippocampus of P3 male rat pups after *in utero* (E10.5) VPA (500 mg/kg) or saline exposure. SYN, PSA-NCAM, v-GLUT and PSD-95 expression profiles during differentiation and synaptic formation were evaluated in hippocampal neuronal cultures (DIV7-14) either from P1-2 VPA or control male pups. Number of functional synapses and vesicular dynamic were measured by FM4-64 loading and unloading assays, respectively. PSA-NCAM expression decreased in the VPA group before the *in vivo* synaptogenic peak. Once synaptogenesis reached its peak *in vitro* (DIV14), reduced SYN total puncta area accompanied with a profound decrease in v-GLUT and PSD95 puncta labelling was found. The PSA-NCAM expression diminution (DIV7) preceded the decrease in SYN puncta labelling seen in neurons from VPA animals (DIV10). FM4-64 experiments showed fewer functional synapses in the VPA group and although the vesicular pool proved to be smaller, unloading vesicle kinetic was conserved. Our results suggest that the early PSA-NCAM reduction seen in hippocampal neurons from VPA animals could affect glutamatergic synapse formation and function by altering both synapse number and vesicular pool size.

Keywords: autism, synaptopathy, PSA-NCAM, hippocampus, glutamatergic synapsis

(1871) **LACK OF $GABA_{B1}$ SUBUNIT OF THE $GABA_B$ RE-**

CEPTOR INCREASES THE AVERSIVE EFFECT ASSOCIATED TO MORPHINE WITHDRAWAL IN MICE

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Previous studies from our laboratory have shown an interaction between the opioidergic and GABAergic systems in a pharmacological approach. The $GABA_B$ receptor agonist, baclofen, was able to prevent the disphoric effect associated to morphine (MOR) withdrawal in mice. In order to continue exploring this interaction we aimed to study this aversive effect associated to MOR withdrawal by the conditioned place aversion (CPA) paradigm in a genetic approach, using mice lacking the $GABA_{B1}$ subunit of the $GABA_B$ receptor ($GABA_{B1}$ *knockout*) and their *wild type* littermates (*WT*). CPA consists in 3 phases: preconditioning, aversive conditioning and post conditioning. After preconditioning, mice of both genotypes were rendered dependent by an intraperitoneal (i.p.) injection of MOR (2 mg/kg), twice daily for 9 consecutive days. A control group received chronic saline (SAL) injections. On the 10th day, the conditioning phase started: 60 min after the last MOR injection, the withdrawal syndrome was precipitated by the administration of the opioid receptor antagonist, naloxone (3 mg/kg, i.p.) and mice were left to explore one of the context of a conditioning box for 30 min. The next day, mice were injected with SAL 60 min after the last MOR injection and placed in the opposite context for 30 min. Post conditioning phase was performed 24 hs after the last conditioning sesion. The results showed that MOR withdrawal was able to induce aversive effect in both *WT* ($p<0.05$) and $GABA_{B1}$ *knockout* ($p<0.001$) mice, but the intensity of this effect was significantly higher in $GABA_{B1}$ *knockout* mice compared to their *WT* littermates ($p<0.05$). Our results support the hypothesis that $GABA_B$ receptors could have a significant role in modulating the aversive state induced by MOR withdrawal. In addition, these $GABA_B$ receptors could be a potential therapeutic target to treat MOR addiction.

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Keywords: conditioned place aversion, naloxone precipitated morphine withdrawal, $GABA_{B1}$ knockout mice.

(188) **LATERALIZED EXPLORATORY ACTIVITY IN MATURING ANIMALS TREATED WITH LOW NON-TOXIC DOSES OF ZN, TE AND ZN+TE.**

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Previously in our laboratory, evidence was presented showing that after systemic administration of ZnTe in non-toxic doses to pregnant mother and its litter rats, several behavioural parameters related to motivated and lateralized exploration in the offspring were affected. Since both Zn and Te have biological effects, the purpose of the present work was to evaluate which of the described effects was due to Zn, Te or both. For this purpose, maturing rats were exposed to ZnCl2 (n=12; Zn), K2TeO3 (n=13; Te) and ZnTe (n=12; Zn+Te), 1.55 nM in the drinking water. Tap water administration was considered control (n=9). At 30 day-old, all animals were tested individually in a Double Lateral Hole-Board Labyrinth (LDHB) to register motivated lateralized exploration induced by novelty; Resident-Intruder Challenge (RIC), to register social behavioural parameters, and Forced Swimming (FS), to register survival motivation. All experiments were videotaped, and behavioural activity was recorded by a digital automatic counter (Counts/3min). Results shown that in the LDHB only Te abolished completely the natural left-biased exploration of rats (50.5±4.7, left; 46.5±7.1, right, n.s., Te group; 52±6, left; 38±5, right, $p<0.05$; Control group). Zn treatment did not affect the lateralized exploration. In the RIC test, Only Te increased significantly the latency to confront the intruder (141.5±32; Te group Vs 32±10; Control, $p<0.01$). Zn had no effect, however, when combined with Te (Zn+Te) a partial increase of latency was observed. In the FS test, Te inhibited the active swimming (176±9, Te Vs 243±12, Control, $p<0.01$), while Zn did not affect this behaviour. In conclusion, results show that the main effect on cognitive behaviours of ZnTe exposition is due to Te and not to Zn or a combined action of Zn with Te.

(1460) **NEURAL MODULATION OF STRESS RESPONSE IN *C. elegans***

María José De Rosa, Tania Veuthey, María Gabriela Blanco, Natalia Andersen, Sebastián Giunti, Mark Alkema, Diego Rayes
INIBIBB

In response to environmental challenges, such as thermal and oxidative changes or nutrient deprivation, cells can trigger widely conserved mechanisms with the aim of maintaining protein homeostasis and minimize intracellular protein aggregation. These mechanisms of stress response include the induction of heat shock protein (HSPs), to prevent protein misfolding, and the up-regulation of enzymes that protect against oxidative stress. Another process that is triggered in stressed cells is the autophagy, which permits the degradation of different biomolecules to satisfying cell energy demands and maintaining the proteostasis.

The coordination of this intrinsic capacity in multicellular organisms is crucial.

Studies in *C. elegans* showed that the nervous system plays a key role in this coordination. However, the signal that integrates stress perception with the response in non-neuronal cells is unknown. Our analysis of the *C.elegans* wiring map reveals that the circuits activated upon stress converge in the tyraminergic neuron, RIM. Tyramine (TA) is the invertebrate counterpart for adrenaline. We found that, even under favorable growth conditions, TA-deficient animals exhibit universal hallmarks of stressed organisms, such as autophagy and HSPs induction. These mutants are resistant to thermal and oxidative stress and starvation. Null mutants of *tyra-3,* a TA-activated adrenergic-like GPCR receptor, are also resistant to stress. Despite tyra-3 is expressed in neurons and intestine, it is only needed in the gut for wild-type stress resistance. Moreover, we show that the insulin receptor DAF-2 is essential for the TA-dependent coordination of stress response. Therefore, inhibition of TA release is a neuroendocrine signal that negatively modulates insulin pathways leading to a coordinated stress response in C. elegans. This study contributes to the understanding of the neurohormonal signaling underlaying stress response regulation in multicellular organisms.

(1521) **ROLE OF INSULIN SIGNALING IN *C. elegans* MODELS OF HUNTINGTON DISEASE**

Natalia Andersen, Gabriela Blanco, Tania Veuthey, Maria José De Rosa, Diego Rayes
INIBIBB

Insulin/insulin-like growth factor signaling (IIS) is an universal conserved pathway, with relevant functions in metabolism, growth, development and longevity. Reduced IIS has been shown to extend lifespan and elevate stress resistance in many animals. On the other hand, it has also been reported that an apparent increase of IIS protects against proteotoxicity. This contradiction leads to the intriguing question of the role of IIS pathway in the development of neurodegenerative diseases.

To shed light into this, we use *C. elegans* models of Huntington disease (HD). These models express expanded polyglutamines (polyQ) repeats coupled to GFP that induce protein aggregation in muscular or neuronal cells, thus mimicking the aggregation that occurs in HD.

Mutants of the mammalian insulin/IGF-1 receptor ortholog, DAF-2, were crossed with HD strains. As previously reported by other groups, we found that *daf-2* mutants are long-lived and extremely resistant to oxidative stress. Unexpectedly, we found that *daf-2* mutant worms expressing polyQ repeats have an increased number of aggregates and reduced mobility when compared with the control. Besides, the lifespan of *daf-2* mutants expressing neuronal polyQ repeats is significantly shorter than control animals. Moreover, polyQ repeats severely impairs the oxidative stress resistance of *daf-2* null

mutants while it does not affect the resistance of wild-type strains.

Taken together, the results reported here show that the inactivation of IIS results in an impairment of the proteostasis network. Further studies are required to elucidate the molecular mechanisms that explains this protective effect of IIS in the progression of HD. Given the conservation of the IIS throughout the animal kingdom, this study will contribute to the understanding of the insulin paradox (protective or harmful) in the context of neurodegenerative diseases.

BIOTECHNOLOGY AND BIOINFORMATICS 3

(276) COMPARATIVE GENOMIC ANALYSES REVEALED NEW *Lactobacillus* STRAINS POTENTIALLY COBALAMIN PRODUCERS

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Vitamin B_{12} (cobalamin-CBL) is an essential corrinoid for cell function in animals. However, only a limited number of bacteria strains and Archaea synthesize CBL *de novo*. Some strains of different *Lactobacillus* (*L.*) species have been described as CBL producers: *L. reuteri* CRL 1098, *L. rossiae* DSM 15814 and more recently *L. coryniformis* CRL 1001 and *L. plantarum* BCF 20 and BHM 10 strains. In this work, genes that encode proteins involved in CBL biosynthesis (Cbi, Cob, Hem) were sought in the genome of different *Lactobacillus* strains sequenced and deposited in the Genbank database. *In sílico* comparative studies between gene cluster of cobalamin producers strains and genomes of *Lactobacillus* strains founded in the database were carried out. Eight genomes of different *lactobacilli* strains possessing the genes for CBL biosynthesis *de novo* were found. Two of them do not possess genes involved in the first steps of the corrinoid biosynthesis (*hem* genes). Other two do not have the genes described for cobalt transporter (*cbiMNQO*). This work highlight that most of the strains have *cblST* genes in their genomes which would make evident that the most common pathway for the activation of the lower ligand is carried out by an α-ribasol kinase. Interestingly, *L. coryniformis* CRL 1001 is the only that possesses the *pduX* gene in its genome, which product is involved in the aminopropanol synthesis, transferring a phosphoryl group to a free L-threonine. This work provides interesting information for the prospection of nutraceutical-producing lactic strains (particularly CBL) as a preliminary step to the use of these strains in the design of functional foods bio-enriched in vitamin B_{12}.

Keywords: *Lactobacillus*, cobalamin, genes, biosynthesys

(385) PUTATIVE QUADRUPLEX SEQUENCE (PQS) DISTRIBUTION IN TRYPANOSOMATID GENOMES: A MARK FOR TRANSCRIPTION OR TRANSLATION TERMINATION?

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Trypanosomatids cause important diseases in humans like Leishmaniosis and Chagas disease. Their genes present an unusual organization in polycistronic transcription units (PTUs) that are transcribed together from a strand switch region (SSR) without canonical promoters. Polycistronic transcripts are processed by *trans*-splicing and polyadenylation to give mature mRNAs.

Trypanosomatids do not rely on typical transcriptional mechanisms to control gene expression. Instead, various post-transcriptional events seem to modulate the final protein content. More recently, epigenetic marks and chromatin state have shown to be important actors in control of gene expression.

Guanine Quadruplexes (G4) are secondary structures formed in DNA or RNA regions rich in guanines. G4 have been implicated in transcriptional regulation and in recombination of immunoglobin and antigenic variation-related genes in several pathogenic organisms.

We performed a bioinformatic analysis of the frequency and distribution of putative guanine quadruplexes (PQSs) in the genomes of *Leishmania* and *Trypanosoma* species in order to evaluate their possible impact on the control of gene expression at transcriptional and post-transcriptional levels.

In *Leishmania*, we observed a higher density of PQSs (concordant with the higher GC content in this genus) compared with *Trypanosoma* species. PQSs are enriched in the terminal end of the PTUs of the template strand, adjacent to convergent SSRs. This pattern suggests a possible relationship between G4s and transcription termination in *Leishmania*. No clear correlation between PQS distribution and possible functions was found in *Trypanosoma* genomes. Then, we analyzed PQSs distribution inside the PTUs of both genera. An outstanding difference was observed between genes and intergenic sequences, with a very low density of PQSs in coding-sequences in relation to intergenic regions, what may suggest a putative role of G4s in RNA maturation, stability and/or translation.

(456) IDENTIFICATION AND CHARACTERIZATION OF DIFFERENTIAL GENE EXPRESSION IN WHITE BLOOD CELLS EXPOSED TO IONIZING RADIATION

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The analysis of gene expression in blood cells has been emerged as a novel method for biodosimetry marker detection. The differential expressed genes could predict the clinical outcome of patients and guide the prevention, early diagnosis and treatment of ionizing radiation induced damage, improving traditional approaches. The aim of this study was to perform a meta-analysis of expression microarray data of irradiated blood cells in order to identify differentially expressed genes with overlapping among studies, which could be proposed as biomarkers of ionizing radiation exposure at 2 Gy and 24 h post-exposure. Data of four expression microarrays obtained from Gene Expression Omnibus (GEO) were evaluated by using the R programming language and Bioconductor packages. Differential gene expression was evaluated by Limma (parameters: lfc=1 and $p<0.005$). The functional classification was done with the Molecular Signature Database from Gene Set Enrichment Analysis (FDR<0.05) and Database for Annotation, Visualization and Integrated Discovery (DAVID). The iRegulon database was used to identify transcription factors (TFs) as master regulators and the gene networks were visualized by GeneMANIA. Nine differentially expressed genes (E2F7, PLK2, TCF4, DRAM1, POLR2A, HRK, NUDT15, C12orf15 and GADD45A) were identified (lfc<0.5 and $p<0.001$) for further validation using quantitative PCR. These genes are associated with regulation after exposure to ionizing radiation, p53 pathway, cell cycle checkpoint involved in G1/S transition, cell cycle arrest and processes induced by the detection of DNA damage. Besides, E2F7 and TCF4 were detected as master regulators TFs with corepressor activity. In conclusion, we identified nine genes which could be relevant as biomarkers of ionizing radiation exposure for biodosimetry studies. Further studies on these genes will relevant to understand the patterns of gene expression in response to ionizing radiation in Argentine subjects.

Keywords: biodosimetry, bioinformatics, biomarkers, ionizing radiation.

(542) PHYLOGENETIC PROFILING OF THE EXOPROTEOME - USING COEVOLUTION OF SECRETED PROTEINS TO IDENTIFY BACTERIAL PROTEIN SECRETION SYSTEMS

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Protein Secretion Systems (SS) are multiprotein complexes that play a pivotal role in the life of bacteria, specially in the processes that require interaction with other organisms such as host invasion, symbiosis and interbacterial competition. A single SS is capable of secreting multiple proteins exhibit high conservation due to the evolutionary pressure on sus-

taining their function, in contrast secreted proteins are highly divergent and poorly conserved in order to be able to interact with different hosts and environments.
Up to this date nine specialized bacterial SS have been characterized and thousands of secreted proteins have been identified in culture supernatants both with and without an assigned secretion mechanism. However, the experimental identification of SS remains challenging.
In this work we validated the detection of a given SS by comparing the evolutionary patterns between secreted proteins and elements of the SS in multiple organisms.
The OMA orthologous database was used to generate phylogenetic profiles (PP) across all the complete bacterial genomes. The PP of secreted proteins were compared against every element of the proteome using mutual information, PPs of protein constituents of the SS were found to score higher than proteins not related to the SS. High scoring proteins were used to conform a graph, where each edge represents a predicted coevolutionary interaction between PPs. The node connectivity is directly related to the number of different proteins secreted by a single SS, and putative complexes were analyzed by clustering the graph adjacency matrix, by doing so we were able to identify and group the core components of the type 2, 3, 4 and 6 SS from an unordered set of secreted proteins. Our results suggest that this methodology would be applicable in the detection of new SS, either by using pre existent data or by supernatant proteomic analysis.

(798) **USE OF VNTRS WITHIN CODING SEQUENCES TO GENOTYPE *TOXOPLASMA GONDII***

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Toxoplasma gondii is an intracellular protozoan with a worldwide prevalence in human and animal populations. Infection occurs as a result of ingestion of resistant forms present in meat products and exposure to cat faeces. In immunocompetent individuals is generally asymptomatic. Severe disease may occur in immunocompromised subjects and in congenital toxoplasmosis, which is caused by transplacental acquisition of Toxoplasma gondii.

Genetic diversity of *T. gondii* has been studied using a PCR-RFLP scheme based on nine molecular markers. These studies led to the description of a clonal population structure with three main lineages, designated as type I, II and III.

The aim of this study was to develop molecular markers that allowed the discrimination of genetic variants within each clonal linage and therefore describe *T. gondii* population variability closer to strain level.

We analyzed the genome of *Toxoplasma gondii* to identify genes containing variable number tandem repeats (VNTRs). The coding sequences of T. gondii ME49 genome (www.toxodb.org) were processed with Tandem Repeat Finder software. A panel of candidate markers was selected based on the following parameters: the repeat period (<9), the number of repeats copies (>20), the repeat module composition (to avoid single and dinucleotide runs) and the absence of introns within the repeat region.

The selected panel of eight molecular markers was analyzed in PRU (type II) and RH (type I) strains. As a first step, the variability of the PCR product size allowed us to differentiate PRU from ME49 (both type II strains) and RH from GT1 (both type I strains). Additionally, amplification products from PRU and RH strains were sequenced to study intra-lineage variability. Polymorphic markers between type I and type II strains presented specific arranges of the VNTR pattern. Nonetheless, those markers that didn´t present size polymorphisms were also conserved at the sequence level.

Keywords:Toxoplasmosis, Epidemiology, Minisatellites

(1027) **LABEL-FREE PROTEOMIC ANALYSIS OF THE HEXACHLOROCYCLOHEXANE-DEGRADING ACTINOBACTERIA *STREPTOMYCES* SP. M7**

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The increment of the global population and subsequently the increasing demand of food has promoted the use of pesticides such as hexachlorocyclohexane (HCH). HCH is a chlorinated pesticide used to protect crops from vector borne diseases. Large-scale production and the extensive use of this compound led to deterioration of environmental quality owing to its persistence in the environment. Over the last years, bacteria-mediated degradation of toxic organic compounds has become in an effective biotechnological process. *Streptomyces* sp. M7 was isolated from polluted sediments in the province of Tucumán, Argentina. Several physiological studies demonstrated the ability of M7 to remove HCH and highlighted the potential of this strain to be used in bioremediation processes. Degradation pathways for HCH and organochlorine compounds in general, are not fully elucidate in Actinobacteria. So far, a complete mechanism for the degradation of HCH was only proposed in *Sphingobium japonicum*. MS-based proteomics have become a powerful tool to elucidate and understand the mechanisms that underlie physiological processes. In the present work we used a MS-based, label-free, and quantitative proteomic approach as a starting point for understanding the degradation pathway for HCH in our strain. M7 proteome showed 293 proteins that were significantly up-regulated in the presence of HCH. Key enzymes involved in the dechlorination of HCH (LinA; LinB and LinC) were identified. In addition, 8 proteins assigned to the xenobiotic degradation category could be involved in the so called downstream degradation pathway of HCH. Proteomic results support the physiological capacity of *Streptomyces* sp. M7 to degrade hexachlorocyclohexane. In turn, the obtained results will allow to postulate a degradative pathway for this bacterium. Keywords: Biodegradation, *Streptomyces*, Proteomic

(1349) **HIGH QUALITY DRAFT GENOMES OF MEMBERS OF ACIDOBACTERIA SUBDIVISION 4 RESOLVED FROM TWO INDUSTRIAL WASTEWATER TREATMENT PLANT METAGENOME**

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The activated sludge (AS) technology, used worldwide for the treatment of municipal and industrial wastewater, relies on the self-assembly of a highly diverse and dynamic microbial community, where bacteria are responsible for the removal of most of the oxygen-demanding pollutants and nutrients. Our general aim is to gain understanding of AS functioning through the ecophysiology of key microbial players.

Shotgun sequencing of samples taken at different time from the aeration basins of two industrial full-scale wastewater treatment plant (WWTP), textile-dyeing (TD) and polymer synthesis (PS), was performed using Illumina HiSeq 1500. A total of 4.38 x 10^7 high quality paired end reads of 150bp (6.57 x 10^9 bp) were assembled. Differential coverage of scaffolds was used as a binning strategy, followed by %GC and tetranucleotide frequency. Paired end reads were tracked and reassembled, and contamination with foreign genomes was checked.

Out of forty-five genomes assembled into near-complete chromosomes, we focused primarily on two genomes of a stable and relatively abundant (ca. 9.8 % and 6.8%) member of this community, according to PCR amplification based on a specific region of the 16S rRNA gene and *in silico* abundance estimation. The genome from TD had 3.6 Mbp and 92.3% of completeness with 0.8% of contamination, while the genome from the PS had 2.7 Mbp with 77.2% of completeness and 0.05% of contamination. Phylogenetic reconstruction using 16S rRNA and 26 conserved genes sequences indicated both genomes belonged to Acidobacteria Subdivision 4,

Blastocatellaceae family.

The resolved genomes are distinguished by the relatively high proportion of genes coding for enzymes related to membrane transport (such as type VI secretion machine) and osmotic stress response (EnvZ-OmpR). These features are consistent with the ability of these bacteria to thrive in a highly competitive, nutrient poor environment of high salt concentration.

(1368) **TDR TARGETS: A CHEMOGENOMICS DATABASE FOR NEGLECTED DISEASES FOR DRUG REPURPOSING AND PROTEIN TARGET PRIORITIZATION**

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The volume of biological and chemical data deposited in the public domain is growing fastly, thanks to next generation sequencing and screening technologies. However, curated, functional and chemical information for most model organisms still vastly exceeds that for neglected diseases. In our laboratory, we have developed a chemogenomics database resource, TDR Targets (www.tdrtargets.org) that aims to organize and integrate heterogeneous large datasets. The resource hosts both for genomic and chemical data with a focus on neglected diseases but also allows users to make use of information available for other organisms. The database facilitates target prioritizations by allowing users to formulate complex queries across diverse query spaces: biophysical properties, predicted annotations, and relevant functional data. Users may also search for bioactive compounds and their annotated targets and navigate the data by taking advantage of existing links (orthology to link targets, chemical similarity to link compounds). Compound searches can be done either by physicochemical features, by their bioactivities or assays, or by drawing a molecules to look for similar compounds. Here we present recent updates to the TDR Targets database resource. The database has been updated to integrate data on >2 million bioactive compounds from different sources; 19 pathogen genomes for WHO/TDR Targets; and 30 complete genomes for model organisms and other related pathogens. Available genomes include those of Trypanosomatids*;* Apicomplexans*;* Mycobateria*;* Metazoans (including flatworms and nematodes); Bacteria; other Early Branching Eukaryotes *(Giardia, Trichomonas); and* Amoebas *(Entamoeba).* We will also present advances in how users can explore drug-target relationships in a more intuitive manner, by taking advantage of chemogenomics data built into a novel network model. *Keywords:* Chemogenomics, Database, Neglected Diseases, Drug Discovery, Target prioritization

(1836) **UTRME: A TOOL TO ANNOTATE UTRS IN TRYPANOSOMATIDS**

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Since post-transcriptional regulation is the main level of gene expression control in trypanosomatides, the co-regulation of functionally related genes is postulated to be mainly achieved through post transcriptional operons. This system relies on signals that are mostly present in the UTRs of mRNAs, and that are recognized by regulatory trans acting factors. To deepen our understanding of the mechanisms and signals that determine these processes we need to correctly determine mRNA boundaries. To this end we developed UTRme (UTR-mini-exon), a program to annotate UTRs in trypanosomatids. Using pair-end or single-end RNA-Seq data and an annotated genome, UTRme annotates the UTRs and assigns them a reliability index, which indicates the confidence with which the polyadenylation and transplicing sites were detected. The program is based on several read filtering steps (through trimming and alignment) in order to obtain a reliable set from which the annotation will be generated. The score assigned to each site depends on the number of reads that support it, the differences between the bases of polyadenylated or transpliced read with its aligned genomic region, the number and percentage of adenines for 3'UTRs, among others. UTRme allowed us to determine the use of preferential sites according to the parasitic stage and search for signals in UTRs, both at the level of primary sequence and secondary structure, that might explain the observed co-regulation of gene family members. UTRme is an open-access, user friendly software that we hope it will be useful for the community working in trypanosomatides.

Keywords: UTRme; RNAseq; Software; Trypanosomatides; UTRs

(166) **CELL CYCLE AND TRANSCRIPTOME ANALYSIS FOR BETTER UNDERSTANDING OF CHO-K1 SUSPENSION CELL CULTURE**

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Chinese Hamster Ovary (CHO) cells are the preferred host for the production of a wide range of biopharmaceuticals. Knowing how these cells behave during bioprocessing has until now relied mainly on empirical analysis with a limited knowledge of the intracellular dynamics. With the latest advances in -omics techniques it is now possible to leverage the genomic resources to better understand and further improve CHO cell bioprocessing. The aim of this work was to study the distribution of cell cycle phases and to perform transcriptome analysis, by RNA-seq, during the culture stages of a typical industrial bioprocess of suspension-adapted CHO-K1 cells.

Three replicates were cultured during 20 days in 1 L bioreactors. Equal operating conditions were used and the temperature was shifted gradually from 37°C to 31°C. For cell cycle study, a daily sample was taken and DNA content was defined by propidium iodide staining followed by flow cytometry analysis and cell cycle modeling. RNA samples were collected on day 1, and during the exponential (days 7 and 8) and the stationary (days 14 and 15) growth phases for strand-specific RNA-seq analysis. Differential gene expression (DE), Gene Ontology enrichment and pathways analysis were performed.

Due to temperature shift, an S phase cell population reduction was observed and G0/G1 arrest over culture time. In particular, during stationary phase nearly 80% of the cells analyzed were in G0/G1. Clustering analysis of RNA-seq data revealed that days 7 and 8 clustered together with day 1, and days 14 and 15 comprised another group, indicating that the major expression changes occur during stationary phase. A number of key regulatory genes and pathways involved in modulating cold stress, metabolic, apoptosis and growth were identified. The DE analysis showed that at 31°C 443 genes were up-regulated and 182 genes were down-regulated. These genes can be used as targets for cellular and metabolic engineering to improve CHO cell bioprocessing.

Keywords: Bioprocess, Cell cycle, CHO cell culture, Flow cytometry, RNA-Seq

REGENERATIVE MEDICINE AND CELL THERAPY 1

(367) **ANGIOGENIC ACTIVITY OF LATE OUTGROWTH ENDOTHELIAL PROGENITOR CELLS IS INHIBITED BY COXSACKIEVIRUS B3**

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Coxsackievirus B3 (CVB3) is a frequent etiological agent of human viral myocarditis, characterized by myocardial inflammation, necro-

sis and subsequent fibrosis. Local or homed stem and progenitor cells are involved in myocardial repair, especially endothelial progenitor cells which have a lead role in new blood vessel formation and tissue revascularization. Here, we aimed to study the effect of CVB3 on the survival and angiogenic activity of late outgrowth endothelial colony forming cells (ECFC).

Cord blood-derived $CD34^+$ cells were seeded in EGM2 medium and ECFC colonies were obtained after 14-21 days. ECFC were incubated with CVB3 at a multiplicity of infection (MOI) 0.1 or 1 for 1 hour at 37°C and then exhaustively washed to ensure virus removal. Cell death (ethidium bromide-acridine orange staining), proliferation (cell count), static adhesion to matrix components (cell count), tubule formation (matrigel) and cell adhesion molecule expression (flow cytometry) were analyzed *in vitro*. Results are expressed as mean ± SEM. Significant differences (*p<0.05) were identified by ANOVA.

Nuclear morphology analysis showed that CVB3 had no cytotoxic effect on ECFC (Mock: 7.2±1.3; MOI 0.1: 6.0±0.6; MOI 1: 10.3±1.5 vs control: 4.7±2.2 % of death, n=3). However, CVB3 significantly decreased ECFC proliferation (Mock: 98.8±5.5; MOI 0.1: 78.3±4.4*; MOI 1: 60.4±3.2* % of control, n=7), adhesion to type I collagen (Mock: 96.5±2.5; MOI 0.1: 73.3±12; MOI 1: 56.7±14.9 % of control, n=3) and tubule formation (MOI 0.1: 66.3±6.1*; MOI 1: 52±7.9* % of control, n=4) in a MOI-dependent manner, whereas adhesion to fibronectin was not affected. TNFα-induced E-selectin expression, but not ICAM, was significantly reduced in the presence of CVB3 (Mock: 7.1±1.5; TNFα 1 ng/ml: 37±13.7 MOI 0.1 + TNFα 1ng/ml: 35.3±11.6; MOI 1 + TNFα 1 ng/ml: 23.3±13* %, n=4). Our results showed that CVB3 caused an inhibition of ECFC angiogenic activity, which is not associated with a viral cytophatic effect.

Keywords: angiogenesis, endothelial progenitor cells, coxsackievirus B3.

(1743) **CARDIOMYOCYTES DERIVED FROM INDUCED PLURIPOTENT STEM CELL PROLIFERATE AFTER CRYOPRESERVATION AND REPLATING.**

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In the human heart, shortly after birth, cardiomyocytes (CM) undergo one last round of division followed by escape from the cell cycle. From then on, the heart develops in absence of CM cytokinesis and generates multinucleated cells. This lack of proliferation is characteristic of CM in the adult mammalian heart accounting for its markedly limited regenerative potential. Production of induced pluripotent stem cell-derived CM (iPSC-CM) is a potentially promising strategy for regenerative therapies, biological studies, disease modeling and drug screening. However, difficulties in generating sufficient quantities of highly purified cardiomyocytes have been a barrier to fully explore their potential.

Therefore, it is necessary to develop a cardiac differentiation protocol that allows obtaining large CMs quantities. We then analyzed the ability of the iPSC-CM to re-enter cell cycle after exposure to stress stimuli, such as cryopreservation and replating (shift to new coated plates). iPSC-CM were obtained implementing a previously reported protocol (Lian *et al.* 2012). Beating cells were cryopreserved in liquid nitrogen with 20% serum and 10% DMSO at day 20 of the differentiation protocol. Proliferative capacity was assessed at two times after thawing, 24 hours and 7 days, and after replating, by incubation with EdU overnight and subsequent analysis by immunofluorescence and flow cytometry.

Our results show that only a small proportion of unstressed CMs were cycling (less than 3%) but this percentage increased when exposed to stressors. In replated CMs we observed that up to 30% of them had re-entered S phase, while cryopreserved CMs showed a 20% of proliferative cells. In all cases, replication activity decreases over time.

These findings suggest that iPSC-CMs are able to re-enter cell cycle under appropriate conditions for a limited period of time. Currently our goal is to develop a protocol that allow us to enhance this capacity to generate large numbers of purified CMs.

Keywords: induced pluripotent stem cell, cardiomyocytes, proliferation.

(859) **CHARACTERIZATION OF IPSCS DERIVED CARDIOMYOCYTES FOR THEIR APPLICATION IN DISEASE MODELLING**

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Cardiomyocyte differentiation is a powerful tool for disease modeling. One cardiomyopathy we are interested in modeling by CRISPR is the arrythmogenic right ventricular dysplasia (C/DAVD), caused by mutations in desmosomal genes including desmoplakin (Dsp), plakoglobin (Pkg), plakophilin 2 (Pkp2), desmoglein 2 (Dsg 2) and desmocolin 2 (Dsc2). The aim of this work was to characterize the cardiomyocyte differentiation protocol from induced pluripotent stem cells (iPSCs) and to analyze the expression of these genes. The differentiation protocol consisted on culturing $3x10^5$ iPSCs in a 24 well with mTeSR medium for 3 days, until when cells were incubated with 9uM CHIR99021 (GSK3 pathway inhibitor) in RPMI+B27 basal medium for 24h (day 0). On day 3, cells were incubated with 5uM IWP2 (Wnt pathway inhibitor) for 48h and on day 7 the medium was changed to RPMI+B27+insulin. To enrich the cardiomyocyte population, the medium was changed to RPMI-glucose+7uM lactate (days 12-18). After that, the cardiomyocytes were maintained with RPMI+B27+insulin. Trizol samples were taken on day 0, day 3.5, day 7 and day 21, from 3 independent experiments. After RNA isolation and RT-PCR, qPCRs were performed for pluripotency genes (*Oct4* and *Nanog*), mesoderm-cardiac differentiation genes (*Brachyury*, *NKX2.5* and *cTnT*), and desmosomal genes (*Dsp, Pkg, Pkp2, Dsg2 and Dsc2*). Student's T test was used for statistical analysis ($p<0.05$). As the protocol advanced, we observed contractile cells by day 8, the expression of the pluripotency genes significantly decreased, and an increase in the expression of brachyury at day 3.5 and NKX2.5 and cTnT at day 21. Respect to desmosomal genes, all of them presented a steady gene expression increase during the differentiation with no statistical differences, though. In summary, we were able to verify a functional cardiomyocyte protocol and the expression of the desmosomal genes of interest during the differentiation protocol for future model of C/DAVD.

Keywords: iPSCs, cardiomyocytes, regenerative medicine.

(1477) **CHARACTERIZATION OF lncRNAs EXPRESSION LEVELS ALONG CARDIAC DIFFERENTIATION**

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Human pluripotent stem cell (hPSC)-derived cardiomyocytes (CMs) are extensively used for the study and modelling of diseases. Long non-coding RNAs (lncRNAs), a diverse group of RNA that have no coding potential, have been shown to actively participate in cardiac differentiation through the modulation of key gene expression networks. This study is focused on three main goals: a) generation of hPSC-derived CMs by the implementation and optimization of a previously reported protocol (Lian *et al.* 2012); b) evaluation of lncRNAs expression levels at three stages of the differentiation protocol -pluripotent (day 0), early mesoderm progenitor (day 3.5) and immature cardiomyocyte (day 21)- by quantitative real-time PCR (qPCR); and c) study the effect of manipulating lncRNA expression levels by CRISPR/Cas9 technology on cardiac differentiation. CMs were generated from two cell lines, an induced hPSCs (hiPSC) line developed in our laboratory (FN 2.1) and an embryonic hPSC (hESC) line (HES3). qPCR analysis was performed with samples collected at the times indicated above. Throughout the differentiation we were able to see a downregulation of the lncRNAs associated with pluripotency (linc-ROR and lncRNA_ES1). Correspondingly,

cardiac differentiation lncRNAs markers showed an upregulation: HOTAIR and MALAT1 (epithelial-to-mesenchymal transition); Hox-Blinc (early mesoderm) and CARMN (immature CM commitment). The next step was to induce or repress these lncRNAs using CRISPR/Cas9 with the purpose of gaining insight in their roles during the cardiac differentiation. Although no results have been produced yet, preliminary data in our group indicates that the strategy is functional. In conclusion, this work provides a simple, efficient protocol for the generation of hPSC-derived CMs, a characterization of lncRNAs expression levels through the differentiation process and contributes to define a clear strategy for modulating these lncRNAs by (epi)genome editing technology.

Keywords: lncRNAs, cardiac differentiation, hPSC

(1010) **CONTRIBUTION OF MESENCHYMAL STEM CELLS TO ENDOTHELIAL REPAIR IN HEMOLITIC UREMIC SYNDROME**

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Mesenchymal stem cells (MSC) are multipotent cells that possess known tissue regenerative properties. Induced Pluripotent Stem Cell-Derived Mesenchymal Stem Cell (iPS-MSC) has similar characteristics as MSC. Endothelial tissue damage in the renal glomerulus is one of the most relevant issues that cause kidney failure in Hemolytic Uremic Syndrome (HUS). Our aim was to investigate if iPS-MSC were able to contribute in the repair of endothelial damage cause by the exposure to lipopolysaccharide (LPS) and Shiga toxin 2 (Stx), the two most important etiological mediators in HUS. For this purpose, we used an *in vitro* model of endothelial damage using the cell line HMEC-1 incubated with LPS and/or Stx for 24 h. We found that iPS-MSC exposed to LPS decreased their migratory capacity measured as the migrated area after a wound in the cell monolayer compared to Control cells, but the combination of LPS+Stx reversed this effect (area cm^2 ($x10^2$), Control:5,10±0,04; LPS:3,60±0,02*; Stx: 3,80±0,03*;LPS+Stx:6,50±0,04#,*vs. Control and #vs. LPS or Stx, p<0,05). Also, LPS+Stx decreased the release of tumor necrosis factor-α (TNF-α) measured by ELISA and increased the expression of the RNAm of Vascular Endothelial Growth Factor (VEGF) assessed by real time PCR (pg/ml TNF-α, Control:27±0,7; LPS:152±3,5*; Stx:75±2,7; LPS+Stx: 79±0,7#; VEGF RNAm expressed in % to Control group, Control:1; LPS: 1,09±0,05*; Stx: 1,60±0,12;LPS+Stx:2,10±0,15#; *vs. Control and #vs. LPS p<0,05). Then, we studied the effect of iPS-MSC conditioned media (CM), obtained from the different groups, on endothelial cells. Only the CM from iPS-MSC treated with LPS+Stx increased the migration of endothelial cells in a wound-healing assay (area cm^2, Control: 68±0,4; LPS:0,3±0,1*; Stx:0,2±0,03*;LPS+Stx:33±0,3 #,*vs. Control and #vs. LPS or Stx p<0,05).In conclusion, whereas LPS and Stx alone seems to be a pro-inflammatory stimuli for iPS-MSC, together LPS+Stx activate a repair endothelial program in iPS-MSC.

Keywords: IPS-MSC, HUS, Shiga toxin, endothelial damage, repair

(645) **EXOSOMAL RESPONSE TO GENOMIC DAMAGE INDUCED BY UV-C RADIATION IN MESENCHYMAL STEM CELLS DERIVED FROM INDUCED PLURIPOTENT STEM CELLS**

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LIAN CONICET, Fleni.

Exosomes are extracellular vesicles, ranging from 30nm-150nm in size that originate from the endosomal pathway; containing proteins, lipids and nucleic acids, implying an important role in cellular communication. This process is regulated and affected by the cell of origin and its physiological condition or state. In our study, we sought to elucidate the effect genomic damage provoked by UV-C may have on exosomal secretion of mesenchymal stem cells derived from induced pluripotent stem cells (iPS-MSC).

In our study, iPS-MSC were irradiated with three different UV-C intensities (0.001 J/cm^2, 0.01 J/cm^2 and 0.1 J/cm^2). Genomic damage was tested by inmunofluoresce against H2AX, p53 and p21. H2AX showed higher, more sustained levels with the highest intensity, while the other inferior intensities had a lower, biphasic response. The highest intensity activated the least percentage of p53 and p21, while the lowest intensity caused the highest p53 nuclear activation, and cytoplasmic p21 activation.

Through RT-qPCR we evaluated the expression of genes involved in the exosomal pathway. Syntenin, Rab27a and ALIX showed a significant increase in expression for the lowest UV-C intensity solely; while CHMP4a, TSG101, TSAP6, Rab3a, Rab5a and Rab7a did not show a significant difference.

In an attempt to corroborate this, exosomes were isolated 16hrs post-radiation and resuspended in PBS or RIPA to measure membrane bound and internal proteins, by the BSA protein quantification kit, which did not show a significant difference between the three intensities.

Taken together, our results suggest that UV-C causes genomic damage to iPS-MSC and that exosomal secretion could be altered due to this. The lowest UV-C intensity causes the highest activation of p53 and p21, and a tentative increase in various genes implied in exosome secretion. However, when total internal or external exosomal proteins were measured, there seemed to be no significant difference.

Keywords: Exosomes, Genomic Damage, iPSC-MSC, Bystander Effect, MSC

(151) **INDUCTION OF HUMAN PLURIPOTENT STEM CELLS APOPTOSIS BY ACUTE SEVERE HYPOXIA IS HIF1A INDEPENDENT**

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Abstract: Human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs) are self-renewing pluripotent stem cells (PSC) that can differentiate into a wide range of specialized cells. Although moderate hypoxia ($5\%O_2$) improves PSC self-renewal, pluripotency and cell survival, we previously demonstrated that severe hypoxia ($1\%O_2$) triggers intrinsic apoptosis. HIF1α, a key hypoxia inducible transcription factor, regulates cell survival in different cellular models. In this study we explored the molecular mechanisms and HIF1α participation in PSC apoptosis induced by acute severe hypoxia. To this end, hESCs (H9 line) and hiPSCs (FN2.1 line) were cultured under physical ($1\%O_2$) or chemical acute severe hypoxia conditions. The latter was generated with $CoCl_2$ (250nM) or dimethyl oxal glycine (1mM) treatments, which stabilize HIF-1α mimicking hypoxia. The apoptotic threshold is governed by the balance between pro- and anti-apoptotic proteins, so we measured MCL-1 (key anti-apoptotic protein in PSC) and BNIP3 (pro-apoptotic factor regulated by hypoxia) abundance by Western blot, 8 and 24 hours after the onset of severe hypoxia. Results show that both proteins products were induced upon hypoxia treatment. Moreover, *mcl-1*, *bnip-3* and *bnip-3L* mRNA expression levels, quantified by RT-qPCR, were also significantly up-regulated. RT-qPCR and Western blot were used to *validate* HIF1α *siRNA-mediated* knockdown in H9 and FN2.1 cells. We found that siRNA-mediated downregulation of HIF1α did not significantly altered, at 48 hours post-transfection, the percentage of surviving cells (Trypan blue dye-exclusion assay) nor the increase in late apoptosis or necrosis (flow cytometry analysis with PI staining) rate induced by severe hypoxia. Importantly, hypoxia mediated up-regulation of *bnip-3* and *bnip-3L,* but not *mcl-1* transcripts levels was reverted by HIF1α silencing. Our findings suggest that apoptosis induction by acute severe hypoxia in PSC is independent of HIF1α.

Keywords: human pluripotent stem cells, hypoxia, HIF1α, apoptosis

(562) **POLY-(L-LACTIC ACID) SHEETS SEEDED WITH DIAPHRAGMATIC MYOBLASTS OVEREXPRESSING CONNEXIN 43 AS A POTENTIAL STRATEGY FOR MYOCARDIAL REGENERATION.**

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Introduction. The diaphragm contracts and relaxes 20 times per minute over a lifespan. Besides, it is a source of muscle stem cells (myoblasts) capable of dividing, differentiating and self-regenerating preserving their phenotype. This makes diaphragmatic myoblasts (DM) a potential candidate for cell-based cardiac regeneration. Methods. To test this hypothesis, we seeded DM modified to overexpress connexin 43 (cx43) on poly-(l-lactic acid) (PLLA) sheets and sutured them on the infarcted zone of sheep undergoing coronary ligation (PLLA-DMcx43 group, n=6). DM were transduced with cx43 to enhance inter-cell connection. Additional sheep received PLLA sheets with non-modified DM (PLLA-DM group, n=6), PLLA without DM (PLLA group, n=6) or no treatment (Placebo, n=6). LV ejection fraction (Fey) (echocardiography) and infarct size (cardiac magnetic resonance) were measured at 2 and 45 days post-AMI. Results. Infarct size decreased by ~25% in PLLA-MDcx43 [8.7±0.6 ml (day 2) to 6.5±0.72 ml (day 45), P<0.01; X±SD, ANOVA -Bonferroni] but not in the other groups. Fey fell in all groups at 2 days vs. baseline. At 45 days, PLLA-DM and PLLA-MDcx43, but neither Placebo nor PLLA, recovered their pre-IAM Fey values (PLLA-MD: 61.1±0.5% vs. 58.9±3.3%, P=NS; PLLA-MDcx43: 64.6±2.9% vs. 56.9±2.4%, P=NS; Placebo: 56.8±2% vs. 43.8±1.1%, P<0.01, PLLA: 65.7±2.1% vs. 56.6±4.8%, P<0.01). Capillary density (cap/mm^2) was higher (P<0.05) in PLLA-MDcx43 (2003±174) than in the other 3 groups (Placebo: 1184±116; PLLA: 1241±130, y PLLA-MD 1580±211). Conclusion: In sheep with AMI, PLLA sheets conveying DM that overexpress cx43 are angiogenic and reduce infarct size, but do not contribute significantly to improve Fey at 45 days post-tretament.

Keywords: acute myocardial infarction, diaphragmatic myoblasts, connexin 43, poly-(l-lactic acid).

(1516) **THE POTENTIAL OF HYPOXIC PRECONDITIONING ON THE ANGIOGENIC PROFILE OF HUMAN ADIPOSE-DERIVED MUSE CELLS**

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Multilineage-differentiating stress-enduring (Muse) cells are a novel type of nontumorigenic endogenous pluripotent-like stem cells that can be isolated from adult tissues. Mesenchymal stromal cells (MSCs) are of great interest as therapeutic agents for regenerative purposes. Several studies have focused on the beneficial effects of hypoxic preconditioning on MSC survival; however, their potential impact on Muse cells remains unclear. The hypoxia inducible factor-1 α (HIF-1α) is known to be a transcription factor that is strongly induced by hypoxia and one of its important functions is to promote angiogenesis. This is done via HIF-1α regulation of vascular endothelial growth factor (VEGF) transcription. VEGF is a major regulator of angiogenesis, which promotes endothelial cell migration.

Our objective was to obtain Muse cells from primary cultures of subcutaneous human adipose tissue (AT), identify their pluripotency, and analyze angiogenic profile changes induced by hypoxic preconditioning.

Muse cells were isolated from human female abdominal lipoaspirates using severe cellular stress procedure. At 6 days Muse-AT cells showed positive expression for pluripotency markers: Nanog, Oct4, and Sox2, as well as stage-specific embryonic antigen-3 (SSEA-3). Hypoxic preconditioning (HP) protocol (6hs, 1.5%O2) or normoxia condition (NC) was applied at 2 or 6 days after isolation. HIF-1α expression was decreased at day 2 (NC: 0.7±0.1 fold; and HP: 0.2±0.04 fold; p<0.001, X±DS, ANOVA Bonferroni) and at day 6 (NC: 0.2±0.03 fold; and HP: 0.1±0.02 fold; p<0.001) with regard to day 0. Contrarily, VEGF gene expression was significantly higher at day 2 HP: 2.0±0.1 fold, p<0.01; and day 6 HP 2.9±0.3 fold; p<0.0001), with regard to day 0. In NC no significant changes were observed.

Conclusions: Muse-AT after 6 days of culture were able to express pluripotency markers and overexpress VEGF post hypoxic preconditioning by a regulatory path presumably different from HIF-1α.

*Keywords***:** hypoxic preconditioning; Muse cells; HIF-1α; VEGF

(1053) **DIFFERENTIAL EXPRESSION OF CELL CYCLE REGULATORY GENES IN FETAL AND ADULT OVINE HEARTS**

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Over the past few years several cardiac regenerative therapies have been investigated. One possible approach would be to promote myocardial self-regeneration by inhibiting the cell cycle brakes that arrest G1/S y G2/M progression. In transgenic mice it has been shown that the Tbx20 gene antagonizes the expression of the cell cycle repressor gene Btg2 and, at the same time, increases expression of the pro-mitotic genes Hand2 and Mef2c. To assess if these cardiomyocyte cell cycle regulatory genes are operative in large mammals more similar to man, we studied the differential expression of Tbx20, Btg2, Mef2c and Hand2 in hearts from fetal and adult sheep. On account that ovine cardiomyocytes are mitotic until approximately day 100 of gestation, myocardial samples from ovine fetuses at 70 days gestation time (F-70, n=4), fetuses at 120 days gestation time (F-120, n=4) and adult sheep (AS, n=4) were harvested. RT-qPCR was performed to assess expression of Tbx20, Hand2 and Mef2c (cell cycle stimulators) and Btg2 (cell cycle repressor). Results: Pro-mitotic genes: Tbx20 expression was increased by 2.1±0.4 fold in F-70 and by 2.5±0.7 fold in F-120 with regard to AS (both p<0.05, X±DS, ANOVA Bonferroni); Mef2c expression and Hand2 were increased only in F-70 with regard to AS (Mef2c: 14.9±9.1 fold, p<0.05; Hand2: 13.3±7.4 fold, p<0.05). Contrarily, expression of the anti-mitotic gene Btg2 was significantly higher (20.4±10.8 fold) with regard to F-70 (p<0.001) and F-120 (1.5±0.3 fold, p<0.01).

Conclusion: As opposed to the results observed in fetal myocardium, adult ovine myocardium shows down-regulated expression of Tbx20. This allows overexpression of Btg2 which, in turn, inhibits the expression of the pro-mitotic genes Mef2c and Hand2. Thus, induction of Tbx20 overexpression may be a potentially useful strategy to encourage adult cardiomyocytes to reenter the cell cycle and advance into mitosis and cytokinesis.

Keywords: Myocardial regeneration, cell cycle, ovine, Tbx20

REPRODUCTION AND FERTILITY 6

(662) **CHARACTERIZATION OF CANNABINOID RECEPTORS IN SPERMATOZOA FROM WILD-TYPE (WT) AND CB1 KO MICE: EVALUATION OF THE INVOLVEMENT OF CANNABINOID RECEPTORS IN SPERM CAPACITATION.**

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The endocannabinoid system, including endocannabinoids and

cannabinoids receptors (ECRs) such as CB1, CB2, TRPV1, has been characterized in most mammalian spermatozoa and at least in boar, bovine and human, plays a crucial role in sperm function. Our previous results indicate the involvement of CB1 and TRPV1 in bovine sperm capacitation. Here we characterized the ECRs in spermatozoa from WT and CB1 (-/-) (KO) mice and investigated its possible activation in sperm capacitation in both groups. First, we analyzed abundance (by Western Blot) and localization (by immunocytochemistry) of ECRs in mice spermatozoa from KO or WT. CB2 and TRPV1 are present in KO and they are localized in the acrosomal region (A) (CB2), or in A and post acrosomal (PA) regions (TRPV1), similarly to WT mice. In addition, an increase of TRPV1 in PA region was found in capacitated spermatozoa from both mice groups.

Subsequently, the involvement of ECRs in capacitation was evaluated in WT and KO mice spermatozoa. In vitro capacitation was performed in the presence of increasing concentrations of CB1, CB2 and TRPV1 antagonists and evaluated by analyzing the levels of phosphorylation of tyrosine residues (pY), an essential event associated with this process.

Results indicated that: 1) the increase in pY levels was similar in capacitated spermatozoa of the two mice groups; 2) the incubation with a CB1 antagonist (10^{-10}-10^{-9}M) produced a partial decrease in pY in WT mice; 3) the incubation with a CB2 antagonist (10^{-10}-10^{-8}M) did not decrease the levels of pY in either WT or KO mice. 4) the incubation with a TRPV1 antagonist (10^{-8} M) decreased ~ 30 % of pY levels ($p<0.05$) in both WT and KO mice.

These results suggest that spermatozoa from KO mice present similar abundance of CB2 and TRPV1 receptors than those from WT. On the other hand, CB2 does not seem to be involved in the regulation of pY levels, but CB1 and TRPV1 might play a role in sperm capacitation in mice.

Keywords: cannabinoid receptors, sperm, capacitation

(1315) **HUMAN SPERM MEMBRANE POTENTIAL AND ITS ROLE IN THEIR FERTILIZING CAPACITY**

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Mammalian sperm cannot fertilize the egg without suffering certain physiological changes triggered during its course through the female reproductive tract. These changes are known as capacitation and involve plasma membrane reorganization, extensive changes in the state of protein phosphorylation and ion membrane-permeability. This ion permeability changes impact on the plasma membrane potential (E_m) of any animal cell, which is typically negatively charged inside. Em hyperpolarization associated to capacitation is well described for mouse sperm, and it is both necessary and sufficient for sperm to undergo the acrosome reaction. However, little is known about Em changes during human sperm capacitation and its relation to fertilizing capacity. To address this question we have set up a fluorimetric population assay for measuring human sperm membrane potential, by means of the cationic carbocyanine DiSC(3)5. Em was calculated for each sperm sample using the Nernst equation and considering an intracellular potassium concentration of 120 mM. Our results show a high dispersion of resting Em values of non-capacitated sperm. However, Em hyperpolarization was observed in most cases upon incubation of 3 to 5 hrs in capacitating media, regardless of the initial value. When analyzing sperm from patients attending to reproductive clinics, a greater dispersion was observed, but Em hyperpolarization was hardly seen. In addition, Em values are compared to Computer Assisted Sperm Analysis data and to fertilization outcome, after assisted reproductive techniques. These data have the potential value to add diagnostic tools to help predict the success of different reproductive techniques.

Keywords: sperm capacitation, membrane potential, fertility

(332) **CHOLESTEROL DISTRIBUTION IN SPERM CELLS SUBMITTED TO TEMPERATURE DECREASE IN THE PRESENCE OF DIFFERENT EGG YOLK COMPONENTS**

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Abstract: Frozen-thawed sperm from several animal species present decreased fertilizing ability, which is related to a low cholesterol/phospholipid ratio in their plasma membrane. In freezing protocols, extenders include lipids to stabilize the cell membrane; being 20% egg yolk (EY) the most widely used. EY low density lipoproteins (EY-LDL) has been proposed as the responsible for the cryoprotective action; however, it was not proved yet. In previous studies, we demonstrated the interaction of EY-LDL with sperm using a spin probe as a cholesterol analogue. The aim of this work was to analyze the sperm cholesterol distribution after temperature decrease using different extenders.

BODIPY-cholesterol was incorporated (50 μM final concentration) to AndrostarÒplus diluent (D), EY, soluble EY fraction (S-EY) and EY-LDL extenders by incubation 30 min at 37ºC. Semen samples in D were stabilized 2 h at 17°C, centrifuged and resuspended ($1.5x10^9$ cells/ml) in the extenders. Temperature was decreased from 17 to 5°C (0.1 °C/min). Controls were carried out at 17ºC. Cells were washed and fluorescence was evaluated by microscopy. Motility was controlled before and after treatments.

Different fluorescence patterns were observed depending on the treatment. Control sperm in D, did not exhibit fluorescence whereas those treated in the presence of complete EY, S-EY or LDL-EY presented fluorescence with high intensity in the head and the main piece, regardless temperature treatment. Fluorescent accumulations were observed when sperm were not washed after treatments with S-EY and LDL-EY. These accumulations were attached mainly to the sperm acrosomal region in samples with S-EY.

These results suggest that the transference of cholesterol from the lipid extenders to sperm was carried out, supporting the proposed protection mechanism attributed to the LDL-EY. Future studies should be performed to determine if the presence of accumulations in S-EY treated sperm could be important against freezing damage.

Keywords: Sperm cryopreservation; egg yolk low density lipoproteins BODIPY-cholesterol; fluorescence microscopy.

(660) **CRISP (CYSTEINE RICH SECRETORY PROTEINS) AS NOVEL REGULATORS OF EPIDIDYMAL EPITHELIUM DIFFERENTIATION AND IMMUNOTOLERANCE**

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Epididymal CRISP1 and CRISP4 associate with the sperm surface during maturation and are key mediators of the fertilization process. Whereas knockout (KO) males for each of these molecules are fertile, double KO (DKO) for CRISP1/CRISP4 exhibit impaired fertility. Histological studies of DKO epididymides revealed the existence of two groups of mice: one with a phenotype identical to controls (Group 1) and one exhibiting epididymal epithelium defects (i.e. vacuoles, evaginations) and abnormal presence of immune cells in both the interstitium and lumen (Group 2) not detected in the single KOs. Based on this, in the present work we investigated the mechanisms underlining the inflammatory phenotype of CRISP1/CRISP4 DKO mice. Analysis of the epididymides from Group 2 showed both an increase in intraluminal pH and a reduction in sperm viability from caput to cauda not observed in controls. RT-qPCR for different immunomodulator molecules revealed higher levels of *Il-6* and *Il-10* as well as a downregulation of *Tgf-β* in Group 2 DKO. None of the described alterations were detected in animals from Group 1. Interestingly, immunofluorescence experiments using specific markers for different epididymal epithelial cells revealed damaged and even lack of principal cells, few and shorter basal cell projections and absence of rows of clear cells in mice from both groups, resembling the phenotype of immature mice. These observations support the idea that initial defects in epididymal epithelium differentiation might be

responsible for the inflammatory response detected in epididymides from Group 2. Altogether, these results revealed the relevance of CRISP for epididymal epithelium differentiation and their key role in maintaining the characteristic immunotolerance of the epididymidis.

Keywords: epididymis, sperm, epithelium, inflammation

(1677) **PRELIMINARY SEMEN SAMPLE MANAGEMENT FROM *PARALICHTHYS ORBIGNYANUS* FOR SPERM MOTILITY OBJECTIVE ANALYSIS BY USING A NOVEL OPEN-SOURCE SUITE OF ALGORITHMS**

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A novel suit of open-source algorithms for sperm motility evaluation was previously reported by our group. The proposed method was successfully compared with the Microptic's Sperm Class Analyzer®. Regarding the precision of the system, number of detected motile trajectories and the percentage of fragmented paths to measure the tracking reliability of ram sperm, our method presented a better performance according to those indicators compared to the commercial system.

Knowledge related to marine fish sperm motility is poor. In view to improve gamete cryopreservation in our country, we performed preliminary assays to standardize the sample collection by stripping and evaluation of semen quality from the autochthonous flounder *Paralichthys orbignyanus*. This species is currently cultured under captivity in the Mariculture Experimental Station of National Institute for Fisheries Research and Development. We performed preliminary motility measurements by videomicroscopy using the recently developed algorithm. Sperm movement was recorded at 30 fr/sec using a videocamera attached to a microscope with a phase-contrast objective. Since fish sperm functionality depend on several factors, firstly, it was necessary to assay sample dilutions in salt water in order to activate the sperm and to find an appropriate sperm concentration that allow detecting individual trajectories. Concentration should not be over $30*10^6$ cells/ml to guarantee the correct tracking. Motility parameters of progressive sperm were within 52.4 to 886.7 μm/s for VAP, 1.7 to 878.1 μm/s for VSL; 201.3 to 885.5 μm/s for VCL; 0 to 4.9 μm for ALH and 0 to 26.6 Hz for BCF, consistent with measurements reported in other marine fishes such as *Sparus aurata*. Even though there is not much published in fish sperm motility parameters, these result are promising to evaluate the quality of fish semen of interest for aquiculture and eventually to progress with fertility studies as well as cryopreservation protocols.

Keywords: sperm, cryopreservation, motility parameters, *Paralichthys orbignyanus,* Open-source motility software

(698) **EFFECT OF ULIPRISTAL ACETATE (UPA) ON POST-FERTILIZATION EVENTS IN MICE**

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UPA is a selective progesterone receptor modulator introduced into the market as the most effective emergency contraceptive pill in several countries. However, the mechanisms underlying its action are not completely understood. Its primary mode of action is to inhibit or delay ovulation only if taken before LH peak. Considering its high effectiveness and previous results in mice showing it does affect neither gamete transport or interaction nor early *in vitro* embryo development, it is possible that this drug prevents pregnancy by another mechanism. Based on this, the aim of this work was to evaluate potential effects of UPA on post-fertilization events in mice. First, females were caged with male breeders and successful mating was confirmed by the presence of copulatory plugs (embryonic day 0.5:E0.5). At E1.5, a group of mated females received 40 mg/kg UPA (i.p., previously shown to prevent ovulation) whereas a control group received vehicle alone (sesame oil). At E14.5, the number of implantation sites and corpora lutea was recorded. Although pregnancy rates were similar in both groups, half of the UPA-treated females presented lower number of implantation sites (UPA: 5.6±0.2 vs control: 11.9±0.5, $p<0.05$) despite similar number of corpora lutea (10.8±1.1 vs 12.4±0.8, NS). The percentage of viable embryos was similar in both treatments. When the procedure was repeated but injecting UPA or vehicle closer to the window of implantation (E2.5), UPA-treated females presented significantly lower pregnancy rates (40% vs 100%, $p<0.05$) and number of implantation sites (1.2±0.8 vs 8±2, $p<0.05$). To evaluate an effect of UPA on embryo transport, embryos were collected by flushing the oviducts and uteri at E3.5. In both cases, all the embryos were recovered only from the uteri and mainly at blastocyst stage. Altogether, these results suggest that administration of UPA after fertilization prevents pregnancy without affecting embryo transport or development.

Keywords: ulipristal acetate; emergency contraception; embryo transport; implantation.

(837) **HUMAN SPERM DECONDENSATION *IN VITRO* IN THE PRESENCE OF SULFATED GLYCOSAMINOGLYCANS: A PREDICTOR OF ALTERATIONS IN CHROMATIN PACKAGING IN INFERTILE PATIENTS?**

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Previous results from our laboratory suggested an interaction between heparin sulfate/heparin (**HS/H**) and dermatan sulfate (**DS**) in chromatin decondensation *in vitro* of spermatozoa obtained from normospermic donors. The aim of this study was to evaluate such an interaction in infertile patients and its possible relationship to sperm protamine content. Sperm samples were obtained from 19 patients undergoing assisted reproduction. Swim-up spermatozoa were decondensed *in vitro* with 10 mM GSH and 46 μM **H** or **DS** for 15, 30 and 60 minutes. % decondensation (**D)** was determined by phase contrast microscopy. In 11/19 patients, sperm nuclear proteins were extracted and analyzed by acid PAGE to determine protamine content (**P1+P2**) and **P1/P2** ratio. There were differences among patients in sperm decondensation kinetics (**D60/D30**) with **H** and **DS**. **H** distinguished 2 groups of patients: **F**ast decondensers (1.07±0.05, n=12), similar to normospermic donors, reached maximum decondensation at 30 minutes and **S**low decondensers (1.86±0.05, n=7, p=0.016, cutoff value 1.5) did not. **DS**, on the other hand, allowed the identification of 3 groups of patients: **F** (0.92±0.07, n=8), **S (**1.66±0.08, n=4) and **V**ery **S**low decondensers (3.46±0.60, p=0.0001 vs **S**low, n=7). In the 11 patients whose protamines were analyzed by PAGE, median **P1+P2** content was 553 (**I**nter**Q**uartile range 356-654) ng/10^6 sperm (normospermic control 680 ng/10^6 sperm) with **P1/P2** = 0.41 (**IQ** 0.29-0.78) (control 1.1). **P1/P2** correlated (Spearman) with **P1+P2** (r=0.697, p=0.02) and **P1** (r=0.957, $p<0.0001$), but not with **P2** (r=0.105, p=0.76). 3/11 were **S** decondensers with **H** and the 3 had diminished **P1/P2**. Among the 8 **F** decondensers with **H**, those with diminished **P1/P2** (5/8) were either **S** or **VS** decondensers with **DS**. These results suggest that differential decondensation of human spermatozoa *in vitro* with **H** and **DS**, which results from structural differences between both GAGs, could be indicative of alterations in sperm DNA packaging in infertile patients.

Keywords: human sperm, chromatin decondensation, protamines, glycosaminoglycans, infertile patients

(765) **EVIDENCE ON THE INVOLVEMENT OF EPITHELI-**

AL AND NEURAL CADHERIN IN MURINE FERTILIZATION

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Successful mammalian fertilization requires a well orchestrated sequence of molecular events leading to gamete fusion and early embryo development. Since gamete interaction involves adhesion events in the presence of Ca^{2+}, we study the participation of the Ca^{+2}-dependent cell-cell adhesion proteins Epithelial (Ecad) and Neural (Ncad) cadherin.

Previously, we reported the expression of Ecad and Ncad in human, bovine and murine gametes and reproductive tissues. Their involvement in human fertilization was evidenced by the ability of anti Ecad or anti Ncad antibodies to impair gamete interaction. However, their role in homologous fertilization was not evaluated for ethical reasons.

Herein, we report results obtained using specific antibodies and blocking peptides toward Ecad and Ncad to test the involvement of both adhesion proteins in murine fertilization. IVF (with Cumulus-Oocyte Complex (COC), denuded Oocytes and denuded ZP-free Oocytes), Sperm penetration of Cumulus, and Sperm-Oolemma binding assays were done.

Firstly, gamete pre-incubation with anti Ecad (ECCD1) or anti NCad (H-63) antibodies resulted in decreased ($P<0.05$) fertilization rate in IVF, using COCs (anti Ecad:53% inhibition; anti Ncad:73%) denuded oocytes (anti Ecad:71% inhibition rate; anti Ncad:76%) and denuded ZP-free oocytes (anti Ecad:58% inhibition rate; anti Ncad: 61%; ECCD1+H63: 72%). Sperm Cumulus penetration was impaired ($P<0.05$) only by anti Ecad (49% Inhibition; anti Ncad: 9%).

To further characterize the involvement of Ecad and Ncad in gamete interaction, specific blocking peptides towards Ecad, Ncad or to both were synthesized and used in the in vitro gamete interaction assays. IVF with ZP-free oocytes was inhibited ($P<0.05$) by all peptides (Ecad:66% inhibition; Ncad:53%; Ecad+Ncad:63%). Sperm-oolemma binding was also impaired ($P<0.05$) (Ecad:55% inhibition; Ncad:62%; Ecad+Ncad:84%).

Altogether, results presented here strongly support Ecad and Ncad involvement in mammalian fertilization.

Keywords: Cadherins, Epithelial-Cadherin, Neural-Cadherin, In Vitro Fertilization, Blocking Peptides

(116) **F-ACTIN LIVE CELL STAINING WITH SIR-ACTIN REVEALS SPECIFIC DYNAMIC CHANGES DURING MOUSE SPERM ACROSOMAL EXOCYTOSIS**

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Acrosomal exocytosis (AE) is an absolute requisite for fertilization in mammals. This complex exocytic process is controlled by several players including changes in the actin cytoskeleton. Most studies to evaluate actin polymerization in mammalian sperm were performed using phalloidins, which are toxic and not capable of crossing the cell plasma membrane. As a result, it is not possible to use this approach to study this dynamic process in real time using live cells. SiR-actin is a novel membrane permeable fluorescent probe that binds to actin filaments *in vivo*. Using this new tool, we aimed to examine actin polymerization dynamics in live mouse sperm. We observed by image-based flow cytometry a capacitation-induced increase in polymerized actin levels ($P<0.05$). We found 6 F-actin structures within the sperm head: perforatorium, lower acrosome, upper acrosome, ventral, septum and neck. The same structures were also observed when we used sperm from transgenic mice expressing LifeAct-EGFP, supporting our *in vivo* observations using SiR-actin. A six positions binary code was used to analyze their possible combination present in the sample. The proportion of five of the structures combinations statistically changed with capacitation ($P<0.05$). We were able to observe in great detail these F-actin structures using super-resolution microscopy. We evaluated the dynamic actin cytoskeletal changes at the onset of AE, by labelling sperm with SiR-actin and FM4-64 (a marker of AE). Interestingly, we found that in all sperm that possessed the septum or the lower acrosome F-actin structure, the actin cytoskeleton depolymerized in those regions prior to ionomycin induced AE, while the rest of the F-actin structures remained unchanged. Thus, we have simultaneously visualized actin dynamics and AE by super resolution *in vivo* and demonstrated for the first time that actin depolymerization occurs in specific regions of the sperm head during the AE.

Keywords: mouse sperm, actin cytoskeleton, acrosomal exocytosis, super-resolution microscopy

(1661) **IN VITRO CAPACITATION AND ACROSOME REACTION IN CRYOPRESERVED PORCINE SPERMATOZOA WITH ALPHA-TOCOPHEROL. PARTICIPATION OF LACTATE DEHYDROGENASE**

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The biotechnology of reproduction in porcine species has poor development mainly due to the limitation in sperm cryopreservation. The availability of reliable markers of sperm function would provide tools to improve the cryopreservation process. In this way, our aim was to determine the activity of the enzyme lactate dehydrogenase (LDH; 1.1.1.27) and study its participation in the processes of the *in vitro* capacitation and acrosome reaction (AR) in cryopreserved porcine spermatozoa with alpha-tocopherol. Spermatozoa were incubated in TBM medium with bicarbonate and follicular fluid as capacitation and acrosome reaction inducers, respectively. Capacitation medium was supplemented with different concentrations of sodium oxamate (competitive inhibitor of the enzyme LDH). Capacitation and AR percentages were determined by the CTC technique and trypan blue combined with DIC, respectively. Sperm viability and motility were evaluated by the eosin/nigrosin technique and optic microscopy, respectively. The LDH activity was determined spectrophotometrically at 600 nm, during 2 minutes, at 37 °C and the enzyme unit (U) was defined as the amount of LDH that oxidizes 1 μmol of NADH/minute. The results were analyzed by ANOVA and Bonferroni test. The addition of the competitive inhibitor of the enzyme significantly ($p<0.05$) diminished capacitation and AR without affecting sperm viability, at different concentrations (1mM for capacitation and 0,5mM for AR). These concentrations also inhibited the activity of LDH (40 ± 5 and 73 ± 10 % of inhibition for 0.5 mM and 1 mM, respectively). Our results demonstrated the differential participation of the enzyme lactate dehydrogenase in the processes that are involved in the acquisition of the sperm fertilizing ability. This information can be useful for the formulation of incubation media to use in biotechnological reproduction techniques.

Keywords: porcine spermatozoa, alpha-tocopherol, lactate dehydrogenase.

ENDOCRINOLOGY 6

(1840) **ASTROCYTE-SPECIFIC DELETION OF PEROXISOME-PROLIFERATOR ACTIVATED RECEPTOR-GAMMA IMPAIRS GLUCOSE METABOLISM AND ESTROUS CYCLING IN FEMALE MICE**

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We previously reported that neuronal PPARg is required for obesity-induced leptin-resistance and female fertility. PPARγ is highly expressed in astrocytes, so we created an inducible, conditional knockout of PPARγ (AKO) by crossing male *Pparg*flox/flox mice with GFAP-Cre-ERT *(GCTF-CRE)* females to generate *Pparg*flox /+:*GCTF-Cre* females, which were bred with flox/flox males to obtain cre+ *Pparg*flox/flox:*GCTF-Cre* mice (AKO) and littermate controls (WT). To induce the CRE allele, AKO and WT females were fed Tamoxifen for 2 weeks after 11 weeks of age. Fifteen-week old mice were fed 60 % high-fat (HFD) or 10% low-fat (LFD) diets. Estrous cycles, body weight, glucose tolerance and insulin sensitivity were analyzed before and after diets. Leptin sensitivity, serum gonadotropins, ovarian histology and ovarian, hepatic and hypothalamic gene expression were analyzed after 18 weeks on diets. AKO showed impaired glucose tolerance and hepatic steatosis that did not worsen with HFD, but maintained leptin-sensitivity. Expression of gluconeogenic genes and also genes involved in lipogenesis, lipid transport and storage were elevated in the liver, what could explain the altered glucose tolerance and liver steatosis ($p<0.05$). Lean AKO had altered estrous cycles and expression of hypothalamic *Npvf* and *Hcrt*, and HFD reversed these effects ($p<0.05$). In AKO, HFD inhibited the increase in LH after Kisspeptin stimulation; however, *Gpr54* hypothalamic expression was increased in AKO regardless of diet. AKO showed altered ovarian histology and *Cyp19a1 Fshr and Star* gene expression, lower serum LH and higher testosterone compared to WT ($p<0.05$). Our data show that astrocytic PPARγ maintains normal hepatic insulin sensitivity and prevents steatosis, and that is important for the correct regulation of the hypothalamic-pituitary axis and estrous cycles.

Funding: NIH grants (HD012303, CA155435, CA023100, CA196853) and a VA Merit Review award (I01BX000130) to N.W.

Keywords: Astrocytes, Obesity, PPARγ, Reproduction

(175) **EFFECTS OF A GABAB ANTAGONIST ON OVARIAN AROMATASE, ESTROGEN RECEPTOR ALPHA AND BETA EXPRESSION IN NEONATAL MICE**

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We have previously shown that neonatal GABAB1KO female mice have decreased Kiss1 expression in the arcuate nucleus (ARC). In addition, the administration of a GABAB antagonist (CGP55845) to neonatal BALB/C mice significantly decreased ARC Kiss1 expression in both sexes. This suggests that a GABAB input can modulate *Kiss1* expression in specific nuclei in neonates, at the time when neuronal connections are being established. The hypothalamic estrogenic system was not involved in this regulation as aromatase, estrogen receptor alpha (ERα) or estrogen receptor beta (ERβ) expression were not affected by this treatment. Nevertheless, we found that ovarian and testicular E2 contents were significantly increased in CGP-treated mice. Therefore, we cannot discard the possible participation of gonadal E2 in the modulation of ARC Kiss1 expression. Here we determined whether aromatase, ERα and ERβ expression are altered by this treatment in the ovary, justifying the increase in estradiol content.

Female neonatal Balb/c mice were injected with CGP55845 (1 mg/kg, sc) or saline as control from postnatal day 2 (PND2) to PND6±1, three times/day (8AM, 1PM, 6PM). Mice were sacrificed at 3PM (after two injections on the last day). One ovary was collected to determine aromatase and ER expression by qPCR whereas the other one was used to analyze the effects of this increase of estradiol on ovarian morphology. Real-time PCR determined that aromatase expression was significantly increased by CGP55845 treatment in the neonatal ovaries (CTRL:0,97±0,32;CGP:2,5±0,55: CGP≠CTRL: $p<0,04$). ERα mRNA expression was also significantly increased in CGP-treated females (CTRL:0,85±0,30;CGP:3,80±0,54: CGP≠CTRL: $p<0,001$) as well as ERβ (CTRL:1,11±0,39;CGP:6,99±1,65: CGP≠CTRL: $p<0,003$).

Our data clearly show that lack of GABAB signaling increases the expression of the ovarian estrogenic system that may have long-lasting effects on reproduction.

(CONICET, ANPCYT, UBA, Fundación René Barón, Fundación Williams).

Palabras clave: KISSPEPTIN – ENDOCRINOLOGY - GABAB

(567) **FIRST EVIDENCES OF PHYSIOLOGICAL ALTERATIONS IN RECENTLY DEVELOPED KISS1-GABAB1KO MICE**

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We have shown that global GABAB1KO mice have alterations in the reproductive axis, especially females, which showed disrupted estrous cycles and reproduction impairment. Moreover, GABAB-1KO mice showed coexpression of GABAB receptors (GABABRs) in Kiss1 neurons and a dramatic increase in Kiss1 expression in extrahypothalamic areas (amygdala, BNST) while their expression did not differ from WTs in AVPV-PeN or ARC. Recent results show constant (morning and afternoon), high kisspeptin neuron activation in a proestrous-like environment and failure of ovulation in these mice. To establish the impact of GABABRs on Kiss1 neurons our aim was to develop a strain of mice with specific deletion of GABABRs in Kiss1 cells/neurons and characterize them from a reproductive and metabolic perspective. Kiss-Cre mice (Jackson's Lab) were crossed with GABAB-floxed mice (donated by Dr. Bettler) to obtain Kiss1-GABAB1KO (KO) mice. Body weight (BW) was evaluated from postnatal day (PND) 7 to PND84. Sexual differentiation was evaluated by ano-genital distance (AGD) on PND7, PND14 and PND21 and relativized to body weight. KO females had increased BW vs WTs [BW (g) PND84: F-KO=29.6±1.2 (n=5) vs F-WT=25.3±1.4 (n=7), $p<0.05$], while no difference in BW was observed in males. In KO males AGD/BW was significantly increased on PND7 [AGD/BW (cm/g)= M-KO=0.067±0.005 (n=5) vs M-WT=0.056±0.003 (n=9), $p<0.05$]. In KO females there was a tendency to decreased AGD/BW at all time points [Repeated measures ANOVA: factor genotype $p=0.07$ (F-KO, n=5; F-WT, n=7)]. These first results from Kiss1-GABAB1KO mice demonstrate a significant increase in BW in females, compatible with kisspeptin's metabolic effects and an alteration in sexual differentiation where KO males are masculinized while KO females show a tendency to feminization. Further evaluation of this colony will increase our understanding of GABAB control of Kiss1 cells/neurons. Funding: CONICET, UBA, ANPCYT, Fund. Williams, Fund. René Barón.

Keywords: Kiss1-GABAB1KO, mice, kisspeptin, sexual-differentiation

(69) **IN-VITRO EFFECTS OF BENZOPHENONE 2 AND 3 IN IMMATURE GNRH NEURONS**

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UV filters, such as benzophenones (BPs), are used as additives in plastics, textiles and personal care products to prevent damage caused by UV radiation. Here we investigated the in-vitro effects of two commonly used benzophenones, BP2 and BP3, on cell proliferation and on basal and kisspeptin-stimulated GnRH release in immature GnRH neurons, GN11 cells.

Cell proliferation was studied using the MTS assay (Promega) in response to 24 hour treatment with BP2, BP3 (10^{-9} and 10^{-7} M,

Sigma), the estrogen antagonist ICI 182780 (ICI, 10^{-6} M, Sigma) and kisspeptin (Kiss1, 10^{-9} M, Phoenix Pharmaceuticals). Results were recorded as Abs490/Abs490 (Control). Basal and Kiss1 (10^{-9} M, 1h)-stimulated GnRH release was studied after 24 h exposure to BP2 or BP3 (10^{-9} and 10^{-7} M). GnRH release was analyzed by RIA. Results were expressed as Mean±SEM, and analyzed by ANOVA.

BP2 and BP3 increased cell proliferation compared to Controls (C) [C: 1.0±0.1, BP2^{-7}: 1.3±0.2, BP2^{-9}: 1.4±0.1, BP3^{-7}: 1.5±0.1, BP3^{-9}: 1.5±0.1; BP2, BP3 different from C, $p<0.05$, n=8]. Co-treatment with the estrogen antagonist ICI did not alter BP2 or BP3-induced proliferation ($p<0.05$, n=8). Kiss1 increased cell proliferation with regards to C and co-treatment with the BPs did not modify this effect [C: 1.0±0.1, Kiss1: 1.8±0.3, Kiss1-BP2^{-7}: 1.8±0.4, Kiss1-BP3^{-7}: 1.5±0.1; Kiss1, Kiss1-BP2, Kiss1-BP3 different from C, $p<0.05$, n=8]. BP3^{-9} M significantly increased basal GnRH release with regards to C, whereas coincubation with BP3 ($1x10^{-7}$ and $1x10^{-9}$ M), Kiss1 ($1x10^{-9}$ M, 1h) failed to further increase GnRH release [C-basal: 8±1, C-Kiss1: 33±9, BP3^{-9}-basal: 18±3, BP3^{-9}-Kiss1: 21±6; C-Kiss1, BP3^{-9}-basal different from C-basal, $p<0.05$, n=9].

Here we show that exposure to BP2 and 3 increase GN11 cell proliferation. In addition, BP3 10^{-9} M increased basal GnRH release. These results show for the first time direct effects of BPs in GnRH neurons.

Funding: CONICET, ANPCyT, UBA, Asoc. ORT Arg., Fund. R. Barón, Fund. Williams.

Keywords: Benzophenones, GnRH, Proliferation.

(327) **L-3,4-DIHYDROXYPHENYLALANINE (L-DOPA) MODULATES THE FUNCTION OF CORTICOTROPHS**

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L-Dopa plus peripheral inhibitors of aromatic L-amino acid decarboxylase (AADC) is the treatment intended to alleviate the motor symptoms of Parkinson's disease (PD). However, in patients this chronic treatment has been described to alter the secretion of ACTH, probably generating an inadequate neuroendocrine stress response. In the present research, we aimed to understand the actions of L-Dopa in the corticotroph function. In first place, we determined the effects of L-Dopa and its decarboxylation to Dopamine (DA) by AADC on ACTH secretion. We observed that L-Dopa decreases ACTH secretion *in vitro* ($p<0.05$, ELISA). As we have shown that AADC is expressed in corticotrophs, we studied the production of DA and DOPAC by monoamine oxidase (MAO) in a corticotroph cell line, AtT20. We found DA and DOPAC production (HPLC) only when cells were incubated with L-Dopa (0-10uM; 2h) ($p<0.05$). Our previous results suggest that L-Dopa conversion to DA induces apoptosis and decreases proliferation in these cells. Therefore, we evaluated the actions of the oxidative metabolism of endogenous DA on the cell turnover of corticotrophs. AtT20 cells were incubated with L-Dopa (1uM, 8h) in the presence or absence of a MAO inhibitor (Pargyline, 10uM, 8h). The apoptotic (TUNEL) and antiproliferative (BrdU) actions of the locally produced DA were not observed when its oxidation was inhibited ($p<0.01$), suggesting that MAO activity is essential to observe DA effects on corticotrophs cell renewal. In summary, exogenous L-Dopa is converted to DA by AADC altering ACTH secretion. Further, DA oxidation by MAO induces apoptosis and decreases proliferation. These results suggest that L-Dopa treatment generates alteration in the function of corticotrophs which could affect the anterior pituitary response to stress.

Keywords: Parkinson's disease, stress, L-Dopa, Dopamine, corticotroph

(490) **MULTIPLE FAILURES IN THE LH SURGE GENERATING SYSTEM IN GABAB1KO FEMALE MICE**

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Kisspeptin and GABA regulate reproduction. Global GABAB1KO (KO) females show impaired ovulation and reproduction, altered cyclicity and GnRH physiology. However *Kiss1* mRNA in AVPV or ARC was unaltered, suggesting that its expression at the mRNA level was not involved. Here we aimed to unravel the origin/s of the reproductive impairment in GABAB1KO female mice. Adult WT/KO females were ovariectomized (OVX), treated with E2 valerate (VAL, 10ug/kg) and sacrificed 7 days post-OVX. Serum LH and E2 were determined (RIA). We evaluated GnRH response to one pulse of Kiss-10 /hour (10^{-7}M) and/or constant E2 (10^{-8}M) in ex vivo incubated hypothalami (HT). Medium GnRH levels were measured (RIA). Anterior and medial basal HT from another set of WT/KO OVX-VAL females were dissected to determine estrogen (*Esr1* and *Esr2*) and kisspeptin (*Kiss1r*) receptors expression (qPCR). Other OVX WT/KO females were given sc E2 implants, which produce an LH surge 2 days later around lights off. They were anesthetized at 10AM or 6:30PM, blood collected to determine LH and PFA perfused. Brains were kept for double IHQ assays (c-Fos/Kisspeptin). OVX+VAL had lower LH compared to OVX and higher E2 than estrous females. GnRH pulse frequency was increased by E2+Kiss-10 in WT HT. Conversely, in KO HT, E2 increased GnRH pulse frequency and this was reversed by E2+Kiss-10 (pulses/h: WT:7.6±0.2 vs KO:5.0±0.3, $p<0.01$). OVX+VAL KOs had increased *Esr1*, *Esr2* and *Kiss1r* expression in HT. Finally, while WT females showed a LH surge in the afternoon, KO females have constant (AM and PM) high LH levels and kisspeptin neuron activation (AVPV: % c-Fos+/Kiss1 cells: WT AM different from all, $p<0.05$) in a proestrous-like environment. These results indicate that HT estrogenic system is upregulated, the GnRH response to E2+Kiss-10 is altered and the increase in PM kisspeptin neuron activation did not occur in KOs, which together induced ovulation failure. Funding: CONICET, UBA, ANPCYT, Fund. Williams, Fund. René Barón.

Keywords: KISSPEPTIN-GNRH-ESTRADIOL-GABAB1KO

(221) **NOTCH INHIBITION IN GH3 PITUITARY CELLS DECREASES PROLIFERATION, HORMONE SECRETION AND CELL MIGRATION**

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The Notch signaling pathway is involved in many processes such as proliferation, migration, differentiation and apoptosis. It participates in tumorigenesis and stem cell self-renovation and is the key in determining cellular fate in diverse types of tumors. Nevertheless, its role in pituitary tumor generation has not been systematically addressed. We therefore studied the functional effect of inhibiting the Notch pathway in GH3 (somatolactotropic) cells in vitro. We focused in cellular aspects such as hormone secretion, cell migration, angiogenesis, proliferation and expression of components of the Notch pathway.

GH3 cells were stimulated in vitro with DAPT (1, 5 y 10 uM), a gamma secretase inhibitor of the enzyme which activates the Notch pathway, during 24 or 48 hours. The secretion of prolactin decreased at 24 and 48hs in a dose-dependent way, while no differences were found in GH secretion. After 48hs of incubation of GH3 cells with different DAPT doses protein levels of components of the Notch pathway were measured. Validating our drug, a decrease in Notch 2 active domain (N2ICD) was detected while no changes were observed in the membrane domains. The protein levels of PCNA (a cell proliferation marker) and phosphoERK were not modified, while HES1 a downstream effector, was also inhibited. DAPT (1 and 5 uM) evoked a significant reduction in *Hes-1* mRNA levels, and no significant alteration in *Notch2* or *Cyclin-d3* mRNA levels.

The migratory capacity of the cells, as assessed by a scratch assay, was reduced with 10uM DAPT after 24, 48 and 72 hours of incubation. And finally DAPT effectively decreased GH3 cell proliferation at 24hs evaluated by MTS assay and cellular area.

These data provide strong evidence of a direct stimulatory role of Notch pathway in the GH3 somatolactotropic cells *in vitro*, and positions this pathway as an alternative therapy in resistant pituitary adenomas.

Keywords: notch, pituitary, GH3, prolactin, DAPT.

(1625) **NOTCH PATHWAY INHIBITION DECREASES PROLIFERATION AND ANGIOGENIC CAPABILITY OF MMQ CELL LINE**

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Pituitary adenomas represent 10% of intracranial tumors. They cause morbidity and mortality due to critical localization, hormone secretion and mass effect. Between pituitary adenomas, prolactinomas are the most frequent. The Notch signaling pathway is involved in the development of various types of tumors. However, its role in pituitary tumor generation require deeper studies.

We previously demonstrated Notch component expression and activation in prolactinoma cell lines, in the *in vivo* GH3 tumors and in rat normal glands. In MMQ cells the NOTCH2 active domain was increased, whereas NOTCH1 active domain was higher in GH3 tumors compared to normal glands. High levels of *Jagged1* and *Dll1* were found in GH3 cells, and *Hes1*, *Hey1* and *Hey2* were expressed in a model dependent pattern. Moreover, the pharmacological inhibition of the Notch pathway decreased GH and prolactin levels *in vitro*, and reduced GH3 migration and proliferation.

Now, we aimed to evaluate the effect of DAPT, a Notch pathway activation inhibitor, in MMQ cells, to determine the role of Notch pathway in prolactinoma tumor development and progression.

We tested the effect of 10uM DAPT treatment *in vitro* for 12, 24 and 48hs in the MMQ cell line and confirmed inhibition of Notch receptor activation. Cell viability determined by MTT assay diminished after 48hs of treatment (N=4, p=0,0032). Moreover, PCNA expression determined by Western Blot analysis showed lower levels at 24hs of DAPT treatment (N=3, p=0,06). Interestingly, angiogenic capability was inhibited by DAPT: the VEGF mRNA levels determined by qRT-PCR in homogenates of MMQ treated cells were lower than control (N=2). We also analyzed the effect of the DAPT treated MMQ cell conditioned media in a scratch assay of plated HMEC endothelial cells and we determined a trend to reduced endothelial cell migration after 48hs of treatment (N=2).

These data provide evidence of a key role of Notch pathways in the MMQ proliferation and prolactinoma development.

Keywords: Pituitary, Prolactinoma, Notch, VEGF

(408) **PITUITARY ACTIVIN SYSTEM DURING POSTNATAL DEVELOPMENT**

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Activin is a known inhibitor of lactotroph, somatotroph and gonadotroph function which action is inhibited by inhibins and follistatin (FST). Activins are homodimers of β chains A and B, giving rise to Activin A (βA-βA), B (βB-βB) or AB (βA-βB). Activin binds to receptors type II (ActRII or ActRIIB) and type I (ActRI/ALK4), inducing direct phosphorylation of Smad2/3. The aim of the present work was to study the ontogeny of pituitary activin system in males and females Sprague Dawley rats at different ages: 11, 23 and 45 days. Adult females were used in diestrus. The expression of the components of the activin system was evaluated by qPCR. Statistical analysis: Two-Way ANOVA, followed by *post hoc* Tukey test.

In general, all components evaluated (βA, βB, ActRII, ActRIIB, and FST) showed a significantly high mRNA expression in 11-day pups, and decreased with age. In addition, we found interesting gender differences; in 11 days old pups, pituitary expression of all components was found significantly increased in female compared to male rats ($p<0,01$). However, in adults, this relation was found inverted: ActRII and βA showed higher mRNA expression in male pituitaries compared to adult females ($p<0,05$). Our results demonstrated that the expression of most components of pituitary activin system is higher in pups and even higher in females, decreasing during postnatal life. This profile correlates inversely with serum PRL levels, which increase during development. As activins play essential roles in tissue homeostasis, the importance of the high expression of pituitary activin system found on the 11th day deserves more in-depth studies.

Keywords: pituitary, activin, postnatal development

METABOLISM AND NUTRITION 2

(1906) **SYNERGISTIC EFFECT OF CHRONIC-CADMIUM EXPOSURE AND SOYBEAN-BASED DIET ON OXIDATIVE STRESS-INFLAMMATION AND APOTOSIS IN THE HIPPOCAMPUS OF RATS**

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Cadmium (Cd) is an environmental and industrial pollutant found in water resources. Experimental and population studies have shown that chronic exposure to Cd causes severe toxic effects in various organs including in the brain, but information on hippocampus (Hp) is limited. Soybean is an important source of protein and isoflavones. Herein we hypothesized that a chronic exposure to low concentrations of Cd in the drinking water can cause oxidative/inflammation changes and apoptosis in the hippocampus (Hp); and whether a soybean-based diet (SBD) may modulate these effects. To accomplish this goal, we fed 4 groups of female Wistar-rats for 60 days as follows: casein-based diet + tap water (CBD); CBD + tap water with 15 ppm Cd (CBD+Cd); soybean-based diet + tap water (SBD); and SBD + tap water with 15 ppm Cd (SBD+Cd). After exposure, markers of oxidative stress/inflammation and apoptosis were measured in Hp. Compared to the Hp of CBD fed rats, the Hp of CBD-Cd showed a reduced cyclooxigenase-2 expression, but did not change Nrf2 expression and apoptotic profile (high p53 and high Bax/Bcl2 ratio). However, a SBD caused a pro-apoptosis pattern, *i.e,* increased p53 and Bax expression, reduced Bcl-2 expression and increased Bax/Bcl2 ratio. Although we did not observe Nrf2 expression in the Hp of SBD fed rats, Nrf2 signaling pathway and genes under ARE control may be induced. Indeed, we observe an antioxidant response in the Hp, which may account for the induction of antioxidant enzyme expression (CAT, SOD and GPX). Regardless increased antioxidant enzymes, Cd exposure in SBD fed rats had a synergistic effect in lipid peroxidation and protein oxidation in the Hp. Taking together our data suggest that a SBD and Cd-exposure have a synergistic effect on oxidative stress/inflammation and apoptosis in Hp. Caution may be taken on using soybean as a source of protein in patients chronically exposed to Cd.

Keywords: Cadmium exposure, soybean, hippocampus, oxidative stress-inflammation, apoptosis

(1462) **FUNCTIONAL MILK FAT ATTENUATED THE LIVER OXIDATIVE STRESS IN RATS FED HIGH-FAT DIETS**

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Non communicable chronic diseases (NCCD) have high prevalence and repercussion in the morbi-mortality of our population and have been related with dietary disorders. In this regards, high intake of fat, including Milk Fat (MF), has been associated with lipid alterations, oxidative stress and cardiovascular disease risk. However, MF can be reduced in the saturated fatty acids and increased the Conjugated Linoleic Acids (CLA) and vaccenic acid (t11-18:1) con-

tents by unsaturated fatty acid supplementation to dairy cow diets leading to a functional milk fat (FMF) with beneficial effects on NCCD risk. Since, the analysis of biomarkers for oxidative stress and lipid alterations is essential for the diagnostic and control of certain NCCD, the aim of this study was to investigate some of these biomarkers involved in hepatic oxidative stress of rats fed diets containing MF and FMF at high levels. Male Wistar rats were fed (60-d) with S7 (soybean oil, 7%), S30 (soybean oil, 30%), MF30 (soybean oil, 3% + MF, 27%) or FMF30 (soybean oil, 3% + FMF, 27%) diets. The ratio of reduced glutathione and oxidized glutathione (GSH/GSSG) by Capillary electrophoresis, lipoperoxidation (LPO) levels by colorimetric reaction of thiobarbituric acid, and the hepatic expression of catalase (CAT), transcription factor (NFK-β) and cytokines involved in the induction of inflammatory status (TNF-α, IL1, IL6) by Real Time PCR were assessed. Statistical differences ($p<0.05$) were tested by ANOVA or T test. Compared with S7, S30 and MF30 diets increased the hepatic biomarkers of oxidative stress: GSH/GSSG 30% and 42%, LPO 27% and 23%, NFK-β 152% and 78%, TNF-α 93% and 239%, and IL6 115% and 221%, respectively. FMF30 decreased all these parameters reaching similar values to the S7 group. In conclusion, the intake of a functional milk fat attenuated the liver oxidative stress in rats fed high-fat diets, which could contribute to reducing the NCCD risk.

Keywords: liver - oxidative stress - Functional Milk Fat – Biomarkers

(1195) **MITOCHONDRIAL RESPIRATORY CHAIN AND KREBS CYCLE ALTERATIONS DUE TO PORPHYRINOGENIC AGENTS IN A MOUSE MODEL OF ACUTE INTERMITTENT PORPHYRIA**

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The mitochondria play a vital role in energy metabolism; inside occurs oxidative phosphorylation in the electron transport chain and the generation of reduction equivalents by the tricarboxylic acid (TCA) cycle. Heme deficiency produced by a reduced synthesis or an accelerated catabolism would trigger severe cell damage. Previously we demonstrated that porphyrinogenic agents affected several brain metabolisms included respiratory mitochondrial chain in encephalon *CF1* and Acute intermittent Porphyria (AIP) genetic modelmice. The aims were to study the effects of volatile anesthetics and other xenobiotics on the activity of fumarase and aconitase in a mouse model of AIP; and to analyze if there are protein and lipid damage due to these treatments. For these purposes we use male knockout mice that have 50% reduced the activity of Porphobilinogen deaminase. Animals were treated with Isoflurane (2 ml/kg), Sevoflurane (1,5ml/kg), ethanol (30%), allylisopropylacetamide (AIA, 350 mg/kg), Veronal (167 mg/kg) or starved (24 hs). The enzymes activities were measured in mitochondria of encephalon. Fumarase activity decreased due to Isoflurane (30%; $p<0.05$), AIA (39%, $p<0.05$), Veronal (87%, $p<0.01$); and in fasted animals (50%, $p<0.01$); while it was augmented due to Sevoflurane (42%, $p<0.05$). Aconitase activity increased due to Isoflurane (93%; $p<0.01$), Sevoflurane (86%; $p<0.01$) and Veronal (170%; $p<0.01$); and it was diminished by AIA (40%; $p<0.05$) and starvation (60%; $p<0.01$). Protein and lipid damage were observed as a consequence of all the xenobiotics studied. The changes observed in the activities of TCA cycle would result in a deficiency of the donor reduction equivalents, NADH and $FADH_2$, and could justify the alterations in the activities of respiratory chain complexes previously reported. Results support our hypothesis that there would be more than one factor to explain the pathogenesis of acute attacks.

Keywords: Cadena_respiratoria, Ciclo_de_krebs, Porfiria_aguda_intermitente

(594) **ACUTE COPPER TOXICITY ASSOCIATED WITH MITOCHONDRIAL DYSFUNCTION, OXIDATIVE DAMAGE AND MULTIORGANIC FAILURE**

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Cytosolic and mitochondrial oxidative damage (OD) is associated with the dose of Cu(II) administered. Previous results shown that Cu(II) doses of less than 5 mg/kg (intraperitoneal (ip) administration), produced cytosolic OD; nevertheless, at slightly higher and toxic doses (6-6.5 mg/kg), the oxidation of mitochondrial phospholipids affects the functionality of mitochondria, and at doses greater than 7 mg/kg animals die before the hour after acute treatment. The aim of this research is to evaluate if acute toxic effects of Cu(II) are associated to mitochondrial dysfunction, OD and multiorganic failure. Sprague Dawley male rats (200 g) received Cu(II) at dose of 6.5-7.5 mg/kg (ip) and were scarified at 1 h and 6 h after treatment. The livers, brains, hearts, and lungs of the rats were excised and the samples were processed according to routine methods for obtaining histopathological preparations that were stained with hematoxylin-eosin. Phospholipid oxidation was measured as thiobarbituric acid reactive substances (TBARS) and mitochondrial function (oxygen uptake, ΔO_2) was evaluated using a Clark type oxygen electrode. Autopsy of these rats indicated that all organs were affected, mainly the heart, in which foci of necrosis were observed in the cardiac tissue. TBARS increased in all the organs evaluated in a dose depended manner (2 to 4 fold at dose 6.5 and 7.5 mg/kg respectively, $p<0.01$) and mitochondrial ΔO_2 decreased with malate-glutamate as substrate mainly in heart and brain (36 and 35% with 6.5 mg/kg dose, and 42 and 27% at dose 7.5 mg/kg, $p<0.05$, respectively), lung (42%, $p<0.05$, at the lowest dose) and liver (41%, $p<0.001$, at the highest dose). With succinate as substrate, the ΔO_2 decreased 30% ($p<0.01$, 6.5 mg/kg) and 70% ($p<0.001$, 7.5 mg/kg) in liver and 30% ($p<0.05$, 7.5 mg/kg) in brain mitochondria. These results indicate that OD and mitochondrial bioenergetic dysfunction are processes associated to the histological damage and precedes the rat death.

Keywords: oxidative stress, copper, lipid peroxidation, mitochondria

(906) **EFFECT OF OXIDATIVE STRESS ON ADIPOCYTE DIFFERENTIATION**

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Oxidative stress (OS) is a major characteristic of adipose tissue in obese patients and its role in adipose hyperplasia/hypertrophy has not been elucidated. The aim of our study was to analyze the effect of OS during adipogenesis as well on differentiated adipocytes. For this purpose, we worked with preadipocytes 3T3-L1 and menadione, a synthetic form of vitamin K known to generate intracellular oxygen species. Two different models were used: the “chronic”, in which OS (5, 10 y 15 μM menadione) was present during the whole process of 3T3-L1 differentiation, and the “acute”, in which differentiated adipocytes were exposed to OS (20 y 50 μM menadione) for 5 h.

Adipogenesis, evaluated by Oil Red O staining as well as by the expression of adipogenic markers (PPARγ, C/EBPα, FAS, FABP4) by Western blot, was observed to be decreased in the chronic model in a menadione concentration-dependent manner ($p<0.01$). The adipogenic markers were also found to significantly decrease upon acute treatment ($p<0.001$). However, no significant morphological changes were observed in differentiated adipocytes acutely treated with menadione. PI3K/Akt and ERK1/2 showed to be inversely regulated: Akt was found to be inactivated whereas ERK1/2 were found

to be activated in both models of menadione-induced OS. Interestingly, experiments in which adipogenesis was evaluated in the presence of LY294002, a pharmacological PI3K inhibitor, but in the absence of menadione showed a significant decreased differentiation. Our results demonstrate that acute menadione-induced OS triggers a gene expression program leading to the decreased expression of transcription factors and enzymes which are crucial for lipogenesis and, on the other hand, chronic menadione-induced OS prevents adipogenesis, presumably in part, in a PI3K-dependent manner.

Keywords: adipogenesis, oxidative stress, lipid metabolism

(797) **INTERPLAY BETWEEN HYPERGLYCEMIA (HG), OXIDATIVE STRESS AND LYMPHOCYTE PROLIFERATION ON PERIPHERAL BLOOD MONONUCLEAR CELLS (PMBC) FROM DIABETIC PATIENTS**

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The association between diabetes and immunosuppression is suggested. HG is the main factor involved in diabetic complications by inducing reactive oxygen species (ROS) and oxidative stress. However, clinically there are patients with normal recovery from infections and others are susceptible. Genetic conditioning could be a factor involved. Previously we observed that lymphocytes isolated from BALB/c mice were sensitive to the deleterious effect of HG, while C57 were resistant, being oxidative stress, the main factor involved. The aim of this study was to analyze on PMBC cells from type 2 diabetic patients the interrelation between HG, oxidative stress and lymphocyte proliferation. Oxidative stress was studied by measuring glutathione content (GSH) and ROS generation. The immune function was studied by proliferation assays. The influence of HG was evaluated by preincubation of PMBC cells in a high glucose-containing medium. 32 type 2 diabetic patients were studied. Regardless of their metabolic control, there are patients who present oxidative stress (high levels of ROS) and others do not. They were divided into 4 groups according to their metabolic control (HbA1c>7,5%) and ROS production (>125%). Most individuals with high ROS, irrespective of metabolic control, age or gender, are patients whose disease is longstanding (over 9 years, $p<0.001$; Chi-square test). We observed that patients with high ROS have a non-significant (NS) lower levels of GSH. While PMBC with poor metabolic control or oxidative stress have a NS lower lymphocyte proliferation that is more marked when patients have both imbalances. Only PMBC cells from patients with HbA1c>7,5% - ROS >125% increased ROS production after the incubation with high glucose medium ($p<0.001$; One-way ANOVA). These results show the importance of studying the presence of oxidative stress and age of diabetes as possible predisposing factors to immune system dysfunction.

Keywords: type 2 diabetic patients, immune function, high glucose, oxidative stress

(214) **VITAMIN E SUPPLEMENTATION IN THE DIET PROTECTS LIVER OXIDATIVE DAMAGE ASSOCIATED TO CHRONIC OVERLOAD OF COPPER**

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Copper (Cu) is a bioelement that responds to the hormesis concept. At physiological concentration is essential for enzymatic reactions (in humans 50±5 μg/g liver and 5-10 μM plasma for diets of 1.4 to 2.0 mg Cu/day; in rats, 7.3±0.5 μg/g liver and 3.6±0.2 μg/g brain) but at pathological levels becomes toxic for living organisms. Chronic pathologies as Wilson, Parkinson and Alzheimer diseases are associated to higher than normal Cu levels in liver (L) and brain (B), oxidative stress (OS) and damage (OD), cognitive and neurological disorders. In L of Wilson patients, the Cu content is higher than 1000 μg/g, and in transgenic rats (ATP7B-/-), 300 μg/g. The aim of this research is to evaluate if vitamin E supplemented diet (Vit.E) prevents the OD associated to toxicity of Cu. Sprague Dawley male rats (200 g) received Vit.E (100 mg/day,5 g/kg food) over 5 days before Cu chronic overload (0.05% P/V water, 0-42 days). Rats survival, Cu content in L and B, phospholipid and protein oxidation (measured as TBARS and carbonyl (CO) protein) and glutathione (GSH) content were determined. Results indicated that chronic overload of $CuSO_4$ decreased rat survival after 28 days of treatment (75%, $p<0.05$) and increased L and B Cu levels after 7 days (105% in L and 10 fold in B, $p<0.01$). At 14 days of Cu overload, in L, TBARS increased 40% ($p<0.01$) and CO 26% ($p<0.05$); in B, TBARS increased 89% and CO 42% (both $p<0.01$). Vit.E pretreatment prevents the OD to phospholipids in L and B: decreased TBARS, 30% and 61% ($p<0.01$) respectively and the OD to proteins in L (decreased CO 40%, $p<0.01$) compared to rats received only Cu load. Nevertheless, GSH content decreased with Cu overload in L and B (both 42%, $p<0.05$) but the intracellular redox dishomeostasis was not prevented with Vit.E. These results indicate that Vit.E protects biomolecules in L from OD (irreversible); but is not effective in preventing B OD, neither cellular OS (reversible, redox homeostasis) associated to chronic Cu toxicity.

Keywords: antioxidants, oxidative stress, thiol, copper, redox

(1290) **NITRIC OXIDE (NO) IN WHITE ADIPOSE TISSUE OF HIGH-FAT FED MICE: EFFECT OF DIETARY (-)-EPICATECHIN**

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Changes in NO metabolism in white adipose tissue cells are common during the development of obesity and metabolic syndrome. (–)-Epicatechin (EC) is a flavonoid present in fruits and vegetables with the potential to avoid or attenuate the alterations in NO production and/or degradation.

The aim of this work was to analyze adipose tissue response to a high-fat diet supplemented with EC, in terms of fat pads remodeling and NO metabolism. For 15 w, two experimental groups of male mice (*C57BL/6)* received control diet, with (CE) or without EC supplementation (C). Two other groups received HFD (60% of calories from fat), with EC (HFE) or without EC supplementation (HF). Food, energy intake and body weight was evaluated during the experimental period. At the end, the increase in body weight (BW) induced by high-fat diet was similar, independently of EC supplementation (4.0±0.3 g). The four fat pads isolated showed significant increases in their weight relative to BW in HF respect to C (47% in perirenal, 72% retroperitoneal, 84% mesenteric and 55% epididymal, $p<0.05$). EC supplementation slightly attenuated this effect in retroperitoneal and mesenteric pads. Expression of endothelial, neuronal and inducible NO synthases (eNOS, nNOS and iNOS) and activator phosphorylation of eNOS in serine 1177 were determined in the fat pads, as well as the levels of nitrotyrosine-containing proteins, a hallmark of peroxynitrite production. NOS expressions were not affected by the treatments. In retroperitoneal pad, EC was able to activate eNOS via its phosphorylation regardless of the presence of high fat content in the diet. Additionally, nitrotyrosine-containing protein levels were significantly lower in HFE group respect to HF (-20%, $p<0.05$).

These results suggest a different response of each fat pad to EC supplementation and a possible role for NO in this variability.

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Keywords: obesity, nitric oxide, flavonoids, adipocytes

(1575) **ROLE OF CDK4 IN THE REGULATION OF WHITE ADIPOSE TISSUE BROWNING**

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Cyclin-dependent kinase 4 (CDK4), has been involved in the adipose tissue (AT) biology through the control of white adipocyte differentiation and insulin response. Previously, we showed that its inhibition *in vitro* induces adipocyte precursor cells (APCs) determination towards the beige adipocyte lineage. In this study, we aimed to evaluate if CDK4 could regulate the *browning* of white AT *in vivo* and *in vitro.* To this end, C57BL/6J male mice were gavaged with CDK4 inhibitor (Palbociclib (PAL), 50mg/Kg) or vehicle (CTR) for 10 days. Caloric intake and body weight were recorded every day and glucose tolerance test (GTT) was performed on day 8. After the treatment, mice were euthanized and plasma, epididymal AT (EAT) and liver samples were collected. EAT was weighted and processed for APC isolation, histology and qPCR analysis. PAL treatment did not induce changes in caloric intake or GTT, however, we observed a significant decrease in body weight and plasma and liver triglycerides ($p<0.05$). PAL mice also showed smaller EAT adipocytes ($p<0.05$) with similar EAT mass. Analysis of beige adipocytes markers in EAT revealed an increase in *Pgc1a* and *Prdm16* expression while *Ucp1* levels remained unchanged. Moreover, when APCs from EAT (CTR and PAL) were cultured and differentiated *in vitro, Ucp1* was found upregulated in adipocytes in the PAL group. Additionally, APCs from EAT of CTR mice were isolated and differentiated with a pro-beige cocktail to mature adipocytes. Then, they were incubated with or without PAL (1μM) for 48 hs. qPCR evaluation of beige related genes (*Ucp1, Prdm16, Pgc1a, Cox8b, CD137*) showed a significant ($p<0.05$) increase in PAL treated cells. Also, PAL treatment induced an increment in *Ucp-1* protein levels measured by immunofluorescence. Overall, these results indicate that CDK4 may be involved in the regulation of *browning* in EAT and its inhibition might promote transdifferentiation from a white to a beige adipocyte phenotype. PICT2013-0930.

Keywords: CDK4, white adipose tissue, browning, beige adipocytes, transdifferentiation.

(858) **INDUCTION OF HEME OXYGENASE REVERTS ENDOPLASMIC RETICULUM STRESS GENERATED IN A RAT MODEL OF NON-ALCOHOLIC FATTY LIVER DISEASE**

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Non-alcoholic fatty liver disease is a common chronic liver disease that has been associated with metabolic syndrome. Under certain stressful conditions, such as those described in the liver of animals fed a sucrose-rich diet (SRD), normal functions of the endoplasmic reticulum become compromised leading to the accumulation of misfolded proteins and inducing the unfolded protein response (UPR). Taking into account that oxidative stress (OS) could trigger an UPR the aim of this study was to evaluate the effects of heme oxygenase-1 (HO-1) induction on the liver of SRD treated rats. Male Wistar rats were randomly distributed into control (C) or SRD groups (30% sucrose in the drinking water over 12 weeks). Hemin treatment (15 mg/kg/48h, ip) was administered during the last two weeks of the dietary modification (H and SRD+H groups). Our results indicate that rats fed a SRD showed an increased liver content of TAG and cholesterol. We also observed higher catalase and SOD activities, and TBARS levels which were attenuated by hemin treatment. Hemin treatment did not modify triglyceride/HDL-c index (insulin resistance parameter), glycaemia or the histological features (analyzed by H&E or Sudan IV) of the livers of C or SRD treated rats, although HO-1 induction was confirmed in these tissues. Nonetheless, hemin was effective in reverting the increase in serum levels of transaminases (ALT), a marker of liver damage, observed in SRD-treated rats (SRD+H $p<0.05$ *vs.* SRD). Analysis of UPR-related markers demonstrated an increase in XBP1s and ATF4 expression (transcription factors associated with UPR) in the nuclear fraction of livers from SRD-rats that was blocked by hemin (SRD+H group). In conclusion, hemin treatment was capable to reverting OS, UPR induction and liver damage (ALT) associated with the long term administration of a SRD. We hypothesize that these effects could be attributed to the antioxidant properties of the HO-1 system.

Keywords: ER stress, NAFLD, Liver, Heme Oxygenase.

PHARMACOLOGY 7/NANOMEDICINE 2

(739) **A QUALITY BY DESIGN STRATEGY TO OPTIMIZE A NOVEL ANESTHETIC AND ANTIMICROBIAL FILM POTENTIALLY USEFUL AS A WOUND DRESSING**

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Films, as dosage forms, have gained relevance in the pharmaceutical field, as novel, patient friendly, convenient products. However, there are no official standardized methods for its evaluation. To ensure quality product with desired characteristics, the aim of this study was apply Quality by Design (QbD) strategies to obtain an optimized film that can be further used as a wound dressing. The desired product quality profile was defined and its critical quality attributes (CQA) were identified. Subsequently, materials were selected: ciprofloxacin hydrochloride (antimicrobial), lidocaine hydrochloride (anesthetic) and sodium alginate, sodium hyaluronate (HNa), carbomer (Cb) y glycerol (Gly) as excipients. Then, through a risk analysis and mitigation matrix the critical components were identified, selected as study variables and subjected to experimental verification through variable-response correlation using *Design-Expert® 10 software.* The response variables were scored to 1 to 5, and were: transparency (absence of precipitate, T> 5); homogeneity (absence of bubbles, H>4); the ease of demold (D=3) and the plasticity (P=3). The selected components allowed to develop a process of elaboration with low levels of criticality and a logical flow of operations. Only HNa, Cb and Gly showed to be critical to obtain the desired profile and were selected as study variables. The 4 variable-response obtained fitted to a R2FI model with an adequate correlation level, indicating that each model can explain the experimental changes. The 3D response surface plot of optimization showed a design space with the relative composition of the components that allows to obtain an acceptable film, and one composition that shows the best global results (desirability 0.96/1.00). The optimized anesthetic antibiotic film obtained by QbD strategies complies with the CQA and could be useful as a wound dressing.

Keywords: Quality by Design, film, polyelectrolytes, ciprofloxacin, lidocaine

(1656) **ANTIBACTERIAL PROPERTIES AND BIOCOMPATIBILITY OF COATED TITANIUM SUBSTRATES**

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Titanium and titanium alloys have excellent mechanical strength, good corrosion resistance and biocompatibility and they have been widely used for producing orthopedic implants. Osteointegration is a critical response to the long-term success of these implants. Focus-

ing the attention on the surface of the biomaterials, a hydroxiapatite (HA) coating on the implant surface can improve a direct implant fixation to bone. Even when the interaction of biomaterials with living tissues is favored, the problems of bacterial infections persist. A possible solution involves the surface modification with an antibacterial coating which maintains good biocompatibility. Incorporating silver nanoparticles (Ag-NPs) into a HA coating is an effective method to impart simultaneously both properties to the surface of the biomaterial. The objective of this work is to evaluate the antibacterial properties and biocompatibility of Ti6Al4V coated with Ag-NP and HA.

To prepare the coatings, a two steps strategy was optimized involving the electrodeposition of HA and the impregnation with Ag-NP. Coated Ti6Al4V substrates were characterized by spectroscopy and microscopy. For the antimicrobial activity assays, the substrates were incubated with a *S. aureus* culture and observed by confocal fluorescence spectroscopy, while the viable bacteria were quantified by serial dilution plates. The biocompatibility was evaluated directly, by culturing human osteosarcoma cells (U-2OS) on the coated substrates and indirectly, by studying the effect of the extract from the coated material on the cell culture. The cell viability was quantified by resazurin assay. HA/Ag-NP coating inhibits completely the bacterial adhesion and diminishes three logs units the number of viable planktonic bacteria with a minimum cytotoxic effect. Therefore, the HA/Ag-NP coating is very promising to develop more effective orthopedic implants exhibiting antibacterial properties without a detriment in the biocompatibility.

Keywords: HA/Ag-NP coating, Ti6Al4V coated substrates, biomaterials.

(1160) **BACTERIAL INACTIVATION AGAINST PSEUDOMONAS AERUGINOSA BY THE EFFECT OF NANOCOMPOSITE BASED ON SILVER NANOPARTICLES**

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In this work, the bactericidal action of the nanocomposite developed through a photochemical synthesis of silver nanoparticles (NPsAg) inside the biocompatible hydrogel was evaluated. The hydrogel was synthesized by copolymerization between N-isopropylacrylamide (NIPAM) and 2-acrylamido-2-methylpropanesulfonic acid (AMPS) (PNIPAM-co-2% AMPS). The antimicrobial activity of the nanocomposite was evaluated using a Gram negative (-) bacterium as the *Pseudomonas aeruginosa* model. This action was studied by means of the diffusion method in agar, which was evidenced by the formation of inhibition halos. The size of the inhibition halos increases whit the incubation time (24-120 h) suggesting an important antibacterial effect of the nanocomposite. This action was confirmed through the viability determination by counting colony-forming units (CFU/mL) and through the MTT technique, which indicates the metabolically active bacteria. From the results of viability and inhibition halos using as a control group the hydrogel and as a group treated the nanocomposite at different incubation times a marked antibacterial action of the nanocomposite (PNIPAM-co-2% AMPS-NPs-Ag) dependent on time was observed. Moreover, it was showed that the PNIPAM-co-2% AMPS-NPs-Ag triggers oxidative stress in bacteria. Furthermore, the concentration of proteins and DNA in the medium was determined through the Bradford method and Uv-visible spectroscopy ($O.D._{260nm}$), respectively. In this way, it was probed that the bacteria treated with the nanocomposite undergo cellular lysis demonstrated through the release of cytoplasmic content (proteins, DNA) which increases with the incubation time (1-4 h). In conclusion, these results reveal that PNIPAM-co-2% AMPS-NPs-Ag can be used as intelligent hydrogel to kill bacteria producing a significant decrease in cell viability triggering cell death by lysis cellular.

keywords: bacterial inactivation, *Pseudomona aeruginosa*, nanocomposite.

(74) **BIOCOMPATIBILITY AND BIODISTRIBUTION OF AMOXICILLIN COATED GOLD NANOPARTICLES EFFECTIVE AGAINST ANTIBIOTIC-RESISTANT BACTERIA.**

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Gold nanoparticles synthesized and stabilized with amoxicillin (amoxi@AuNP) have shown, in our previous experiments, to be bactericidal when irradiated with a LED panel. Their use in Photodynamic Therapy is very promising because they were effective even in antibiotic-resistant strains. The goal of this work was to evaluate their effect on eukaryotic cells and their biodistribution, under the same conditions in which they produced the photothermic effect: 1.5 µg / mL, 30 minutes of irradiation. *In vitro* experiments have proven that amoxi@AuNP did not produce significant hemolysis nor reduce fibroblast viability, according to the MTT assay, even after 24 hours. In addition, amoxi@AuNP completely killed prokaryotic cells, without affecting eukaryotic cells integrity. This was observed through a novel methodology using Fluorescence Lifetime Imaging System developed to study co-cultures of fresh blood cells with bacteria. On the other hand, TEM images of fixed tissue samples from male Wistar rats administered with amoxi@AuNP showed that most of them were found in the kidneys (samples 2 hours post intraperitoneal injection), after internalization through the microvilli and subsequent degradation in vesicles. They were also present in smaller amounts inside phagocytic cells in the liver and the spleen, but did not pass through brain blood barrier. Although 5% of the nuclei showed pyknosis 5 hours after administration, all tissues recovered normal structures the next day. This findings and spectrophotometrically analized urine samples collected along 24 hours, indicate that they are rapidly eliminated from the body, mainly by renal clearance. Overall, the amoxi@AuNP seem to be non-toxic to mammals cells in their bactericidal conditions.

Keywords: Amoxicillin, Gold, Nanoparticles, Bactericidal, Biocompatibility

(832) **NEBULIZABLED ARCHAEOLIPID NANOVESICLES: AZITHROMYCIN INCORPORATION AND INTERACTION WITH PULMONARY SURFACTANT**

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Archaeosomes (ARC) are nanovesicles prepared with archaeolipids (TPA) extracted from the archaebacteria *Halorubrum tebenquichense*. ARC have higher colloidal and chemical stability than conventional phosphatidylcholine liposomes over a wide range of temperature and pH, as well as in the presence of surfactants.[1] Furthermore, ARC are more endocytosed by different types of macrophages and endothelial cells than conventional liposomes.[2]In this work we propose to incorporate the macrolide antibiotic azithromicyn into ARC to perform an active targeting to macrophages as a new inhalable treatment for intracellular lung infectious diseases. Nanovesicles with a composition of HSPC:cholesterol:AZ 0.75:0.25:1 w:w (L-AZ) and TPA:AZ 1:1 w:w (ARC-AZ) were prepared by the film hydration method. The nanovesicles had sizes between 200 and 300 nm, low polydispersity indexes and ζ potential of -5 and -40 mV for L and ARC respectively. These formulations did not affect cellular viability of human macrophages after 24 hours incubation up to 50 µg/mL of AZ and to 150 µg/mL of total lipids. When cells were co-incubated with lipopolysaccharide their viability levels decreased.However, this was completely reversed with the addition of ARC-AZ. The stability of nanovesicles upon nebulization was evaluated by changes in size and aqueous content retention. After nebulization both formulations kept their size and retained at least 50

% of their aqueous content. The interaction between nanovesicles and a monolayer of pulmonary surfactant (PS) was evaluated after compression-expansion cycles in a Langmuir balance. After the first cycle both formulations increased PS surface tension, although ARC continued increasing it during the cycles, indicating a greater absorption on monolayer PS without function loss.1. Caimi, AT, et al. Colloids and Surfaces B: Biointerfaces 152 (2017): 114-123.2.2 Altube, MJ, et al. Nanomedicine 11.16 (2016): 2103-2117. Keywords: archaeosomes*, macrophages.*

(1281) **SILVER NANOPARTICLES/GELATIN NANOCOMPOSITES: NEW APROACH TO OVERCOME ANTIMICROBIAL RESISTANCE.**

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The main objective of this study is to develop an economical and biocompatible biomaterial with antimicrobial activity for tissue engineering applications. The nanocomposites were obtained employing gelatin as biopolymer scaffold and silver nanoparticles as a wide spectrum antimicrobial agent. Water and glycerol have been used as solvents for the gelatin hydrogel synthesis. This solvents mixture led to a biomaterial with improved thermal properties. Indeed, an increase in the melting point of the gelatin polymer of 46°C was observed by using glycerol and water, therefore, a material with excellent rheological properties has been made.

Nowadays, multiple drug resistance has developed due to the use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases. Silver nanoparticles have been studied as an antimicrobial agent as an alternative to antibiotics. The release of silver was analyzed by atomic absorption spectroscopy. The maximum release of silver was reached at 12 hours showing good performance of the nanocomposite as a metal ion and silver nanoparticle delivery system. The antibacterial activity of the nanocomposite against *S. aureus* and *P. aeruginosa* was tested, showing a decrease in bacterial growth higher than 99,99%. In conclusion, a AgNp/gelatin nanocomposite, with good rheological and improved thermal properties and silver nanoparticles as a source of silver ions has been synthesized. These properties of the nanocomposite with controlled silver delivery result in a more efficient topical pharmaceutical form for wound healing applications.

Keywords: Antimicrobial resistance, silver nanoparticles, tissue engineering, nanomedicine, sustained release

(426) **STUDY OF pH-RESPONSIVE BIOPOLYMER-SILICA COMPOSITES WITH POTENTIAL BIOACTIVITY FOR PERIODONTAL TISSUE REGENERATION WITH ANTIOXIDANT ACTIVITY**

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Temperature and pH are some of the variables in which materials can respond differently and therefore they are able to modulate their behavior. Particularly, chitosan (Chi) polymer amino groups, carboxylic acid groups from carboxymethylcellulose (CMC) and silanol groups from silica nanoparticles may play a role in the development of pH-responsive composites. We present pH-responsive composites with a differential mucoadhesive profile throughout periodontal disease. Saliva from patients with gingivitis presents a higher pH than saliva from patients with periodontitis. Accordingly, the swelling profile of Chi-SiO2-CMC and Chi-SiO2 composites shows a greater affinity for water at a pH of 7.5 in contrast to a pH of 6.5, encouraging their mucoadhesion.

Besides, we performed an EDS-SEM analysis from Chi-SiO_2-CMC and Chi-SiO_2 composites after soaking in simulated body fluid for 14 days. Results revealed that only Chi-SiO_2 composites showed an accumulation of Ca^{++}. Silanol groups could provide an environment for possible biomineralization. The supersaturation of Ca^{++} near a negative surface could trigger the nucleation of hydroxyapatite. The addition of CMC discouraged Ca^{++} accumulation. The development of bioactive composites could promote the regeneration of periodontal tissue in periodontitis. Although the mucoadhesion of both composites at pH 6.5 is lower, Chi-SiO_2 could possibly be used for regeneration of osseous tissues.

We also incorporated *Larrea divaricata* aqueous extract to the Chi-SiO_2-CMC composites and evaluated its release profile at a pH of periodontitis saliva. *L. divaricata* is a plant with antioxidant and anti-inflammatory activities, thus it can be used in the treatment of periodontal disease. *L. divaricata* release was measured by HPLC. In parallel, DPPH scavenger activity was assayed in order to evaluate antioxidant activity. The extract was released from the composites for over 48 hs and conserved its antioxidant activity.

keywords: chitosan, composite, antioxidant activity

(1035) **INNOVATIVE NANOTECHNOLOGICAL PLATFORM BASED ON POLYELECTROLYTES OF DERMATAN SULFATE AND CHITOSAN FOR THE TREATMENT OF VASCULAR DISEASES**

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Vascular diseases, particularly atherosclerosis, are one of the principal causes of morbid-mortality in the world. Recently, we reported the production of polyelectrolyte complexes (PECs) by a simple and reproducible method between low molecular mass dermatan sulfate (DS) (polyanionic polysaccharide) and chitosan (CS) (polycationic polysaccharide), which specifically interact with injured endothelial cells through CD44 receptor. The aim of the present work was to employ these complexes as a drug delivery platform of an anti-inflammatory egg white ovotransferrin-derived tripeptide Ile-Arg-Trp, (IRW).The tripeptide was obtained on solid phase employing Fmoc chemistry in Rink-Amide-MBHA resin. Then, protector groups from the lateral chains were cleaved and IRW was released with trifluoracetic acid. DS/CS PECs-IRW were prepared by polyelectrolyte complexation method in the presence of IRW (5, 25, 50 μM). The hydrodynamic diameter (D_h), size distribution (PDI) and zeta potential (Z-potential) of PECs were determined by dynamic light scattering (DLS). The presence of free IRW in particle suspensions was evaluated by spectroscopy at 390nm. Unloaded DS/CS PECs exhibited a D_h (±SD) of 729 nm (11), PDI (±SD): 0.322 (0.055) and Z-potential (±SD) of 49.4 mV (0.8) (n= 3). DS/CS PECs- IRW (5 μM) exhibited a D_h (±SD) of 510 nm (77), PDI (±SD): 0.495 (0.114) and Z-potential (±SD) of 29.9 mV (2.6) (n= 2). Finally, DS/CS PECs-IRW (50 μM) exhibited a D_h (±SD) of 550 (131), PDI (±SD): 0.475 (0.021) and Z-potential (±SD) of 35.6 mV (1.6) (n= 2). In all cases, the presence of free IRW was undetectable. In conclusion, DS guarantee PECs specific uptake by endothelial cells; while CS limits lysosomal degradation and support the gradual release of IRW inside the cell.Ongoing studies are oriented to explore the capacity of DS/CS PECs-IRW to modulate the endothelial inflammatory response associated with early stages of vascular diseases.

Keywords: Dermatan sulfate, Chitosan, Polyelectrolyte complexes, Anti-inflammatory peptide, Vascular disease

(131) **LIPOSOMAL FORMULATIONS WITH MUCOLYTICS AS DELIVERY SYSTEMS FOR COMBINED TREATMENTS AGAINST RESPIRATORY DISEASES**

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Respiratory diseases are a major health issue around the world. Despite easy access to the respiratory tract, the local delivery of drugs to the lung tissue is complicated due to the unique architecture of the respiratory system and its natural defense mechanisms. New therapeutic strategies are needed in order to overcome these issues. Liposomal delivery systems that encapsulate mucolytics are an alternative; these substances break down the gel structure of mucus, helping to overcome barriers and increasing the effectiveness of different therapies.

Based on this, the main goal of this work was to improve effectiveness of stem cell therapy in chronic lung diseases. In order to achieve this, mucolytic transporters were developed with phospholipids of the lung surfactant so as to improve their interaction with the lung tissue.

To carry out this work, liposomes were obtained by the dehydration/rehydration method. Afterwards, extrusion was applied to obtain smaller vesicles, which were combined with different mucolytics. Microscopy and dynamic light scattering described large unilamellar vesicles with a hydrodynamic ratio of 100 nm. Finally, lung mechanics were improved after intranasal inoculation of the formulations in a murine model of silicosis.

Keywords: **liposomes, mucolytics, respiratory diseases**

(753) **NANOTOXICOLOGICAL STUDIES OF CARBON NANOTUBES IN ZEBRAFISH**

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Carbon nanotubes are graphene sheet structures with a striking mechanical strength, chemical stability, exceptional electrical and thermal conductivity. Its application in constantly growing, ranging from printer inks to drug delivery systems. Since the physicochemical properties of the compounds vary when they are produced at nanoscale, and that the environmental systems are dynamic, it is not possible to predict the hazards associated with the release of nanomaterials into the environment both for human and ecosystem health. So, it is necessary to carry out a comprehensive analysis of the toxicity of water and effluents by studying the behavior of new technologies in ecosystems.

In this work, toxicological assays of multiple wall carbon nanotubes (MWCNTs) of different dimension were done (MWCNT A is short and width, MWCNT B is large and thin). Zebrafish were used as biomarkers due to their short development times. Tests done were acute toxicity (mortality), teratogenicity (hatching rate and morphology), general developmental anomalies and tissue-specific toxicity (morphology and functioning of brain, heart and liver).

Zebrafish embryo were incubated with 0.005-50 ppm MWCNTs, then mortality, hatching rate and morphology were evaluated. Zebrafish larvae were incubated with 0.005-50 ppm MWCNTs, next morphology (bent spine, jaw malformation, head opacity, liver opacity, yolk opacity, small head, tail malformation, and uninflated swim bladder) and functioning of brain and heart and were analyzed. No MWCNT was found to be lethal or teratogenic at the concentrations evaluated, although MWCNT A caused morphological abnormalities in larval development. On the other hand, both MWCNTs reduced the swimming activity of the zebrafish at different concentrations, whereas no changes in heart rate were observed.

Results were different for both carbon nanotubes, indicating a potential dissimilar toxicity between MWCNTs, which could be due to the different dimensions.

Keywords: carbon nanotubes, nanotoxicology, zebrafish

INFECTOLOGY 6

(412) **6B6 BINDING PROTEIN: A NOVEL ANTIGENIC PROTEIN OF *TRYPANOSOMA CRUZI* WITH DIAGNOSTIC POTENTIAL**

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Chagas disease (ChD), caused by the protozoan *Trypanosoma cruzi*, affects approximately between 6 and 7 million people around the world and remains a major public health concern throughout much of Latin America. During its chronic phase, the diagnosis relies on serological methods, being the most extensively used Indirect Hemagglutination Assay (IHA), Indirect Immunofluorescence Assay (IFA) and Enzyme-Linked ImmunoSorbent Assay (ELISA). Given that none of these assays render 100% specificity and sensitivity (*gold standard method*), the World Health Organization (WHO) recommends two tests in parallel with the use of a third one in case of discordances, for reaching a precise diagnosis of *T. cruzi* infection. In this context, the objective of our work is to establish the diagnostic utility of a *T. cruzi* protein, which was discovered as the target molecule to a single chain recombinant antibody (scFv 6B6), isolated from an antibody library made from B cell of patients with chronic Chagas heart disease. This 6B6 binding protein (6B6-BP), of 175-250 KDa, is found in the cytoplasm of the three morphological forms of the parasite belonging to different Discrete Typing Units (DTUs), but not in other trypanosomatids, like *T. brucei* and *Leishmania spp*, or in mammalian cells. After isolating 6B6-BP from *T. cruzi* lysate by immunoprecipitation, the reactivity of sera from patients with chronic ChD, with cutaneous leishmaniasis, and non-infected individuals was assessed by Western-Blot. Results showed that only sera from chronic ChD and one from a patient with mixed *T. cruzi-Leishmania* infection recognized 6B6-BP. Although its identity is under current investigation, our preliminary data positions 6B6-BP as a potential specific diagnostic marker for this disease. We expect that our research will contribute to overcome this issue, enabling the effective serologic discrimination of ChD from other trypanosomiasis.

Keywords: diagnosis, new specific antigen, infectious diseases.

(115) **DIFFERENTIAL DIAGNOSIS OF *TRYPANOSOMA CRUZI* INFECTED POPULATIONS USING THE TRYPOMASTIGOTE SMALL SURFACE ANTIGEN**

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Chagas disease is caused by *Trypanosoma cruzi*. Available drugs are mostly effective during the acute phase and display less advese effects during childhood. New serological, easy-to-assess markers able to distinguish between 1) acute and chronic infections and 2) endogenous and maternal IgG in congenital infections, are needed. In this study we analized the antigenic core (from T-24 to S-62) of the previously validated TSSA (*T*rypomastigote *S*mall *S*urface *A*ntigen) protein in ELISA assays. Using three 15-mer deletional variants (TSSA30-44: 30-TSSTPPSGTENKPAT-44, TSSA36-50: 36-SGTENKPATGEAPSQ-50, TSSA42-56: 42-PATGEAPSQPGASSG-56) expressed as GST-fusion proteins, we analized serum samples from 2 *T. cruzi* infected populations: 8 from acutely infected patients (vectorial) and 86 from chronic patients (>8 years of infection). Serum samples from infected acute patients showed equivalent reactivity against 2 or 3 deletion variants. In contrast, serum samples belonging to a chronic population showed specificity against the TSSA30-44, predominantly. On the other hand, in the context of a project that aims to evaluate TSSA24-62 as a serological marker of treatment efficacy we identified a child with particular features. Briefly, a newborn that clarified maternal anti-TSSA24-62 antibodies at 4.2 months and conventional – whole parasite based-ELISA (tELISA) at 7.2 months showed an increase in TSSA24-62

and SAPA reactivity at 10.5 months. Later, at 19.2 months, tELISA and HAI became positive and this child was diagnosed as *T. cruzi* infected and treatment was initiated. TSSA24-62 showed better sensitivity compared to tELISA and, most interestingly when evaluated against TSSA deletion variants, this child showed differential immune signatures compared to its mother. In this study we showed that the use of small antigenic sequences inside TSSA can reveal different immune signatures in *T. cruzi* infected populations which can be exploited as a differential diagnosis tool.

Keywords: Chagas disease, Trypomastigote Small Surface Antigen, differential diagnosis.

(1042) **MOLECULAR DETECTION OF *Trypanosoma cruzi* IN A PATIENT WITH SYSTEMIC LUPUS ERYTHEMATOSUS AND REACTIVATION OF CHAGAS DISEASE**

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Abstract: The objectives of this work were amplify specific DNA of *Trypanosoma cruzi* by conventional PCR and measure the parasitic load using PCR in real time in blood of patient with reactivation of Chagas disease acquired by transfusion. Indirect Hemagglutination and Ig G-ELISA test were performed. The DNA was purified with CTAB (Hexadecyltrimethyl Ammonium Bromide) as previously reported. PCR based detection of the 330-bp minicircle variable region of parasitic kinetoplastid DNA (kDNA-PCR) was carried out in blood samples using primers 121 and 122. Parasitic loads were determined by means of TaqMan Real Time PCR (qPCR) targeted to a 166-bp segment from *T. cruzi* satellite DNA (SatDNA).The results for anti-*T. cruzi* serodiagnosis were IAH: 1/128 and ELISA positive. The Microhematocrit analysis reveal protozoan forms, while kDNA-PCR allowed detection of *T. cruzi* DNA in peripheral blood and the Real Time PCR revealed a mean of 120 par eq/ml of blood. The role of *T. cruzi* parasitemia on the onset of chagasic reactivation due to immunosuppressive treatments is poorly explored. The PCR provided a rapid differential and sensitive diagnosis of *T. cruzi* reactivation prompt administration of specific chemotherapy.

Keywords: *Trypanosoma cruzi,* DNA, PCR, Chagas.

(1074) **QUANTIFICATION OF *TRYPANOSOMA CRUZI* EPIMASTIGOTES: A COMPARATIVE STUDY OF FLUOROMETRIC METHODS**

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Cell counting with Neubauer chamber is a traditional tool of great utility and precision in the quantification of parasitic forms of *T. cruzi.* However, this method has some limitations, including long-operating times and subjectivity in counts, which makes it impractical for testing antiparasitic compounds at medium or large scale. With the objective of validating a technique that allows us to test a library of compounds, two alternative fluorescent methods of quantification of parasites were tested: transgenic GFP epimastigotes and a resazurin (Rz) reduction-based assay. GFP transgenic and wild type epimastigotes of the Y strain were grown under standard culture conditions and treated with the reference drugs (Benznidazole-BZ- and Nifurtimox -NFX-). Fluorescence was recorded on a plate fluorometer (BMG Fluostar Optima). Neubauer chamber count was performed in parallel. We confirmed a linear correlation (r2 = 0.99) between the number of parasites and the fluorescence signal, with both Rz and GFP. The linearity, sensitivity, limit of detection, limit of quantification and range for both methods were analyzed. The accuracy and precision of the two techniques were evaluated using the EJCR (Elliptic Confidence Region) test. Although both methods showed good correlation, GFP showed a better correlation with the standard method.

(1557) **EFFECT OF THE MECHANISM OF DEGRADATION OF TRYPTOPHAN IN THE INFECTION OF HUMAN PLACENTA BY *Trypanosoma cruzi***

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L-Tryptophan(Trp) is catalyzed by Indoleamine 2,3-dioxygenase (IDO) in the kynurenine (Kyn) pathway (KP). IDO is highly expressed in the placenta. Local depletion of Trp and/or the presence of metabolites of the KP mediate immunoregulation and exert antimicrobial functions, which is involved in the inhibition of intracellular pathogen replication. This system has been described to participate in the infection of Chagas disease, but the interaction of placental tissue with Trypanosoma cruzi (T. cruzi), the causal agent of congenital Chagas transmission, is not yet studied. Objective: To analyse the effect of the degradative mechanism of tryptophan in the infection of human placenta by T. cruzi. Methods: Explants of human placenta were co-cultured with 10^5 trypomastigotes of T. cruzi (Infected, n=3) or without (Control, n=3) for 4, 24 and 96hs. Placental explants were treated with 50 μM and 100 μM of L-Trp (n=3). We used paraffin-embedded placentas from Chagasic pregnant women (Infected, n=12) and normal placentas at term (Control, n=5). Kyn production were measured. Parasitic load was determined by qPCR. Immunohistochemistry were used to determination of IDO protein expression. Significance was determined at $p<0.05$. Results: Levels of Kyn were significantly decreased in 96hs of co-culture infected group ($p<0.001$) as compared to control group. Parasitic load was no modified with different concentration of L-Trp ($p>0,05$). IDO expression was decreased in Chagasic human placentas ($p<0.05$) compared to normal ones. Conclusion: T. cruzi modifies the catabolic tryptophan pathway in human chorionic villi in vitro and in vivo. This pathway could participate in the process of infection of placental tissue in the congenital transmission of Chagas. Grants: PICT2012-1061, MINCyT-PID-2014, SECyT-UNC, UNVM, PICT-V-2015-0074. Keywords: Trypanosoma cruzi, Human placental, Tryptophan, Indoleamine 2,3 dioxygenase, Kynurenine

(1497) **EFFECT OF *Trypanosoma cruzi* INFECTION IN TROPHOBLAST CELLS (BEWO CELL LINE)**

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Congenital Chagas has become a global health problem due to the migration of chagasic mothers from endemic to non-endemic countries. During congenital transmission, the parasite breaks down the placental barrier. It has been shown that placental immune response exerts a deleterious effect on *Trypanosoma cruzi* (*T. cruzi).* Nitrosative/oxidative stress and cytokine profile are mechanisms that prevent microorganism invasion and fetal infection. This study aimed to evaluate the role of trophoblast cells (BeWo cell line) on *T. cruzi* infection. We induced BeWo syncytialisation with forskoline and tested it by immunofluorescence assays and staining nucleus. Cytotrophoblast (CT) and Syncytiotrophoblast (ST) were co-cultured for 24 hs with trypomastigotes of Tulahuen strain (ratio 1:1) and reinfections were done at 12, 24 and 48 hs.*T. cruzi* survival capacity in supernatant media (SN) from BeWo CT and ST cell conditioned cultures were studied. Culture media quantifications: nitric oxide (NO; Griess assay), reactive oxygen species (ROS; fluorescent probes) and hCG and IL-6 (ELISA). NO and hCG production increased more

than 1.5 fold (p<0.05) in infected BeWo ST, but no differences were observed in IL-6 production. ROS production of infected BeWo ST increased more than 1.8 fold (p<0.05). There were not significant differences between reinfected and control groups respect to the survival of the parasite after 96hs of culture. Mobile parasites incubated in SN from BeWo ST decreased 75% (p<0.05) compared with fresh medium and SN of BeWo CT. These results suggest that trophoblast cells are able to modulate *T. cruzi* infection, independently of their reinfection, by forming syncytiotrophoblast and producing harmful metabolites for the parasite. Grants: PICT2012-1061/2015-0074,SECyT-UNC, UNVM.

Keywords: *Trypanosoma cruzi,* BeWo cells, Nitrosative/oxidative stress, cytokine.

(1884) **IMPLEMENTATION RESEARCH TO MONITOR ACUTE TOXOPLASMOSIS SCREENING IN PREGNANT WOMEN AT PUBLIC HOSPITALS FROM JUJUY PROVINCE, ARGENTINA: IMPLICATIONS FOR CONGENITAL TOXOPLASMOSIS PREVENTION**

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The major problem in public health related to Toxoplasmosis is when women of childbearing age acquire Acute Toxoplasmosis (AT) infection during pregnancy. In these cases, congenital toxoplasmosis can be vertically transmitted to the fetus, and causes serious congenital defects. At the Ingeniero Carlos Snopek Public Hospital (Jujuy province, Argentina) a retrospective population-based study was conducted to analyze the seroprevalence of toxoplasmosis infection in almost 1600 pregnant women, who requested prenatal screening. According to the detection of immunoglobulin (IgG, IgM) and IgG avidity testing, the patients were classified as: susceptible, with chronic infection, and with suspected or confirmed AT. The study objectives were: to characterize the seroprevalence of toxoplasmosis according to the age of the patients; to monitor the efficacy of AT screening at the hospital, and to identify factors that could make difficult the early diagnosis of AT. The results show that toxoplasmosis infection was observed in 44.2% of pregnant women and its prevalence increased with the age of the patients up to 67% between 36 and 40 years old. Within the group with positive immunity, 15 (0.94%) were diagnosed with suspected AT. The high IgG avidity, during the first trimester, discarded AT in 9 women. In one case (0.06%), AT was confirmed, and in the 5 remaining cases the diagnosis was not completed. On the other hand, the follow-up of susceptible patients showed that 77.1% (n = 602) received only one serological control throughout the pregnancy, 27.82% (n = 133) received two controls, and 1.45% (n= 9) one control per trimester. We concluded that active toxoplasmosis in pregnant women attending at the mentioned public hospital is among the highest values reported in Argentina. However, the algorithm of AT screening confirmed a single case at the hospital. We will discuss about possible problems that hinder the efficient implementation of the algorithm for early diagnosis of AT in pregnant women living in vulnerable contexts.

(1733) **VALIDATION OF A REAL TIME PCR KIT PROTOTYPE FOR EARLY DIAGNOSIS OF CONGENITAL CHAGAS DISEASE IN A MULTICENTER FIELD STUDY**

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Congenital transmission of *T. cruzi* occurs in approximately 4% of newborns to infected mothers. Treatment with trypanocidal drugs has an effectivity close to 100%, so it is essential to provide an early diagnosis of infection. The low sensitivity of the current microhematocrit method (MH) determines that around 75% of the babies must return for confirmatory serological diagnosis 10 months after birth. In practice, many patients cannot complete the algorithm and remain without diagnosis. In this context, we developed a novel duplex Real Time PCR kit prototype, based on TaqMan probes, to improve sensitivity (Se) of early detection of congenital infection. Analytical Se of the prototype was 0.2 parasite equivalents/mL. The present work shows the prospective evaluation of the kit prototype in the framework of a multicenter field study. A total of 560 seropositive pregnant women were enrolled in five Health centers located in Buenos Aires, Chaco, Tucumán and Santiago del Estero, after written informed consent. Umbilical cord blood was collected at delivery and/or venous blood samples at 4-8 weeks of life to be analyzed blindly by PCR and MH. Additionally, venous samples were drawn at 10 months of life for final serodiagnosis. A total of 359 newborns got final diagnosis and 13 were infected, as detected by gold standard diagnosis. PCR was carried out on duplicates from each blood sample. It showed a Se of 66.7% and a specificity (Sp) of 95.2% from umbilical cord blood, and a Se of 63.6% and Sp of 98.9% in venous blood. Considering that samples had to be transported from the endemic areas to a core molecular biology laboratory for DNA extraction and PCR amplification (INGEBI), the prototype could detect a higher proportion of infected newborns than routine parasitological examination. It is expected that implementation of the PCR kit in trained centers located at the endemic areas may achieve a better performance and provide a more sensitive diagnosis.

Keywords: Congenital Chagas Disease, Multicenter field study, *Trypanosoma cruzi,* Real Time PCR.

(1793) **EVALUATION OF THE PERFORMANCE OF A LAMP PROTOTYPE KIT FOR DETECTION OF *Trypanosoma cruzi* DNA IN CLINICAL SAMPLES**

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The protozoan *Trypanosoma cruzi* (*T. cruzi*) causes Chagas Disease (ChD), with 7 million infected people worldwide. Without treatment, the disease evolves from an acute phase with nonspecific symptoms, high parasitaemia and slow seroconversion to a chronic phase (CCD), frequently asymptomatic, with low parasitaemia and serodiagnosis as the gold standard. Immunocompromised CCD patients can reactivate (RCD), sometimes with severe clinical symptoms. For Congenital Chagas (CI) low cost microscopy, which lacks sensitivity and is operator-dependent, is routinely employed because serodiagnosis is only useful around 10 months of age. Real Time quantitative PCR (qPCR) has been validated for molecular detection and monitoring of *T. cruzi* infection, but it must be performed in specialized laboratories. Recent Target Product Profiles for ChD diagnosis recommended the use of easier methods for point-of-care applications. The aim of this study was to evaluate a kit prototype based on Loop mediated isothermal amplification (LAMP) for *T.cruzi* DNA detection in human blood samples. The LAMP prototype contains dried reagents in the caps of microtubes and targets satellite DNA repeats (Analytical sensitivity=0.1 par. equivalents/mL from EDTA-blood, extracted using a commercial kit). The reaction is completed in 40 minutes and visualized by naked-eye. LAMP sensitivity (*Se*) and specificity (*Sp*) were compared to qPCR in 221 EDTA-blood samples from 172 patients of three clinical groups: Congenital (CI), Chronic (CCD) and Reactivated Chagas Disease (RCD). The Non Infected Control group (NIC) included 87 samples, 21 from babies. For the CCD group (n=109), *Se* was low and *Sp* was 98.5% (IC 0.93-0.99), resembling qPCR results. For RCD (n=26), the Newmar test (p<0.005) did not show significant differences between qPCR and LAMP. For the CI group (n=37), LAMP *Se* was 100% (IC 0.725-1.00) and *Sp* 92.6% (IC 0.824-0.916).This exploratory study showed high accuracy values of LAMP for CI and RCD groups.

Keywords: Chagas disease, LAMP, qPCR, diagnosis, *Trypanosoma cruzi*

(1623) **ADAPTING A MOLECULAR ISOTHERMAL AMPLIFICATION REACTION TO DEVELOP A SIMPLIFIED *Try-***

***panosoma cruzi* DETECTION METHOD.**

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Chagas disease, originally from Latin America, is caused by *Trypanosoma cruzi*. It is estimated that 15000 babies are born with congenital Chagas around the world each year. Early detection and treatment of congenital Chagas disease increases the therapy´s effectiveness. However, serological methods of diagnosis are effective only after 9 months of age, when maternal antibodies have been completely removed. On the other hand, molecular diagnostic strategies, applicable for newborns, require infrastructure, skilled personnel and expensive equipment, factors that restrict their use to few health centers.

The objective of this work was to develop a molecular amplification technique to detect *T. cruzi* DNA with high sensitivity and specificity that would be simple and stable enough to be used on point of care ("POC") conditions.

The first step consisted in selecting target sequences and primer design, using specific bioinformatics software. Then, the in vitro reaction was set up testing: a- the reaction conditions; b- sensitivity with different types of samples; and c- specificity, using different strains of *T. cruzi*, other phylogenetically related parasites, human cells and cells unrelated to the parasite or host. Finally, the reaction was adapted to make it simpler and suitable for all health care conditions (different equipment, infrastructure, etc.). The read-out methods were simplified, showing a changing color on a lateral flow dipstick (LFD) system.

Two of the 6 sets of designed primers, from repetitive DNA fragments, showed high specificity (with no cross reactions) and high sensitivity (~1fg of template), along different types of templates (DNA, parasites, artificially inoculated samples, etc.) and using analytical read-out (gel electrophoresis), color changes and LFD.

These results encourage us to continue developing a test for Chagas disease as well as for other infectious diseases whose current diagnosis methods also need improvement and simplification.

Keywords: Chagas; Trypanosoma Cruzi; Point Of Care Conditions; Molecular Isothermal Amplification.

TOXICOLOGY 4

(975) **ACUTE AIR PARTICULATE MATTER EXPOSURE TRIGGERS VASCULAR DYSFUNCTION IN SENESCENT RATS**

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Epidemiological evidence supports an important association between air particulate matter (PM) exposure and cardiovascular risk. Acute 10 μg/m3 increase elevates cardiovascular mortality by 1%. Cardiovascular detrimental consequences due to PM could be explained by PM translocation from the respiratory epithelium towards circulation and next initiate cardiovascular toxicity. The aim of this study was to determine if pulmonary exposure to a PM surrogate, such as residual oil fly ash (ROFA), affects systemic vascular function. Objective: Evaluate vascular changes, lung, heart and aorta histology, blood smears and inflammatory serum cytokines after acute exposure to ROFA. M&M: Senescent (17 months old) female BDIX rats were divided in two groups and exposed by intranasal instillation to saline solution (Control) or ROFA 1mg//kg BW (ROFA). Euthanasia was performed 24h post-exposure and the respiratory tract, heart and aorta were excised, formalin fixed and process for routine histology. Previous to fixation a section of the aorta was placed in Krebs solution and cut into 3-4 mm wide rings. Rings vessel were suspended in a water-jacketed organ bath and the contractile response evaluated by cumulative noradrenaline (NA) addition (10-9 to 4x10-6 M) or Acetilcholine 10-6M (Ach). Complete blood count (CBC) was performed in blood smears. Serum TNFa, IL-6 and IL-10 levels were analyzed by ELISA. Results: Acute ROFA exposure induced histological changes in lungs and hearts and markedly systemic PMN augmentation when compared to Controls but no changes in cytokine levels between groups. Functionally, the maximal contractile response to NA increased in arteries from ROFA-exposed compared to Controls (39.4%, $p<0.001$). Even more, the response to Ach showed a decreased (32.4%, $p<0.001$). Conclusions: ROFA induces organ histological changes and triggers vascular dysfunction causing a lower capacity relaxation and a greater capacity contraction in senescent rats.

Keywords: particulate matter; aorta, heart, lung, vascular function

(1331) **PERINATAL EXPOSURE TO GLYPHOSATE OR ITS COMMERCIAL FORMULATIONS: UTERINE MOLECULAR MECHANISMS INVOLVED IN EMBRYO IMPLANTATION FAILURES**

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Glyphosate is the active ingredient in a wide variety of broad-spectrum non-selective herbicides. Commercial formulations of glyphosate include other compounds which act as adjuvants. Recently, we found that perinatal exposure to either glyphosate (Gly) or a glyphosate-based herbicide (GBH) caused subfertility in female rats associated with implantation failures. In this work, we studied whether these alterations might be induced by a defective uterine functional differentiation during the pre-implantation period. Pregnant rats (F0) were orally exposed to Gly or a GBH through food, in a dose of 2 mg of glyphosate/kg/day (RfD, EPA), from gestational day (GD) 9 until weaning (lactational day 21). Sexually mature F1 females were pregnant and uterine samples collected on GD5 (pre-implantation period) for morphological and mRNA analysis. Uterine sections were stained with hematoxylin and eosin to analyze the following morphological features: luminal epithelial height, number of glands, and thickness of myometrium and subephitelial stroma. The expression of implantation-associated genes, such as progesterone receptor (PR), the homeobox Hoxa10 and leukemia inhibitory factor (LIF), was assessed by RT-qPCR. At the morphological level, a lower number of uterine glands was detected in Gly- and GBH-treated rats. These results are in accordance with the lower expression of LIF in both groups, since it is mainly secreted by glands. A downregulation of PR was found in glyphosate-treated rats, which correlated with a decreased expression of Hoxa10 detected in both exposed groups. In conclusion, perinatal exposure to Gly or GBH induced uterine morphological and molecular alterations during the pre-implantation period, which might explain, at least in part, the implantation failures triggered by Gly and GBH treatments. These results also suggested that the active principle, glyphosate, is the responsible of the observed effects.

Keywords: Glyphosate, Uterus, Subfertility, Implantation

(1655) **ASSESMEMT OF THE POTENTIAL HEALTH IMPACT OF SILVER NANOPARTICLE EXPOSURE**

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Silver nanoparticles (AgNP) are commonly used in different commercialized products, such as disinfectant sprays, personal hygiene products, and several biomedical applications. Along with the development of AgNP, the question of their possible toxicity has increasingly gained attention. Since oxidative stress has been postulated

to play a major role in AgNP toxicity, the aim of this work was to evaluate the oxygen metabolism in the lung after AgNP inhalation. Characterization of AgNP by TEM showed a spherical shape and the absence of NP aggregation. Hydrodynamic diameter and zeta potential, as assessed by dynamic light scattering, was 17 **± 6 nm and -40 ± 7 mV** respectively. Desorption studies showed a 24% release of Ag from AgNP after 1 h of incubation. The ability of AgNP to participate in Fenton chemical reaction was also evaluated, showing that AgNP was able to induce the production of reactive oxygen species in a concentration dependent manner. For *in vivo* studies, female Balb/c mice (25 g) were intranasally instilled with a AgNP suspension (0.1 mg Ag/kg body weight). The control group was treated with saline solution. Samples were collected after 1 h. Biodistribution studies showed that the main organ of Ag deposition was the lung (450 ± 50 ppb Ag/g tissue). In lung tissue, the O_2 consumption increased by 31% (control group: 300 **± 20** ng-at O/min g tissue, $p<0.05$), probably due to an increase in mitochondrial active respiration (55%, $p<0.001$). Moreover, H_2O_2 production rate by isolated mitochondria was also increased by 39% (control group: 1.1 ± 0.1 nmol/min mg protein, $p<0.05$). Taken together, these results show that AgNP remain in the lung, leading to alterations in oxygen metabolism, probably due to changes in mitochondrial function.

Keywords: nanoparticles, silver, oxidative stress, lung.

(1831) **TIME COURSE OF LUNG INFLAMMATION AND CARDIAC OXYGEN METABOLISM IN A MICE MODEL OF CONTINUOUS EXPOSURE TO URBAN AIR POLLUTION**

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The exposure to air pollution leads to increased morbidity and mortality rates from cardiorespiratory diseases. In has been suggested that local and systemic oxidative stress and inflammation play a central role in this scenario. The aim of this work was to characterize a mice model of continuous exposure to urban air pollution, by the assessment of different markers of pulmonary, systemic, and cardiac oxygen metabolism and inflammation. 8-week-old balb/c male mice were exposed to urban or filtered air (control) inside a chamber located in a highly populated area of Buenos Aires City. Animals were exposed for 8 h/day, 5 days/week, for up to 3 months. After 2 months, mice exposed to urban air pollution showed a 53% increase in total leucocytes in bronchoalveolar lavage (BAL) samples in comparison with the control group (control: 1.0±0.2 x10^5 cells, $p<0.001$), together with a 104% increase in BAL protein concentration (control: 0.15±0.03 mg/mL, $p<0.05$). Both BAL leucocytes and protein concentration were still significantly increased after 3 months in exposed mice in comparison with the control group. NADPH oxidase activity was increased in lung homogenates by 25% (control: 1.33±0.03 AU/mg protein, $p<0.05$) after 2 months. While no differences were observed in total lung oxygen consumption at any evaluated time point, cardiac oxygen consumption decreased by 37% in exposed mice in comparison with the control group after 2 months (control: 980±50 nmol O_2/min g tissue, $p<0.05$), and by 32% after 3 months (control: 1100±90 nmol O_2/min g tissue, $p<0.01$). A slight, yet not significant increase was observed in plasma TNF-α and IL-6 levels in exposed mice. The present data suggest that a continuous exposure to urban air leads to lung inflammation and decreased cardiac oxygen metabolism, which may explain some of the adverse health effects associated with the exposure to air pollution.

Keywords: air pollution, inflammation, oxidative stress, mice.

(735) **BIOMARKERS OF ACUTE TOXICITY IN PATIENTS POISONED WITH CARBON MONOXIDE IN BUENOS AIRES CITY**

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Carbon monoxide poisoning (CO) is preventable and avoidable. In our country, about 200 people die annually from CO poisoning. In this opportunity, we characterized a cohort intoxicated with acute CO and search for a prognostic biomarker. Data were collected from the clinical records, including the Folstein minimental status exam (MMSE), of patients received in the J. A. Fernández Hospital throughout 2016. In a group of patients, peripheral blood samples were extracted to evaluate genetic damage performed by the comet assay, expressed as TailDNA%, before (prett) and after (postt) receiving the established treatment. The most frequent source of exposures were boiler (59%) and water heater (25%). The main clinical manifestations were headache (30%), nausea (18%) and confusion (13%). About 59% of patients had blood carboxyhemoglobin levels (COHb) below 10%. Of all patients, 24 received normobaric oxygen therapy (ONB), 12 received hyperbaric oxygen therapy (HBO) and the rest received no treatment. Regarding the MMSE, the main errors were attention and denomination (23%), followed by recall (16%). Some patients were followed throughout hospitalization and TailDNA% (prett and postt) according to the treatment was compared. Data are expressed as mean±SEM and statistical analysis was t test or two way ANOVA with Bonferroni post-test. No significant differences were found between the groups (ONB: prett 10±3, postt 6±1; HBO: prett 3.8±0.1, postt 6±1). We found two prediction models which had lineal regression between: i) COHb with gender, age, headache and syncope (R^2 0.75, Raj 0.24); and ii) TailDNA% with gender, age, COHb (R^2 0.67; Raj 0.4). In conclusion: i) the most important prevention measures should be focused on home; ii) it is necessary to apply a more specific cognitive test to improve the evaluation; and iii) the treatment did not decrease the DNA damage caused by CO. It is necessary to include more patients in the protocol.

Keywords: carbon monoxide, DNA damage, biomarker prognostic

(1222) **EVALUATION OF AIR POLLUTION FROM BUENOS AIRES CITY EFFECTS ON THE MICE GENICULATE NUCLEUS**

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Abstract: The exposure to polluted air is an important cause to the development of adverse health effects in urban population. The aim of the present study was to evaluate oxidative stress markers on the geniculate nucleus on a mice model exposed to external ambiental air for 1, 2, and 4 weeks.

8-week-old Balb/c male were exposed to urban air or filtered air (control) in an exposure chamber located in a highly populated area of Buenos Aires city. Air particulate matter levels in this area range between 20-60 $\mu g/m^3$. The animals were exposed for 8h/day, 5days/week, up to 4 weeks. The local committee for animal care approved every experimental procedure. The following parameters were eval-

uated: The activity of Superoxide dismutase (SOD) and NADH Oxidase (NOX), lipid and protein oxidation (LO and PO, respectively). One-way ANOVA test and Dunnett's test as post hoc test were used for statistical analysis.

In the group of 1 week exposure, there was no significant difference between control and exposed groups in the parameters evaluated. We found a significant increase in the activity of SOD in the groups of 2 and 4 weeks exposure (47% and 19%, *p<0.01 and p<0.05, respectively*), compared to the control group, an increase in the activity of NOX (30%, *p<0.01*) and LO (55%, *p<0.01*) after 2 weeks of exposure. Meanwhile, there was no significant difference between control and exposed groups on PO.

The increase in the activity of SOD observed in this study after a 2-week exposure, could be an adaptive response to an increasing pro-oxidant environment triggered by the polluted air exposure. However, after a 4-week exposure, this increase in NOX and LO levels retuned to control values, suggesting that the antioxidant mechanism seems to be enough to restore oxidative damage.

Keywords: Air pollution, environmental health, geniculate nucleus, oxidative stress, antioxidants.

(938) **OXIDATIVE BALANCE IN BRAIN AFTER EXPOSURE TO ARSENIC IN *EX VIVO* AND *IN VIVO* MODELS**

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The hypothesis of this work was that radical generation after exposure to As may contribute to its toxic effects in the brain tissues. Oxidative status of brain was studied, both using *ex vivo* and *in vivo* protocols of exposure to the toxic. The generation rate of lipid radicals ($LR^{\bullet}$), and ascorbyl radical ($A^{\bullet}$) content, were measured by Electron Paramagnetic Resonance (EPR). In the *ex vivo* model control brain homogenates were exposed to As, and a 2-fold increase was detected in the $LR^{\bullet}$ generation rate (Studen't t-test, $p<0.5$), with no changes in $A^{\bullet}$ radical content. A significant decrease of 33% and 30% in the content of glutathione (GSH) was measured after exposure to 3.3 and 4.0 pmol As/mg FW (Studen't t-test, $p<0.5$), respectively. In the *in vivo* model, As was ip injected to rats to get in brain similar As concentration studied *ex vivo* and the rate of generation of $LR^{\bullet}$ by homogenates of brain tissue, was increased by 81 and 122%, as compared to control animals after the injection of 3.0 and 5.8 mg As/kg (Studen't t-test, $p<0.5$), respectively. Neither GSH, nor α-tocopherol (α-T) nor ascorbate (AH^-) content was affected in As-treated rats, as compared to the values from control animals. Even though $LR^{\bullet}$/α-T content ratio was significantly increased in As-treated animals as compared to control brains (Studen't t-test, $p<0.5$), the $A^{\bullet}/AH^-$ content ratio was not affected. The content of total Fe showed non-significant differences between control (1.4 ± 0.2 pmol/mg FW) and rat brains after 24 h of As administration (1.5 ± 0.2 pmol/mg FW). The data presented here showed new evidence on the generation of specific radical species by As treatment employing EPR methodologies in both, *ex vivo* and *in vivo* models. The data suggested the triggering of different pathways leading to some reactive species generation may occur accordingly to the via As reaches the brain, even when the same concentration of the toxic was achieved by the tissues.

Keywords: Antioxidants, Arsenic, Brain, EPR Detection, Oxidative Stress Ratios.

(1500) **PLASMA MEMBRANE LIPID COMPOSITION AND DOMAIN ORGANIZATION AFFECTS ORGANOPHOSPHATE PESTICIDES ACTION ON SECONDARY TARGETS.**

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Although acetilcholinesterase (AChE) is the primary target of organophosphate pesticides (OP), secondary targets such as oxidative stress enzymes become relevant at sublethal concentrations. Plasma membrane (PM) changes in cholesterol (Chol) content or fatty acid (FA) insaturation may alter its domains and affect cell functions. OP have been suggested to compete with Chol for partition domains at the PM and cause structure perturbations. Our objective was to analyze the effects of the OP chlorpyrifos (CPP) on oxidative stress enzymes catalase, glutathione S-transferase and glutathione reductase in transfected CHO-K1 cells, which do not express AChE, differing in lipid composition and PM fluidity. One cell line (SCD) shows higher monounsaturated FA content but keeps fluidity by increasing Chol content, while other (FADS) has higher polyunsaturated FA and lower Chol content with increased PM fluidity. The different cell lines were exposed during 24h to 0.5 and 250 μM CPP and the enzymes were measured kinetically by spectrophotometry. Data were analyzed by ANOVA and Tuckey *post hoc* test. Glutathione S-transferase and reductase activities decreased significantly in control cells exposed to CPP (50 and 40% respectively) ($p<0.05$) while remained unchanged in the transfected lines with modified PM. In turn, catalase was significantly increased in SCD line (25-40%) and reduced by low CPP concentration in FADS line (40%) ($p<0.05$). Thus, PM FA composition and fluidity differentially modulates CPP interaction with molecular targets as shown in modified CHO-K1 cell lines. A protective role by higher unsaturated FA content is suggested, probably in relation with toxicant absorption kinetics and its effects on some antioxidant enzymes susceptible to reactive oxygen species. The induction of catalase protecting activity in FADS line suggests in turn other mechanisms involving signaling through Nrf-2 or AhR transcription factors, previously described for CPP actions.

Keywords: CHO-K1 cells – cell toxicity – antioxidant enzymes - membrane fluidity – unsaturated fatty acids

(1653) **THE ROLE OF MITOCHONDRIA IN THE OXIDATIVE RESPONSE OF RAW 264.7 MURINE MACROPHAGES EXPOSED TO AIR POLLUTION PARTICULATE MATTER**

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The exposure to environmental particulate matter (PM) is associated with pulmonary oxidative stress and inflammation. Alveolar macrophages are suggested to play a central role in this scenario, since they produce inflammatory mediators and reactive oxygen species. The aim of this work was to study the redox status and the role of mitochondria in macrophages after the exposure to Residual Oil Fly Ash (ROFA), a PM surrogate rich in transition metals. The murine cell line RAW 264.7 was exposed to ROFA at 100 µg/mL for 24 hs. Cell viability was not significantly affected under these experimental conditions. Intracellular redox status was assessed by flow cytometry as dichlorofluorescein mean fluorescence intensity (MFI), which was increased by 30% following ROFA incubation compared to the control group ($p<0.05$). Mitochondrial $O_2^{\bullet-}$ production was determined by MitoSox fluorescent probe, resulting in an increase of 167% (control: 9±4% $MitoSox^+$ cells, $p<0.05$). Consistently, mitochondrial inner membrane potential decreased by 41% (control: 80±6% $TMRM^+$ cells, $p<0.05$). Mitochondrial functionality was also assessed by Seahorse XF Analyzer, resulting in an 85% decrease in respiratory control ratio ($p<0.05$), as well as a 33% decrease in the ATP-O ratio ($p<0.05$), an indicator of oxidative phosphorylation efficiency, and a 2-fold increase in the H^+ leak ($p<0.01$), an indicator of the mitochondrial inner membrane integrity, following ROFA incubation. Given that we observed a different response with Concentrated Ambient Particles (CAPs), another source of PM, which differs in elemental composition from ROFA, we suggest that PM chemistry

might play an important role in the observed effects. These findings indicate that redox status of RAW 264.7 cell line is affected by the exposure to ROFA, probably due to changes in mitochondrial function.

Keywords: Air Pollution - Oxidative Stress - Mitochondria

(1461) **URBAN AIR POLLUTION ALTERS THE REDOX BALANCE OF MICE CORNEAS**

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The exposure to air pollution leads to adverse health effects at levels that are currently experienced by urban population, in which oxidative stress is suggested to play a central role. The aim of the present study was to evaluate the redox balance in mice corneas after the exposure to urban air pollution.

8-week-old Balb/c male mice were exposed to urban air or filtered air (control) in an exposure chamber located in a highly populated area of Buenos Aires city. Air particulate matter levels in this area range between 20-60 µg/m^3. The animals were exposed for 8 h/day, 5 days/week, up to 4 weeks. The local committee for animal care approved every experimental procedure. Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) activities, as well as protein oxidative damage were evaluated in corneal lysates.

The animals exposed to urban air pollution showed an increase in SOD (115%) and CAT (218%) activities after 1 week of exposure ($p<0.05$), while GPx activity remained unchanged. After 2 weeks, SOD activity remained increased in exposed mice (56%,$p<0.001$), but this level was lower when compared to the 1-week exposed group ($p<0.001$). CAT levels returned to control values after 2 weeks of exposure. After 4 weeks, SOD and GPx activities decreased compared to the control group (42% and 34 %,$p<0.05$) and to the earlier time points evaluated ($p<0.05$). In addition, protein carbonyls were found increased ($p<0.05$) at this time point.

The corneas of exposed mice showed an increase in enzymatic antioxidant defences after a short exposure, suggesting an early adaptive response to air pollutants. After longer exposures, this antioxidant response is shut-down and leave the cornea more susceptible to oxidative damage, which was evidenced by the increased protein oxidation. These results suggest that the corneal antioxidant defences play a central role in the toxicological mechanisms triggered by the exposure to urban air pollutants.

Keywords: air pollution, cornea, oxidative stress, antioxidants, redox balance

CELL SIGNALING 6

(522) **ALTERNATIVE SPLICING OF MKP-3 (OR DUSP6) GENE PRODUCES TWO ISOFORMS WITH DIFFERENT EFFECTS ON CELL PROLIFERATION**

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Mitogen-activated protein kinases (MAPK) are activated by dual phosphorylation in response to several stimuli and regulate multiple cellular functions. MKP-3 (or DUSP6) is a MAPK phosphatase induced only by proliferative stimuli which specifically dephosphorylates ERK1/2. The human MKP-3 gene generates the full length transcript, variant L, and an alternative splice product, or variant S. MKP-3S protein, encoded by the S transcript, is expressed only in human cells, mostly of tumoral origin. MKP-3L is a cytosolic protein regulated by phosphorylation. MKP-3S protein is catalytically active but lacks the nuclear export signal (NES) and a target residue for ERK phosphorylation involved in MKP-3L stability. This work focuses on the analysis of potential differences between isoforms and their effects on cellular localization, proliferation and cell cycle progression. Previous immunocytochemistry studies have shown that Flag-tagged L isoform is barely detected in the nucleus, while Flag-tagged S is evenly spread between cytosol and nucleus. Here we confirmed this distribution by subcellular fractioning and Western blot in HEK293-transfected cells for Flag-L or Flag-S expression. Cell proliferation was measured as BrdU incorporation (expressed in arbitrary units as mean ± SEM, values relative to control) in cells transfected with either one of the plasmids encoding flag tagged proteins or the empty vector. Compared to empty vector-transfected cells, proliferation rates were decreased in L-transfected cells (0.79 ± 0.07 $P< 0.05$), but not in S-transfected cells (1.01 ± 0.06). Flow cytometry analysis showed a 25% ($P < 0.05$) reduction in the percentage of L-transfected but not S-transfected cells in the G2/M stage regarding empty vector transfection. Cyclin D levels, assayed by RT-PCR, were also different across groups. In conclusion, these differences observed in MKP-3 isoforms suggest that expressing different L/S ratios could shape cells proliferative behavior.

Keywords: MKP-3, Cell Proliferation, ERK, Alternative Splicing

(599) **EFFECTS OF CHOLATE AND DEOXICHOLATE SODIUM SALTS ON MCF-7 CELL LINE**

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Biliary acids (BA) are bioactive molecules with multiple metabolic actions and pharmaceutical applications. High levels of BA were associated with colon, stomach, pancreas and also breast tissue tumorogenesis. Considering BA association with cancer pathology and their use in pharmaceutical technology, it is highly relevant to study their biological effects. The objective of this work was to analyse the molecular and cellular effects of sodium cholate (SC) and deoxycholate (SDC) on the epithelial breast cancer cells MCF-7. For this purpose, effects over cell proliferation, migration, membrane fluidity and activation of signaling molecules, involved in cell cycle and survival promotion, were studied. Moreover, SC and SDC preparations used for in vitro assays were characterized by electron microscopy and dynamic light scattering.

MCF-7 cells were incubated with different concentrations of each bile salt for 48 hours and cell proliferation was assessed by BrdU incorporation. Results showed that SC 10 uM and SDC 5 uM induced an increase in cell proliferation. Assessment of Akt and Erk1/2 phosphorylation and Cyclin D1 expression at different time points by Immunoblotting evidenced that SC 10 uM and SDC 5 uM induced a slight activation of Akt and an increased in Cyclin D1 protein levels. Wound healing assay showed no significant effects on cell migration for those BS concentrations. PA-DPH fluorescence anisotropy revealed that low concentrations of SC and SDC had no effect on membrane fluidity during the first hour and after 24 hours treatment with the biliary salts. Morphological characterization of BS preparations revealed particles in the nanoscale.

In conclusion, biliary salts have pro-proliferative effects over the MCF-7 cell line while no significant action over cell migration was observed. BS cellular effects might be consequence of specific receptor activation/modulation as unspecific effects of the nanostructures over membrane fluidity were not observed.

(588) **NEW INSIGHTS INTO THE MOLECULAR MECHANISMS THAT PROVOKE THE DEVELOPMENT OF PRENEOPLASTIC ALTERATIONS IN A HEPATOCYTE CELL LINE**

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Previously, it was demonstrated that the lack of aminoacids (Aa) in the culture medium of an immortalized mice hepatocyte cell line (parental line = P) induces apoptosis, and that cells that are able to survive (selected line = S) showed a more proliferative phenotype and resistance to apoptotic stimuli, reminiscent of a preneoplastic phenotype. Recent data proposes an essential role for the NADPH oxidase NOX4 as a liver tumor suppressor, so the aim of this work was to deep into the molecular mechanisms that support such preneoplastic alterations focusing on growth factors, inflammatory signals and NOX4, in the cross-roads of these pathways. Cell proliferation was measured using violet crystal, at 0, 24 and 48h of cell culture. Different concentrations of cells were employed. We performed qPCR to quantify the expression of NOX4, TGF-β, TGF-α, CXCL-1 and CXCL-2. The levels of NOX4 and pEGFR proteins were determined by Western Blot. S showed higher proliferation vs P, which was more marked at 48h and using the highest concentration of cells per well (10 x10^6) (P vs S, mean±SD: 718±42.77 vs 1317±130.8; p<0.001). These data support the previous results and show that S exhibits a pro-tumor profile. We found a higher level of pEGFR protein (p<0.05), and higher expression levels of TGF-α (p<0.05), an EGFR ligand. Levels of CXCL-1 (p<0.001) and CXCL-2 (p<0.001), both pro-inflammatory cytokines, were also higher in S vs P. TGF-β levels decreased in S (p<0.01), where an increase in NOX4 expression (p<0.01) was also evident. S tended to show higher levels of NOX4 protein, although this increase was not statistically significant. These results indicate that Aa deprivation provokes an imbalance in growth factor and inflammatory signals. Contrary to the hypothesis, levels of NOX4 are not decreased, even increased. Because it is a transitional stage between an untransformed line and another one with pre-neoplastic characteristics, NOX4 may play a differential role in these cells.

(265) **CONTINUOUS PTHrP ADMINISTRATION IN COLORECTAL CANCER CELLS XENOGRAFTS INCREASES POSITIVE REGULATORS LEVELS OF CELL PROLIFERATION**

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Abstract: PTHrP has been widely studied because this hormone has an important role in fetus development and adult tissues homeostasis. Today, it is recognized for its endocrine, paracrine and autocrine modes of action. PTHrP was found to be expressed in numerous types of cancer and in more than 90% of colon cancer patients. It has been shown that HCT116 cells, derived from human colorectal carcinoma (CRC), are tumorigenic in nude mice and we previously demonstrated that the continuous administration of PTHrP (40 ug/kg) in HCT116 cells xenografts of these animals promotes the formation and tumor growth and also increased the expression of cyclin D1, a positive regulator of cell cycle progression. The aim of this study was to investigate if the levels of other mitogenic markers changed in response to PTHrP administration employing the same murine model. We found by immunohistochemistry analysis that the hormonal exposure increased the immunoreactivity scores of the active form of RSK kinase and the activated and total forms of ERK 1/2 MAPK, which are involved in the progress of many cancers, including CRC. Moreover CREB/ATF-1 expression was incremented by PTHrP and as expected, the localization of these transcription factors was nuclear whereas active RSK and active and total ERK 1/2 MAPKs were observed in the cytoplasm and nucleus of HCT116 cells. Finally, the expression of the pro-angiogenic factor VEGF was not modified by the peptide at least at the times studied. The present investigation provides, to our knowledge, additional evidence demonstrating that exogenous PTHrP regulates signaling pathways associated with proliferation in animal models. Taken together, these findings emphasize the endocrine/paracrine action of the hormone in *in vivo* models.

Keywords: PTHrP, colorectal cancer, cell proliferation, mitogenic markers

(641) **AN ALTERNATIVE SPLICE VARIANT OF MKP-2 TRANSCRIPT IS EXPRESSED IN A NON-SMALL-CELL LUNG CANCER CELL LINE**

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MAP kinases (MAPK) ERKs, JNKs and p38 are activated by dual phosphorylation and regulate several processes such as proliferation, differentiation and apoptosis. Given that MAPK phosphatase (MKP) family members inactivate MAPK, they are potential modulators of MAPK-dependent processes. MKP-2 (or DUSP4) is a nuclear dual activity phosphatase able to dephosphorylate JNK1/2 and ERK1/2 and induced by stress conditions. An alternative splice variant of MKP-2 transcript and protein were found in PC3 prostate and MDA-MB-231 breast cancer cells and human prostate biopsies. This variant, generated by exon skipping and referred to as the S or short variant, displays phosphatase activity but lacks several motives involved in the subcellular localization and regulation. Thus, the L (complete) and S variants could have different biological roles, even though the characterization of the S transcript has not been fully elucidated yet. We analyzed transcript expression in non-small-cell lung cancer cell line A549. RT-PCR analysis showed a time-dependent increase in L and S transcripts after cell stimulation with 0.1 μg/mL LPS (bacterial lipopolysaccharides), a stimulus able to induce MKP-2 L. The L transcript rendered a three-fold increase vs. control after 1 h (P<0.01) and reached 8-fold increase after 4 h (P<0.001). LPS also increased mRNA- MKP-2 S levels after 2 h (P<0.05). Our study also showed that the predominant transcript is the L variant. Transcription inhibitor Actinomicyn D and PD98059, which impair ERK activation, both blocked the effect of LPS on L and S transcripts. In sum, we report for the first time the expression of the alternative splice variant of MKP-2 in the A549 cell line. As S isoform lacks important sites involved in its regulation, such as a nuclear localization sequence, a different L/S ratio could differentially modulate MAPK-dependent events. Also, we show that L and S transcripts are induced by LPS at transcriptional level by an ERK-dependent mechanism.

Keywords: DUSP4; MKP-2; splice variant; lung cancer cell line.

(267) **PARATHYROID HORMONE-RELATED PEPTIDE TREATMENT OF COLON CANCER CELLS ENHANCES ANGIOGENESIS THROUGH VEGF**

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Abstract: Angiogenesis plays a critical role in tumor growth and involves multiple processes including the secretion of angiogenic factors by tumor cell and the increase of endothelial cell mobility and its differentiation. We found that Parathyroid Hormone-related Peptide (PTHrP) induces the proliferation and migration of Caco-2 and HCT116 cells, two cell lines from human colon tumors. Using both cell lines, the objective of this work was to study if the hormone is also implicated in tumor angiogenesis. qPCR assay revealed that mRNA levels of VEGF, HIF-1alpha and MMP-9, which are factors involved in angiogenesis, are increased after PTHrP treatment for 20 h in both cell lines. To test tumor angiogenic potential of PTHrP *in vitro* we evaluated the migratory properties of the human endothelial cell line HMEC-1 employing transwell inserts and a chemoattractant source, which is conditioned cultured media (CM) from Caco-2 or HCT 116 cells exposed to PTHrP for 24 h. Results showed that CM

from cells treated with the hormone markedly increases the migration of endothelial cells. Similar data were obtained using co-culture. In addition, we performed tube formation assays using growth factor-reduced Geltrex. HMEC-1 cells incubated with CM from non-treated Caco-2 or HCT 116 cells formed few tube-like structures on geltrex whereas the CM from colon cancer cells treated with PTHrP increased tube formation of HMEC-1 cells. In contrast, by direct treatment the hormone not stimulated migration neither tube formation of HMEC-1 cells. Finally, studies with neutralizing antibody against VEGF revealed that augmented angiogenic response of endothelial cells exposed to CM from colon cancer cells treated with PTHrP was associated with enhanced production of VEGF. In summary, the results obtained from this study show that PTHrP treatment of colon cancer cells promotes pro-angiogenic signaling.

Keywords: PTHrP, colon cancer, angiogenesis, VEGF

(1263) **ACYL-COA SYNTHETASE 4 LEVELS ARE DEPENDENT ON PROTEASOME ACTIVITY AND MODULATE MITOCHONDRIAL METABOLISM REGULATORY PROTEINS EXPRESSION IN BREAST CANCER CELLS.**

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Acyl-CoA synthetase 4 (ACSL4) is an enzyme that catalyzes acyl-CoA synthesis from long chain fatty acid, being arachidonic acid its preferred substrate. ACSL4 is overexpressed in triple negative breast cancer cells correlating with tumor aggressiveness. We demonstrated that ACSL4 expression is regulated by transcriptional mechanisms in breast cancer cells. But other mechanisms involved in regulating ACSL4 levels might be taken place. It is known that in cancers exists a strong mitochondrial metabolism deregulation. Computational data showed that ACSL4 is a possible candidate for modulating mitochondrial master regulatory genes. Then, our goal is to study stability of ACSL4 by post translational mechanisms and to study the role of ACSL4 in the regulation of mitochondrial function. ACSL4 stability was tested on MDA-MB-231 breast cancer cell line with cycloheximide (CHX) treatment. Immunoblot showed a time-dependent decrease in ACSL4 levels after CHX treatment, significant at 4h of CHX (without vs. with CHX 4h: 1 vs 0.5, relativized to control *p<0.05). The treatment with MG-132 (a potent proteasome inhibitor) promoted a clear increase of ACSL4 levels. Finally, we used a stable and inducible MCF-7 breast cancer cell line overexpressing ACSL4 (MCF-7tet-off/ACSL4) and MCF-7tet-off as control. Levels of mitochondrial proteins as Complex III and VDAC1 and nuclear factor NRF1 were evaluated. A slight increase in Complex III and a marked increase of NRF1 levels was observed in MCF-7tet-off/ACSL4 respect to control, meanwhile VDAC1 levels were unaffected. Downregulation of ACSL4 expression by Doxycycline treatment decreased NFR1 levels. These results demonstrate that, in breast cancer cells, half-life of ACSL4 is regulated post-translationally and degraded by the proteasome and that ACSL4 expression positively regulates NRF1, which induces some key nuclear genes required for mitochondrial respiration, DNA transcription and replication.

(164) **ANTINEOPLASCTIC EFFECTS OF BORTEZOMIB IN CELLULAR SPHEROIDS FROM ENDOTHELIAL CELLS TRANSFORMED BY KAPOSI SARCOMA-ASSOCIATED HERPES VIRUS G PROTEIN COUPLED RECEPTOR**

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The Kaposi's Sarcoma-associated Herpes virus G Protein-Coupled Receptor (vGPCR) is a key molecule in the pathogenesis of Kaposi sarcoma. Persistent expression and activity of vGPCR is required for NFκB pathway activation and tumor maintenance in endothelial cells. We have previously demonstrated that Bortezomib (BTZ, 0.5 nM) inhibits vGPCR cell growth by MKP-3 accumulation, a specific phosphatase that attenuates ERK1/2 signaling. The activity of this phosphatase reduces ERK1/2 and FOXO1 phosphorylation. In turn, ERK1/2 inhibition reduces VEGF expression and FOXO1 activation promotes p21 induction. All these events converge in the inhibition of cell proliferation. Many types of mammalian cells can aggregate and differentiate into 3-D multicellular spheroids (MCS) when cultured in suspension or in a non-adhesive environment. Compared to conventional mono-layer cultures (2D-cultures), MCS resemble real tissues better in terms of structural and functional properties. Multicellular spheroids formed by transformed cells are widely used as avascular tumor models for metastasis and invasion research and for therapeutic screening. In the present work, we develop the technique to obtain MCS from vGPCR cells in order to test whether MCS respond similar to 2D-cultures. To that end, MCS were treated with BTZ (0-2.5 nM) for 48 h. Results from Western blot analysis showed that BTZ decreases ERK1/2 protein phosphorylation, while MKP-3 protein levels are increased. Moreover, qRT-PCR studies revealed that p21 gene expression is also increased. All together, these results suggest that MCS of vGPCR cells treated with BTZ respond likewise those treated in 2D-cultures of vGPCR cells, but a higher dose.

Keywords: Spheroids, vGPCR cells, Bortezomib, antiproliferative effects.

(1764) **CYCLOOXYGENASE-2 MEDIATES KSHV G PROTEIN COUPLED RECEPTOR *vGPCR* ANGIOGENESIS AND IT IS EXPRESSED IN KAPOSI'S SARCOMA.**

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Kaposi's sarcoma associated herpesvirus (KSHV) vGPCR is a constitutively active G protein-coupled receptor that subverts proliferative and inflammatory signaling pathways to induce cell transformation and angiogenesis in Kaposi' s sarcoma. For this reason, identifying and targeting pathways leading to vGPCR angiogenesis could have therapeutic significance. Cyclooxygenase-2 (COX-2) is an inflammatory mediator involved in tumor angiogenesis that can be targeted by non-steroidal anti-inflammatory drugs. Our aim is to determine if COX-2 could be a target for KS therapy. We demonstrate by an enzyme immunoassay that vGPCR upregulates COX-2 activity and expression, and we show that COX-2 activity is critical for vGPCR pathogenicity *in vivo*. Using an intradermal angiogenesis assay, we found that treatment with NS398 before inoculation with vGPCR-transformed NIH3T3 abolished the angiogenic response induced by vGPCR expression ($P<0.01$). We also observed that treatment with the COX-2-selective inhibitory drug Celecoxib produced a significant retardation in tumor growth ($P<0.05$). Tumors from animals treated with Celecoxib showed a significant decrease in tumor cell VEGF production ($P<0.001$). We conclude that vGPCR regulates angiogenicity and tumorigenicity via COX-2 activation. Consistent with a role in KS pathogenesis, we found that vGPCR upregulates COX-2 activity in endothelial cells and that COX-2 is overexpressed in KSHV-infected KS lesions. Based on these results we are currently committed to identify COX-2 gene expression regulators at the molecular level. We conclude that these facts pinpoint COX-2 as one of the molecular components of the vGPCR angiogenic switch and a potential target for KS chemoprevention and therapy.

Keywords: Cyclooxygenase-2, KSHV, vGPCR, angiogenesis, tumorigenicity.

(1060) **ACYL-COA SYNTHETASE 4 INHIBITION DECREASES ADRENOCORTICAL HUMAN CELL PROLIFERATION SUSTAINED BY ANGIOTENSIN II.**

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Angiotensin II (Ang II) is one of the most important stimuli of adrenal glomerulosa cells. Ang II stimulates proliferation of adrenocortical cells *in vivo* and primary bovine glomerulosa cells *in vitro*, although rat glomerulosa cells *in vitro* exhibit hypertrophy rather than proliferation by Ang II. Then, proliferating effects of Ang II rely on multiple factors. In adrenal cells, proliferation depends on the activation of the PI3K/Akt/mTOR pathway, which is overactive in adrenocortical tumor and others. The enzyme acyl-CoA synthetase 4 (ACSL4) activates mTOR, promoting phosphorylation of its components and inducing proliferation in breast cancer cells. ACSL4 is highly expressed in adrenal gland regulated by Ang II and involved in aldosterone synthesis, in H295R adrenocortical human cells. The aim of this work is to study a possible role of ACSL4 in Ang II-modulation of mTOR pathway and proliferation in human adrenocortical cells. We used H295R human adrenocortical cell line that responds to Ang II stimulation. We observed by immunoblot that Ang II promotes a time-dependent phosphorylation of Ribosomal protein S6 (RpS6), activated downstream mTOR pathway. RpS6 phosphorylation is significantly decreased when cells were treated with Triacsin C (T), a potent ACSL4 inhibitor (Ang II vs. Ang II + T; 4h: 4.71 vs. 0.66; 6h: 5.41 vs. 1.05 ***p<0.001, relativized to control). Then, we assessed proliferation of H295R Ang II-treated cells with or without Triacsin C. H295R cells were subjected to BrdU incorporation assay using an ELISA kit. We observed that Ang II-sustained proliferation at 72h was diminished by Triacsin C, while basal cell proliferation was unaffected (Ang II vs. Ang II + T; 0.86 vs. 0.41 OD 450 nm **p<0.05). ACSL4 inhibition showed a decreased redox cellular activity of Ang II-treated cells, measured by MTT assay. These results suggest a role for ACSL4 in Ang II-sustained proliferation, possibly mediated by mTOR pathway activation in adrenocortical human cells.

BIOPHYSICS 6

(728) A NOVEL NANOFORMULATION OF VISMODEGIB FOR TOPICAL SKIN THERAPY: DESIGN AND BIOPHYSICAL CHARACTERIZATION

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The aim of this work was to obtain a Vismodegib-loaded nanoformulation for topical treatment of skin precancerous lesions. Vismodegib (Vis) is a new generation drug, recently approved by the FDA for the treatment of basal cell carcinoma, with several secondary effects. Encapsulation into ultradeformable liposomes (UL) could be useful to reduce the side effects by specific-site delivery. UL were selected because are capable to penetrate the stratum corneum (SC) of the human skin.

UL were prepared with Soy Phosphatidylcholine, Sodium Cholate and Vismodegib by rotary evaporation. Vesicles were extruded and non-encapsulated Vismodegib was removed by molecular exclusion chromatography. Size and stability in the time of UL-Vis were determined by DLS, zeta potential and Turbiscan Lab® Expert analysis. Lamellarity was corroborated by TEM. The interaction drug-lipid was assessed by DSC and Merocyanine 540 probe. Drug-lipid ratio and encapsulation efficiency were determined. A test of deformability was performed to study the elastic properties. Ex vivo penetration studies were performed on a Saarbrücken Penetration Model device with human skin explants. Penetration profile was quantified in the SC after tape stripping. Intact skin and transversal sections of 20 μm were studied by CLSM.

A unimodal population of UL-Vis with a mean size of 116.40 ± 2.09 nm and zeta potential of -19.30 ± 0.76 mV, without significant variation up to 30 days, was obtained. There were not observed events of coalescence, sedimentation or creaming, thus the formulation was stable in time. Vismodegib was retained in the liposomal membrane and modified its deformability and temperature and enthalpy of lipid transition. UL-Vis was capable to penetrate the SC of intact human skin. These results allow to continue in the design of a future topical therapy, maximizing the availability of Vismodegib in the area of the lesion and reducing systemic side effects.

Keywords: ultradeformable liposomes – Vismodegib – topical therapy

(1475) STRUCTURAL STABILIZATION OF AN OLIGOMERIC SCAFFOLD AT ACIDIC PH FOR THE PRODUCTION OF ARTIFICIAL CELLULOSOMES AND THE DEGRADATION OF LIGNOCELLULOSE

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The polymeric display of molecules is an emerging technology for many applications. It could be used to increase the immunogenicity of antigens, the interaction strength of protein domains for polymeric ligands, and the production of multifunctional particles. We use a oligomeric protein scaffold for the polymeric display of protein targets. Coupling to the scaffold is produced through a non-covalent strategy with high affinity heterodimeric modules complementary fused to the scaffold and the target proteins. We are using this method for the development of artificial cellulosomes based on the colocalization of lignocellulolytic enzymes and cellulose binding domains. This co-localización would increase cellulose degradation activity through enzyme proximity and targeting effects and would be valuable for the production of biofuels. However, the decameric structure of the scaffold dissociates into pentamers below pH 6, a condition in which certain cellulolytic enzymes exhibit maximum activity. The objective of this work is the stabilization of the pentamer-pentamer interface of the scaffold by means of protein engineering to improve its properties as a structural scaffold of artificial cellulosomes at acidic pH. The dissociation of pentamers at acidic pH seems to be induced by protonation of histidines at the pentamer-pentamer interface, which would decrease the level of colocalization of the enzymes coupled to the scaffold. To design stabilizing mutations, we bioinformatically explored with FOLDX software all possible single mutants of the scaffold and their effect on its stability at pH 5 and pH 7. From this analysis we selected those single mutants which showed a significant stabilization at the interface at pH 5, no destabilization at pH 7, and no destabilization of the pentamer structure at pH5 or pH7. The combination of the selected mutations and their effect on the stability of the scaffold as a function of pH is also presented.

Keywords: scaffold, pH, stability, foldx, cellulosome

(513) ASSIGNMENT AND SECONDARY STRUCTURE OF THE LOWER STEM REGION OF PRI-MIR172A FROM *Arabidopsis thaliana*

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Plant microRNAs (miRNAs) are processed by the RNase III-like enzyme DICER-LIKE1(DCL1) acting principally in concert with the double-stranded RNA-binding protein HYPONASTIC LEAVES1 (HYL1) and the zinc finger protein SERRATE. Together, they excise a miRNA/miRNA* duplex with a 2 nucleotide 3′ overhang from the primary miRNA (pri-miRNA) transcript. pri-miRNAs include a partially self-complementary foldback or stem loop, which gives rise to the mature miRNA.

The secondary structure of pri-miR172a in *Arabidopsis thaliana* shows a CU mismatch between the 3' base corresponding to the mature miRNA (U) and its complementary base on the other

RNA strand (C) of the duplex. A mutant that has a CC mismatch in this position showed an impaired processing. We propose that the presence of a CC mismatch in this position of pri-miR172a affects the binding of the processing machinery and/or its catalytic activity due to alteration of the 3D structure of the stem. We produced by *in vitro transcription* unlabeled and ^{13}C-^{15}N labeled lower stem constructs of wild type pri-miR172a and it CC mutant. A first approach to prove this hypothesis is to assign resonances of both samples. We obtained assignments for the imino protons of the wild type construct. The imino proton of the unpaired U is not detected and the imino protons of the basepairs above and below the mismatch are visibly broadened. The use of ^{13}C-^{15}N labeled samples allowed the application of heteronuclear scalar and dipolar correlated experiments to obtain assignments in the crowded aromatic region of the construct. These assignments will allow us to establish the differences between the CU and CC mismatches that result in the impaired processing of the latter.

Keywords: pri-miR172, secondary structure, resonance assignment.

(1339) **CRITICAL MICELLAR CONCENTRATION AND SURFACE PROPERTIES OF SUCROGLYCERIDES (ESTEN-80) IN DIFFERENT AQUEOUS SOLUTIONS**

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Esten-80 is composed mainly of monoesters of transesterified fatty acids of glucose and fructose, mono and di glycerides of methylated soybean oil and potassium saponified fatty acids and disaccharides. This compound is a surfactant that may be used to encapsulate and deliver chemical species with potential interest in agrochemistry and foods. A critical micellar concentration (CMC) of 9.005 x 10^{-3}% P/V was determined in water by means of light scattering. The formation of the micelles at different concentrations of glucose gives higher values of CMC with respect to H_2O control. In contrast, for 1M urea a significant decrease in CMC was observed. Due the known effect of urea on water structure, it may be thought that the stabilizing forces are not hydrophobic interactions. The analysis of the surface properties of the aggregates by means of particle size distribution before and after the filtration and with spectrophotometry using Merocyanine 540 (MC540) as a surface probe indicates that particles are highly hydrophilic even at high curvatures (low particle size). It is likely that hydrogen bonding interaction could be the main structural factor to stabilize these aggregates.

Keywords: sucroglycerides, surfactant, critical micellar concentration, surface properties.

(1901) **LIPID DROPLET BIOGENESIS AND HYDROPHOBIC BLISTERS INSIDE BILAYERS STUDIED USING MONOLAYER WETTING BY LIQUID LENSES.**

Benjamín Caruso

The biogenesis of triglyceride (TG) Lipid Droplets and the encapsulation of apolar substances, such as vaseline (V), in bilayers involve a dewetting process and the formation of blisters containing a bulk phase of the hydrophobic molecule (HM). To gain insight into the factors affecting the stability of the bulk structure in contact with phospholipids (PC) we studied Langmuir films of PC with HM which form (TG) or do not form (V) stable monolayers. Then, we evaluated TG-blister formation using microscopy, calorimetry (DSC) and Fluorescence Anisotropy (FA). PC/TG and PC/V monolayers collapse at a composition-dependent surface pressure (SP) producing micrometric lenses (observed by BAM and fluorescence with Nile red probe) interpreted as bulk HM phase. PC/TG compression isotherms are reversible after collapse (lenses disappeared at SP below the collapse pressure). On the contrary, in PC/V, once formed, V lenses remain on the surface. To analyze the monolayer wettability, HM lenses height was calculated assuming refractive indexes similar to those for bulk TG and V. We found that TG lenses were thicker than V lenses. Knowing the thickness allowed estimating contact angles, interfacial tensions and spreading tendencies, and thus obtaining coefficients which, together with wettability arguments, indicated that segregated TG would form discrete structures whereas V, alike other hydrocarbons (e.g. n-decane and squalene), would tend to spread along the intrabilayer space. Free standing bilayers of PC/TG did not exhibit microscopic blisters. However, DSC of PC/TG vesicles at compositions where TG remains in the bilayer showed a thermotropic behavior similar to that of pure PC, suggesting a phase separation inside a global bilayer structure. FA of PC/TG vesicles using DPH and TMA-DPH (hycrocarbon chain and polar head regions probes) are coherent with X_{TG}~TG solubility defined in monolayers and suggest TG segregation inside the bilayer (blisters) up to X_{TG}=0.25.

Keywords: Lipid Droplets, Triglycerides, Vaseline, Monolayers

(1291) **WATER AND HYDROGEN BONDS IN DMPA AND DMPC MEMBRANES SEEN FROM NEAR INFRARED SPECTROSCOPY**

Jorge Javier Wenz

By exploiting the sensitivity of the NIR spectrum to hydrogen bonds, particularly in the first overtone of water around 1450/55 nm, the influence of temperature and phospholipid head group on the H-bond network and water polymerization in membranes of DMPA and DMPC was studied. Principal components analysis of the spectra was used to disclose subtle spectral changes that mirror alterations in the vibrational energy of the water O-H bonds, as a measure of the H-bond network. Increasing temperatures drastically diminished the number of strongly H-bonded water molecules and increase the number of weakly H-bonded waters. This dominating effect of temperature was missing after the subtraction of the pure water spectra from the lipid-containing ones, suggesting the presence of two water populations. An intriguing secondary effect of temperature was also revealed. At the water:lipid interface, phospholipids exhibited an effect qualitatively similar to that of the temperature. DMPA, and particularly DMPC, disrupted the H-bond network in the neighboring water, reducing water polymerization and strengthening the water O-H bonds. The type of the polar head affects the H-bonds more than duplicate the concentration of the lipid from 250 to 500 µM. A relation between head group structure and the effect on the H-bonds network and the existence of two populations of water molecules are discussed.

Keywords: Membranes; Water; Near Infrared Spectroscopy; Principal Component Analysis.

(1850) **WATER BEHAVIOR ON LIPOSOMES WITH PRO AND GB IN HYDRIC STRESS CONDITIONS BY FTIR-ATR**

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Glycine Betaine (GB) and Proline (Pro) are two major organic osmolytes that accumulate in a variety of plant species in response to environmental stresses such as drought, salinity and extreme temperatures. Although their actual roles in plant osmotolerance are thought to be as osmolytes their effects on membrane integrity along with adaptive roles in mediating osmotic adjustment in plants grown under stress conditions remain controversial. In particular, it is of interest to analyze the effects of these aminoacids on membrane structure and hydration.

In this context, the interaction of GB and Pro with the hydration centers of DPPC membrane phosphates, carbonyls and acyl chains was evaluated at controlled hydration and temperature by FTIR-ATR was analyzed. In the same direction, in order to evaluate the water behavior different populations of water bands were also studied.

No significant changes are observed in the frequency of phosphate groups in the presence of GB and Pro at different RH.

In contrast, at low hydration degrees (11% RH), DPPC carbonyl groups in the presence of Pro show an increase in the bounded population compared to pure DPPC. In the presence of Gb no significant

differences are observed between both populations and pure DPPC. The most noticeable changes were observed at the methylenes groups at different RH indicating that water between aliphatic chains (confined water) remain at low hydration degrees in the presence of high concentrations of both aminoacids. A similar behavior in the water content and confined water was observed in ETERPC MLV´s.

These results gave suggest that the aminoacids enhance the water entrapment in the membrane, a response that may be helpful to interpret their preservative properties in hydric stress process.

Keywords: Liposomes, Hydration, FTIR-ATR, Aminoacids.

(339) **PLASMA MEMBRANE POTENTIAL (PM_V) AND ITS ROLE IN THE RECRUITMENT OF CYTOSOLIC PROTEINS TO THE PLASMA MEMBRANE**

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Previously, our group showed that erythrocytes from diabetic and hypertensive patients displayed more tubulin associated with the plasma membrane than erythrocytes from normal subjects. This association results in the inhibition of plasma membrane P-ATPases, as Na^+,K^+-ATPase and Ca^{2+}-ATPase. Consequently, the intracellular concentrations of Na^+ and Ca^{+2} increases and it has been stablished a relationship between the content of membrane tubulin, the changes in the intracellular concentration of some ions and the alteration of some hemorheological properties of these cells, as deformability and osmotic fragility. Since the PM_V is generated as a result of the distribution of electrical charges on both sides of the membrane, the inhibition of ionic pumps could affect the PM_V. Given some physicochemical properties of tubulin, and using biochemical tools and the eletric-sensitive fluorescent dye $DiBAC_4(3)$ we designed experiments to know whether changes in the PM_V could influence the migration of tubulin to the plasma membrane. For this purpose, we modified pharmacologically the PM_V in erythrocytes from healthy individuals, and then we quantified tubulin in isolated plasma membranes. We observed a marked increase in membrane tubulin in erythrocytes that were depolarized, whereas in hyperpolarized erythrocytes, the amount of tubulin in membrane was relatively minor and was mostly of the acetylated isotype. On the other hand, and using flow cytometry and $DiBAC_4(3)$, we determined the PM_V in erythrocytes from spontaneously hypertensive rats (strain SHR) and control mates (Wistar Kyoto strain). The results showed than erythrocytes from SHR rats displayed a 17% higher fluorescence compared with control animals (which theoretically represents a variation of around 2 mV), suggesting than this erythrocytes are depolarized when compared whit normal erythrocytes. These results suggest that there exists a relationship between PM_V and the association of tubulin to the plasma membrane.

Keywords: tubulin, membrane potential, erythrocyte.

(1415) **ALLOSTERIC ACTIVATION OF THE HUMAN 5-HT_3 RECEPTOR**

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The serotonin type 3 receptors (5-HT_3) are cation-selective channels that belong to the Cys-loop receptor family. They are involved in fast excitatory transmission in central and peripheral nervous systems and are implicated in gastrointestinal and neurological functions. Five different subunits (A-E) have been identified in humans, and the A subunit is the only one capable of forming functional homopentameric receptors (5-HT_3A). These receptors are activated by agonist binding to the orthosteric sites located at the interfaces between two adjacent subunits at the extracellular region. Ago-positive allosteric modulators (Ago-PAMs) are ligands that bind to an allosteric binding site and mediate a receptor response in the absence of an orthosteric agonist and also potentiate its response. We here used the high-conductance form of the receptor (5-HT_3A_{HC}), which allows detection of single-channel openings from patch-clamp recordings, to determine the molecular basis underlying its activation and modulation by two ago-PAMs, thymol and carvacrol. From cell-attached recordings, we observed that both ligands activate the receptor in a similar way, eliciting openings in quick succession that are grouped in episodes (bursts) of high open probability (>0.9). The combined application of orthosteric agonists (full or partial) with the allosteric ligands elicits single channel events whose mean open and burst durations are intermediate between those obtained when orthosteric or allosteric agonists are applied individually.

Our results reveal the mechanistic basis underlying activation and modulation of the 5-HT_3A receptor by two ago-positive allosteric modulators, thus providing new information that is essential for the design of more efficacious and specific therapeutic compounds.

Keywords: serotonin, positive allosteric modulators, single-channel.

(576) **CONTRIBUTION OF ACCESSORY SUBUNITS TO HETEROMERIC SEROTONIN TYPE 3 RECEPTOR FUNCTION**

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Abstract: $5HT_3$ receptors are members of the Cys-loop receptor family that mediate fast excitatory transmission in central and peripheral nervous system. Five different subunits (AE) have been identified in humans, and most of them have multiple isoforms. It is known that the A subunit can form functional homomeric ($5HT_3$A) and heteromeric receptors with the B subunit ($5HT_3$AB). Here we combined single-channel and macroscopic current recordings to determine if the other $5HT_3$ subunits (C, D or E) can also combine with the A subunit to form heteromeric receptors. After co-expression of the A subunit with each of the tested subunits, single-channel events with different amplitudes to that of $5HT_3$A receptors were detected. From the analysis of the single-channel amplitudes, the stoichiometry of each heteromeric receptor was inferred. The activation patterns of the heteromeric receptors elicited by 1030 μM 5HT showed long-activation episodes composed by openings in quick succession. No statistically significant differences in the open durations were observed among the homomeric and the heteromeric A/C, A/D, A/E receptors. However, the EC_{50} values for 5HT, which were determined by whole-cell macroscopic recordings, were statistically different between heteromeric and $5HT_3$A receptors. *In silico* studies provided insights into the contribution of the different subunits to the 5HT binding site.

Our results demonstrate that all the $5HT_3$ subunits can combine with the A subunit to form heteromeric receptors, thus leading to a wide variety of receptors showing different functional properties. The functional characterization of different heteromeric $5HT_3$ receptors, which are expressed in different tissues, contributes to the development of selective therapies targeting this receptor family.

Keywords: serotonin, single-channel, heteromeric receptors.

STRUCTURAL AND FUNCTIONAL BIOCHEMISTRY 6

(137) **ACIDIC PH IS A SIGNAL THAT TRIGGERS THE PHOSPHORYLATION OF THE RESPONSE REGULATOR NTRX IN ALPHAPROTEOBACTERIA**

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Caulobacter crescentus is a gram-negative bacterium that grows in dilute aquatic environments and is a member of the alpha-subdivision of proteobacteria. Much attention has been given to the study of *C. crescentus* signaling pathways to describe how they control cellular development and cell-cycle progression. A system-level study of two-component systems (TCS) described that the gene that codes for the response regulator (RR) NtrX was conditionally essential because it was only possible to obtain a deletion strain of this gene in a minimal medium. However, the signal to which NtrX responds and its role in *C. crescentus* biology remains elusive. On

the other hand, NtrX and its cognate histidine kinase NtrY have been extensively studied in the pathogen *Brucella abortus*, where it has been reported that they participate in the bacterial adaptation to low oxygen tension.

Here, we show conditions required for NtrX expression in *C. crescentus*, as well as a signal that triggers NtrX phosphorylation, and we describe the relevant role of this RR during growth in minimal media. We found that NtrX expression is induced by high concentrations of phosphate, despite the fact that the system does not respond to it. Instead, we demonstrate that acidic pH leads to NtrX phosphorylation and that this signal is physiologically relevant because *C. crescentus* produces the acidification of the medium upon entry to stationary phase, causing NtrX phosphorylation at this stage of the growth curve. Besides, we show that *ntrX* deletion produces a decreased viability at stationary phase and a reduced resistance to acidic stress. Finally, we prove that NtrX is also phosphorylated by acidic pH in *B. abortus*, pointing out to a potential conserved role across the alphaproteobacteria class.

Keywords: NtrY/X two component system, Caulobacter crescentus, Brucella abortus, acidic pH sensing

(289) **STRUCTURAL ANALYSIS OF THE BLUE LIGHT PHOTORECEPTOR FROM *BRUCELLA***

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Light modulates the virulence of the bacterium *Brucella abortus* through a histidine kinase containing a light-oxygen-voltage domain sensitive to blue light (LOV-HK). *Brucella* LOV-HK exhibits the spectroscopic changes corresponding to the typical adduct formation of LOV domains, between the C4 of the isoalloxazine ring and S of a strictly conserved cysteine. After illumination, *Brucella* LOV-HK does not return to the dark state. *Brucella* LOV-HK increases its autophosphorylation upon absorption of blue light.

Brucella LOV-HK comprises an N-terminal blue-light sensor (LOV) domain, followed by a central PAS domain and a C-terminal histidine kinase (HK) domain. We have performed a structural characterization of the isolated LOV and HK domains.

The LOV domain consists of a globular core and N- and C-terminal flanking regions. The core of the LOV domain adopts the typical α/β PAS domain fold, consisting of a β-sheet and α-helical connector elements. The β-scaffold is a central element in the light activation. The N-terminal region consists of an α-helix and plays an essential role in dimerization.

The HK is a parallel dimer. It is comprised of the dimerization/histidine phosphoacceptor subdomain (DHp) and the catalytic and ATP-binding subdomain (CA), which are connected by a flexible linker.

Based on the information from the study of the isolated LOV and HK domains now we intend to investigate the mechanism of light activation, that is, how the light sensing by the LOV domain is coupled to the increase of the autophosphorylation activity of the kinase domain.

Here, we will show our recent progress in the project, describing the crystal structure of a construct comprising the LOV, the PAS and the connecting hinge in its dark state. The structure was solved at 2.74Å by molecular replace. It is a parallel dimer with the N-helix, both from the LOV and the PAS domain intertwined.

Keywords: Blue light activated histidine kinase; *Brucella*; Protein X-ray crystallography

(677) **STRUCTURAL BASIS FOR LONG CHAIN UNSATURATED FATTY ACIDS AS SIGNALS OF THE *Salmonella* PhoP/PhoQ VIRULENCE SYSTEM**

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Salmonella is an enteropathogen that causes a wide range of diseases in humans and animals. In *S. typhimurium*, the PhoP/PhoQ two-component system (TCS), composed by PhoQ, the histidine kinase sensor, and PhoP the cognate transcriptional regulator controls key virulence phenotypes such as the invasion and proliferation within host cells. As signal transduction in mammals does not involve TCS, the inhibition of histidine kinases is an attractive target to develop new antimicrobial agents. By using a bio-guided strategy, we have previously discovered that long chain unsaturated fatty acids (LCUFAs) repress the transcription of PhoPregulated genes by inhibiting the PhoQ autokinase activity. The aim of this work is to analyze the specificity of the inhibition and the molecular basis for this action. Performing fluorescence-based thermal shift assay (FTS) we characterize LCUFAs as ligands that bind to the purified sensor domain of PhoQ (PhoQp). The analyzed data from the thermal denaturation curves revealed a shift in the melting temperature (Tm) indicated a direct interaction of LCUFAs with PhoQp. To further understand LCUFAs-PhoQ dynamic interaction we collected a series of (1H, 15N)-HSQC NMR spectra of PhoQp as a function of LCUFAs and $MgCl_2$ concentrations. NMR data indicate that two different types of conformational changes can occur in the PhoQ sensor domain, depending on the addition of divalent cation or LCUFAs. Assignments for the chemical shift perturbations of backbone amide allowed us to identify residues in the PhoQp structure that experience LCUFAs-dependent changes. The majority of the residues that experience significant peak shifting and broadening during LCUFAs titration were found in β-strand 2, loop-1 and β-strand 3 of PhoQp. Taken together, our results provide the structural basis for LCUFAs as PhoQ signals and lead us to conclude that divalent cations and LCUFAs act at different sites of the PhoQ sensor domain.

Keywords: *Salmonella typhimurium, PhoP/PhoQ* system, long chain unsaturated fatty acids, ligand-protein interaction

(866) **FUNCTIONAL ASSESSMENT AND STRUCTURAL ANALYSIS OF AN ANTI-TFR1 ANTIBODY FRAGMENT THAT BLOCKS INTERNALIZATION OF NEW WORD HEMORRHAGIC FEVERS ARENAVIRUSES**

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Abstract:

New World Hemorrhagic Fevers (NWHF) are grave diseases because of their infectivity and mortality. Their etiological agents are the arenaviruses Chapare (CHAV), Machupo (MACV), Guanarito (GTOV), Sabiá (SABV) and Junín (JUNV), but only is available a vaccine and an effective therapy against JUNV. The entry of these arenaviruses into human cells occurs via binding of the viral glycoprotein (GP1) to the human transferrin 1 receptor (hTfR1). Previously we established that the anti-hTfR1 IgG3 chimeric antibody ch128.1 inhibits the internalization of NWHF viruses. However, ch128.1 presents risks of inducing crosslinking of the receptor and inflammatory reaction in healthy tissues. The goal of this work is to generate a monovalent Fab fragment of ch128.1 without Fc effector function (Fab128.1), to assess whether it can block internalization of NWHF arenaviruses, to analyze its structure, and to model its interaction with hTfR1. Monovalent Fab128.1 fragment was obtained by proteolytic digestion of ch128.1 with papain. We determined that it binds to the extracellular domain of hTfR1 by ELISA and size exclusion chromatography. Flow cytometry functional studies showed that Fab128.1 significantly blocks internalization into HEK-293T cells of pseudoviruses expressing eGFP decorated with GP1/GP2 of JUNV or MACV. Structural analysis of Fab128.1 was performed in crystals generated by hanging drop. X-ray diffraction was collected from a synchrotron beam and the atomic structure solved by molecular replacement with 2.69Å resolution. With the Fab128.1 structural data we modeled the interaction with sTfR1 using computational docking analysis to obtain a structural model of the Fab-antigen complex. These studies confirm that monovalent Fab128.1 can block the entry of NWHF arenaviruses and provide insights in the structure of the variable region that interacts with hTfR1. The results shown here will contribute to the rational design of a safe and effective biotherapy against the NWHF.

Keywords: antibody fragment, transferrin receptor 1, arenavirus, hemorrhagic fever, crystal structure

(1412) **ANALYSIS OF SUGAR PARTITIONING IN ACTINOBACTERIA: STUDIES OF DUPLICATED GENES IN *Rhodococcus jostii***

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The study of enzymes determining the use of glucose-1P (Glc-1P) for the production of different carbohydrates is critical for a better understanding of developmental biology, physiology, and metabolism of biotechnological microorganisms, such as *Rhodococcus jostii* and *Streptomyces coelicolor*. The genome from *R. jostii*, an oleaginous bacteria belonging to actinobacteria, shows many gene duplication such as those coding for UDP-glucose pyrophosphorylase (GalU), glycogen synthase or trehalose-6P synthase. Albeit the organism presents single gene coding for ADPglucose pyrophosphorylase and the bifunctional protein GlmU. We performed the molecular cloning of *R. jostii galU1* and *galU2* genes respectively encoding the enzymes *Rjo*GalU1 and 2, which share more than 78% identity. Also, in preliminary structural analysis *Rjo*GalU2 behaved as a trimer, differing from other prokaryotic GalUs. After recombinant expression and purification, the comparative kinetic characterization showed that *Rjo*GalU2 has 25-fold more activity than *Rjo*GalU1 regarding UDP-Glc synthesis; although both enzymes depicted $S_{0.5}$ values for both substrates (UTP and Glc-1P) in the same order of magnitude. Both GalUs from *R. jostii* showed a high degree of promiscuity towards sugar-1Ps. Remarkably, *Rjo*GalU2 depicted one order of magnitude higher activity with glucosamine-1P (GlcN-1P) than with Glc-1P, thus suggesting this enzyme should be reannotated according to its principal activity rather than GalU. Sugar-1P is substrate of the NDPsugar pyrophosphorylases that produces different NDP-sugar, which are used by different enzymes (i.e., glycosyl transferases) leading the monosaccharide to carbohydrates multifaceted routes. In this regard, our results reinforce the importance of deepen the structure to function analysis of carbohydrate related enzymes from bacteria to further understand evolutive mechanisms, physiological behaviors and even identify tools for future biotechnological applications.

Keywords: UDP-glucose pyrophosphorylase; sugar-1P; promiscuity; Actinobacteria; glucosamine1P.

(1365) **STUDY OF THE EXPRESSION AND TOPOLOGY OF BlaR1 AND MecR1 OF *Staphylococcus aureus***

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Methicillin-resistant *Staphylococcus aureus* (MRSA) has emerged as a globally important pathogen that is resistant to all classes of β-lactam antibiotics and some strains also are resistant to glycopeptides, the last resort antibiotics used to treat MRSA infections. Resistance to β-lactam antibiotics in MRSA is due to the inducible expression of an accessory transpeptidase, PBP2a, with low affinity for most β-lactams and of the serin-β-lactamase PC1. The sensor/transducer proteins MecR1 and BlaR1 regulate the level of expression of PBP2a and PC1, respectively, in response to the presence of the β-lactam antibiotic. Little is known about the intramolecular events that lead to activation of BlaR1 and MecR1, and the search for inhibitors has been limited by the lack of high resolution structural information on the full-length proteins.

In order to gain insight into the molecular details of the activation of BlaR1 and MecR1 we have evaluated the expression of full-length BlaR1 and MecR1 and also of shorter versions of these two proteins (with fewer transmembrane helixes) as fusions to Mistic. We succeeded in overexpressing Mistic-BlaR1-JH1 in *E. coli* BL21 Star™ (DE3) membranes and we were able to purify it to homogeneity using affinity chromatography. We conducted Proteinase K susceptibility assays in spheroplasts to evaluate the topology of BlaR1-JH1 when fused to Mistic. We also verified the extracellular localization of the sensor domain of BlaR1-JH1 in this construct by incubation of spheroplasts with the fluorescent antibiotic Bocillin-FL. We expressed MecR1-E205A (no auto-proteolytic activity) with a C-terminal His-6x tag in *E. coli* BL21 Star™ (DE3). However, MecR1-E205A was still prone to proteolysis by native *E. coli* proteases. In order to identify the later site of proteolysis by MALDI-TOF/TOF, we purified MecR1 from membranes by affinity chromatography for analysis of the full-length protein and of the proteolysis bands by MALDI-TOF/TOF.

Keywords: Staphylococcus aureus, β-lactam antibiotics, sensor/transducer proteins, topology.

(1693) **EXPLORING THE CIS/TRANS AUTOPHOSPHORYLATION MECHANISM OF A SENSOR HISTIDINE KINASE FROM *Mycobacterium tuberculosis***

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One of the mechanisms that contributes to persistent infection by *Mycobacterium tuberculosis* is the bacillus capacity to enter a latent, dormant state, that renders antibiotics inefficient while reducing clinical manifestations of the disease. The DosS-DosT/DosR two-component sensor system consists of the DNA binding element DosR that induces expression of the ~48 gene dormancy regulon and the signaling heme histidine kinases (HK) DosS and DosT. These signaling HKs can be activated by hypoxia, presence of NO, CO and ascorbic acid. Following activation, HK autophosphorylate by transferring a phosphate moiety from an ATP molecule to a conserved histidine residue; this phosphate is then transferred to an aspartic acid residue in DosR. DosS is a HK consisting of an heme binding domain (GAF1), a GAF2 domain with unknown function and a dimerization-phosphorylation (DhP) domain. Structurally, dimeric HKs can autophosphorylate in a *cis* or *trans* intradimer mechanism. It has been posited that the key determinant is the loop that connects two alfa-helices at the base of the DhP domain four-helix bundle. In this case, the DosS DhP tertiary structure has not been solved yet. To explore this mechanism in DosS, we engineered mutants removing the sensor domains leaving just the DhP domain to ensure constitutive activity of the enzyme. We also engineered an impaired ATP-binding mutant to act as a possible phosphate acceptor but unable to transfer phosphate to a histidine residue. We verified structural integrity and tertiary and quaternary structure of our constructs by circular dichroism, size exclusion chromatography and dynamic light scattering. Autophosphorylation activity was verified by incorporation of radioactive phosphorus from ATP-γ-^{32}P. Using these protein constructs and the information regarding their activity and quaternary structure we will present biophysical/biochemical evidence that will contribute to elucidate the mechanism of DosS autophosphorylation.

Keywords: Histidine Kinases, *Mycobacterium tuberculosis*, autophosphorylation

(1781) **THE INTRINSICALLY DISORDERED PROTEIN-PROPERTIES OF THE PLANT PROTEIN ASR1 ARE CLOSELLY RELATED TO ITS FUNCTION AS A DROUGHT STRESS-RESPONSIVE TRANSCRIPTION FACTOR**

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Plant species in arid zones are constantly exposed to drought stress. The ASR (Abscisic, Stress, Ripening) protein family- a subgroup of the late embryogenesis abundant (LEA) superfamily- is involved in the water stress response and adaptation to dry environments. Tomato ASR1, as well as other members of this family, is predicted to be an intrinsically disordered protein (IDP). In this context, we employed biophysical techniques to perform a deep *in vitro* characterization of ASR1 as an IDP protein and showed how both

environmental factors and *in vivo* targets are related to its folding.

We present evidence supporting that ASR1 is an IDP and exhibits the plasticity to easily adopt different conformations like the α-helix or polyproline (PII) depending on the surroundings (ΔG~1 kcal/mol). We also show that environmental changes like low temperatures and low pH promote ASR1 to be partially folded in a PII conformation. Interestingly, the addition of agents that mimic abiotic stress conditions causes different effects on ASR1 secondary structure. While NaCl diminishes PII content, PEG and glycerol stabilize the α-helix conformation. In addition, we found that Zn^{2+} binding to ASR1 with a defined stoichiometry promotes its folding to α-helix [Kd= (1.3 ± 0.2) μM]. Extra Zn^{2+} binding promotes dimerization. Moreover, the binding of stoichiometric Zn^{2+} is necessary for binding its specific target DNA with a 1:1 stoichiometry [Kd= 216± 10) nM], with a 5-fold preference over the scrambled sequence oligonucleotide.

Furthermore, we designed a FRET reporter to sense ASR1 folding *in vivo*. In order to assess its effectiveness, we expressed this protein sensor in E. *coli* and tested it under saline and osmotic stress.

Overall, this work supports the notion that plasticity of ASR1 is a key feature that facilitates its response to drought stress and the interaction with its specific targets.

Keywords: ASR1; intrinsically disorder proteins, FRET sensor

(1806) **EFFECT OF GHRELIN ON THE EXPRESSION AND ACTIVITY OF TYROSINE HYDROXYLASE, AN ENZYME INVOLVED IN HYPERTENSION**

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Ghrelin, is involved in several metabolic and cardiovascular processes. In the brain, the receptor for ghrelin has been shown to localize in the main cardiovascular control centers in neurons of the nucleus tractus solitaries. Tyrosine hydroxylase (TH), the enzyme that catalyses the first and rate-limiting step in catecholamines biosynthesis, is involved in the development and maintenance of hypertension. The aim of this study was to examine the effect of ghrelin on TH expression and activity. PC12 cells were incubated in the presence or absence of 100 nM ghrelin during different times and TH expression and phosphorylation (as an index of TH activity) were evaluated by Western-blot and immunofluorescence. Our results showed that ghrelin modulate TH expression in PC12 cells in a time-dependent manner. TH expression showed an increase of 30±5% above basal after 30 min incubation but a decrease of 25±5% below basal after 24 h incubation in the presence of ghrelin ($P<0.05$). TH phosphorylation in Ser-40 was increased by 3 and 2 fold above basal in cells treated with ghrelin during 15 and 30 min respectively ($P<0.05$). In accordance to that observed in PC12 cells, ghrelin induced an increase of 50±2% respect to basal in PSer40TH expression after 15min incubation in primary neuronal cultures from hypothalamus and brain stem of spontaneusly hypertensive rats (SHR). Given that TH expression and activity are regulated by the UPS we tested whether proteasome activity could be modulated by ghrelin. A significant decrease in proteasome activity was observed after 15 min incubation of PC12 cells with ghrelin. In conclusion, our results suggested that ghrelin may play a role in hypertension by modulting TH protein level and activity.

Keywords: Ghrelin, Tyrosine hydroxylase, Ubiquitin proteasome system, Hypertension.

ONCOLOGY-ONCOIMMUNOLOGY 13

(345) **CREL AND RELB HIERARCHIES DEFINE A DNA-BINDING NETWORK IN NON-HODGKIN AND HODGKIN LYMPHOMAS.**

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NFkB is a pleitropic family of transcription factors known to play major roles in cell proliferation, apoptosis and inflammation. It comprises two signaling arms: canonical (mediated by dimeric combinations of RelA, cRel and p50) and non canonical or alternative (mediated by RelB and p52). Despite considerable evidence supporting the role of the REL members in lymphomagenesis, it is not clear whether specific NFKB dimers control a particular set of genes in distinct germinal center (GC) derived lymphomas.

To address this issue we analyzed the genome-wide distribution of the NFkB factors by ChIP-Seq in human cell lines model of different lymphomas that arise from the GC. We included BJAB and HBL1 that represent respectively GCB (Germinal Center B Cell) and ABC (Activated B Cell), the two Diffuse Large B Cell Lymphoma (DLBCL) subtypes, the most frequent Non Hodgkin Lymphoma (NHL). We performed the same assay in UH01 a HL cell line, as well as in primary human centroblasts. The ChIP-Seq data was merged with microarray results from the same cell lines before and after knocking down each NFkB factor by specific shRNAs.

This analysis shaped two network architectures: a RelB hierarchical network in HL and a cREL hierarchy in NHL. As an example: we selected 5,888 RelB peaks (that fulfilled the statistical criterias and localized +/- 2kb from the TSS) that were distributed on 4,581 genes. The signature analysis revealed that the set of RelB bound genes was enriched for cell cycle control, cell death and DNA repair genes among others. Single locus ChIP confirmed RelB and p52 binding on CCND3, CDK6, BCL2 and $BCLx_L$ genes in HL cells. The switch from RelB in HL to cRel in NHL on the mentioned genes was found and it repeated when other gene sets were analyzed.

These data suggests each REL factor has specific targets as we predicted from the lack of similarities in their transactivation domains. It also defines a RelB controlled network in HL and a cRel controlled network in NHL.

Keywords**:** Non Hodgkin Lymphoma, Hodgkin Lymphoma, RelA, RelB, cRel.

(106) **LONG INTERGENIC NON-CODING RNA 885 IS INVOLVED IN NON-INVASIVE AND INVASIVE STAGES OF BREAST CANCER PROGRESSION**

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Abstract: Long non-coding RNAs (lncRNAs) are defined as RNA transcripts longer than 200 nt which do not encode proteins. Although our understanding is limited, lncRNAs have been shown to be important players in normal development, organogenesis, multiple cellular processes and human disease. Multiple lncRNAs have been reported associated with breast cancer progression. In a recent study, RNA-Seq analysis allowed us to identify a group of novel lncRNAs as differentially expressed between normal and ductal carcinoma *in situ* (DCIS). The aim of this study was to characterize the *in vitro* and *in vivo* effects of LINC885 in non-invasive and invasive models as a novel breast cancer progression driver lncRNA. First, we performed a phenotypic characterization of the effects of overexpressing LINC885 in a prototypic normal and DCIS cell lines (MCF10 and DCIS.COM) and vice-versa; we analyzed the effects of silencing the target lncRNAs in invasive breast cancer lines displaying their overexpression. Second, transcriptomic (RNA-Seq) and RNA-pull down (TAP-MS) analyses were also applied to identify the signaling pathway modulated by LINC885 among stable transfected breast cell lines. LINC885 stable overexpression or transient silencing affected colony formation, cell proliferation and migration. Functional enrichment analysis of deregulated transcripts between LINC885 stable transfected cells vs. control cells revealed specific bioprocess related to TP53 signaling pathway and also deregulated expression of genes associated with a proliferative signature (EGFR and FOXM1 pathways). In addition, analysis of TCGA data showed an association between high LINC885 expression and decreased overall survival ($p=0.02$) in patients with primary invasive breast carcinomas. Based on these studies, we conclude that LINC885 overexpression represent a novel oncogenic lncRNA associated with early stage breast cancer progression.

Keywords: long non-coding RNA, breast cancer progression, RNA-Seq

(1843) **POLA-SEQ: A PIPELINE FOR THE ANALYSIS OF MRNA ISOFORMS FROM ALTERNATIVE POLYADENYLATION IN CANCER.**

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More than half of human genes contain alternative polyadenylation (pA) sites, which allow the creation of various 3' untranslated regions (3'UTRs). Alternative pA sites can be localized in internal introns or exons, allowing the formation of different protein isoforms, or they may be localized in the 3'UTR resulting in transcripts with variable 3' UTRs encoding the same protein. It has been reported that cancer cells often express substantial amounts of mRNA isoforms with shorter 3'UTRs than differentiated non transformed cells. It is also known than higher gene expression is tightly linked to cellular proliferation and, generally, short 3'UTR isoforms are more abundant in proliferating cells. Here, we report a pipeline named PolA-Seq to analyze the differential expression of genes than present alternative pA sites based on RNA-seq data. This new tool allows the use of transcriptomic public data for searching specific mRNA isoforms involved in tumor development. The pipeline consists in the implementation of Galaxy tools and its processing capability, R/Bioconductor and deeptools packages, for data analysis and visualization. This pipeline allowed us to identify different 3'UTRs of previously reported and unreported genes with alternative polyadenylation based on RNA-Seq data. With the use of the deeptools packages, we took an alignment of reads and generated a coverage track for each 3'UTR in the different genes of interest and compared their levels between normal differentiated mammary glands and mammary tumors. We have successfully tested this pipeline comparing the levels of short and long Beta 1 integrins mRNA isoforms in tumors Vs lactating mammary glands from RET transgenic mice. Our results confirm the over-expression of the shorter form, which has been previously discovered by our group, in mammary tumors. In addition, preliminary data allowed us to verify this pattern in other relevant genes with alternative pA sites.

Keywords: Alternative polyadenylation, Mammary Gland, RNA-seq, Bioinformatics

(282) **ROLE OF RSUME IN THE FUNCTIONAL IMPACT OF TYPE 2 VHL MUTATIONS**

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von Hippel–Lindau (VHL) disease is characterized by development of highly vascularized tumors. VHL protein drives the degradation of hypoxia-inducible transcription factors (HIFs) in normoxia. Mutations of VHL lead to a deregulation of HIFs promoting tumor development. The molecular mechanisms describing VHL mutation loss of function are still an open question. We found that RSUME is expressed in VHL-derived tumors. At molecular level, we demonstrated that RSUME interacts with VHL leading to HIFs-α stabilization. The functional inhibition was also found in representative Type2 mutants (VHLY112H; VHLR167Q; VHLL188V), in which RSUME potentiates their loss of function activity. In this work we aimed to determine the mechanism of action of RSUME over VHL Type2 mutants. By co-transfecting RCC-786-O cells (VHL -/-) with RSUME expression reporter vector (RSUME-Luc) and VHL or VHL representative mutants we demonstrate that VHL exert an inhibitory action on RSUME expression which is not produced by VHL mutants, highlighting a permissive setting for the RSUME levels deregulation. In addition by co-immunoprecipitation we demonstrated RSUME interacts with each VHL type 2 mutant and HIF-2α in the same complex and displaces the binding between HIF-2α and each VHL mutant. To understand if RSUME actions are mediated by summoylation, we generated VHL_{171} mutant, unable to be sumoylated and we evidence that RSUME interacts with VHL_{171} mutant and leads HIF-2α stabilization as seen for VHLwt. Additionally we observed the interaction RSUME - non-sumoylated VHL in cellular extract treated with Gam enzyme (inhibitor of generalized sumoylation). Even more, we establish that RSUME interacts with non-sumoylables Type 2 VHL mutants (VHL mutants carrying additional 171 mutation) and allows HIF-2α stabilization. Taken together these results support a role of RSUME participation in Type2 VHL mutations functional impact.

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Keywords: HIF2α, Type 2-VHL-Mutations, RSUME.

(149) **ROLE OF THE TRANSCRIPTION FACTOR P63 DURING BLADDER CANCER PROGRESSION**

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Nowadays, it has been proposed a new classification of muscle-invasive tumors of the bladder which remains similar to those established for breast cancer. Based in previous results of our team, where p63 has been demonstrated as an essential factor for basal type breast tumor progression (Lodillinsky, 2016) and the literature, where it has started to classify bladder cancer related its gene expression pattern, (Choi, 2014; Dambauer, 2014) we propose in this project to evaluate the role of p63 in bladder cancer progression.

Knocking down for p63 in human bladder cancer cell lines MGHU3 and UMUC14 was developed using a TET-ON system where the p63 depletion is induced externally under doxycycline (DOX). Cells were treated for 96 and 120 hours with DOX and p63 expression was analyzed by western blot. We could observe an almost complete abrogation of p63 expression under DOX in MGHU3 and a 60% of depletion in UMUC14 cells. Gelatin degradation assay was performed in MGHU3 cells. We could observe that while DOX has no effect in the degradation ability of Non-Targeting cells, shp63_i cells under DOX degraded 60 percent less compared without DOX. *($p \leq 0,01$;* ANOVA Tukey between with and without DOX). Migratory ability was tested using the wound healing assay. p63 depleted cells presented a significantly lower potential of 70% to close the wound 24 h after initiation of the wound healing assay ($p < 0.0001$; t test Mann Whitney, DOXY vs untreated shp63_i cells).

Based in all previously exposed we can conclude that, first technically we were able to obtain p63-depleted clones inducible under exogenous treatment such as DOX observed by western blot and gelatin degradation assay as a method to evaluate the invasive ability of cancer cells. In a biological level, our results indicate that p63 is an essential protein through which the cell mediates its invasive mechanism.

(1019) **STUDIES ON THE EXPRESSION OF A NOVEL SPLICE VARIANT OF HUMAN E-CADHERIN IN ENDOMETRIAL CANCER**

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Endometrial cancer (EC) is the 6th most common cancer in women worldwide. Endometrioid Endometrial Carcinomas (EEC) are ~80% of EC cases. Myometrial invasion (MI) is a key event in EC dissemination and deep MI is critical to define EC recurrence risk and associated with poor prognosis. Epithelial Cadherin (Ecad)-mediated cell-cell adhesion is altered, and Epithelial to Mesenchymal Transition (EMT)-related events occur during tumor progression in EEC, but the molecular basis are not fully known. We have identified a novel E-cadherin splice variant (Ecadvar) mRNA that induces an

EMT phenotype in a breast cancer model. The present study aims to evaluate Ecadvar mRNA expression levels in EEC.

Materials & Methods: QRT-PCR assays were run to quantify Ecadvar expression relative to Ecadwt in (A) In vitro studies with Hec1a cells (A1) stably transfected with ETV5 transcription factor, found in the invasive front of EC, (A2) treated with TGF-β1, a cytokine that induces EMT, (A3) transiently transfected with Ecad siRNA to downregulate the wild type Ecad. (B) Tissue endometrial biopsies from EC patients and controls.

Results: (A1) Hec1a-ETV5 cells showed higher ($p<0.05$) Ecadvar levels than control cells, (A2) Hec1a cells treatment with TGF-β1 induced EMT-related changes and increased ($p<0.05$) Ecadvar mRNA, (A3) Hec1a cells treatment with Ecad siRNA increased ($p<0.05$) Ecadvar mRNA. (B) Ecadvar mRNA levels were higher ($p<0.05$) (B1) in tumor samples (n=59) compared with atrophic endometria (n=7), (B2) in biopsies from the tumor invasive front compared to matched superficial biopsies (n=20), (B3) in Stage IB (MI>50% 2009 FIGO) (n=23) compared to Stage IA (MI<50%) tumor samples (n=28), (B4) in the invasive front of Stage I tumor samples compared to matched superficial biopsies (n=9).

Conclusion: Assessment of Ecadvar mRNA levels may contribute to EC early detection and to determine disease progression to deep MI, helping patients who may benefit from fertility preserving options.

Keywords: endometrial cancer, myometrial invasion, Epithelial cadherin, splicing, Hec1a cells

(83) **THE EXPRESSION LEVELS OF RAC3 COACTIVATOR IN THE BREAST ADIPOSE TISSUE ARE ALTERED IN THE TUMORAL CONTEXT**

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Several types of tumors grow in the adipose tissue anatomical neighborhood. It is well-known that there is a communication between tumors and adipocytes around them, that involves the secretion of different factors.

RAC3 coactivator is over expressed in a broad variety of cancers, including breast cancer. Previously, in the L-929 fibroblastic murine line, we demonstrated that the decrease of RAC3, using an interference RNA, favors the adipogenesis through an increase of autophagy and a decrease in the proliferative rate.

In this work, we aimed to determine RAC3 expression levels in L-929 cells during the differentiation. The transcript levels of RAC3 were characterized by qPCR at different times after induction with 0.1 mg/ml insulin, 1 μM dexamethasone, 0.1 mM indomethacin and 0.5 mM 3-isobutyl-1-methylxanthine (DI3).

Notably, the levels of RAC3 mRNA decrease in the studied temporal range (1.0 ± 0.1 at 0h; 0.3 ± 0.1 at 30h; 0.2 ± 0.1 at 48h, $p<0.001$). Likewise, we determined the transcripts levels of an adipocyte marker, Perilipin, by qPCR after treatment with DI3. We observed that its levels increase (1.0 ± 0.0 0h, 4.4 ± 0.8 30h $p<0.05$).

In view of the role of the coactivator during the adipogenesis, we decided to study if its levels were altered in the adipose tissue around breast tumors of *Instituto de Investigaciones Médicas Dr. A. Lanarî* s patients. We measured RAC3 mRNA expression by qPCR in the adipose tissue and according to the characteristics of breast tumors, we obtained the following levels: 5.3 ± 1.9 for those with ER, PR and Her2 negatives (TN) and; 4.5 ± 1.6 for ER and PR positives but Her2 negative (ERPR+Her2-) versus 1.0 ± 0.0 for benign tumors ($p<0.01$ y $p<0.05$, respectively).

In conclusion, it is necessary that RAC3's levels diminish during adipogenesis, although in a breast tumoral context these limited values could be affected in accordance with the tumoral characteristics.

Keywords: RAC3, ADIPOSE TISSUE, BREAST CANCER

(1818) **THE GALECTIN-*N*-GLYCAN AXIS MODULATES RESPONSES TO TRASTUZUMAB THERAPY IN HER2+ BREAST CANCER**

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Galectins decode glycan information contained in cell surface receptors. In particular, Galectin-1 (Gal-1), binds to terminal LacNAc residues on glycosylated proteins in the absence of α2-6 sialic acid capping, modulating several cellular functions. Tumors secrete Gal-1 to evade immune responses and to promote aberrant angiogenesis. In this work, we explored the glycan profile of HER2+ breast cancer cell lines and investigated whether Gal-1 could mediate resistance to anti-HER2 targeted therapies such as Trastuzumab (TZ). Previously, we demonstrated by lectin-binding assay and WAX-UPLC that TZ-resistant JIMT-1 cells exhibited a Gal-1 permissive glycophenotype. Herein, we analyzed the expression of galectins and glycosyltranferases essential for biosynthesis of Gal-1 ligands. We found that the JIMT-1 TZ resistant cell line expressed higher levels of Gal-1 than its sensitive counterparts (BT474 and SKBR3) both at the mRNA ($p<0.05$) and protein levels ($p<0.001$). Moreover, TZ sensitive BT474 cell line expressed higher ST6GAL1 mRNA levels ($p<0.01$), while TZ resistant cell lines exhibited higher amounts of ST3GAL1, favoring a Gal-1 permissive glycophenotype, an effect which was further confirmed by confocal microscopy (exogenous and endogenous Gal-1 binding to JIMT-1 cells). To further evaluate Gal-1 implications in TZ resistance, we knocked down Gal-1 in JIMT-1 cell line by shRNA strategies. Knocking down Gal-1 sensitized JIMT-1 cells to *in vitro* TZ-inhibition ($p<0.05$). Finally, *in silico* analysis of public databases (GSE44272 and GSE62327) revealed that patients with poor response expressed higher amounts of Gal-1 mRNA than TZ-long term responders, who also expressed higher levels of ST6GAL1, reinforcing our hypothesis from a clinical standpoint. In summary, we conclude that TZ-resistant HER2+ human breast cancer cells display a particular "glycosylation signature" which, in association with Gal-1, may control and predict the clinical outcome to anti-HER2 therapies.

Keywords: Galectin-1, Trastuzumab, HER2, sialic acid.

(1109) **PHARMACODYNAMIC AND SITE-DIRECTED MUTAGENESIS STUDY OF THE INTERACTION OF 1A-116 WITH THE GTPASE RAC1**

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Rho GTPases are key molecular switches controlling transduction of external signals to cytoplasmic and nuclear effectors. In the last few years, the development of genetic and pharmacological tools has allowed a more precise definition of the specific roles of Rho GTPases in cancer. In this context, our group has been studying Rac1, one of the most studied Rho GTPases, as a therapeutic target in several types of cancer, including breast cancer, glioblastoma and myeloid acute leukemia. Our preview data showed the potenciality of using 1A-116, a Rac1 inhibitor developed by our group, as an anticancer therapeutic drug. The main objective of this work was to study the pharmacodynamics characteristics of the interaction of 1A116 with its main molecular target Rac1. Using the Serum Response Element (SRE)-Luc reporter system we identified which upstream interactions of Rac1 with different guanosine exchange factors (GEFs) were inhibited by 1A-116. We showed that 1A-116 inhibited GEF-mediated activation of SRE by Vav1, Vav2, Vav3, Tiam1 and DBL. Moreover, different schemes of pull-down assays confirmed that 1A-116 acts at the GEFs-Rac1 interaction level. 1A-116 also inhibited activation of SRE by the GTPases Rac1, RhoA and RhoG, but not by Cdc42. We also carried out a site-directed mutagenesis to evaluate the specificity of 1A-116. We generated the Rac1 W56F mutant, changing the tryptophan (W) 56 of Rac1 to a phenylalanine (F), since W56 is a key residue in Rac1 recognition by

different GEFs. Interestingly, when we treated cells transfected with the Rac1 W56F mutant, 1A-116 lost its activity, showing that W56-Rac1 residue is required for 1A-116 inhibitory effects. Finally, when we treated cells expressing a fast cycling version of Rac1, 1A-116 inhibited Rac1-regulated processes, such as ruffles and lammelipodia formation. These results contributed to a better understanding of the inhibitory effects of 1A-116, a therapeutic option to be tested in a clinical research setting.

Keywords: GTPases, Rac1 inhibitor, preclinical study,

(1065) **PHOTODYNAMIC THERAPY MODULATES A NOVEL PATHWAY IN TUMOR MICROENVIRONMENT MEDIATED BY GLI 1**

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Tumor microenvironment (TME) is a complex system whose dynamic lead to tumor evolution. In this context, Cancer Associated Fibroblast (CAF) represent stromal cells with ability to promote malignant behavior. This stimulation from TME is regulated by chemokines and transcription factors activity. In this sense, we identify a novel axis conformed by GLI and the SDF-1 cytoquine in CAF. Firstly, to mimic TME complexity, we implement a 3D heterotypic co-culture of melanoma cells (SKMel-2) with CAF (F88.2) or tumor cells alone (homotypic spheroids). On one hand, this 3D cultures showed fibroblast necessity to be well-structured. On the other hand, the cellular migration from heterospheroids is higher than homotypic ones ($p< 0.05$), demonstrating CAF influence. Secondly, we analyzed if this behavior could be attribute to ability of GLI to regulate SDF-1 cytoquine in fibroblast. Through set of tests, like luciferase (SDF-1 promoter reporter activity increase between 25-50 % with GLI transfection) and ChIP assay (percentage of imput increase 100 % respect to IgG control), we stablished that GLI 1 either GLI 2 can potentially regulate SDF-1 gen activity. Finally, we demonstrated that CAF could be a suitable target to potentially prevent melanoma recurrence using photodynamic therapy (PDT). Primarily, we adjusted a combination of photosensitizer (cationic porphyrin) and light to targeting CAF (4 mM plus 3 J/cm^2) which constitute therapeutic components. In addition, we exposed heterospheroids to PDT doses. As a result, cellular migration was delayed even for 72 h (50 % respect to control group. $p< 0.05$). Furthermore, it was evidenced that PDT exerted a global impact on GLI 1 and SDF-1 status in fibroblast. On balance, GLI 1 or GLI 2 are competent to lead malignant progression from TME, but at the same time becomes in a prospective pathway to be regulated in order to redefine our concept in melanoma phototherapy.

Keyword: Tumor microenvironment, GLI, SDF-1, stromal fibroblast

ONCOLOGY-ONCOIMMUNOLOGY 14

(91) **AKT1 REGULATES AKT2 EXPRESSION AND SUBCELLULAR LOCALIZATION IN A HUMAN BREAST CANCER MODEL**

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The aim of this work was to study the regulation between AKT1 and AKT2 isoforms and its possible implications in breast cancer progression. Previously, in a cohort of 46 advanced ductal invasive breast carcinomas we found that high nuclear AKT1 was associated with high Ki67 index, whereas either high nuclear AKT1 or high cytosolic AKT2 were associated with earlier disease progression. Furthermore, using T47D and IBH-6 human breast cancer cell lines we selectively downregulated AKT1 (shAKT1) or AKT2 (shAKT2) isoforms. In xenograft assays we found that AKT2 inhibition (shAKT2) induced non-invasive tumors, whereas AKT1 inhibition (shAKT1) reduced tumor growth, but induced an invasive phenotype and lung metastasis. Surprisingly, we found increased AKT2 protein levels in shAKT1 cells. For this reason, we analyzed how the AKT isoform regulation could be involved in breast cancer progression. We found that T47D shAKT1 tumors, that grow with lower rate but are more invasive, express higher levels of both nuclear and cytosolic AKT2, than control (shco) tumors; while shAKT2 tumors express lower levels of nuclear and higher levels of cytosolic AKT1 than shco tumors. These results suggest a switch in AKT isoforms expression and localization that may be regulated by the isoforms themselves, along tumor progression. Moreover, data from from 825 invasive breast carcinomas from "The Cancer Genome Atlas" (TCGA) demonstrated that alterations in AKT2 mRNA expression, more than in AKT1, are associated with worse patient outcome.In conclusion, AKT1 and AKT2 level and their subcellular localization might be used as biomarkers of breast cancer progression early in the diagnostic process to discriminate which subset of patients could progress sooner and consequently have potentially worse clinical outcomes.

Keywords: Breast Cancer, Cancer Progression, AKT Isoforms

(295) **BENEFITS OF METRONOMIC THERAPY TARGETING MUSCARINIC RECEPTORS IN BREAST CANCER AS A SENSITIZER OF CELLS TO CLASSIC CHEMOTHERAPY WITH PACLITAXEL**

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Abstract: Resistance to chemotherapy is a critical problem in cancer treatment and is believed to be due to the presence of a sub-population of cells named cancer stem cells (CSC). It has been postulated that metronomic therapy (administration of low doses of drugs with short inter-dose intervals) could be a useful strategy to over-come the resistance to traditional chemotherapeutic regimens. Here, we analyzed the effect of a combination of subthreshold concentrations of carbachol (CARB) (10^{-11}M) with paclitaxel (PX) (10^{-9}M) on MCF-7 breast cells, derived from a human luminal tumor. The unique addition of the combination during 40 h increased cell death by 47±6 % ($p<0.001$ vs. control) measured by MTT assay, in a similar manner than 10^{-6}M PX, that also induced death in the non-tumorigenic cell line MCF-10A, an undesirable effect in cancer treatment. In addition, the combination reduced necrosis ($p<0.05$ vs. control) and increased apoptosis ($p<0.05$ vs. control) measured by flow cytometry using annexine-V and 7-AAD. These effects could be mediated by a reduction of 92±3% ($p<0.001$ vs. control) in the expression of ABCG2 protein, measured by Western blot, that mediates the resistance to chemotherapy. We also detected a reduction in CSC population (CD44+/CD24-) by flow cytometry after this first cycle of treatment ($p<0.01$). Moreover, we determined that one cycle treatment with CARB+PX is useful as a neo-adjuvant therapy before PX 10^{-6}M. We can conclude that the metronomic administration of this new combination of anti-tumor drugs could be useful not only to promote cancer cell death per se but also to sensitize tumors cells to traditional chemotherapy.

Keywords: metronomic therapy; muscarinic receptor; paclitaxel; cancer

(438) **CORRELATION BETWEEN THE PROGESTERONE RECEPTOR ISOFORM RATIO DETERMINED IN BREAST CANCER BIOPSY CORES AND SURGICAL SAMPLES**

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Abstract: Seventy percent of breast cancers are susceptible to endocrine therapy currently aimed to target the estrogen receptor alpha (ERα). We have recently shown that breast cancer tissue cultures with higher levels of progesterone receptor (PR) isoform A (PRA) than isoform B (PRB) were inhibited by mifepristone whereas different responses were observed in those with the opposite ratio. This highlights the relevance of determining the PR isoform ratio to

identify patients that may benefit from this therapy. The aim of this study was to evaluate the correlation between the PRA/PRB ratio determined by western blots (WB) in core biopsies and those determined in surgical samples from the same breast cancer patients from the Hospital "Magdalena V Martínez" from General Pacheco (n=20). A similar trucut core was used for diagnostic purposes. The protocol has been approved by Institutional Review Boards. We determined the PRA/PRB ratio by WB using nuclear extracts from frozen tissues (biopsy and surgery) and the percentage of cells expressing PR, evaluated by immunohistochemistry (IHC), was obtained from the clinic records (surgical samples). WB and IHC data, regarding total PR expression, both obtained from surgical samples, were coincident in 90% of the cases. The few ones that differed, were samples in which the PR value determined by IHC was less than 20 % and the WB gave negative results. If the analysis is performed considering the WB data obtained from biopsy samples, the percentage decreases to 65 %, probably due to scant tumor cells in some biopsy cores. When the PRA/PRB ratios were compared between biopsy and surgical samples considering all cases in which WB biopsy data matched with the IHC data obtained in surgery, a 95% of coincidence was found. We conclude the PR isoform ratio in biopsy cores reflects the PRA/PRB ratio in the tumors in cases in which PR values are higher than 20%. We are still recruiting patients to confirm this data in a larger cohort.

Keywords: progesterone receptor isoforms, western blot, ratio, inmunohistochemistry, antiprogestins.

(1367) **DNA DAMAGE, γH2AX AND 53-BP1 STATUS AS PREDICTIVE FACTORS OF RESPONSE TO PLATINUM ANALOGUES-BASED CHEMOTHERAPY**

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Cisplatin (cisPt) constitute a widely used chemotherapeutic drug in the treatment of solid tumors. Its cytotoxic mode of action is based on the formation of DNA-interstrand and -intrastrand crosslinks with purine bases, which interfere with normal DNA function. We have reported that alkaline comet assay may constitute a useful technique to study the DNA damage and repair status and to predict response to chemotherapy in peripheral blood leukocytes (PBL) from cancer patients. γH2AX phosphorylation has been proposed as a sensitive marker of DNA double-strand breaks (DSBs). 53-BP1 is DNA damage response factor. The aim of our work was to determine DNA damage and repair rate, and to stablish their predictive value in peripheral blood leukocytes (PBL) from cancer patients treated with cisPt. We isolated PBL from 5 healthy persons and 12 cancer patients before chemotherapy. PBL were *in vitro* exposed to cisPt (200 μM, 1 h) or hyperthermia (42°C, 1 h), 24 h before cPt treatment (H+cisPt). The cells were harvested at: T0 (immediately after cisPt) and T24 (24 h after recovery). DNA damage/repair were evaluated by alkaline comet assay and by immunocytochemistry using specific antibodies against γH2AX and 53-BP1. At basal conditions, healthy individuals showed larger number of γH2AX ($P<0,05$) and 53-BP1 foci ($P<0,05$) and a significant increase in the percentage of γH2AX-positive cells ($P<0,05$) than chemotherapy-resistant cancer patients. There were no statistically significant differences between patients with complete clinical response to chemotherapy and those with partial response, stable or progressive disease with respect to DNA migration mean and number of γH2AX foci per nucleus. Our preliminary findings indicate that immunocytochemical detection of 53-BP1 and γH2AX in PBL from cancer patients could potentially be a useful tool for predicting cisplatin-based chemotherapy response.

Palabras claves: DNA damage, cancer, cisplatin, 53-BP1, γH2AX

(1520) **EFFECT OF ALLOPREGNANOLONE, A PROGESTERONE METABOLITE, ON THE PROGRESSION OF HUMAN OVARIAN CANCER CELL LINES: POTENTIAL USE AS THERAPEUTIC TOOL.**

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Ovarian cancer is one of the most common cause of gynecologic cancer death. Allopregnanolone (ALL), a progesterone (P4) metabolite, modifies ovarian physi-pathological processes. Changes in ALL levels during cycle or under stress situations can conduce to alterations in ovarian development. This was the first evidence that ALL induces ovarian morpho-physiological changes altering proliferation, apoptosis and angiogenesis, crucial for cancer progression. Epidemiologic and *in vitro* studies have shown controversial data about P4 in cancer. The effect of P4 metabolites over ovarian cancer is relevant and require more in-deep trials due to it could be involved on cancer progression. The hypothesis is that changes in ALL concentrations affect ovarian tumor progression. We investigated proliferation, apoptosis, clonogenic capacity and migration of human ovarian cancer cell lines IGROV-1 and SKOV-3. Cell lines were exposed to a range of P4 and ALL concentrations (10^{-11}-10^{-5} M) for 72 h. We showed that ALL increased proliferation in a concentration dependent manner by MTT, with a maximum effect of 44.5 ± 13.5 % on IGROV-1 ($p<0.001$ vs. control: untreated cells). The IGROV-1 expression of the antigen Ki67 showed similar values to proliferation assay. Expression of cleaved caspase 3 did not change in any line studied. IGROV-1 clonogenic capacity was increased by ALL treatment (10^{-11} and 10^{-8} M; $p<0.001$; $p<0.01$ vs. control respectively). P4 and ALL increased IGROV-1 migration, the maximal effective concentration was 10^{-11} M (148 ± 14 % and 175 ± 21 % respectively; $p<0.001$ vs. control), measured by wound assay. None of the steroids tested modified SKOV-3 progression. These results showed different responses to ALL that may be due to the heterogeneity present in the tumor lines. Overall, we found that ALL could stimulate malignant progression in human epithelial ovarian cancer, thus inhibiting the ALL synthesis could be used as a potential anti-neoplastic agent in the future.

(834) **IMPLICATIONS OF HEME-OXYGENASE 1 MODULATION OF INTERFERON INDUCIBLE ANTIVIRAL (MX1) IN PROSTATE CANCER**

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The principal goal currently within prostate cancer (PCa) research is to unveil markers for early detection of aggressive tumors that are predestined to invade and metastasize. We have previously reported that heme oxygenase-1 (HO-1) exerts an anti-tumoral role in PCa, ascertaining it as a logical target for therapy intervention. HO-1 over-expression impairs tumor growth and angiogenesis *in vivo*. Considering the cross talk between inflammation, angiogenesis and cancer progression, our next step sought to identify signaling pathways by which HO-1 could be operating. Towards this end we performed and analysed RNAseq data on PCa cells overexpressing HO-1 pharmacologically or genetically. Of note HO-1 significantly up-regulated the human myxovirus resistant protein A (Mx1). In vitro studies revealed that forced expression of HO-1 in PCa cells, significantly up-regulated MX1 mRNA and protein levels and shifted its localization towards the perinuclear area.

To address the relevance of MX1 in PCa we searched the public cancer microarray database, *Oncomine*. MX1 was ranked by its P-value for every analysis scoring a *gene rank*. We then obtained a *median rank* (Median P-value rank across datasets) for MX1. The expression profile for MX1 showed a significant down-regulation (fold change 1.5, $P<0.05$) comparing prostate adenocarcinoma *vs.* normal prostate gland, lying within the 2-19 % of the most consis-

tently low-expressed genes across this comparison.

We extended the bioinformatics analysis, using *cBioportal, assesing* whole exome and RNAseq data. The most frequent genetic alteration found for MX1 was deletion. RNASeq data also confirmed a significant down-regulation for MX1 ($P<0.05$). Moreover, Kaplan-Meier analysis also showed in PCa patients that MX1 loss was associated with decreased overall and disease-free survival ($P<0.05$).

Overall, HO-1 potentially operates through Mx1, whose expression inversely correlates with PCa, depicting its critical role in prostate carcinogenesis.

Keywords: prostate, cancer, heme-oxigenase 1, Mx1.

(596) **MOLECULAR CHARACTERIZATION OF MELANOMA CELLS THAT PRESENT PLASTIC RESISTANCE TO MAPK INHIBITORS**

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Nearly one-half of melanoma patients harbor the BRAFV600 driver mutation, the most common being BRAFV600E, which leads to the activation of proliferative and survival pathways. The development of BRAF V600 and MEK inhibitors constitutes a breakthrough in the treatment of patients with BRAF-mutated metastatic melanoma. However, although there is an increase in overall survival, these patients generally confront recurrence, and several resistance mechanisms have already been described. We studied the effects PLX4032 (BRAFV600 inhibitor) and GDC-0973 (MEK inhibitor) long-term treatment on sensitive V600E BRAF-mutated melanoma cell lines. After several weeks of long-term *in vitro* treatment with PLX4032 and/or GDC-0973, the majority of the melanoma cells died whereas some remained viable and quiescent. We named this population SUR cells. Strikingly, discontinuing treatment of SUR cells with MAPK inhibitors allowed the population to regrow and these cells retained drug sensitivity equal to that of parental cells. SUR cells had increased expression levels of CD271 and ABCB5 and presented senescence associated characteristics. In order to characterize SUR cells we performed Whole Exome Sequencing, RNAseq experiments, expression microarrays and reverse phase protein arrays (RPPA) comparing parental and SUR cells. Even though SUR cells do not present mutations associated with resistance, consistently with their plastic phenotype, RNA seq experiments showed that SUR cells present changes in the expression of 1509 genes ($p<0.05$): 684 are upregulated and 825 downregulated. GSEA analysis revealed that gene ontology signatures related with proliferation and cell cycle are downregulated in SUR cells, while there is an increase in signatures related with cellular adhesion and DNA packaging ($p<0.01$, FDR<25%). We propose quiescent plasticity as a mechanism of resistance to BRAF and MEK inhibitors.

Keywords: melanoma, BRAF, PLX4032, GDC-0973, plastic resistance

(1686) **ONCOLYTIC ADENOVIRUS-LOADED MENSTRUAL MESENCHYMAL STEM CELLS OVERCOME THE BLOCKADE OF VIRAL ACTIVITY EXERTED BY OVARY CANCER PATIENTS' ASCITES**

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Ovary cancer patients present peritoneal ascites at recurrence as a marker of disseminated disease and dismal prognosis. Oncolytic immunotherapy is an emerging approach for the treatment of disseminated cancer. In the present work we show the construction of a novel oncolytic adenovirus, AR2011, to target malignant ovary tumors. The survival curves were made by infecting malignant cells with AR2011 at different MOIs and showed that AR2011 exhibited a clear lytic effect *in vitro* in human ovary cancer cell lines and malignant cells obtained from ovary cancer patients´ ascitic fluids (AFs). By mixed the oncolytics adenovirus and crescent dilutions of AFs we demonstrated that AR2011 activity was neutralized by antibodies present in 31 samples of patients´-derived AFs. However, this blockade was overridden by preloading menstrual blood stem cells (MenSC) with AR2011 (MenSC-AR) since under these conditions AFs exerted no *in vitro* inhibitory effect on viral lytic activity. We performed cytokines and chemokines arrays of AF to study the composition of the factors present in the patient samples. Moreover, we perfomormed migration assays and observed that soluble factors present in AFs act as MenSC chemoattractants. MenSC-AR treatment of nude mice carrying established peritoneal carcinomatosis following administration of human ovarian cancer cells was able to inhibit tumor growth at levels similar to those observed with AR2011 alone. This study demonstrates that MenSC can be used to override the blockade that ascitic fluids exert on viral oncolytic effect.

(970) **STUDY OF THE BROWNING PROCESS OF BREAST CANCER ADIPOSE TISSUE**

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Abstract: Adipose microenvironment is involved in signaling pathways that influence breast cancer progression. Although adipocytes have been shown to promote breast cancer development, adipocyte characteristics involved in this process remain poorly understood. The aim of this work was to investigate the effects of factors derived from conditioned media (CMs) from human breast cancer adipose tissue explants (hATT) or normal breast adipose tissue explants (hATN) on induction of white adipocyte cell line browning. Morphology changes and brown adipose tissue (BAT)-related markers (UCP1, PRDM16, PGC1α and TBX1 among others) expression were evaluated following exposure of 3T3-L1 adipocytes to hATT or hATN CM. Increased expression of UCP1, PRDM16 and PGC1α was observed in adipocytes 3T3-L1 exposed to hATT CM in comparison to hATN CM. Interestingly, adipocytes exposed to hATT CM displayed characteristics that morphologically resembled brown adipocytes. In contrast, adipocytes exposed to hATN CM increased lipid droplet size, characteristic features of white adipocytes. In summary, these findings suggest that hATT secrete a different set of factors compared to hATN, which may induce browning of white adipocytes. This simple experimental approach suggests that hATT attached to the tumor could induce white adipocyte browning present in its microenvironment, for paracrine signaling.

*Keywords: A*dipose tissue, Browning, Breast cancer

(720) **A NOVEL ROLE OF KLF6 ACTIVITY IN THE INDUCTION OF CELLULAR SENESCENCE AND GENOME INTEGRITY MAINTENANCE**

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Krüppel-like factor 6 (KLF6) is a transcription factor involved in the regulation of relevant biological processes as cell proliferation, differentiation and apoptosis. In addition, KLF6 have a tumor suppressor activity and, accordingly, loss-of-functions mutations of *klf6* gene have been found in many human malignancies. We have demonstrated that KLF6 knockdown leads to spontaneous transformation of fibroblast cells whereas forced KLF6 expression provokes a marked cell cycle arrest. In this regard, we hypothesized whether KLF6-mediated cell cycle arrest could be associated with induction of cellular senescence. In fact, this process limits proliferation of potentially detrimental cells, preventing tumorigenesis and restraining tissue damaging helping to avoiding neoplastic transformation. In

this study, we observed that increased expression levels of KLF6 in HeLa cervical carcinoma cells was able to promote cellular senescence (Fisher Test, p<0,05), as determined by higher levels of senescence associated β-galactosidase activity. Then, cellular senescence induced by H_2O_2 treatment of NIH3T3 fibroblast cells was markedly reduced upon KLF6 downregulation by stable shRNA transduction (p<0,05). In addition to cellular senescence bypass, these cells also shown signs of genome instability as micronuclei and chromosome rings formation, suggesting that KLF6 may be involved in genome integrity maintenance. These surprising findings, along with the cytostatic function of KLF6 upon oncogenic activation is suggesting that its tumor suppressor activity could be mediated by cellular senescence as an alarm signal in response to certain stimuli producing exacerbated proliferation or cell transformation.

Palabras clave: Krüppel-like factor 6, Tumorigenesis, Cellular Senescence, Genome integrity.

GENETICS AND MOLECULAR BIOLOGY 13

(557) ANALYSIS OF THE INTERACTION BETWEEN THE GLUCOCORTICOID AND THE PROGESTERONE RECEPTORS

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Glucocorticoid (GR) and progesterone (PR) receptors are members of the steroid receptors family. The active PR is associated with cell proliferation and mammary tumors progression. GR activation promotes cell differentiation. Thus, the relative abundance of both receptors may modulate the proliferative response of the mammary epithelium. In view of these precedents, the aim of this work was to test the ability of both receptors to be part of the same complex and to study if both receptors are able to be recruited at the same binding regions in the genome. To assess if PR and GR have the potential to form complexes in vivo, T47D cells were transfected with expression vectors encoding for both receptors fused to fluorescent proteins (eGFPPR and mCherryGR) and incubated with their ligands R5020 and/or Dex, respectively. Then, fluorescence correlation spectroscopy (FCS), which allows obtaining quantitative parameters related to the mobility of fluorescent molecules and their interaction with fixed targets in living cells was used. From these analyzes it was observed that when both receptors are activated, they move in the nucleus, simultaneously. Upon activation with their respective ligands, both receptors are also recruited to a large fraction of specific binding regions. This result led us to inquire whether receptor co-binding at these regions could occur upon simultaneous treatment with their specific hormones. Thus, sequential ChIP for the PR/GR was performed in T47D/A1-2 cells which express comparable levels of both receptors. We found that indeed GR and PR co-bind to specific regions located in the CD44, GREB1, STAT5A, SNAI1 and ELF5 genes. Altogether these results suggest that PR and GR could be part of the same protein complex and play an important role in the cellular response of the mammary epithelium.

Keywords: Glucocorticoid receptors; progesterone receptors

(953) GLUCOCORTICOIDS AND ITS RECEPTORS IN RETINAL PIGMENT EPITHELIAL CELLS

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Glucocorticoids are required for photoreceptor survival and retinal protection, but little is known about their signaling pathways in the retinal pigment epithelium (RPE). Several glucocorticoid receptors (GRs) are known, including GRα and GRβ. Therefore, we studied the presence of these receptors in mice and ARPE-19 cells, a human RPE cell line. In addition we studied their modifications after treatment with dexamethasone (DEX, a GRα agonist) and mifepristone (MFP, an inhibitor of GRα and a possible agonist of GRβ).

Balb-c mice eye sections were immunostained with GRα and GRβ antisera. ARPE-19 cells were treated with DEX and/or MFP. Controls with or without ethanol were used. After 24 h, apoptosis was measured with acridine orange and ethidium bromide (AO/EB) staining, and viability with MTT. Immunofluorescence, Western blots or qPCR were used for GRα and GRβ assays.

Immunochemistry showed that GRα and GRβ appeared in retinal cell nuclei, whereas in the RPE, GRβ showed a strong cytoplasmic localization. Control ARPE-19 cells exhibited GRβ in a perinuclear localization. In these cultures, DEX induced a concentration-dependent increase of necrotic/apoptotic cells and a decrease of cell viability. MFP did not induce significant alterations in these parameters. Co-treatment with MFP did not avoid the cytotoxic effects of DEX. GRα mRNA was not significantly modified by 0.32 mM DEX, but it was significantly increased by 10 μM MFP. DEX did not affect GRβ mRNA. 50 μM MFP only induced a slight increase of GRβ mRNA, but the combination of 50 μM MFP with 0.08 or 0.32 mM DEX induced a ten-fold increase.

DEX cytotoxicity was not antagonized by MFP, indicating that the effects would not be mediated by GRα. The strong expression of GRβ in RPE cells, together with its localization and upregulation under DEX and MFP exposure suggests that this receptor might play an important role in retinal cell survival. However, further studies are required to understand the involved mechanisms.

Keywords: Glucocorticoids, Glucocorticoid receptors, retinal pigment epithelial cells

(852) ELUCIDATING THE MECHANISMS UNDERLYING NA^+/I^- SYMPORTER TRANSPORT TO THE PLASMA MEMBRANE

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The Na^+/I^- symporter (NIS), a 643 amino acid-long glycoprotein expressed at the basolateral plasma membrane of thyroid follicular cells, mediates iodide accumulation for thyroid hormones biosynthesis and radioiodide transport for the diagnosis and treatment of thyroid cancer. Most differentiated thyroid cancers showed a surprisingly NIS overexpression as compared to the surrounding normal tissue but intracellularly retained. Considering the clinical relevance to elucidate the mechanisms underlying NIS transport to the plasma membrane, we investigated the importance of short linear motifs in the transport to the protein to the cell surface under physiological conditions.

In silico computational analysis revealed several putative short linear motifs involved in transmembrane proteins transport to the cell surface. These are mainly placed in the second intracellular loop and the intracellular carboxy-terminus. We generated human NIS carboxy-terminus deletion mutants and site-directed mutants of putative sorting motifs. Functional studies were performed on transfected MDCK cells. The tyrosine-based motif NIS mutant Y120A and the mutant Δ546-643 lacking the entire carboxy-terminus are intracellularly retained. The mutant Δ546-578—containing a tryptophan-acidic motif ($W^{565}D^{566}$)—and W565A/D566A NIS are intracellularly retained. Although the mutant Δ578-583—containing a di-leucine motif ($I^{582}L^{583}$)—and I582A/L583A NIS shows defective cell surface transport in non-polarized cells, they were apically targeted in polarized cells. Moreover, the mutant missing the PDZ binding-motif TNL^{643} is properly targeted to the cell surface in non-polarized cells; however the mutant is intracellularly retained in polarized cells.

Although the molecular mechanisms that determine NIS intracellular retention in thyroid cancer cells remain elusive, here, we provide evidence regarding the importance of short linear motifs for NIS functional cell surface expression under physiological conditions.

Keywords: Thyroid cancer, Radioiodide therapy, Na^+/I^- symporter, Plasma membrane transport, Polarized traffic.

(365) COMPARISON OF PCR-RFLP OF gap AND groEL GENES AND MATRIX-ASSISTED LASER DESORPTION IONIZATION–TIME OF FLIGHT MASS SPECTROMETRY

(MALDI-TOF MS) FOR IDENTIFICATION OF COAGULASE NEGATIVE Staphylococcus

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Abstract: Coagulase-negative *Staphylococcus* (CNS) are the predominant pathogens causing bovine mastitis. Correct identification is essential to understand the involvement of CNS in intramammary infections (IMI) and make appropriate management decisions. The aim of the present study was to evaluate the performances of *gap* or *groEL* genes PCR-restriction fragment length polymorphism (PCR-RFLP) as reliable reference methods for *Staphylococcus* species identification in comparison to matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS), which can differentiate microorganisms based on their protein profiles. In this study, we identified 114 CNS isolates from bovine IMI at species level by MALDI-TOF MS as the definitive test. Based on previous studies, a cut-off score >1,7 was considered for species level identification. The identification obtained using this method was compared to results from the *gap* and *groEL* PCR-RFLP analysis. The partial *gap* and *groEL* genes (approximately 931 and 550 bp, respectively) were successfully amplified by PCR from ATCC control strains and CNS isolates from IMI. The size and number of the fragments obtained by *Alu*I digestions made possible to form distinctive *gap* and *groEL* PCR-RFLP patterns. Only 49% (56/114) of the species identification results obtained by *groEL* PCR-RFLP matched those obtained by MALDI-TOF MS, whereas 96,5% (110/114) of the species identification results obtained by the *gap* PCR-RFLP analysis matched those obtained by MALDI-TOF MS. Only three strains were misidentified, two *S. chromogenes* strains were assigned as *S. haemolyticus* and *S. warneri*, and one *S. xylosus* strain as *S. chromogenes*. In this way, the *gap* PCR-RFLP analysis could be a useful and reliable alternative method for the species identification of CNS isolates from bovine IMI and appears to be a more accurate method of species identification than the *groEL* PCR-RFLP.

(410) **STUDY OF THE JOINT ACTION OF STEROID HORMONES RECEPTORS IN THE MECHANISMS THAT CONTROL INFLAMMATORY RESPONSE**

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Inflammation is a vital defense mechanism against infections or damaged tissue. While this response is active, homeostatic processes that balance pro- and anti-inflammatory signaling pathways are induced. If unbalanced, these control mechanisms lead to deregulated inflammation. Studies about inflammatory response allowed the development of therapies against several disorders. Glucocorticoids have been widely used as immunosupressors and anti-inflammatory drugs. However, other steroid ligands and their receptors (e.g. LXRs) have been proved to act as modulators of the inflammatory response. Steroid receptors share structural and functional properties. Cross action between some of them was found in several tissues and cellular response. Nevertheless, there are currently no studies that evaluate whether GR and LXRs interact when activated in immune cells. In this work, we use as model the pro-monocytic human myeloid leukaemia cell line U937, which can be differentiated into macrophage-like cells in the presence of phorbol myristate acetate (PMA). Treatment with PMA stimulates the expression of several pro-inflammatory genes such as CD11b, IL6, IL8, TNF-α and COX2 ($p<0.05$). The presence of LXRs agonist GW3965 would not seem to affect the expression of these genes whereas the treatment with synthetic glucocorticoid dexamethasone (Dex) inhibits PMA-induction of these markers of differentiation ($p<0.05$). When U937 cells were treated simultaneously with PMA and both hormones we observed a lower inhibition of CD11b and IL6, both target of GR and LXRs, with respect to the treatment with Dex alone. However, inhibition of genes responding to Dex only (COX2 and TNF-alpha) was not affected by the presence of GW. When analyzing the abundance of receptors, we found that the expression of GR and LXR-β increased in presence of PMA. In contrast, LXR-α diminished under these conditions ($p<0.05$). Our results suggest a possible interference of LXRs, especially LXR-β, on the action of GR.

Keywords: Glucocorticoid Receptor, Liver X Receptor, Inflammatory response

(232) **FUNCTIONAL INTERACTION BETWEEN AQUAPORIN-1 (AQP1) AND EPITHELIAL SODIUM CHANNEL (ENaC). THEIR ROLE IN CELLULAR VOLUME AND BIOMECHANIC**

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AQP1 and ENaC are both expressed in vascular endothelium. Their functions in this tissue would not be related to their canonical functions. It has been published that ENaC acts as mechano-sensor stimulated by a non-genomic action of aldosterone hormone. In addition, we have reported that AQP1 permeability (P_f) is modulated by changes in membrane tension. Our aim was to study a possible functional interaction between AQP1 and ENaC which regulates cellular volume and biomechanic. The experimental model was *Xenopus laevis* oocyte expressing AQP1, ENaC or both protein by mRNA injection. We measured the osmotic response (OR) and the intracellular pressure (P_{IC}) of these phenotypes challenged by different hypotonic gradients during short times (minutes). Also, these phenotypes were treated with amiloride, an ENaC inhibitor, or aldosterone. In all conditions, intra and extracellular Na^+ remains in balance. We estimated the two classical parameters, P_f and water flux (J_w), to describe the OR obtained for the defined phenotypes. Both parameters were significantly greater in the AQP1-ENaC phenotype than the AQP1 one ($p<0,05$) and this phenomenon was independent of the value of osmotic gradient (OG). Interestingly, the experiments with amiloride showed that the OR-raise only occurred with a functional ENaC. Furthermore, aldosterone enhanced the OR and different P_f and J_w values were obtained comparing to the control ($p<0,05$). Regarding to the P_{IC}, we estimated the elastic module (ε), that reflects membrane stiffness. A significant difference ($p<0,0001$) in ε values between both AQP1 phenotypes was observed. Evenmore, since AQP1 P_f behaves as a variable parameter for high OGs, we observed an enhanced deviation from linearity in the AQP1-ENaC phenotype. As a conclusion, we proposed a functional interaction between AQP1 and ENaC where the sodium channel softens cellular membrane, turning it more permeable to water. This phenomenon is improved by aldosterone short action.

Keywords: AQP1-ENaC co-expression, osmotic response, membrane stiffness, non-genomic aldosterone action.

(422) **DUX4 NEGATIVELY REGULATES THE ACTIVITY OF THE HUMAN PROGESTERONE NUCLEAR RECEPTOR**

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The retro-transposed gene *DUX4*, at the human chromosome 4q35, encodes a transcription factor that regulates the expression of zygote activated genes in placental mammals. Our laboratory has demonstrated that DUX4 is a toxic pro-apoptotic protein underlying the pathogenesis of facioscapulohumeral muscular dys-

trophy (FSHD), the third most common form of inherited myopathy in humans. FSHD females, less affected than males, dramatically deteriorate after menopause or pharmacological treatment with tamoxifen. Interestingly, the C-terminal region of DUX4 possess a LLXXL amino acid motif, present in co-regulators of hormone nuclear receptors, suggesting a potential endocrine function of DUX4. In this report we explored if DUX4 is a co-regulator of the progesterone nuclear receptor (PNR). The activity of PNR was studied on cultured T47D cells, which endogenously expresses PNR, as well as in a reconstituted system in cultured HepG2 cells, transfected with a plasmid expressing the PNR. Two alternative reporters of the activity of the PNR were used: MMTV-Luc and 2XPRE-Luc. The potential co-repressor activity of DUX4 in these experimental systems was assayed using co-transfection with a plasmid expressing either wild-type, GFP fusions or mutant versions of DUX4. We found that DUX4 dramatically inhibits the transcriptional activating function of PNR in T47D and HepG2 cells expressing PNR. DUX4 mutants affecting its nuclear localization signals (NLS-1-2), or its homeodomains 1 and 2 (H1/H2-IWF), loose the repressor activity on the PNR. Taken together, our results indicate that DUX4 is a strong co-repressor of the PNR and that its nuclear location and/or its N-terminal region contribute to this activity. Although DUX4 is mostly considered a transcriptional activator, our results indicate that this protein could indirectly modulate gene expression by repressing the activity of a sex hormone NR.

Keywords: FSHD, DUX4, nuclear receptor, progesterone, co-repressor

(423) **SEX HORMONE PROTECTION OF DUX4-MEDIATED CELL TOXICITY IS OBSERVED IN DUX4-TRANSFECTED CELLS BUT NOT IN CELLS ENDOGENOUSLY EXPRESSING DUX4**

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DUX4 is a nuclear transcription factor that regulates the expression of zygote activated genes in placental mammals. We have proposed that aberrant expression of DUX4 contributes to the pathogenesis of facioscapulohumeral muscular dystrophy (FSHD) because: i) the *DUX4* gene is located at the FSHD1A, and ii) DUX4 is pro-apoptotic and cytotoxic when expressed in transfected cells. It has been observed that FSHD females, clinically less affected than males and presenting a higher proportion of asymptomatic carriers, show a clinical worsening of symptoms when underwent a rapid decline in estrogen levels (i.e., early menopause or anti-estrogenic treatment for breast cancer). Based on this observation, we hypothesized that sex hormones may be natural FSHD protective factors *in vivo*. In this report we explored the potential protective effect of sex hormones on DUX4-mediated cell death. A GFP-based co-transfection assay previously developed in our laboratory was used in this study. We found that physiological levels of estrogens in the culture medium protect HepG2 cells from the toxicity of DUX4. The protective effect was also observed when testosterone and progesterone were analyzed. We also explored if estrogens and progesterone show a protective effect on cells endogenously expressing DUX4. In these experiments, we used cultured immortalized human myoblasts (LHCNM2) expressing a DUX4 transgene under the control of the Tet-On/Off system. The potential protective effect of sex hormones was indirectly determined, measuring cellular contents of ATP (i.e., cell viability). In these studies, we observed that neither estrogens nor progesterone protect human myoblasts from the toxic effect of endogenously expressed DUX4. Physiological differences between HepG2 and human immortalized myoblasts models and/or between DUX4 expressed from transfected vectors versus endogenous transgene-mediated expression of DUX4, may explain these apparent contradictory findings.

Keywords: FSHD, myopathy, DUX4, myoblasts, sex hormones

(478) **CFTR CHLORIDE CHANNEL MODULATES THE MITOCHONDRIAL DYNAMICS**

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Cystic Fibrosis (CF) is caused by mutations in the gene *CFTR,* encoding a cAMP-activated chloride (Cl^-) channel. Previously, we have reported that the impairment of the CFTR channel activity leads to a variety of alterations, including differential gene expression and mitochondrial dysfunction. We found a reduced expression of the genes *MTND4* (mitochondrial gene) and *CISD1* (nuclear gene) in CF models that was later associated to a reduced mitochondrial complex-I activity (mCx-I). The aim of this work was investigating if the CFTR activity was involved in the mitochondrial dynamics regulation. The mitochondrial network morphology was studied in the CF cell line IB3-1 and compared with S9 or C38 cells that are IB3-1 cells with the CFTR activity restored. Mitochondria of these cells were labelled by the mitochondrial probe TMRM or transfecting plasmids pMito-YFP and pMito-Cherry. Mitochondria morphology was analyzed in cells with basal or AMPc stimulated for 24 h in serum-free medium by confocal microscopy. An increased ($p < 0.05$) small mitochondria population was observed in CF cells compared to control cells. The CFTR activity role in mitochondrial dynamics was then tested in C38 cells expressing pMito-YFP treated with two specific CFTR inhibitors (GlyH101 and CFTR(inh)-172) and analyzed by time series confocal microscopy in live-cells. The treatment with CFTR inhibitors showed an early (30 seconds) mitochondrial fragmentation induction ($p<0.05$). These data suggest that CFTR activity might be regulating the mitochondrial fission-fusion balance. Thus, the impairment of the CFTR activity could impair the renewal of the damaged mitochondrial components, explaining the mitochondrial disorders observed in CF. Supported by PIP 2015-2017, PUE 22920160100129CO and PICT 2012-1278 to TASC and PICT-2015-1031 to AGV.

Keywords: mitochondrial dynamics, CFTR, Cystic Fibrosis

GENETICS AND MOLECULAR BIOLOGY 14

(644) ***Serratia marcescens* TYPE VI SECRETION SYSTEM IS TRANSCRIPTIONALLY UPREGULATED BY RcsB IN RESPONSE TO THE ATTACK OF BACTERIAL COMPETITORS**

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Serratia marcescens (*Sma)* is an opportunistic human pathogen that represents a growing problem for public health. It has been reported that the type VI secretion system (T6SS) of *Sma* has a role in bacterial competition. We have previously showed that the T6SS of *Sma* is transcriptionally regulated by RcsB. The aim of this work is to analyze the RcsB-dependent regulation. Performing killing assays between *Sma* Db10 and *Sma* RM66262, we have determined that both strains were able to kill their wild-type counterparts but unable to kill the T6SS mutants (*tssM)*. Using a fusion to GFP as a reporter of the transcriptional activity of T6SS promoter, we have demonstrated that this activity increased when *Sma* RM66262 was challenged by *Sma* Db10. In contrast, *Sma* Db10 *tssM* was not able to induce the T6SS transcriptional activity. Lack of RcsB expression resulted in the inability of the T6SS promoter to be induced when challenged by *Sma* Db10. We also performed inter-species competition assays using *Acinetobacter baumannii* or *A. nosocomialis* as attackers. The wild-type strains were able to kill *Sma* and to induce the transcriptional activity in a T6SS-dependent manner. To determine the signal that activates the T6SS in *Serratia,* we designed different approaches to detect if there is a danger signal detected by the prey. Finally, we analyzed whether the action of bacterial envelope damaging agents were able to promote T6SS expression. None of these challenges induced T6SS activity, indicating that non-specific envelope damage or the presence of endogenous molecules released as the result of bacterial lysis or T6SS-provoked death does not suffice to

induce T6SS expression. Taken together, our results demonstrate that in *Serratia*, RcsB-controlled up-regulation of the T6SS over basal expression levels would constitute a survival strategy triggered by specific lethal threats posed by interbacterial competition.

Keywords: *Serratia marcescens,* Type VI secretion system, Rcs system, gene regulation.

(460) ***S. marcescens* EXPRESS THE FLAGELLUM INSIDE EPITHELIAL CELLS**

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Serratia marcescens (Sma) is an opportunistic human pathogen that represents a growing problem for public health, particularly in hospitalized or immunocompromised patients. Despite its clinical prevalence, factors and mechanisms that contribute to *Sma* pathogenesis remain unclear. *Sma* ability to adapt to and survive in both hostile or changing environments also relates to the bacterial capacity to express a wide range of secreted enzymes, including lipases, phospholipases, hemolysin, proteases and nucleases. In our previous work, we have shown that *Sma* is able to invade, persist, and multiply inside non-phagocytic cells, residing in nonacidic, nondegradative, autophagosome-like vacuoles. In these host cells *Sma* elicits an autophagic response. We determined that the hemolysin ShlA is responsible for the autophagic response that is promoted previous to the bacteria internalization in host epithelial cells. Recently, our group demonstrated that *Sma* is able to escape from infected non-phagocytic cells in a ShlA-dependent manner. We demonstrated that flagellar expression was essential for the bacteria to adhere to epithelial cells and that this contact was required for subsequent internalization in nonphagocytic cells, suggesting that *Sma* flagellar apparatus components can act as adhesins. In this work, we analyse the expression of flagella in the invasion process of *Sma* in non-phagocytic cells. Results of indirect immunofluorescence assay using either confocal or fluorescence microscopy showed that *Sma* is able to express flagella inside of epithelial cells. We here also analyse the ability to re-infect epithelial cells of the bacteria that escape from infected cells. Our results show that the bacteria that egress from infected cells increase up to 2-fold the capacity to invade naïve epithelial cells than planktonic bacteria. The results suggest that flagellar expression within infected cells are involved in the capacity of *Serratia* to successfully spread to new cells.

Keywords: *Serratia marcescens,* epithelial cells, flagella, bacterial spread.

(511) **PrtA METALLOPROTEASE EXPRESSION IS REGULATED BY CpxR AND CONTRIBUTES TO *SERRATIA MARCESCENS* BIOFILM FORMATION**

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Serratia marcescens (Sma) is an environmentally ubiquitous bacterium also acting as an opportunistic pathogen. Its ability to adapt and survive in either hostile or changing environments can be related to the expression of a myriad of secreted hydrolytic enzymes, including proteases. Genomic analysis of *Sma* clinical strain RM66262 identified four zinc-metalloprotease-encoding genes. Amongst them, we previously showed that PrtA is prominently secreted and its expression depends on the bacterial growth temperature, being transcriptionally upregulated at 30°C in comparison with 37°C. In this study, we found that CpxRA -a canonical TCS typically involved in counteracting envelope stress- represses PrtA expression in a temperature dependent manner. Secreted proteolytic activity by azocaseinase assay was 50%-increased in a *cpxR* background at 37°C of growth temperature, but no significant difference was observed at 30ºC. *In trans*-expression of CpxR restored the activity of the mutant strain to wild type levels. This modulation at 37°C is attained at the transcriptional level, as shown by comparative fluorescence measurements from a P_{prtA}-*gfp* reporter plasmid between wild type and *cpxR* mutant. Over-expression of outer membrane protein NlpE, a CpxRA-activating condition, diminished proteolytic activity at both growth temperatures (-64% at 30°C; -90% at 37°C) when compared with the control strain. Furthermore, we performed EMSA and DNAse I protection assays with radiolabeled DNA fragments and purified CpxR, and found that the regulator specifically binds to a previously *in silico*-predicted CpxR-binding site within *prtA* promoter region. Lastly, we examined if PrtA expression could influence biofilm control in *Sma.* Cristal violet assays indicated that *prtA* mutant is defective in biofilm production (-57% at 30°C; -30% at 37°C). In correlation with CpxR inhibitory action at 37 °C, *cpxR* inactivation enhanced 40% biofilm formation at 37 ºC, but no difference was observed at 30°C.

Keywords: *Serratia*; PrtA metalloprotease; CpxRA TCS; biofilm

(1154) REGULATION OF *Salmonella* BIOFILM DEVELOPMENT BY DIFFERENT MECHANISM ACTIVATION OF RCSCDB SYSTEM

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The RcsCDB regulatory system has an important role in the bacterial physiology, controlling the capsule synthesis and motility behavior. Previously studies showed that the RcsCDB system participate in biofilm development. Bacterial biofilms are complex communities consisting of microorganisms embedded in a self-produced extracellular matrix. The RcsCDB phosphorelay system participates in the response to extracytoplasmic stress signaling that affect the cell envelope and are responsible for the system activation. In this work we analyzed if different RcsCDB activation conditions affect the biofilm formation in *Salmonella* Typhimurium. We here investigated whether some of the RcsCDB activation conditions have the ability to regulate the synthesis of the pellicle in LB medium, producing red dry and rough (RDAR) morphotype on Congo red and brilliant blue agar plates, and in crystal violet binding assay. For these propose, we studied the biofilm phenotype in strains harboring the p*rcsB* and p*rcsC* plasmids, and in *tolB* and *rcsC11* mutants as RcsCDB activation conditions. Moreover, we measured the transcription levels of *bapA* gene in these strains in order to investigate whether the activation condition of RcsCDB phosphorelay could somehow be affecting the expression of this biofilm reporter gene. Taken together, our results demonstrated that the RcsCDB system activation modulates the *bapA* gene in a direct pathway, and consequently the *Salmonella* biofilm development.

Keywords: biofilm, RcsCDB system, *Salmonella*

(1165) **NEW FINDINGS ON THE INTERACTION BETWEEN RCSB AND SLYA TO CONTROL Salmonella Typhimurium VIRULENCE**

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The *Salmonella* Typhimurium RcsCDB system regulates the synthesis of colanic acid and flagellum as well as the expression of numerous virulence genes. We previously demonstrated that the *rcsC11* mutant, which constitutively activates the RcsB regulator, attenuates *Salmonella* virulence in an animal model. In addition, this attenuated phenotype could be also produced by deletion of the *slyA* gene. In this work we investigated if this antagonistic behavior is produced by the modulation of the expression of both regulator-encoding genes. We demonstrated that SlyA overproduction negatively regulates *rcsB* transcription. A bioinformatic analysis allowed us to identify a putative SlyA binding site on each of both promoters, P_{rcsDB} and P_{rcsB}, which control *rcsB* levels. In addition, we determined that SlyA is able to recognize and bind to these predicted sites to modulate the activity of both *rcsB* promoters. According to these results, SlyA represses *rcsB* transcription by direct binding to specific sites located on the *rcsB* promoters, thus accounting for the above antagonistic behavior. Moreover, we showed that the opposite effect between both regulators also physiologically affects the *Salmonella* motility phenotype. In this sense, we observed that under SlyA overproduction the P_{rcsB} is repressed and consequently bacterial motility

is increased. On the basis of these results, we suggest that during infection the different RcsB levels produced act as a switch between the *Salmonella* virulent or attenuated form. Thereby, we propose that higher concentrations of RcsB tilt the balance towards the attenuated form, while absence or low concentrations resulting from SlyA overproduction does it towards the virulent form.

Keywords: SlyA, RcsCDB system, *Salmonella*

(1401) **NOD1 AND ITS ROLE IN THE SURVIVAL OF S. TYPHIMURIUM INSIDE THE HOST CELL.**

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NOD1, a member of NOD-like receptor protein family and is a close relative of NOD2, is an intracellular pattern recognition receptor involved in recognizing bacterial peptidoglycan fragments that localize to the cytosol. NOD1 activation triggers inflammation, antimicrobial mechanisms and autophagy in both epithelial cells. FYCO1 (FYVE and coiled-coil protein 1) is a transport adaptor that binds to LC3 to mediate transport of late endosomes and autophagosomes along microtubules in the plus end direction. *S. enterica* serovar Typhimurium (S. Typhimurium) is an intracellular bacterial pathogen that infects phagocytic and nonphagocytic eukaryotic cells and resides in a specialized phagosomal compartment named the SCV. In previous work, the role of autophagy during *Salmonella* survival within the cell has been demonstrated. To determine the role of NOD1 in relationship with FYCO1 and LC3 in the survival of *Salmonella*, NRK-49F cells were transfected with pEGFP or pEGFP-NOD1, pEGFP-LC3, pEGFP-FYCO1 and the negative mutants and then infected with *Salmonella*. For Indirect Immunofluorescence cells were processed and following parameters were determined: number of infected cells, *Salmonella* intracellular multiplication and *Salmonella* intracellular distribution. The results showed that wild type or mutants forms of overexpressed proteins significantly affecting these parameters. In conclusion, the results presented here try to approximate a mechanism for the survival of *Salmonella* during its interaction with the autophagy process of the host cell.

Keywords: Salmonella, NOD1, Autophagy

(1744) ***Salmonella* PRESENT POPULATIONS WITH DIFFERENT METABOLIC STATES DURING INFECTION IN MACROPHAGES**

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(1) IBR. (2) CIML-Francia.

S. typhimurium is a Gram-negative pathogen that causes various host-specific diseases. During their life cycle, *Salmonellae* survive frequent exposures to a variety of environmental stresses, e.g. carbon-source starvation. The virulence of this pathogen relies on its ability to establish a replicative niche, named *Salmonella*-containing vacuole (SCV), inside host cells. However, the microenvironment of the SCV and the bacterial metabolic pathways required during infection are largely undefined. The objective of this work was analyses the metabolic state of the pathogen in different stages and conditions during infection. For this we developed different biological probes whose expression is modulated by the environment and the physiological state of the bacterium. We constructed transcriptional reporters by fusing promoter regions to the *gfpmut*3a gene, to monitor the expression profile of genes involved in glucose utilization and lipid catabolism. The induction of these probes by a specific metabolic change was first tested *in vitro*, and then during different condition of infection in macrophages, using flow citometry analysis. We were able to determine that Entner-Doudoroff is the main metabolic pathway utilized by *Salmonella* during infections in mouse macrophages. Furthermore, we found sub-populations of bacteria expressing genes involved in pathways for the utilization of different sources of carbon, including beta-oxidation and glycolysis. These populations are modified in presence of different metabolizable substrate and the initial metabolic state of the bacteria, suggesting the coexistence of *Salmonella* with diverse metabolic states during the infection.

Keywords: Salmonella, lipid metabolism, fluorescence probes, FACS, infection in macrophages

(642) **ENVIRONMENTAL AND GENETIC FACTORS AFFECTING THE EXPRESSION OF A BIOFILM-INDUCING TRANSCRIPTIONAL REGULATOR IN *Salmonella***

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Salmonella is a food-borne pathogen associated with animal and human infections ranging from gastroenteritis to enteric/typhoid fever, constituting an important problem for public health and causing important economic losses. The persistence, both inside and outside the host, relies on its ability to adjust its lifestyle to the different environmental conditions it faces. Many microorganisms, including *Salmonella*, respond to changes in their surroundings by switching their lifestyle from planktonic, motile, individual cells to a sessile, multicellular bacterial community embedded in a self-produced extracellular matrix, a biofilm, that allows them to support harsh conditions including nutrients limitation, resistance to antimicrobial agents, desiccation and disinfectants. In *S. enterica*, the two major components of the extracellular matrix are curli, a thin, coiled and aggregative fimbriae, and the exopolysaccharide cellulose. Production of these components depends on the master transcriptional regulator CsgD, which in turn is affected by cell growth stage and environmental stimuli through the action of other transcriptional factors, 3',5'-cyclic diguanylic acid, and small RNAs. We have previously identified a *Salmonella*-specific transcription factor important for both biofilm production and *csgD* transcription. Furthermore, a recent report shows that this factor is of relevance for the intestinal colonization of food-producing animals. To gain insight into this factor's expression, we generated a *lacZ* transcriptional fusion of its gene and analyzed its expression under different environmental conditions. Also, using the T-POP system to carry out a general screen for loci affecting its transcription, we identified 3 chromosomal regions whose mutations either activate or repress this gene. Altogether, these results define host-related conditions that trigger the activation of this regulator to induce biofilm and *Salmonella* intestinal colonization.

Keywords: *Salmonella*, biofilm, pathogenesis, transcriptional regulator

(523) **A *Salmonella*-SPECIFIC TRANSCRIPTIONAL REGULATOR CONTROLLING BIOFILM FORMATION AND VIRULENCE**

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Salmonellosis is among the most common foodborne diseases, with millions of human infections occurring worldwide every year. A key aspect of *Salmonella*'s life cycle that contributes to its high prevalence is its ability to form biofilms, bacterial communities embedded in a self-produced extracellular matrix that allows them to adhere to each other and to diverse surfaces. This multicellular behavior facilitates persistence and transmission between hosts, and survival in the environment. In *Salmonella*, the extracellular matrix is composed mainly by cellulose and curli fimbriae whose synthesis are controlled at transcriptional level through the expression of its master regulator, CsgD. Expression of this transcriptional activator is in turn finely regulated by several transcription factors that integrate different environmental signals. We identified a previously uncharacterized *Salmonella*-specific transcription factor, MlrB, that, according to *in silico* analysis, could participate in the control of biofilm-formation. Overexpression of the gene coding for this factor in different genetic backgrounds provoked marked differences in the characteristic *Salmonella* biofilm morphotypes, as well as on the expression of its target genes, including *csgD,* determined by the use of chromosomal reporter fusions to *lacZ*. Our results demonstrate that under specific conditions this *Salmonella* transcription factor affects biofilm-formation by controlling the induction of the Csg regulon, and hence in switching between planktonic and sessile lifestyles. In addition, we determined that it expression is maximal under conditions relevant

to the infection, which is in agreement with the regulation observed for members of the SPI-2 locus, allowing us to postulate it as a link between the biofilm formation and *Salmonella* pathogenesis.

Biofilm – *Salmonella* - Transcription Factors

IMMUNOLOGY (ADAPTATIVE IMMUNITY) 4

(1326) MATERNAL STRESS DURING PREGNANCY ENHANCES ALLERGIC AIRWAY INFLAMMATION RISK IN THE OFFSPRING

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RATIONALE: Allergic asthma is increasing worldwide. The presence of atopic diseases in the mother propagates the onset of allergic diseases in the offspring considerably stronger than atopic diseases of the father. Such observation challenges genetic predispositions as the sole cause for allergic diseases. Epidemiological studies suggest that caregiver stress in the perinatal period may predispose offspring to asthma. At the moment only three groups have studied the link between prenatal stress and asthma severity or susceptibility in murine models. To further study this phenomenon, we aimed to establish a mice model of maternal stress and neonatal asthma susceptibility. METHODS: Pregnant BALB/c mice (day 15) were subjected to a single restraint stress exposure. On day 4 after birth, pups were treated with a suboptimal sensitization protocol (single i.p. injection of ovalbumin (OVA) in alum) prior to antigen aerosol challenge (days 12-14) and evaluation of allergic airway inflammation (day 16). Negative controls included pups of non-stress dams subjected to the same suboptimal protocol or i.p. sensitized and aerosol challenged with PBS. RESULTS: Offspring of stress, but not control dams, showed increased eosinophils infiltrate in bronchoalveolar lavage ($p<0.05$). Perinatal stress resulted in pathological changes of pulmonary allergic inflammation. These changes included eosinophils and mononuclear cell infiltration around airways and vessels and goblet cell hyperplasia. An increase in serum anti-OVA IgE antibodies was detected in pups of stress, but not of control dams ($p<0.05$). This increase was accompanied by high levels of IL-4 and IL-5 in bronchoalveolar lavage fluid ($p<0.05$). CONCLUSIONS: Maternal stress during pregnancy resulted in an increase of litter susceptibility to develop allergic lung inflammation.

Keywords: asthma risk, stress, maternal effect

(178) IMMUNIZATION SCHEDULES AND AVIDITY ASSAY AGAINST RESPIRATORY BOVINE DISEASE BACTERIA IN CATTLE

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Livestock production is exposed to many adverse conditions which threaten animal health as the Respiratory Bovine Disease (RBD). Although commercial vaccines are employed, protective immune responses are not well understood and immunodiagnostic methods are not well defined. This work aimed to employ an indirect in-house ELISA and develop an avidity assay to study bovine immune response against the bacterial agents: *Pasteurella multocida* (PM), *Mannheimia haemolytica* (MH), *Histophilus somni* (HS).

Freshly weaned 2-month calves were employed in 2 different immunization schedules. The 1st plan (n=10) received 3 doses (every 21 days) of the commercial vaccine BiopoligenHS (CV) or an experimental vaccine (EV) formulated with inactivated culture supernatant of PM and aluminum. The 2nd plan (n=7) was immunized with 2 doses (days 0 and 21) employing the CV or an EV either with PM or MH formulated with a polymeric adjuvant, Montanide Gel01. A control cow inoculated only with adjuvant was included in both schedules. Calves were bled on days 0, 21, 42 and 60, depending the schedule. Specific IgG and IgM were measured by ELISA, and for the avidity assay an adaptation was tested employing urea as dissociating agent.

In the 1st plan, IgG levels were higher for CV compared to PM supernatant and with the placebo ($p<0,05$). Avidity assay showed differences between immunized groups and placebo ($p<0,05$). In the 2nd plan, Montanide group showed higher tendency IgG levels against PM and MH than the CV (ns). PM Montanide showed higher avidity index than the rest ($p<0,05$). Surprisingly, anti-HS IgG of vaccinated calves were similar to control cows. No differences were observed in IgM levels for the 3 bacteria in any plan. Our results show that different formulations and immunization schedules could be used to improve the immune response against bacteria causing BRD.

Analyzing bovine immune response would help to understand how vaccines could be modified and reduce new RBD outbreaks.

Keywords: Respiratory Bovine Disease, ELISA, Antibodies, Avidity, Vaccines

(357) INTERLEUKIN 17A (IL-17A) SHOULD BE INVOLVED IN CHANGES OF ANTIBODY SPECIFICITY

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Introduction**:** Lactate dehydrogenase elevating virus (LDV) is a persistent and non pathogenic RNA arterivirus that induces NK, macrophages and B- cell activation in mice. It was found that LDV-infection modified Ab specificity to different antigens. This effect was correlated with the release of various cytokines after viral infection.

Objectives**:** The purpose of this work was to explore the role of interleukin 17A (IL-17A) on the changes of Ab specificities.

Methods**:** C57BL/6 mice were inoculated subcutaneously with 25 µg of OVA emulsified in phosphate-buffered saline (PBS) and Complete Freund's Adjuvant. At day 15, the mice were boosted with the Ag in Incomplete Freund's Adjuvant. At days 4, 7 and 11, half of the animals were inoculated intraperitoneally with 150 μg of anti-IL-17A MAb (MM17F3) in 200 μl of PBS. Another mouse group were treated as before but infected with 2×10^7 50% infectious doses of LDV in saline at day -1. Bleeding was performed at days 8, 21, and 30. Serum lactate dehydrogenase (LDH) was determined enzymatically. The titer of Ab anti-OVA was determinated by ELISA, whereas the proportion of Ab directed to native OVA epitopes was calculated by ELISA competition assays.

Results**:** MAb to IL-17A decreased the plasmatic LDH levels induced by LDV (3668±547 and 2900±175 U/L, for control and MAb treated mice, respectively, $P<0.05$).

Titers of anti-OVA Ab in LDV-infected animals decreased by MAb treatment (1/167000±1/12600 to 1/68000±1/4700, for control and MAb treated mice, respectively, $P<0.001$). Besides, percent of native anti-OVA Ab in non-infected mice increased by treatment with the MAb (48 to 61%, respectively). Same effect was shown in LDV-infected mice (65 to 87%, for control and treated animals, respectively)

Conclusions: Results suggested that IL-17A is involved in mechanisms leading to changes of Ab specificity to a given antigen.

Keywords: LDV, IL-17A, Ab specificity, Native epitopes

(894) *LACTOBACILLUS RHAMNOSUS* ENHANCES ADAPTIVE IMMUNE RESPONSE ELICITED BY INFLUENZA VACCINE AND INFLUENZA VIRUS INFECTION IN MICE

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We demonstrated previously that nasal administration of viable (Lr05) or heat-killed (HK05) *Lactobacillus rhamnosus* CRL1505 protects mice against influenza virus (IFV) infection by stimulating respiratory innate immune response. In this study, we evaluated whether Lr05 and Hk05 treatments modulated respiratory and systemic anti-IFV adaptive immune response. We also studied the induction of humoral responses after intranasal immunization of mice with H1N1 split vaccine using Lr05 and HK05 as adjuvants. 6-week-old BALB/c mice were treated with Lr05 or HK05 nasally for 2 days (d). Treated and untreated control mice were nasally challenged with IFV. A 2nd group of mice were nasal immunized with H1N1 split vaccine added with Lr05 or HK05 as adjuvants. In the infection model, levels of respiratory and serum IFN-γ, IL-4, IL-17, IL-10, anti-IFV IgG and IgA were evaluated after IFV challenge. As expected, IFV increased the levels of several cytokines in serum and BAL (Bronchoalveolar lavage). Lr05 and HK05 increased IFN-γ and IL-10 when compared to controls, being Lr05 more effective than HK05 to induce those effects ($p<0.05$). Levels of IgA, IgG were detected from 5d post-infection and they increased until d15. Lr05 and HK05 treated mice showed higher levels of respiratory anti-IFV IgA than control animals ($p<0.01$). Further, both treatments significantly enhanced BAL and serum specific IgG after IFV challenge. In the nasal vaccination model, neither Hk05 nor Lr05 increased IgG serum levels but Hk05 increased IgA levels in BAL after vaccination. These results demonstrate that nasally administered Lr05 and HK05 may improve humoral respiratory adaptive immune responses against IFV infection. Hk05 is as effective as Lr05 as an adjuvant, which offers advantages such as its safer use in immunocompromised hosts, in which the use of live bacteria might be dangerous, a longer product shelf-life, and easier storage and transportation.

Keywords: Influenza virus, nasal, adjuvant.

(1137) **IMPACT OF GALECTIN-GLYCAN INTERACTIONS IN THE PHYSIOLOGY OF FOLLICULAR HELPER T LYMPHOCYTES**

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Galectin-1 (Gal1), a glycan-binding protein that recognizes N-acetyllactosamine residues in membrane glycoproteins and the extracellular matrix, is a key regulator of immune cell homeostasis. It has been demonstrated that different effector profiles of helper T cells, exhibit a specific glycosylation profile and a differential susceptibility to Gal-1 binding; being those of pro-inflammatory nature (Th1 and Th17) preferential targets of this lectin and those of a Th2 or Treg nature, resistant to this endogenous lectin. However, it is still unknown how this lectin affects the fate and function of follicular helper T cells (Thf), key cells in the development of the humoral responses. These cells are essential for germinal center formation, production of high affinity antibodies and differentiation of long-lived plasma cells and memory B cells. Here we aimed to study the role of Gal-1-glycan interactions in the biology of Thf. An *In vitro* differentiation protocol was set up by stimulating splenic naïve CD4+ T cells from WT and Gal1-deficient (*Lgals1* -/-) mice, with recombinant IL-6 and IL-21 and neutralizing antibodies against IL-4, IL-12, IFN-γ, and TGF-β. To study Thf cells *in vivo*, we immunized mice with BSA-TNP subcutaneously and, after 7 days, analyzed the Thf cell population as CD4+ CXCR5+ PD1+ cells in draining lymph nodes. We found that after immunization, *Lgals1-/-* mice displayed higher percentage of Thf cells than WT mice. In addition, Thf cells showed a particular glycosylation profile characterized by increased poly-lactosamine residues and beta-1,6-branched complex N-glycans, consistent with a glycophenotype permissive for Gal1 binding. These results suggest that Gal1 may control the fate and function of Thf cells during the development of antibody-mediated responses.

Keywords: Galectin-1, Follicular T helper cells, Glycobiology.

(1173) **LIMITED IL-2 BIOAVAILABILITY DURING PEDIATRIC RSV INFECTION**

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It is well known that Respiratory Syncytial Virus, despite having slight antigenic variation, repeatedly infects throughout life reflecting a defect in the development of immunological memory. In addition, we have reported that severe RSV infection of infants induced a dramatic reduction in the frequency of peripheral blood regulatory T cells (Tregs). Because IL-2 is crucial for both, the development of T cell memory and the maintenance of Tregs, we analyzed whether the production and/or the availability of IL-2 was affected in RSV-infected children (RSV-ch).

Here, we aimed 1) to analyze if IL-2 addition increased the expression of FOXP3 in CD4+ T cells from RSV-ch; 2) to determine the ability of CD4+ T cells from RSV-ch to produce IL-2 upon *in vitro* stimulation; 3) to analyze the serum levels of soluble IL-2Rα (sCD25); and 4) to explore the response of CD4+ T cells from RSV-ch to sCD25.

We observed that incubation of CD4+ T cells from RSV-ch with IL-2 (20 ng/ml) markedly increased the frequency of CD4+FOXP3+ T cells ($p<0.001$; n=29). This effect was associated to a significant enhancement of Stat5 phosphorylation ($p<0.01$ for untreated vs IL-2-treated CD4+ T cells; n=10). Moreover we detected that CD4+ T cells from RSV-ch produced lower amounts of IL-2 compared with healthy donors ($p<0.01$; n=11). We also found not only a significant higher expression of CD25 on CD4+ T cells ($p<0.01$) but also elevated serum levels of sCD25 in RSV-ch ($p<0.001$; n=20). Finally we observed that recombinant sCD25 significantly ($p<0.01$) blocked the IL2-dependent Stat5 phosphorylation and decreased FOXP3 expression in CD4+ T cells from RSV-ch.

Collectively our results show that CD4+ T cells from RSV infants were sensitive to IL-2 action. However a deficient ability of these cells to produce IL-2 joined with high levels of sCD25 might affect the bioavailability of IL-2 explaining, at least in part, the depletion of Tregs and the fail to induce long lasting immunity.

Keywords: Immune response, Children, RSV, Tregs cells, IL-2.

(678) ***B. abortus*** **RNA INDUCES MHC-I RETENTION IN THE GOLGI APPARATUS VIA TLR8 AND BY DISRUPTING THE ACIDIFICATION OF THIS COMPARTMENT**

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Despite the cytotoxic $CD8^+$ T cell responses elicited by *Brucella abortus*, this pathogen is able to survive inside macrophages and generate a chronic infection. *B. abortus* infection of human monocytes down-modulates the IFN-γ-induced MHC-I cell surface expression by retaining these molecules in the Golgi apparatus (GA). We have recently demonstrated that *B. abortus* RNA is the bacterial component involved in this phenomenon. Thus, the aim of this study was to further characterize the receptor and mechanisms implicated in MHC-I down-modulation. Endo/phagolysosomal Toll-like receptors (TLR) 3, 7 and 8 are the most known receptors capable of recognizing RNA. We had previously discarded TLR3 consequently, to study whether TLR7 and/or TLR8 were involved in the *B. abortus* RNA-mediated MHC-I down-modulation, THP-1 cells or murine bone marrow macrophages (BMM) were treated with human TLR-7 or TLR-8 agonists in the presence of IFN-γ for 48 h. Then, the expression of MHC-I molecules was evaluated by flow cytometry. Surpringsily, TLR8 ($p<0.05$) but not TLR7 was the receptor involved in this phenomenon. Mice do not have a functional TLR8 instead TLR7 performs its function. To confirm that TLR7/8 was the receptor linked to MHC-I down-modulation, TLR7 KO BMM were infected with *B. abortus* or treated with its RNA. In both cases, MHC-I down-modulation was abolished as well as the antigen presentation

to CD8+ T cells. Concerning the retention mechanism, we confirmed that neither MHC-I protein degradation nor a modification of its mRNA expression was involved. Rather, we demonstrated that the ionophore monensin (which impedes the proper acidification of GA cisternae) mimicked *B. abortus* RNA-induced retention of MHC-I in GA ($p<0.05$). Overall, these results indicate that *B. abortus* RNA, viaTLR8 and probably due to an inhibition of Golgi acidification, inhibits MHC-I expression. Thus, bacteria can hide within infected cells and avoid the immunological surveillance of cytotoxic CD8+ T cells.

Keywords: *B. abortus*, RNA, MHC-I, Golgi apparatus, Evasion strategies

(1438) **NONALCOHOLIC FATTY LIVER DISEASE: RESISTIN DIFFERENTIALLY REGULATES T CELL ACTIVATION AND REACTIVE OXYGEN SPECIES LEVELS**

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Resistin (RES) is a cytokine which plasma concentration has been found elevated in Nonalcoholic Fatty Liver Disease (NAFLD) patients. Although human RES is produced by immunological cells, its effect on them is poorly understood. We have previously demonstrated that RES decreases CD69 expression in activated T cells from controls (Co) but not from NAFLD patients. We aimed to evaluate RES-mediated modulation of CD25 in T cells from NAFLD patients and Co and RES ability to modulate reactive oxygen species (ROS) production in peripheral blood mononuclear cells (PBMC). PBMC were obtained from NAFLD (n= 9) patients and Co (n= 14). Isolated T cells were activated with coated anti-CD3 (3 ug/ml) +/- RES (10 ng/ml) for 72 h, stained with anti-CD4, -CD8 and -CD25 mAbs and evaluated by flow cytometry (FC). To evaluate ROS levels, PBMC were incubated with or without RES (20 ng/ml) for 24 h, stained with 2´7´-dichlorofluorescin diacetate (DCFH-DA) and anti-CD3, -CD4, -CD8, -CD14 mAbs and studied by FC. As a preliminary approach, monocytes oxidative burst was stimulated with PMA (100 ng/ml) +/- RES and ROS production was evaluated by DCFH-DA. Mann-Whitney and Wilcoxon paired tests were used. RES decreased CD25 expression in activated T cells from Co but not from NAFLD patients. As a result, CD25 expression is higher in CD4+ ($p<0.01$) and CD8+ ($p<0.05$) activated T cells from NAFLD patients. RES decreased ROS levels in monocytes ($p= 0.031$), CD4+ ($p= 0.014$) and CD8+ ($p= 0.008$) T cells only from Co. NAFLD patients showed higher ROS levels than Co in CD4+ ($p<0.05$) and CD8+ ($p<0.05$) T cells. The presence of RES prevented ROS production when oxidative burst was induced by PMA stimulation. Similar to CD69 activation marker, RES can modulate CD25 expression and ROS levels in T cells from Co but not from NAFLD patients. Thus, NAFLD patients may have an alteration in RES signaling pathway which might contribute to NAFLD progression through ROS production and/or T cell-mediated injury.

Keywords: Resistin, Nonalcoholic Fatty Liver Disease, T cell activation markers, ROS.

IMMUNOLOGY (IMMUNOTHERAPY) 3

(68) **THE GLYCOSYLATION OF A CHIMERIC anti-rhIFN-α2b ANTIBODY PRODUCED IN DIFFERENT CELL LINES INFLUENCES ITS NEUTRALIZING ACTIVITY**

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The monoclonal antibodies constitute a large subset of the marketed biotherapeutics, most of which are glycosylated, and thus produced in mammalian cells. These molecules are bifunctionals, since the variable (V) regions are responsible of antigen binding and the constant (C) regions confer effector properties. However, this immunological dogma is in revision because several studies suggest that C regions of different class or subclasses of antibodies with identical V regions, influence the antigen binding activity Also, despite the glycosylation pattern strongly influences the antibody effector functions, this feature always was considered not to be important for binding antigen ability. In this work, we studied the impact of the different cell lines on the the affinity constant and antigen neutralizing ability of a chimeric anti hIFN α2b murine single chain Fv fused to Fcg1 (scFv Fc). The proteins, produced by CHO K1, HEK293 and NS0 cells showed no significant differences in the affinity constant measured by competitive ELISA. In spite of this parameter, the in vitro IFN neutralizing ability of the antibodies was higher for the molecule produced by CHO cells. In fact, the neutralizing activity of the same deglycosylated protein was considerably reduced. The present study invites us to critically discuss the choice of the cell line to produce biotherapeutic antibodies.

Keywords: antibody constant region; neutralizing activity; affinity constant; different producing cell lines, glycosylation

(341) **THE ANTI-MELANOMA THERAPEUTIC VACCINE CSF-470 CAN BE CRYOPRESERVED WITH TREHALOSE PLUS HUMAN SERUM ALBUMIN FOR LONG TERM STORAGE**

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The therapeutic vaccine CSF-470 is a mixture of four lethally irradiated melanoma cell lines administered with BCG and GM-CSF, that has demonstrated a significant benefit in the distant metastasis-free survival for high risk melanoma patients in a phase II Clinical Trial as compared to IFN-α2b (CASVAC-0401). Currently, CSF470 vaccine irradiated cells are frozen using dimethyl sulfoxide as a cryoprotectant and stored in liquid N_2 (DMSO) until its use. Prior to inoculation, vaccine doses must be thawed under sterile conditions, washed to remove DMSO and resuspended until clinical administration. *Methods:* To facilitate CSF-470 pharmaceutical production and distribution we designed an alternative preservation of CSF470 while keeping its biological and immunogenic properties. We tested comparatively different freezing conditions using cryopreservants suitable as excipients in the final product, and analysed the feasibility of vaccine freeze-drying. *Results*: Loading with trehalose during 5h culture in serum-free medium and the use of 0.2M trehalose in phosphate buffer saline plus 30 mg/ml human serum albumin (TRE) allowed cryopreservation of CSF-470 at -84ºC for at least 1 yr. After thawing, DMSO and TRE vaccine doses conserved cell integrity (trypan blue exclusion; electron microscopy). Persistence of melanoma associated Ags (FACS; western-blot) and the proportion of apoptotic/necrotic cells (Annexin-V/ IP staining) were also comparable. TRE and DMSO vaccine doses were equally captured by dendritic cells (DC), induced their maturation and allowed vaccine-derived Ags cross-presentation to specific T cells clones to release IFN-g. CSF-470 vaccines release ATP for up to 48h after thawing, a DAMP that may have an impact on DC activation. Freeze–drying of TRE vaccine doses did not allow conservation of vaccine properties. *Conclusion:* CSF-470 vaccine doses can be properly cryopreserved by freezing in TRE medium for future production.

Keywords: melanoma, vaccine, immunology.

(808) **KUNITZ TYPE MOLECULE-BASED VACCINE ELICITS ELEVATED SPECIFIC IGGS AND IGA ANTIBODIES AND TH1/TH17 RESPONSES AGAINST *FASCIOLA HEPATICA***

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Fasciola hepatica is a helminth parasite with a worldwide distribution, which can cause a chronic liver disease: fasciolosis, leading to economic losses in the livestock and public health in many countries. The prevailing control strategy based on anthelmintic drugs is unsustainable due to widespread resistance; hence vaccination appears as an attractive option to pursue. In our laboratory we study a vaccine formulated with Kunitz type molecule (KTM), an inhibitor of serine proteases with a key role in the parasite survival, and CpG-ODN/Coa-ASC16, an adjuvant with capacity to induce Th1 and Th17 responses.

Previous results showed the efficacy of the vaccination in mice which generates a reduction in the liver damage produced by the infection. The aim of this work was to investigate the immune response developed during KTM/CpG-ODN/COA-ASC16 immunization

Animals were allocated in four groups of 4 each. BALB/c mice were subcutaneously inoculated with 10 µg of KTM in CpG-ODN/Coa-ASC16 on days 0-7-14. Control animals were inoculated the same days with CpG-ODN/Coa-ASC16 or PBS. On day 21, all animals were orally infected with 6 *F. hepatica* metacercariae. An additional group without treatment or infection was including as control. After 4 days of challenge the mice were euthanized.

Mice vaccinated with KTM/CpG-ODN/Coa-ASC16 showed high levels of specific IgG1, IgG2a and IgA in plasma (Student test <0,05) compare with another groups. In addition, Increased IgA titers in intestinal lavage and feces (student test p<0,05) were observed. Igs detection were performed by ELISA

Splenocytes from KTM/CpG-ODN/Coa-ASC16 immunized mice, KTM restimulated, secrete significant amounts of IL-17 and IFN-γ (Student test, p<0,05) by ELISA.

These data suggest that the presence of elevated Th1-Th17 mixed responses and the high levels of IgGs and mucosal IgA antibodies, would be involved in the protection observed by KTM/CpG-ODN/Coa-ASC16 vaccination.

Keywords: *Fasciola hepatica*, Vaccine, Kunitz type molecule, CpG-ODN/Coa-ASC16, IgA

(817) **NANOEMULSION OF *Minthostachys verticillata* ESSENTIAL OIL. EVALUATION OF BIOCOMPATIBILITY AND ADJUVANT ACTIVITY OF THE IMMUNE RESPONSE**

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This study determined the morphology, biocompatibility and adjuvant effect of *M. verticillata* essential oil (EO) nanoemulsion. Morphometric parameters were studied by transmission electron microscopy (TEM). Wistar rat lymphocytes were exposed to EO nanoemulsion (0.3, 0.6, 30 and 60 ug/ml) for 24 h and viability was determined by trypan blue test. Balb/c mice (n=4 per group) were immunized (day 0) and revaccinated (day 14 and 28) as follows: Group 1:100 µl PBS; Group 2: 100 µl ovalbumin (OVA) 0.2 mg/ml; Group 3: OVA + $Al(OH)_3$ 0.5 mg/ml; Group 4: OVA+EO(2.5 mg/ml) nanoemulsion. Seven days after revaccination, serum samples were collected and analyzed for antigen-specific total antibodies (Ab) by indirect ELISA. For delayed-type hypersensitivity (DTH) test, animals (n=2 per group) were immunized by subcutaneous injection on days 0 and 14. Seven days after immunization animals were challenged with OVA by intradermal injection in the left footpads. The thickness of hind footpads was measured before and 24 and 48 h after the OVA injection. The difference in thickness of footpads after and before the inoculation was analyzed. TEM images show regular spheres of approximately 8.33±1.1 nm. EO nanoemulsion was not cytotoxic for rat lymphocytes at any evaluated concentration. A significant increase in Ab levels was observed in the group 3 respect to group 2 (p<0.001). However, in the group 4 no significant levels of Ab were detected. A significant increase in footpads thickness was observed in the groups 3 and 4 respect to group 2 (p<0.001; p<0.01, respectively) at 24 h by DTH test. A significant increase in footpads thickness of group 4 respect to groups 2 and 3 was observed (p<0.01) at 48 h. EO nanoemulsion at this concentration did not acts as an adjuvant of humoral immune response. However, it activated cellular immunity with greater potency than $Al(OH)_3$. Higher doses of EO nanoemulsion are being tested to evaluate the optimal dose as adjuvant.

Keywords: *Minthostachys verticillata*, essential oil nanoemulsion, biocompatibility, adjuvant, humoral and cellular immunity

(985) **SYNERGISTIC ANTITUMOR EFFECT OF ANTI-PD-1 WITH AN IMMUNOTHERAPY BASED ON STAT3 BLOCKADE**

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Stat3 is constitutively activated in diverse cancers and acts as a critical mediator of tumor immune evasion. We described in murine breast cancer (BC) models, that blockade of Stat3 activation induces senescence and that immunization of mice with irradiated Stat3-blocked BC cells inhibits tumor growth. Our objectives were to study the secretome induced by Stat3 blockade and to develop an immunotherapy (IT) based on the supernatant (SN) from Stat3-blocked cells. We observed that knockdown of Stat3 with siRNA increased senescence markers in BC, colon cancer and melanoma models. Then, we used the serum-free SN from 4T1 (BC) or B16-OVA (melanoma) cells transfected with Control siRNA (SN-Control) or Stat3 siRNA (SN-Stat3) as an adjuvant of a cellular vaccine with irradiated wild-type tumor cells. Therapeutic IT with SN-Stat3 in mice bearing 4T1 or B16-OVA tumors decreased tumor growth (51%, p<0.001 and 65%, p<0.0001 respectively vs. SN-Control). In 4T1 tumors, we also observed a decrease in pulmonary metastasis (70%, p<0.05 vs. SN-Control) and an increase in activation of NK cells and CD4 T cells vs. SN-Control (P<0.05). To enhance the effect of the SN-Stat3 IT we combined it with immune checkpoint inhibitors. The combination of SN-Stat3 with an anti-PD-1 antibody improved the antitumor effect by decreasing 55% B16-OVA tumor growth vs. SN-Stat3 (p<0.05) and 84% vs SN-Control (p<0.001). Next, we characterized the components of SN-Stat3. With a multiplex cytokine array in SN-Stat3 of 4T1 and B16-OVA cells, we detected an increase in many cytokines including IP-10, MIP1αβ, RANTES and TNFα (P<0.05 vs. SN-Control). Using SILAC-based quantitative proteomics in secreted proteins, we found enrichment in proteins involved in cell adhesion, metabolic and immune system processes. Altogether, these data demonstrate that cytokines and proteins released from Stat3-blocked tumor cells can be used to formulate an effective adjuvant to enhance the antitumor effect of PD-1 antibodies.

Keywords: Stat3; Senescence; Immunotherapy; PD-1

(986) **M-TYROSINE EFFECT ON IMMUNOSUPPRESSION MURINE MODELS**

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IMEX-CONICET-ANM

Meta-tyrosine (m-tyr) is an isomer of tyrosine with anti-metastatic effects. This could be explained, in part, by the modulation of the inflammatory context in an immunosuppression (IS) state. We have studied the m-tyr effect on the immune response and the associated inflammatory context in two IS murine models. In the first one, BALB/c mice were immunosuppressed with increasing doses of LPS and treated with m-tyr. The second model consisted in the administration of m-tyr in advanced metastatic LMM3 tumor-bearing mice when IS was established and the subsequent challenge with a single dose of LPS. Humoral immune response, after immunization with sheep erythrocytes, was evaluated through a hemagglutination assay, citokines production by ELISA and splenic cell populations by flow cytometry. M-tyr enabled a significant increase of the anti-

bodies titre when it was administered simultaneously and at the end of the schedule of LPS-induced IS. [Mean ± SEM, IS=4,78±3,1%; IS-mtyr=68,25±34,6% p<0,05]. M-tyr did not modify neither the % nor the number of splenic lymphocytes, monocytes, and neutrophils in an IS context. M-tyr induced a slightly but not significant reduction of PDL-1 expression in CD11b+ population [Mean±SEM, IS=132,5±24,1*; IS-mtyr= 71,35 ± 19,7#; control=72,49±4,5 *p<0,05 vs control #p=0,09 vs IS]. Mice treated simultaneously with m-tyr and LPS showed a significant decrease of TNF-α in plasma post the first dose of LPS [Mean ± SEM, TNF-α (pg/ml): LPS=2425±678,2; M-tyr-LPS=392,8±46,3; p<0.05] and a significant increase of IL-10 [Mean ± SEM, IL-10 (pg/ml): LPS=1912±362,1; M-tyr-LPS=4366±641,1; p<0.05]. Conversely, m-tyr induced a significant increase of TNF-α post LPS in tumor-bearing mice [Mean ± SEM, TNF-α (pg/ml): LMM3-LPS=266,6±2,2; M-tyr-LMM3-LPS=4009±255,1; p<0.001]. M-tyr treatment not only prevents IS, but disarticulates IS already established. M-tyr could have a dual role as a modulator of inflammatory context and, consequently, on the immune response.

Keywords: Immunosuppression; Meta-tyrosine; LPS; Tumor**;** Inflammatory response.

(1199) **ORAL ADMINISTRATION OF SOLUBLE B-GLUCANS EXTRACTED FROM GRIFOLA FRONDOSA MUSHROOM (MAITAKE D-FRACTION PRO4X) INDUCES IMMUNE T CELLS RECOVERY AND REVERTS IMMUNOSUPPRESSION IN BALBC MICE.**

Diego Máximo Aguilera Braico, Gabriela Andrea Balogh
CONICET

In order to investigate if Maitake D-Fraction Pro4X, rich in beta-glucans, are able to

recover the immunosupression state in the immune cells population, we perform a

study in female imunodepleted-BALBc mice employing flow cytometry. We detected immune cells expressing CD3ε, CD19, CD105 and Ly6G markers. After immune depletion by oral administration of Dexametasone (0.15mg/kg) during 8 days, BALBc mice were separated into 3 groups: one group were used as control (immunodepleted) and the other two receive oral administration of 5mg/kg Maitake -Fraction (Maitake Pro4X) (Mushroom Wisdom, Inc, N.J, USA) alone or in combination with Verapamil (10mg/kg) during 4 weeks. After treatment, mice were sacrificed and immune cells were isolated from spleen and lymph nodes. Using flow cytometry, we study T-Lymphocytes (CD3ε), B-Lymphocytes (CD19), granulocytes (Ly6G) and monocytes/macrophages (CD105) immune cells population. Results shown that Maitake Pro4X significantly recover the level of depleted CD3ε cells from 6.80 ± 7.08 % to 21.09 ± 12.39 %, with p<0.01 in lymph nodes as in spleen as well from 16.68 ± 9.17% to 27.79 ± 3.77%, with p<0.01. No significant differences were found with Verapamil treatment compared to Maitake (27.04 ± 29.96%, p>0.05). However, Maitake Pro4X was not able to recover the level of B-Lymphocytes in lymph nodes from 0.00 to 1.10 ± 1.67% or in spleen from 0.953 ± 0.767 to 1.34 ± 0.43% with p>0.05. By another side, we found that Maitake Pro4X was able to recover significantly the level of granulocytes (from 3.88 ± 3.55 to 27.18 ± 9.57 with p<0.05) in lymph nodes but not in spleen. Not significant differences were observed in the level of monocytes/macrophages (CD105) at any assayed condition. In conclusion, the study indicates that oral administration of Maitake Pro4X enhanced the level of T-Lymhocytes and granulocytes recovery after immunosupression. These effects were extracellular calcium-independent.

Keywords: Maitake D-Fraction, immunosuppression, T-lymphocytes, immunomodulation.

(1402) **STUDY OF 7-OXO-DEHYDROEPIANDROSTERONE AS POTENTIAL ADJUVANT FOR THE TREATMENT OF PULMONARY TUBERCULOSIS.**

Maria Belen Vecchione

Tuberculosis (TB) affects one-third of the world's population, but BCG vaccine, the prophylactic available against *Mycobacterium tuberculosis* (*Mtb*), does not prevent pulmonary TB in adults. Understanding the immunopathogenesis of TB would facilitate the choice of new immunotherapeutic agents. We focus on the role of DHEA and its metabolite 7-oxo-DHEA in the context of HIV-TB coinfection. We previously reported HIV-TB patients exhibited upper plasma levels of these hormones and that 7-oxo-DHEA positively correlated with absolute CD4+ T cell count, CD4+ T cell nadir values and with TB restricted to the lungs. Additionally, 7-oxo-DHEA increased lymphocyte proliferation and the production of IFN-γ of peripheral blood mononuclear cells from coinfected individuals.

We aimed to study the use of 7-oxo-DHEA as a modulator of *Mtb*-specific immune responses. Human THP-1 and alveolar murine MH-S macrophage cell lines were infected with *Mtb* H37Rv in presence of DHEA or 7-oxo-DHEA. Macrophages were stained to measure *Mtb* phagocytosis and were lysed to quantify colony forming units (CFU) as a measure of mycobacterial growth. Additionally, male BALB/c mice were infected and treated with 7-oxo-DHEA (15, 50 or 200 μg/day, 3 times a week) by subcutaneous and intratracheal administration. Animals were killed at 90 and 120 days post-infection. Lungs and spleen from each mouse were used for counting CFU and for histological and morphometric analyses.

Cultures exposed to 7-oxo-DHEA displayed higher phagocytic capability (p<0.05) and increased ability to control bacterial growth (p<0.001). Moreover, we found that 7-oxo-DHEA at 15 μg subcutaneously and 200 μg intrathecally administered significantly reduced bacterial burden in lung and spleen from infected mice (p<0.05). Our data support the idea of using 7-oxo-DHEA as an adjuvant to therapy to improve TB treatment, shortening treatment times and improving quality of life in patients, particularly those immunocompromised as HIV+ individuals.

Keywords: 7-oxo-dehydroepiandrosterone, tuberculosis, adjuvant therapy, immunomodulatory compound.

(1458) **N-TERMINAL TRANSIALIDASE EXPRESSED IN RECOMBINANT BCG CONFER PROTECTION IN MURINE MODEL OF *Trypanosoma cruzi* INFECTION**

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In a previous work, we have expressed different antigens of *Trypanosoma cruzi* (*T. cruzi)* in recombinant bacilo calmette y Guerin (BCG) and assessed the constructions as vaccine candidates in a murine infection model. The more promising candidate corresponded to the amino terminal fraction of the trans-sialidase (nTS). In this work we compared the immunogenicity of nTS when it is expressed in two BCG plasmids, which differ in their expression promoter in BCG; the nTS-Pus977 and the nTS-Pus2000. Additionally, we assessed the protection of these constructions in a highly virulent infection of *T. cruzi*.

BALB/c mice (n=6) were immunized every 30 days twice with nTS-Pus2000, nTS-Pus977, or BCG and PBS as controls groups. 30 days after the last immunization, significant delayed-type hypersensitivity (DTH) reactivity of nTS constructions were obtained compared to controls groups (p<0,05, immunized groups vs control group; Mann Withney test). Moreover, mice groups were challenged with 1000 *T. cruzi* parasites. After 21 days post infection (dpi) only the 16 % of PBS group were still alive whereas nTS-Pus2000 and nTS-Pus977 groups had survivals of 83%. Finally after 120 dpi, nTS-Pus2000 had a survivals of 67 % however BCG and PBS groups presented survivals of 16% and 0% respectively (p <0.05; nTS-Pus2000 vs PBS; Mantel Cox test). Furthermore, nTS-Pus2000 group had lower parasitemia and weight loss compared with the controls groups. Finally we observed lower fibrosis at 120 dpi in nTS-Pus2000 group compared to the control group, associated with decreased inflam-

matory infiltrate and lesions in the chronic phase. These results confirmed that recombinant BCG is a suitable platform for designing vaccines against *T. cruzi* infections, and showed that the construction nTS-BCG is a very promising vaccine candidate to use in future assessments.

Keyword: *T. cruzi*, VACCINE, TRANS-SIALIDASE, BCG

(1674) **ORAL ADMINISTRATION OF LIPOTEICHOIC ACID PROMOTES SKIN INNATE AND ADAPTIVE IMMUNITY WITH ABILITY TO PREVENT UVB-INDUCED IMMUNOTOLERANCE**

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Abstract: The local and systemic effects of microbiome-immune system interaction, relevant for the development of immunotherapies, are just starting to be elucidated. We have previously reported that oral administration of *Lactobacillus rhamnosus* GG-derived lipoteichoic acid (LTA) reverts the immunosuppressive effects caused by UVB skin irradiation. In the present work, we analyzed innate and adaptive immune responses exerted in the cutaneous microenvironment by oral LTA, which prevents the tolerogenic effect of UVB irradiation. To this end, we used the model of delayed type hypersensitivity (DTH) to oxazolone (OXA), applied to C56.BL.6 mice previously treated with eight doses of oral LTA (100μg /dose) followed by UVB irradiation (150 mJ/cm2) for 3 days. The innate immunity to OXA was analyzed 48 and 96 hs after sensitization by characterization of the inflammatory infiltrate in the abdominal skin and the homing of skin migratory dendritic cells (smiDCs) subsets in local draining lymph nodes (sDLN) by histology and FACS. The skin inflammatory infiltrate induced by OXA composed by polymorphonuclear cells and macrophages, was significantly abrogated by UVB and the diminished inflammatory response was prevented by oral LTA. Analysis of DC subsets in sDLN demonstrated an increased activation and proinflammatory phenotype of all DC subsets as determined by the expression of CD86, IA^b, and Ly6c. Likewise, the number of activated $CD44^+$ T cells in sDLN after sensitization, and of the effector CD4 and CD8 T cells homing to the skin following elicitation was significantly higher in mice treated with UVB/OXA/LTA vs. those treated with UVB/OXA. We conclude that the effect of oral LTA to prevent UVB induced tolerance involves stimulation of innate immunity capable of generating inflammatory DCs that activate effector CD4 and CD8 T cells with skin homing ability.

Keywords: Microbiome, Dendritic cells, Skin, Ultraviolet irradiation, contact hypersensitivity.

PLANT BIOLOGY 3

(1584) **AtAzg*1* IS A HIGH AFFINITY PURINE CELL IMPORTER OF *A. thaliana* WITH A ROLE IN CYTOKININ MEDIATED ROOT DEVELOPMENT**

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Abstract: To coordinate developmental processes plants have established a complex signalling system involving different hormones, including auxins and cytokinins (CK). Auxin research shows the importance of hormone transport to successfully drive morphogenetic processes. However, little is known about the transport of CKs. For many year our group has been studying the AtAZG transporter family. *In vitro* essays showed that AZGs are able to transport not only purines, but also a set of structurally similar compounds including CKs with high affinities. To further investigate the potential role of AZGs in CK transport, experiments in *Arabidosis thaliana* were performed. Plant overexpressing AZG1, took up significative more radiolabeled CKs from the media than wildtype (Wt) and knock-out (KO) plants. To investigate the expression of *Azg1*, a promoter fusion with GUS was constructed. Promoter activity was found in distinct plant organs, but was particularly intense in roots. Interestingly, *Azg1* has a different expression pattern whether expressed in the main root meristem or in lateral root meristems. To address the subcellular localization, different reporters fusions (e.g. pU10::AZG1-GFP) showed that AZG1 is localized to the plasma membrane. If AZG1 is a physiologically important transporter, its lack should lead to a morphologic disorder. In this direction, two independent KOs, and two overexpressor (OE) lines are being described. First approaches, pointed out that KOs are partially insensitive to exogenous CKs and OEs are more sensitive ($p>0,0001$). To know how AZG1 expression could affect root architecture, different experiments were performed, and they suggest that Azg1 is an important gene in root development regulation. These results together support the hypothesis that AZG1 is a CKs transporter playing a role in hormonal regulation of root development.

Keywords: cytokinin transport, purine transport, phytohormones, development, lateral root.

(1392) ***AZOSPIRILLUM BRASILENSE* AZ39 AND *PANTOEA* SP.: GFP- LABELLING AS A METHOD TO STUDY ROOT COLONIZATION**

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Plant growth promoting rhizobacteria (PGPR) are associated with the roots of a wide variety of plant species leading to an increase of water and nutrients uptake, through diverse mechanisms.

To obtain a tool for the study of root colonization by microorganisms, *Azospirillum brasilense* Az39 (a PGPR widely used in commercial formulations) and a strain of *Pantoea* sp. isolated from wheat were labelled with the green fluorescent protein (GFP). To demonstrate that bacterial labelling does not affect other bacterial properties, the dynamics of growth in liquid medium, biofilm formation (by violet crystal assay) and indole acetic acid (IAA) production (by Salkowski method) of wild type and GFP-labelled strains were studied. Preliminary results would indicate that GFP labelling did not affect IAA production of *A. brasilense*, whereas biofilm formation was lower in the transformed strain in LB medium. Great variability was observed in Nfb medium. The dynamics of in vitro multiplication, the growth in semisolid Nfb medium and the morphology of the colonies remained similar.

Additionally, an inoculation assay of *Arabidopsis thaliana* plants was carried out. After 6 days of inoculation with *A. brasilense*, the plants showed an evident fluorescence in the roots denoting the presence of the bacteria. Fluorescence microscopy allowed to observe a great colonization of the surface root and a certain number of fluorescent cells arranged in a row inside a root segment, apparently colonizing a vascular bundle. While plants inoculated with labelled *Azospirillum* promoted root growth, inoculation with labelled *Pantoea* sp. negatively affected *Arabidopsis* plants and a significant chlorosis in the leaves was observed. Labelled strains are important tools to advance in the knowledge of the interaction between rhizospheric microorganisms and diverse crops of high economic impact for our country.

Keywords: Azospirillum brasilense Az39, Pantoea sp., GFP, Arabidopsis thaliana.

(1722) ***BACILLUS AMYLOLIQUEFACIENS* $MEP_2$18 PROMOTES THE GROWTH OF TOMATO PLANTS AND PROTECTS AGAINST BACTERIAL SPOT CAUSED BY *XAN-***

THOMONAS AXONOPODIS PV. VESICATORIA

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Some species of the *Bacillus* genus are of interest in agronomy and pharmacy because they produce a wide variety of metabolites with biotechnological applications. The major fractions of the pathogens-suppressing antibiotics are cyclic lipopeptides (CLPs) with a structural diversity that influences their physicochemical properties and antimicrobial activities.

Bacterial spot disease produced by *Xanthomonas axonopodis* pv. *vesicatoria* (Xav) representing a worldwide risk to production of Solanaceae crops, especially tomato. An enhanced antibacterial activity against Xav was observed in CLPs fractions from *B. amyloliquefaciens* $MEP_2$18 (a strain isolated and characterized by our lab) grown in an optimized medium MMOLP. The application of CLPs significantly prevented ($p \leq 0.05$) the formation of biofilm by *Xav* on biotic and abiotic surfaces and the foliar application of CLPs resulted in the disruption of pre-formed biofilm of *Xav* on tomato leaves. Because fengycins were the major CLP from $MEP_2$18 detected in active HPLC fractions against Xav, we suggest that this CLP could have a protective role in avoiding biofilm formation and/or disrupting the preformed Xav biofilm.

Tomato plants sprayed with $MEP_2$18 and later infected with Xav showed a decreased incidence and severity of the lesions at 15 days post-infection. Negative controls were plants sprayed with *B. subtilis* JH642 (a strain non-producer of CLPs) and uninoculated and uninfected plants. A protective effect of $MEP_2$18 against Xav infection was observed, and the plants showed a better general appearance in terms of foliage and vigor. Tomato roots inoculated with $MEP_2$18 increased the dry weight of foliage ($p \leq 0.001$) indicating a plant-growth promoting effect of *Bacillus* inoculation.

Our results suggest that application of $MEP_2$18 could be an efficient and ecological alternative for controlling the development of biofilms and pathogenesis caused by Xav in tomato plants and for promoting plant growth.

Keywords: Cyclic lipopeptides, plant growth promoting bacteria, *Xanthomonas,* biofilm

(87) **EXPRESSION OF THE ARABIDOPSIS *ABF4* GENE IN POTATO ENHANCES DROUGHT AND SALT TOLERANCE AND INCREASES TUBER YIELD**

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ABF/AREB (ABRE-binding factor/ABA-response element binding factor) proteins are bZIP transcription factors (TF) that play key roles as regulators of abscisic acid-dependent gene expression during abiotic stress. It has been shown that StABF1, an ABF transcription factor of cultivated potato, not only regulates the responses to abiotic stress, but may also act as a positive regulator of tuberization. We developed transgenic potato plants (*Solanum tuberosum* cv. Spunta) expressing the Arabidopsis *ABF4* gene (35S::ABF4). 35S::ABF4 plants exhibited an enhanced tuberization induction *in vitro*, suggesting that *ABF* genes might be good candidates for crop improvement. The aim of the present study was to assess the performance of the 35S::ABF4 plants in soil, determining the tuber yield and the tolerance to drought and salt stress. 35S::ABF4 plants showed normal vegetative growth and exhibited an increased tuber yield (grams of tuber obtained per plant) with respect to wild type plants under normal conditions; the number of tubers produced per plant was higher in 35S::ABF4 lines. The leaves of 35S::ABF4 plants showed reduced water loss, and higher relative water content (RWC) after exposure to dehydration or 250 mM NaCl. Transgenic plants presented higher proline content than wild type plants under normal conditions or after exposure to stress, and higher chlorophyll content after salt stress. 35S::ABF4 plants showed higher tuber yield under drought or salt stress. The results obtained indicate that the Arabidopsis *ABF4* gene is a good tool to improve abiotic stress tolerance and tuber yield in potato plants.

Keywords: potato, ABF/AREB transcription factors, tuber yield, abiotic stress

(1174) **CADMIUM TOXICITY IN ARABIDOPSIS PLANTS WITH ALTERED POLYAMINE METABOLISM**

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Cadmium is a non-essential element normally present in soils at low concentrations but now widespread due to industrial activities and fertilizers applications. Nitrogen is a crucial nutrient, for what nitrogen fertilization is a strategy to increase shoot biomass but also rises metal extraction from soils, adding the risk of Cd accumulation in food. Polyamines are vital nitrogen-containing compounds related to abiotic stress tolerance. To study the importance of N levels in alleviating Cd toxicity, transgenic Arabidopsis plants with increased Put levels were used. Wild type, 7.2 and 2.1 transgenic seeds were germinated and grown for 10d in a controlled climate room at 22±2°C in pots with a mixture of organic substrate:perlite:vermiculite (1:1:2). Thereafter, they were irrigated either with Hoagland (C) or with Hoagland added with 100 μM Cd Cl_2 for 10 d. Rosettes were used for experiments. Biomass and RWC did not change, chlorophyll content decreased around 30% whereas lipid peroxidation was not modified after Cd treatment in all cultivars. The antioxidant enzymes catalase (CAT) and guaiacol peroxidase (GPOX) were constitutively increased in the transgenic 7.2 and 2.1 cultivars. Catalase activity was reduced 60% in WT plants and between 30 and 40% in transgenic plants after Cd exposure. GPOX activity was not modified in WT plants but increased around 40% with Cd in both Put overproducers. Nitrate reductase (NR), a crucial enzyme in N metabolism, was 18% higher in transgenic cultivars compared to WT plants without Cd, and was reduced around 20% only in 7.2 transgenic line upon Cd exposure. Despite growth was not affected and no toxicity symptoms were observed either in WT or in transgenic cultivars after Cd treatment, some antioxidant enzymes as well as NR were constitutively modified in Put overproducers plants and were differentially affected by the metal, suggesting a relationship between Cd stress, N metabolism and the antioxidant status in plants

Keywords: cadmium, nitrogen metabolism, polyamines, Arabidopsis

(1062) **PLC2 REGULATES MAMP-TRIGGERED IMMUNITY BY MODULATING ROS PRODUCTION IN ARABIDOPSIS.**

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Abstract: The activation of phosphoinositide-specific phospholipase C (PI-PLC) is one of the earliest responses triggered by the recognition of several microbe-associated molecular patterns (MAMPs) in plants. The Arabidopsis PI-PLC gene family is composed of nine members. Previous studies suggested a role for PLC2 in MAMP-triggered immunity (MTI) as it is rapidly phosphorylated *in vivo* upon treatment with the bacterial MAMP flg22. Here we analyzed the role of PLC2 in plant immunity using an artificial microRNA to silence *PLC2* expression in Arabidopsis. We found that *PLC2*-silenced plants are more susceptible to the type III secretion system-deficient bacterial strain *Pseudomonas syringae* pv. tomato (*Pst*) DC3000 *hrcC*- and to the non-adapted pea powdery mildew

Erysiphe pisi. However, *PLC2*-silenced plants display normal susceptibility to virulent and avirulent *P. syringae* strains. Ion leakage analysis showed that silenced lines conserve typical hypersensitive response features. In response to flg22, *PLC2*-silenced plants maintain wild-type MAPK activation and PHI1, WRKY33 and FRK1 immune marker gene expression.In leaf disc using peroxidase/luminol based method we found that silenced lines have reduced reactive oxygen species (ROS) production. ROS -dependent responses such as callose deposition and stomatal closure were analyzed by optical microscopy showing a compromised response in the silenced lines. FLAG-tagged immunoprecipitation of NADPH oxidase (RbohD) showed that PLC2 was associated, suggesting its potential regulation of a branch of MTI and non-host resistance that involves early ROS-regulated processes. We are currently analyzing whether PLC2 is specific for flg22 responses or it is also involved in the perception of other MAMPs such as chitin or Elf18. Additionally, we are also measuring Ca^{2+} cytosolic increases upon MAMPs treatments in PLC2-silenced lines using transgenic plants expressing an aequorin reporter.

Keywords: Arabidopsis thaliana, phospholipase C, reactive oxygen species, NADPH oxidase, *Pseudomonas syringae*

(1676) **EFFECT OF A GLYPHOSATE-BASED HERBICIDE ON THE HYDROPHILIC REDOX BALANCE OF THE FRESHWATER ALGA *Chlorella vulgaris* CPCC90 DURING DEVELOPMENT**

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The objective of this work was to study the effect of analytical glyphosate (GLY) and a commercial formula (RUP) on the hydrophilic oxidative state of the freshwater alga *Chlorella vulgaris* CPCC90. The ascorbyl radical (A•) is the final radical product from the oxidative transformations of ascorbate (AH^-). The ratio $A^•/AH^-$ content has been described as a quick and simple method of diagnosis of stress in the hydrophilic cellular medium. To study the effects of GLY on oxidative balance, axenic cultures of *C. vulgaris* CPCC90 were prepared in BBM medium supplemented with analytical and commercial GLY to a final concentration of 8.9 μM. The cultures were grown at 20ºC and light/dark cycles of 12/12 h, for either 7 (exponential (Exp) phase) or 15 days (stationary (st) phase). Algal growth was monitored by spectrophotometry (λ=600 nm) and cell count. A• and AH^- content were measured using paramagnetic resonance spectroscopy (EPR) and HPLC, respectively. The treatments with neither GLY nor RUP affected the growth rate when compared to control, in both phases ($p>0.05$). No statistically significant differences were observed in the content of AH^- during Exp phase in either of the treatments, when compared to control ($p>0.05$). However, there was a significant increase in the content of A• by 3-fold and 8-fold for GLY and RUP, respectively, as compared to non-treated algae ($p<0.05$). Thus, a 10-fold and 20-fold increase was measured in the ratio $A^•/AH^-$ for GLY and RUP, respectively. During st phase there were no significant changes neither in the content of AH^-, nor A• nor in the $A^•/AH^-$ ratio for any of the treatments when compared to control ($p>0.05$). Freshwater algae keep the $A^•/AH^-$ ratio in a narrow range, suggesting adaptability to the habitat conditions. However, during active growth in Exp phase an increase in the oxidative balance was produced at this moderate concentration of GLY. $A^•/AH^-$ ratio returned to control values in st phase probably by antioxidant activity.

Keywords: glyphosate, oxidative balance, *Chlorella vulgaris*.

(886) **POLYAMINE CONCENTRATIONS ARE DYNAMICALLY REGULATED DURING PLANT-PSEUDOMONAS SYRINGAE INTERACTIONS**

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Due to their abilities to bind polyanions such as nucleic acids and proteins, the polyamines putrescine, spermidine and spermine are involved in several cellular responses. For instance, these amines participate in the activation of plant defense responses and are required for virulence of plant pathogens. Thus, the modulation of their metabolism plays essential roles in plant-pathogen interactions. In this work, we challenged plants with different pathovars of the bacteria *Pseudomonas syringae* to evaluate the changes in the concentrations of polyamines occurring in contrasting pathogenic interactions. Our analysis demonstrated that polyamines are increased in tomato in response to a virulent strain, which was associated to the induction of several polyamine metabolic genes from plant and bacteria. These results suggest that the metabolism of both organisms modulates polyamine contents in infected tissues. Interestingly, the rise in the concentration of putrescine was particularly higher in apoplastic fluids. A further exploration of the polyamine profiles using *Arabidopsis* plants demonstrated that non-pathogenic strains inducing the hypersensitive response provoked a remarkable increment in their concentrations, which was not observed by using an avirulent non-host bacteria. Thus, specific regulatory mechanisms may modulate polyamine metabolism in different biological interactions.

(905) **N-METHYLENEPHOSPHONIC CHITOSAN (NMPC) EXERTS CITOTOXICITY IN FUNGAL CELLS.**

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The aqueous solubility of chitosan depends on the degree of protonation of the NH_2 group by acid treatment, which limits its versatility in biological fluids. However, N-methylenephosphonic chitosan (NMPC) is a chitosan derivative which hydrosolubility is improved due to the addition of aminoalkylphosphonic ligands. The aim of this study was to study whether NMPC has antifungal properties on phytopathogenic fungus *Fusarium solani* f sp. *eumartii* (*F. solani*). Firstly, we analyzed the effect of NMPC on germination of *F. solani* spores. *In vitro* quantitative assays showed that NMPC exerted antifungal properties in a dose dependent manner, displaying an estimated IC_{50} value=2.5 μg/ml. To investigate whether NMPC has a lethal effect on fungal spores, different experimental approaches were performed. *F. solani* spores were incubated in the presence of different concentrations of NMPC for 24 h. Fungal viability was calculated counting the number of colony forming units (CFU). In addition, the effect of NMPC on cell plasma membrane validated citoxicity based on the uptake of the fluorogenic dye SYTOX Green. ROS production was *in vivo* measured by DAB staining in fungal spores upon 4 h post incubation with 2.5 μg/ml NMPC. Beside, tomato cell cultures were incubated with 2.5 and 5 μg/ml NMPC, and cell viability was tested with Evans blue dye. The results showed that at fungicide doses, NMPC did not produce tomato cell death. Simultaneous analysis of cell viability, cell permeabilization and activation of ROS generation explained integrative citotoxic properties mediated by NMPC on fungal cells.

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Keywords: NMPC, *Fusarium solani*, antifungal activity.

NEUROSCIENCE 10

(1678) **TRPV1 OSMOSENSITIVE CHANNEL INVOLVEMENT IN THE CONTROL OF SODIUM APPETITE**

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There is a temporal dissociation between sodium depletion (SD) and the appearance of sodium appetite (SA) behavior. After an acute SD, the natremia decreases immediately however SA takes at

least 16 h to appear. Our recent results demonstrated in Wistar rats that the transient receptor potential vanilloid type 1 (TRPV1) channel, required for normal osmoregulation, is involved in SA control. The TRPV1 mRNA expression was increased in the kidney and previously involved brain nuclei, during the delay of SA (2 h after SD) and significantly decreased during the appearance of SA (24 h after SD), possibly allowing the hypertonic sodium consumption. The aim of the present work was to evaluate in TRPV1 knockout mice (KO), sodium intake and the urinary pattern of renal excretion at different times after SD. In particular, we analyzed the sodium and water intake and the renal response at 2 h and 24 h after SD induced by furosemide (50mg/kg) in combination with low sodium diet in wild type (WT) and KO mice.

After SD, the KO animals showed an increase in the sodium preference (F=8.49; p=0.006) and consumed a higher hypertonic cocktail (F=8.49; p=0.0059) in relation to WT animals, independent of the time after SD. These data suggest that KO animals, when stimulated to drink water and sodium, make a hypertonic cocktail instead of the isotonic one usually made by the control animals. The urinary volume (F=5.45; p=0.0003) and sodium excretion (F=3.99; p=0.028) induced by Furosemide at 30 minutes were both reduced in KO animals in comparison to WT. There was no change in plasma osmolality between the groups 2 h and 24 h after SD.

In sum, these data suggest that the TRPV1 channels are involved in the osmoregulatory behavioral and renal responses after acute body SD.

Keywords: sodium depletion, sodium appetite, TRPV1, osmoregulation.

(1276) **THE HYPOTHALAMIC ACTION OF GHRELIN IS REQUIRED FOR A FULL RESPONSE OF THE HYPOTHALAMIC–PITUITARY–ADRENAL (HPA) AXIS TO FASTING**

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Ghrelin is a stomach-derived hormone that regulates food intake and neuroendocrine axis via its action on the GHSR (*growth hormone secretagogue receptor*). The administration of ghrelin actives corticotropin-releasing factor (CRF) neurons of the hypothalamic paraventricular nucleus (PVN) and, as a consequence, the HPA axis via inhibition of the local GABA tone and independently of the neuropeptide Y (NPY)/GABA neurons of the arcuate nucleus (ARC). Under fasting ghrelin levels increase; however, the impact of endogenous increments of plasma ghrelin on the HPA axis is currently unknown. Here we studied the response of the HPA axis to fasting of wild-type (wt) mice, GHSR-deficient mice and mice expressing GHSR only in GABA neurons. In addition, we quantified if fasting affects the NPY and GABA inputs to the PVN in an *in vitro* setting and in mice expressing tdTomato fluorescent protein in GABA neurons. Results: As compared to wt mice, GHSR-deficient mice showed an impaired fasting-induced increase of both plasma corticosterone and the marker of neuronal activation, c-Fos, in the PVN (corticosterone: 204±30 vs. 113±30 ng/ml; c-Fos: 44±9 vs. 13±6 cells/side, respectively; p≤0.05, 2-way ANOVA). In contrast, mice expressing GHSR only in GABA neurons displayed a full response to fasting. As compared to *ad libitum* fed mice, fasted mice showed an increase of the NPY-fiber density and an increase of the area of tdTomato positive fibers in the PVN (NPY-fibers: 0.09±0.02 vs. 0.17±0.02 OD; td-Tomato:28.0±3.1 vs. 38.1±1.5 % of total area; respectively; p≤0.05, T-test). As compared to PVN explants of *ad libitum* fed mice, PVN explants of fasted mice showed a reduction of basal and KCl-stimulated GABA release (basal:4.5±0.3 and 3.1±0.5; KCl:6.1±0.3 and 4.3±0.8 % of total incorporated tracer, respectively; p≤ 0.05, two-way ANOVA). Thus, these data indicate that ghrelin signaling in GABA neurons is relevant for the normal response of the HPA axis to fasting.

Keywords: GHSR, GABA, NPY,CRF

(1295) **THE CEREBROSPINAL (CSF)-BLOOD BARRIER TRANSPORTS CIRCULATING GHRELIN INTO THE BRAIN**

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Ghrelin is a stomach-derived orexigenic peptide hormone that mainly acts in the central nervous system. The mechanisms transporting plasma ghrelin to the brain are yet to be elucidated. The goal of the current study was to test the hypothesis that the CSF-blood barrier transports circulating ghrelin to the brain. First, we centrally infused a fluorescent ghrelin analog to mice and found that the tracer labeled the ependymal cells of the choroid plexus and the hypothalamic tanycytes, which form the CSF-blood barrier. Then, we performed a time course study by subcutaneously infusing the fluorescent ghrelin to mice. In this experiment, we found that the probe labeled the tanycytes and the choroid plexus at early time points (~5 min) while the hypothalamic periventricular brain parenchyma was labeled ~15 min after the injection. Notably, the ghrelin analog increased food intake in the mice when injected both centrally and peripherally (p≤0.01). In a subsequent experiment, we tested the effect of peripherally-injected ghrelin in mice that had been centrally-injected with a scrambled ghrelin peptide. We found that the ability of ghrelin to both increase food intake and activate the marker of neuronal activation, c-Fos, in the hypothalamus was impaired in mice previously injected with the scrambled peptide, as compared with the control animals (analyzed by two way anova, $p<0.0001$), suggesting that the accessibility of ghrelin to the brain depends on a saturable mechanism. In order to test whether the access of circulating ghrelin to the brain parenchyma requires its passage thru the CSF, we centrally injected an anti-ghrelin antibody 15 min before a subcutaneous injection of ghrelin. Interestingly, the anti-ghrelin treatment decreased food intake (analyzed by two way anova, $p<0.05$). Thus, we conclude that the cells of the CSF-blood barrier are involved in the blood to brain transport of ghrelin by a saturable mechanism, which would require passing through the CSF.

Keywords: ghrelin, tanycytes, choroid plexus, accessibility.

(226) **SHIGA TOXIN 2 (STX2) FROM ENTEROHEMORRHAGIC *Escherichia coli* (EHEC) PRODUCED ANOREXIA THROUGH REDUCED GHRELIN EXPRESSION AND DAMAGE IN HYPOTHALAMIC ARCUATE (ARC) AND PARAVENTRICULAR (PVN) NUCLEI**

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Anorexia and weight loss are observed in patients by Stx2 intoxication from EHEC which may leadto coma and death. Loss of appetite may be due to a neurotoxic effect of Stx2 on hypothalamic orexigenic neurons or failure of orexigenic signals from the gut. Ghrelin is an orexigenic hormone produced by enteroendocrine cells that acts through its hypothalamic Arc and/or PVN neuronal receptors. The aim of this study was to determine whether Stx2: i) induces loss of weight, ii) changes the expression of ghrelin in the gut, and iii) affects the parenchyma of Arc and PVN. NIH mice (n=4) were treated intravenously with vehicle (100μl of saline solution) or Stx2 (1ηg/mice). Animal body weight and food intake were monitored daily for 4 days. Another control group (pair fed) was included to determine whether the loss of weight was only caused by an anorexigenic effect or by a metabolic component. The amount of food provided to

this group was the exact amount of food eaten by the Stx2 treated mice. Then, the animals were fixed and their stomachs and brains were subjected to immunofluorescence with anti-Ghrelin, anti-GFAP and anti-NeuN. After 4 days, the Stx2 treated mice significantly lost weight, as compared to the vehicle ones (3.05±0.58g control and -1.01±0.32g Stx2). Weight loss was accompanied by a significant decrease of food intake (5.04±0.46g control and 3.10±0.28g Stx2) and in the number of ghrelin immunopositive cells in the stomach (13.97±0.68 cells control and 7.73±0.95 cells Stx2). GFAP expression (30.43±1.73 AU control and 44.65±1.40 AU Stx2, in IOD) and the number of immunopositive NeuN abnormal neurons(0 cells control and 3.18±1.83 cells Stx2) were increased in the ARC and PVN Of Stx2 treated mice ($p<0.05$).These results suggest that Stx2 produced anorexia through ghrelin signaling.

Keywords: neurodegeneration, infection, encephalopathy

(1558) **NEW MUTANT OF MOUSE GHRELIN RECEPTOR (GHSR1aA203E) AS A TOOL TO STUDY THE RELEVANCE OF GHSR1A CONSTITUTIVE ACTIVITY IN NEURONS.**

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The ghrelin receptor (GHSR1a) is a G protein coupled receptor expressed in the brain that controls fundamental functions such as food intake and energy balance (Hypothalamus), mood behavior (limbic system) and memory and learning (hippocampus). Recently we found that this receptor is capable of controlling presynaptic voltage gated calcium channels (VGCC). Interestingly, GHSR1a has the singularity of having high level of activity in absence of its agonist, the hormone ghrelin. We hypothesized that GHSR1a constitutive activity is relevant in brain areas with limited access to ghrelin, a hormone produce by stomach cells. In order to test this hypothesis in mouse neurons we developed an experimental tool: the murine version of a natural human GHSR1a mutant that lacks constitutive activity (GHSR1aA204E). We mutated the analog alanine in the mouse sequence of GHSR1a (GHSR1aA203E) and we tested it by patch clamp and imaging experiments where we found that this mouse GHSR1a mutant behaves identically to the human version. Our experiments measuring VGCC currents in HEK cells transfected with the VGCC subunits and the receptor demonstrated the lack of effect of GHSR1aA203E on basal calcium currents, utilizing the wild type mouse GHSR1a as a positive control of basal current inhibition ($p<0.05$ in comparison with VGCC currents in absence of receptor). Moreover we performed imaging experiments to confirm that GHSR1aA203E does not modify VGCC membrane density in contrast with wild type mouse GHSR1a ($p<0.05$ in comparison with VGCC membrane density in absence of receptor). These experiments are fundamental to proceed to obtain a mouse line where the GHSR1a is mutated and so to investigate the relevance of its constitutive activity in the mammal brain.

Ghrelin Receptor, Voltage Gated Calcium Channels, G Protein–Coupled Receptor, Neurons

(482) **GHRELIN MODULATES HIPPOCAMPAL PLASTICITY BY INDUCING INCREASED BDNF EXPRESSION AND INCREASED DENSITY OF DENDRITIC SPINES.**

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Ghrelin (Gr) is a peptide involved in the modulation of several biological processes. In a previous work we have shown that Ghr administration in rats, either intracerebroventricularly or directly into brain areas such as the hippocampus (hp), amygdala or dorsal raphe nucleus, enhances memory retention in tests such as step-down and object recognition in a dose-dependent. In addition we have also demonstrated (electrophysiological studies) that intra-hippocampal Gr administration decreases the threshold to induce long term potentiation (LTP), a process underlying memory formation) and also that gr increases glutamate release and the expression of the NMDA receptor NR2B subunit.

A neurotrophin BDNF (Brain-derived neurotrophic factor), the most abundant in the nervous system and in Hp, is an important regulator of synaptic plasticity, dendritic arborization and memory.

Taking into account the effects of Gr and those described for BDNF, we propose to investigate A) if Gr-induced memory facilitation correlates with modifications in BDNF expression in young and old mice. Hp slices were incubated in absence and in presence of Gr and the BDNF mRNA from the activation of promoters I, II, IV and VI were evaluated; B) the effect of Gr on the density and morphology of dendritic spines (ED): Gr was added to hp cell culture. Results show that Gr increased levels of BDNF mRNA by almost 5 fold compared to untreated animals: the transcriptional activity of specific promoters increased selectively: I and II in young mice and I and IV in old animals. The number and density of ED also increased after Gr treatment. Our data showing that Gr administration increases BDNF transcription as well as the density of the EDs suggest that probably some processes involved on the memory facilitation induced by the peptide could be mediated by BDNF.

Keywords: Ghrelin, Memory, Bdnf, Hippocampus, Synaptic plasticity.

(366) **GHRELIN RECEPTOR CONSTITUTIVE ACTIVITY REDUCES SURFACE EXPRESSION OF VOLTAGE-GATED CALCIUM CHANNELS IN A $CA_V\beta$** DEPENDENT MANNER

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Voltage gated calcium channels (Ca_V) couple plasma membrane voltage changes to calcium influx, triggering calcium dependent processes. This sequence of fact is crucial for shaping neuronal activity. Several Ca_V subtypes exist and they have different time- and place-specific functions in neurons. Thus, great effort has been devoted to determine what elements can control Ca_V activity in neurons. In this context, many G protein coupled receptors (GPCR) activated cascades have been detected as powerful Ca_V activity modulators. On the other hand, data about the control of trafficking and density of Ca_V subtypes at specific location on the neuronal plasma membrane are scarce. Here we describe that a GPCR that is constitutively active (GHSR1a) in absence of its agonist (ghrelin) significantly inhibits the forward trafficking to the plasma membrane of several Ca_V subtypes (low- and high- voltage activated Ca_V) and consequently Ca_V currents. We found that the mechanism implies retention of the channel complexes at the endoplasmic reticulum and the requirement of auxiliary subunit $Ca_V\beta$. This was demonstrated in both hypothalamic primary neuronal cultures and in heterologous expression systems, using patch-clamp electrophysiology, live fluorescent- and confocal-microscopy to examination the subcellular locations of tagged proteins. We reported significant differences when calculated p-value <0.005. P-values were calculated from one- or two-sample t tests or Mann–Whitney test, and multiple comparison one way- ANOVA with Tukey's post-test or Kruskal–Wallis test with Dunn's post-test. Since the solely expression of GHSR1a is enough to control Ca_V trafficking and this receptor is highly expressed in brain areas controlling food intake, reward and learning and memory, our finding could have a great impact in animal behavior. Further experiments focusing on the function of each Ca_V subtype function are required to deeply understand the scope of GHSR1a effect on neurons.

Keywords: Voltage-gated calcium channels, GPCR, $Ca_V\beta$.

(1274) **CONSTITUTIVE GHRELIN RECEPTOR SIGNALING MODULATES THE MAGNITUDE OF THE COMPENSATORY HYPERPHAGIA TRIGGERED BY AN EVENT OF FASTING**

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Ghrelin is the only peptide hormone known to stimulate food intake. Ghrelin acts through the growth hormone secretagogue receptor (GHSR), which is a G protein coupled receptor highly expressed in the central nervous system. Notably, GHSR displays the highest known constitutive activity. The physiological relevance of the constitutive GHSR signaling is uncertain. Our goal here was to study if the ghrelin/GHSR system modulates the magnitude of the hyperphagia that follows an event of fasting. First, we characterized the food intake and body weight responses of wild-type (WT) mice that have been exposed to a 48-h fasting event and then refed. We found that refed WT mice display a robust hyperphagia after fasting that continues for 5 days after refeeding and changes its food intake daily pattern. Fasted WT mice show an increase of plasma ghrelin levels as well as the GHSR levels in the hypothalamic arcuate nucleus (ARC), indicated by both a ghrelin binding assay and gene expression analysis. Then, we compared the fast-refeeding response of WT, ghrelin-KO and GHSR-deficient mice in our protocol. In contrast to ghrelin-KO mice, only GHSR-deficient mice showed a significantly smaller compensatory hyperphagia than the observed in WT (14.4±3.5%, unpaired t test). Then, we tested the compensatory hyperphagia of WT mice intracerebroventricularly-treated during the fasting period with either a GHSR antagonist (D-Lys_3-GHRP-6) or a GHSR inverse agonist (K-(D-1-Nal)-FwLL-NH_2). The compensatory hyperphagia was significantly smaller only in the inverse agonist-treated group (14.8±3.8%, unpaired t test) as compared to vehicle- and antagonist-treated mice. Thus, the constitutive GHSR signaling modulates the magnitude of the compensatory hyperphagia triggered by an event of fasting.

Keywords: Ghrelin, GHSR constitutive activity, arcuate nucleus, fasting

(185) **CHANGES IN OXIDATIVE STRESS MARKERS BY DIETARY SUPPLEMENTATION WITH NATURAL OILS**

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Uncontrolled maternal hyperglycemia during pregnancy produces an increase in oxidative stress causing alterations in the developing nervous system of the offspring. The aim of this work was to evaluate the effect of an early dietary supplementation with natural oils on the oxidative stress state at adulthood. Adult male Sprague-Dawley rats born from control mothers (CO) and mothers with experimental diabetes (DO; Streptozotocin, 30 mg/Kg iv.) were orally supplemented with corn oil (Mz; used as control) or with either extra virgin olive oil (Ol) or pistachio oil (Ps) from day 2 to 62 (8µl /15g). At 8 months old, oxidative markers like the activity of superoxide dismutase (SOD) and Malondialdehyde (MDA) concentrations were evaluated in plasma (P) and hypothalamus homogenates (HT). DO-Mz animals showed reduce SOD activity, and increased MDA respect CO-Mz animals in P and HT (SOD=48%,15%; MDA=20%; 92% respectively; $p<0.05$). Early supplementation with Ol and Ps improved the enzymatic activity of SOD compared to Mz in plasmatic samples from DO animals (Ol=230%, Ps=190%, $p<0.05$) and reduce MDA values (Ol=17% Ps=30%; $p<0.05$). In HT the SOD activity was increased 18% by Ol and 46% by Ps compared to DO-Mz ($p<0.05$). This change was also associated with a significant reduction of MDA in HT of DO animals supplemented with natural oils (Ol=6%, Ps=32% vs. Mz; $p<0.05$). CO animals showed increase in SOD activity in both tissues (10%) mainly by Ps supplementation ($p<0.05$) meanwhile no significant changes were observed in MDA concentration. Thus, early dietary supplementation with natural oils like Ol and Ps improves the expression of central and peripheral antioxidant systems, reducing the lipid peroxidation in the progeny of diabetic mothers. In addition, in this case the supplementation with Ps seems to be more effective than the Ol. (PIP0243, PICTO/UCCuyo-0158- CICITCA UNSJ-IDeA1400.0107/2012).
Keywords: gestational diabetes, hyperglycemia, hypothalamus. lipid peroxidation, superoxide dismutase.

(733) **BASAL AND HYPEROSMOLALITY-INDUCED AVP EXPRESSION IS AFFECT BY EARLY MATERNAL SEPARATION**

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We assess the implication of an adverse early environment such as the maternal separation (MS) on the regulatory response to osmotic stress during adulthood. Since the interplay between an early psychological stress and late osmotic stress both associated with the activity of vasopressinergic circuits within the paraventricular (PVN) and the supraoptic (SON) nuclei would result in a differential osmoregulatory response in terms of vasopressin (AVP) mRNA levels during adulthood. The aim of this work was to evaluate whether early MS may induce a differential programming in the adult offspring vasopressinergic system in particular the hyperosmolality-induced AVP expression. Male Wistar rats were subjected to daily maternal separation for 4.5 hours during the first three weeks of life. At postnatal day 75, rats were intravenously infused with isotonic or hypertonic saline solution during 20 minutes. After 10 minutes, animals were decapitated. Thereafter, the PVN and SON were identified and collected by micropunch technique. Then, we determined the relative AVP expression by qPCR. In the PVN, as we expected, non-separated animals responded to hypertonic stimulation with a threefold increase in AVP levels ($p<0.05$). Surprisingly, maternally separated rats did not respond to hypertonic solution, showing similar mRNA levels in PVN compared to rats infused with isotonic solution. Besides, separated rats showed higher AVP levels in the SON than non-separated rats ($p=0.045$).Our data indicate that early maternal separation induces a long-term effect on vasopressinergic system involved in the control of hydroelectrolyte balance. These results also suggest that psychological stress during the neonatal period may impair the vasopressin system activity provoking a reduced response in the offspring after an osmotic challenge. This could be the consequence of alterations in the central osmosensitive mechanism or in the activity of brain circuits involved in hydroelectrolyte homeostasis

BIOTECHNOLOGY AND BIOINFORMATICS 4

(611) **DEVELOPMENT OF IMPROVED ENZYME-BASED AND LATERAL FLOW IMMUNOASSAYS FOR RAPID AND ACCURATE SERODIAGNOSIS OF CANINE BRUCELLOSIS**

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Brucellosis is a major zoonotic disease caused by *Brucella spp.* that affects animals and humans. *Brucella canis* is the etiological agent of canine brucellosis, a disease that can lead to sterility in bitches and dogs causing important economic losses in breeding kennels. The underestimation of the clinical relevance of *B. canis* coupled with the results of several reports of human brucellosis cases confirmed by isolation of *B. canis,* urge the need to develop new high-performance diagnostic tests that allow an early and accurate diagnosis of canine brucellosis. Here, we aimed to develop and validate enzyme-based (*B. canis*-iELISA) and lateral flow (*B. canis*-LFIA) immunoassays for improved serodiagnosis of canine brucellosis using as antigen the *B. canis* rough lipopolysaccharide (rLPS), obtained by a hot-saline modified method. To validate the assays, 284 serum samples obtained from naturally infected dogs and healthy animals were analyzed. This panel included 71 positive reference samples obtained from dogs in which the infection was confirmed by bacteriological culture and serology (RSAT and AGID positive) and 213 samples obtained from healthy animals with negative serology results. For the *B. canis*-iELISA (cut-off = 45 8%) and *B. canis*-LFIA the diagnostic sensitivity was of 98.6%, and the specificity 99.5% and 100%, respectively. These findings demonstrate that both assays have an excellent diagnostic performance. In order to improve the detection and control of the disease and lower the risk of transmission of brucellosis to humans, we propose the implementation of the *B. canis*-LFIA as a rapid, easy to use screening test in combination with the *B. canis*-iELISA as a confirmation test. Finally, a blind study including 1,040 serum samples obtained from urban dogs showed a prevalence higher than 5% highlighting the need of performing a more comprehensive serological survey to determine the magnitude of *B. canis* circulation among the urban dog population in Argentina.

Keywords: *Brucella canis*; canine brucellosis; diagnostics; iELISA; lateral flow.

(977) **DEVELOPMENT OF A PLATAFORM FOR DESIGNING, PRODUCTION AND PURIFICATION OF RECOMBINANT CYCLIC BETA GLUCANS BY GENETIC GLYCOENGEENERING**

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Cyclic beta-1,2-glucan (CBG) are ring-shaped bacterial polysaccharides synthesized by *Rhizobiaceae* and *Brucellaceae* members. CBG can be purified as complex mixtures of molecules with varying rings size; they can be neutral or decorated with different molecules that confers a negative charge. Due to their particular structure, CBG can be used for drug solubilization and stabilization, enantiomer separation, catalysis, synthesis of nanomaterial and even immunomodulatory properties. Since there is no way to produce CBG by chemical synthesis thus, CBG are still considered a "natural product" which hampers their potential industrial applications. To circumvent these problems, we have recently developed a technological platform for the standardized production and purification of "recombinant CBG". The platform is based on the modular expression of different engineered enzymes involved in CBG synthesis in a specially designed *E. coli* strain. By means of this technology, we are able to produce homogeneous batches of CBG of different ring sizes and different molecular decorations depending on the chosen enzyme variants. In this way, we can produce CBG "á la carte" in a simpler, economic and standardized manner suitable for several industrial applications.

(107) **EVALUATING APTABLOTS AND SOUTHWESTERN-BLOT ASSAYS AS ALTERNATIVES FOR THE SENSITIVE DETECTION OF ALL HISTONES IN TOTAL CELL LYSATES.**

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Abstract: Histones PTMs are markers of epigenetic events and extracellular histones modulate inflammatory, autoimmune and innate immune responses. It is useful to detect histones by sensitive assays. Since they evolved to bind DNA, several biotinylated oligonucleotides and one aptamer were evaluated as reagents for the colorimetric or chemiluminescent detection of endogenous histones bound to nitrocellulose. Many assay variables were systematically adjusted. These south-western or aptablot techniques resulted superior alternatives to western-blot with antibodies. Cell lines or organs were lysed to study the oligo-histones binding profiles resolved by SDS-PAGE. For the TOTAL DETECTION of the 5 histones, biotinylated DNA was an optimal, cost-effective tool, complementary or supplementary of antibodies. We characterized oligonucleotide-histones interactions modulating the components of solutions in the steps of membrane blocking, DNA binding or washing and controlled which histone was detected preferentially adjusting the ionic strength. When needed, histone detection was completely prevented by adding polyanionic reagents (p-value ≤0.01). Testing 1 ssDNA aptamer published as H4 specific but not selected/validated in a lysate context, it reproducibly failed (p-value ≤0.01) to differentiate its H4 target from the other core histones indicating that the selection environment is important in specificity determination. Finally, we developed novel, reliable, non-proteic, coloured MW trackers for better histone resolution in gels. Our procedures showed reproducible analytical advantages and may aid the design of histone bioassay platforms. With a single reagent at picomolar concentration all histones can be sensitively detected for signal normalization in membranes. Our strategies are expected to improve DNA aptamer selection protocols and to facilitate studies of histones for epigenetic, proteomic or immunity research. *Keywords***:** histones, epigenetic, proteomics

(401) **DESIGN AND SIMULATION OF A MICRO-DEVICE FOR PULSATILE AND CONTROLLED RELEASE OF GROWTH HORMONE**

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Drug delivery has posed numerous challenges in optimizing release of adequate drug amount in the right place and time with low invasiveness and high automation. The development of micro-electro-mechanical devices (MEMS) can offer solutions to these challenges. In particular, patients suffering from growth hormone (GH) deficiency are treated with a daily injection replacement therapy. Although considered mostly successful, there are drawbacks associated with drug dosing and administration, as GH presents a sexual differentiated pulsatile secretion pattern. In this context our aim is to develop an implantable MEMS device for GH release in a pulsatile and externally controlled manner. To that end we are developing and have performed engineering simulation (ANSYS Mechanical 17) of a device where GH will be loaded in multiple micro-reservoirs and release by an electrothermal actuation mechanism. Device materials were chosen considering biocompatibility and current microfabrication processes. The best achieved design of a 1 cm^2 device included 60 individual reservoirs of 1.5 μL each, conformed by a truncated pyramid fabricated in a silicon wafer attached to a rectangular polyhedron fabricated in polydimethylsiloxane. These reservoirs will be capped by a Pt/Ti/Pt thin suspended layer which, according to simulation experiments, can be melted in 10-40 microseconds by applying 1.2 V using gold contacts. Current intensity (0.9A) and Joule heating (1W) are maximum in the suspended membrane owing to resistivity differences between titanium and gold. Moreover, thermal gradient simulation experiments indicate good heat dissipation towards the surrounding tissue and the interior of the reservoir. These results indicate this device could be used

for loading and releasing of GH by melting the capping membranes in a very short time and using a low amount of energy supplied by an embedded conventional battery. This is of great relevance for optimization of GH replacement treatments.

Keywords: micro-electro-mechanical systems, drug delivery, growth hormone deficiency

(418) **STEROID-RELEASING NANOSTRUCTURED BIOMATERIAL COATING FOR CONTROLLING CELL PROLIFERATION AND HORMONE SECRETION**

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The development of functional biomaterials at the frontiers of cell biology, chemistry and materials sciences is of great research interest. The possibility of organizing matter in the nanoscale has great relevance in designing biomaterials for the regulation of specific cell functions. This could be achieved through molecules loaded within the material that can be delivered to cells with high efficiency. In this context, the aim of this work was to develop new biomaterials using titanium dioxide mesostructured thin films containing biocompatible reservoirs for storage and release of steroid hormones, capable of modifying cells behavior. These films were synthesized on glass slide pieces by sol-gel and molecular self-assembly methodology to facilitate the inclusion of estradiol within the template micelles during film preparation. The release of loaded hormone to culture media was evaluated in vitro by RIA. Biocompatibility and functionality of as prepared film coatings was assessed by culturing GH3 cells on them for 48 h and by evaluating the effects of the released steroid on cell proliferation and PRL secretion. Estradiol release from film coatings did not depend on the amount of loaded hormone assayed, showing a progressive pattern and reaching 500 nM at 48 h. Proliferation of GH3 cells and PRL secretion was stimulated (Relative proliferation index: 1.37 vs 0.98; PRL: 4.46 mg/mL vs. 3.52 mg/mL; $p<0.05$) by estradiol released from film coatings in a way similar to what was observed in control cells exposed to exogenous estradiol. These results demonstrate that a nanostructured biomaterial film coating with controllable steroid releasing features can be produced. The ability of these films to load key molecules and allow their release to exert differential effects on cells, would convert them into multifunctional biocompatible reservoirs.

Keywords: Biomaterials, Mesostructured film coatings, Drug release, Sex steroid hormones.

(434) **FOLIC ACID ENCAPSULATION IN A CARBOXYMETHYL CELLULOSE MATRIX: EVALUATION OF ITS BIOAVAILABILITY USING THE CELLULAR MODEL CACO-2**

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Abstract: Folic Acid (FA) is a water soluble vitamin belonging to the vitamin B complex. FA is particularly sensitive to the acidic pH of the stomach and this leads to a low bioavailability in the small intestine epithelium. Due this, new approaches for improving FA bioavailability and stability are required. In this study we proposed that FA can be encapsulated into a carboxymethyl cellulose matrix (CMC), an innocuous polysaccharide, which can behave as dietary fiber. This strategy could allow a controlled release of FA in the small intestine cells. Here, we characterized the physical-chemistry interaction between CMC and FA, and the effect of this complex on an intestinal model, using the differentiated cell line Caco-2. Firstly, CMC-FA was characterized using fluorescence and Infrared spectrometry (IR). Fluorescence spectra showed an increased in the polysaccharide signal in response to increased FA concentration. On the other hand, IR spectra showed an increased in the carbonyl group signal of the CMC in response to FA concentration. Taken together, these results suggest that both compounds interact. The cell line Caco-2 can differentiate to enterocytes and emulate the physiological conditions of nutrient absorption in intestinal epithelium. Using a *Transwell* system we assessed the transport capacity of encapsulated FA. Finally, the effect of CMC-FA complexes was assessed by viability (**$p<0.01$ AF vs CMC-AF and $p>0.05$ CMC-AF vs control, n=3; One Way ANOVA, LDH and MTT assays) and fluorescence microscopy assays. Our results showed that the complex does not affect cell viability neither the integrity of the differentiated cell monolayer. These results are promising for understanding the possibility of encapsulating and protecting the FA from injurious conditions in the body. In addition, this complex does not produce cytotoxic effects in our cellular model, proposing it as a possible application of these complexes for increasing the bioavailability of folic acid.

(1383) **NEW RED-SHIFTED FLUORESCENT BIOSENSOR FOR MONITORING INTRACELLULAR REDOX CHANGES IN MAMMALIAN CELL LINES**

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Mammalian cell lines have become the dominant system for the production of recombinant proteins for clinical applications. During cell culture, cells experience oxidative stress, which hampers proliferation and productivity. Monitoring of intracellular redox changes can be useful to guide intervention strategies aimed at optimizing cell-based production processes. Also, reporter animal cell lines are of great interest in drug discovery to test the properties of different compounds.

Redox-sensitive variants of green fluorescent proteins (roGFP2 and rxYFP) able to report the pool of reduced and oxidized glutathione are already available. In this work we develop and characterize a new red-shifted redox biosensor that allows the detection of redox changes in cells in a non-invasive, dynamic, real-time and in-situ manner. Based on theoretical models a new variant of mRuby2 was designed incorporating two cysteine residues that confer sensitivity towards redox potential. The new rxmRuby2 biosensor was expressed, purified and characterized biochemically and biologically in different cellular systems (HeLa and HEK-293 cells). Spectrofluorimetric analysis reveals that the biosensor responds in a reversible manner to redox stimuli at different pH along the physiological range. HeLa and HEK293 reporter cell lines for the red-redox biosensor showed a sensitive and reversible response to different redox stimuli as assessed by flow cytometry and confocal microscopy.

The new redox biosensor will turn of great utility for multiparametric and high-content analysis of cells. Future studies with these reporter cell lines aim to detect metabolic deficiencies associated with cellular redox balance that may help optimizing industrial processes, test its usefulness for *in vivo* or deep-tissue imaging applications as well as to develop test to study the properties of antioxidant compounds.

This project is funded by ANII-FMV1-2014-1-104000.

(1614) **DESIGN AND INSTRUMENTATION OF A TRANS-ABDOMINAL UTERINE ELECTROMYOGRAPHIC DEVICE FOR CLINICAL RESEARCH**

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Trans-abdominal uterine electromyography (TUE) is a technique that measures the electrical activity of the uterus through the abdominal wall, using surface skin electrodes. The electrical signal obtained can be analyzed and correlated with the mechanical activity, especially with the synchronized uterine contractions. Despite being non-invasive and easy to apply, it is not used during the practice of obstetrics in Uruguay, due to high cost of available equipments. A possible use of the technique is to predict labor and delivery, as well as different pathological situations. In the present report, we propose a simple processing model for TUE signals, using a low cost, two electrodes uterine electromyograph designed in the Ion Chan-

nels Laboratory, School of Medicine (UdelaR). Our results show that with our device it is possible to obtain and isolate TUE signals from other noise signals. The power spectrum frequency obtained from the burst of uterine electrical activity during contractions, shows ergodicity when compared in the same pregnant women, as well as with different pregnant women in the same gestational age (ANOVA test). The power spectrum of non-pathological pregnancies shows centered modal pikes in frequencies between 0.2 to 1 Hz, that discriminate significantly between gestational ages ($p<0.001$). In obstetric pathologies like pre-eclampsy, or during pharmacological labor induction, the modal pike is greater in amplitude and in frequency than in the control situation. Our results show that with our economic device it is possible to obtain and isolate TUE signals from other noise signals. They also suggest that TUE is a useful technique to predict labor, as well as to monitor some clinical conditions in pregnancy. Thus, our device can be applied as a paraclinical approach for recording TUE, helping clinical decisions.

Keywords: trans-abdominal uterine electromyography, pregnancy, labour, power spectrum analysis

(1903) **GENERATION OF A SNV DATABASE FOR *NKX2.5*, A GENE ASSOCIATED WITH CONGENITAL HEART DISEASE**

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Linking the effects of Single Nucleotide Variants (SNVs) to their functional outcomes is a major issue in the field of human genetics, especially now that next-generation sequencing projects generate millions of previously unknown single nucleotide variants. Congenital Heart Diseases (CHD) are structural problems originated during prenatal heart development and stand out as a cause of prenatal, neonatal and infant mortality and morbidity. In 5-10% of the patients, CHD is caused by a single gene variation. Several genes with observed associations to CHD have been described, one of the most frequently involved is *NKX2.5*.

The aim of the present work was to compile sequence information regarding the SNVs of NKX2.5 into a single database. The information of the SNVs was obtained and curated from different databases (DBs), among them Uniprot, dbSNV (NCBI), EVS (NHLBI-ESP), SwissVar (ExPASy), GWAS Central and ExAC, as well as our own data. To get uniform SNV names and assign unique IDs, we developed an algorithm in the Python 2.7 programming language to relativize the SNV's positions to that of the GRCh38.p7 scaffold.

A total of ~1260 SNVs were compiled: ~1257 from the different DBs. From these, ~397 affected coding regions and ~860, non-coding ones. We also retrieved 3 novel SNVs found in CHD patients from our cohort.

The tools developed to acquire and clean-up the data for these genes could be used to build a DB which, in conjunction with state of the art prediction tools, could offer health professionals useful information regarding genetic disorders.

Keywords: NKX2.5, database, congenital heart disease, SNV

REGENERATIVE MEDICINE AND CELL THERAPY 2

(442) **ACIDIC PRECONDITIONING OF ENDOTHELIAL PROGENITOR CELLS IMPROVED SURVIVAL AND BLOOD FLOW RESTORATION AFTER ISCHEMIA IN TYPE II DIABETIC MICE.**

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We demonstrated that acidic preconditioning exacerbates angiogenic responses of human late outgrowth endothelial progenitor cells (EPC). We now aimed to analyze whether this strategy also improves EPC survival and functionality in the presence of damage signals (DAMPs) highly augmented in ischemic milieu or in diabetes, where tissue regeneration is compromised.

Cord blood-derived EPC were exposed at pH 6.6 for 6 h (preconditioned) or pH 7.4 (control) and then pH was restored at 7.4. N=5-7, $^{*}p<0.05$ vs control at pH 7.4, $^{\#}p<0.05$ vs same treatment at pH 7.4, one-way ANOVA.

While several DAMPs like MSU crystals (150mM), TNFα (50ng/mL), histones (3μM) or their combination induced EPC death at pH 7.4 (30±4*, 25±3*, 24±3* or 52±5*% of death, nuclear morphology), a significantly lower effect was observed in preconditioned EPC (15±3$^{\#}$, 5±3$^{\#}$, 7±2$^{\#}$ or 18±6$^{\#}$%). At non-toxic concentrations, high glucose (25mM), TNFα (10ng/mL) or their combination reduced proliferation (80±2, 54±4*, 29±2*% of control, cell count), SDF1-driven migration (83±4, 70*±4, 62*±2%, transwells) and tubule formation (77±6, 68±6*, 51±6*%, matrigel) at pH 7.4, whereas almost no inhibition was observed in preconditioned EPC (proliferation: 102±3, 87±3$^{\#}$ or 93±4$^{\#}$; migration: 135±9$^{\#}$, 121±6$^{\#}$,108±5$^{\#}$%; tubule formation: 108±10$^{\#}$, 89±5$^{\#}$ or 79±12$^{\#}$%). In type II diabetic mice, blood flow recovery after induction of hind limb ischemia was significantly improved by transplantation of preconditioned EPC (0.79±0.04* perfusion index at 14 d post-ischemia, doppler), but not control ones (0.66±0.07), when compared with PBS-treated group (0.55±0.07). Perfusion index of non-ischemic limb: 0.98±0.02.

Preconditioned EPC showed improved survival and angiogenic activity in the presence of DAMPs and efficiently restored blood flow after ischemia in diabetic mice, suggesting that acidic preconditioning is an effective strategy to improve tissue regeneration despite the stressful conditions associated with inflammation and diabetes.

Keywords: Endothelial progenitor cell, inflammation, angiogenesis, acidic preconditioning, type II diabetes.

(1449) **ANALYSIS OF SINGLE CELL DYNAMICS AND THEIR RELATIONSHIP WITH THE CELL CYCLE AND CELL FATE IN MOUSE EMBRYONIC STEM CELLS**

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Mouse embryonic stem cells (mESCs) are pluripotent cells that resemble the preimplantation epiblast of the embryo. They can give rise to all the cells of the organism, which makes them an interesting cell type in the field of regenerative medicine. Recently, the cell cycle has gained attention as a key determinant of their properties, based on the observation that cells only respond to differentiation cues during the G1 phase. In addition, it has been reported that mESCs possess a cell cycle length (CC-L) significantly shorter than their differentiated counterparts. With the onset of differentiation, it has been reported that CC-L increases. However, most of the research came from experiments that address cell populations as a whole, without considering the underlying single-cell dynamics. In this work, we aimed to characterize the dynamics of proliferation in mESCs, both while maintaining the pluripotent state and during differentiation. With this objective, we performed 48 h time-lapse videos of mESCs expressing the FUCCI cell cycle reporters. We developed a bioinformatic pipeline to extract single-cell dynamics of thousands of cells, with information about their lineage relationships. Analysis of this database confirmed the short G1-L and CC-L of mESCs. It also showed that G1-L and CC-L are greatly correlated between sister cells, implying that these properties are partially inherited from the parental cell. We also show that during the first steps of differentiation, G1-L and CC-L decrease rather than increase, and that this is

also accompanied by a decrease in S/G2/M-L. Along these lines, we applied Grassberger-Proccatia algorithm, that showed that the overall CC-L dynamics is a highly deterministic process where each CC-L can be well predicted from each cell's lineage. By addressing the great complexity of single-cell dynamics of mESCs, we believe our work contributes to a greater understanding of the connection between the cell cycle and cell-fate decisions.

(308) **CYCLIN E1 IS PERIODICALLY EXPRESSED IN HUMAN PLURIPOTENT STEM CELLS AND IN ITS DIFFERENTIATED NEURONAL PROGENY**

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Abstract: Embryonic or induced human pluripotent stem cells (hPSCs) are self-renewing cells that can differentiate into a wide range of specialized cells. hPSCs exhibit an atypical cell cycle profile compared with other cell lines, showing a longer S phase and shorter G1 phase. These unique properties suggest that the cell cycle machinery plays a role in hPSCs stemness. Previously, through synchronization of cells in early G1/S with PD0332991 and in G2/M with Nocodazole, we determined a periodic expression of almost all Cyclins mRNAs in hPSCs and derived neuronal progenitors (NP) with the exception of Cyclin E1 mRNA, which appeared to be constitutively expressed. These findings prompted us to deepen our knowledge about Cyclin E1 regulation in hPSCs and NP. In this regard, we observed by RT-qPCR analysis that MEK/ERK1/2 and c-Myc signaling pathways are involved in *cyclin E1* mRNA regulation in hPSCs as its expression levels were significantly reduced 24 hours after treatment with the specific inhibitors UO126 20μM and 10058-F4 100μM, respectively. Moreover, inhibition of E2F transcription factors with HLM006474 40μM also diminished *cyclin E1* mRNA expression levels in hPSCs. Then, we analyzed *cyclin E1* mRNA and protein expression levels by RT-qPCR and western blot in hPSCs and NP arrested in late G1 phase with Aphidicolin (10μg/ml, 20h) and in G2/M with Nocodazole (100ng/ml, 24h) and Vincristine (250nM, 24h). We observed that both mRNA and protein were present at peak levels in G1 declining to a low in G2/M. Finally, as GSK3β phosphorylation mediates rapid degradation of Cyclin E1, we treated hPSCs and NP with GSK3β inhibitor CHIR99021 (10μM) during G2/M synchronization and found no significant changes in the abundance of Cyclin E1. In conclusion, Cyclin E1 mRNA and protein are periodically expressed through hPSCs and NP cell cycle. Moreover, E2Fs, MEK/ERK1/2 and c-Myc regulate *cyclin E1* gene expression and Cyclin E1 degradation does not require a functional GSK3β.

Keywords: human pluripotent stem cells, cyclin E1, neuronal progenitors

(794) **EFFICIENT DETECTION OF CELL POPULATION USING SYNTHETIC RNA SWITCHES.**

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Synthetic RNA-switches are a novel tool for cell identification using the Dicer/Drosha/RISC pathway. They consist in a synthetic mRNA that encode a fluorescent protein and contain in the 5' UTR, a complementary sequence to a specific microRNA of the cellular lineage of interest. If the transfected cells contain the specific microRNA it will hybridize with the synthetic RNA-switch and the construction will be degraded by RISC complex, otherwise the synthetic mRNA will be traslated and the cells will express the fluorescent protein, allowing their identification and subsequent separation. The objective of this work was identify a cell population with the endogenous microRNA Let7a using sythetic RNA-switches. The cell lines HeLa and HEK293 express the microRNA Let7a so the synthetic construction was design containing the sequence complementary of miR-Let7a, a blue fluorescent protein (BFP) and a 85bp polyA sequence. The constructs were synthesized as gBlocks (IDT), amplified by PCR with specific primers and subsequently transcribed *in vitro* (MegaScript IVT Kit, Ambion). The resulting synthetic RNA switch was lipotransfected (Lipofectamine 2000, Invitrogen) in HeLa and 293T. mRNA-GFP obtained by IVT, was used as transfection efficiency control and an inhibitor of the RNA-switch was added as a control of specificity. Fluorescence was observed under fluorescence microscopy at 18-20hr post transfection. We observed 100% and 30% of transfection efficiency in the HEK293T and HeLa, respectively. The absence of the BFP signal indicate that none of the cells expressed the synthetic RNA-switch suggesting the presence of the microRNA Let7a in both cell lines. These results were confirmed when we observed expression of synthetic RNA-switches (BFP protein) in the inhibitor group. These results show the functionality of synthetic RNA-switch as an efficient cell lineages detection methods based on the presence/absence of endogenous microRNAs.

(1182) **GENERATION OF REPORTER CELL LINES TO STUDY THE DINAMICS OF INTERACTIONS OF THE TRANSCRIPTION FACTORS OCT4 AND SOX2 WITH THE CHROMATIN DURING CELL REPROGRAMMING**

Camila Vazquez Echegaray, Maria Soledad Cosentino, Claudia Solari, Paula Verneri, Camila Oses, Maria Victoria Petrone, Marcos Francia, Jesica Canizo, Ricardo Alberio, Valeria Levi, Alejandra Guberman

Abstract. Due to the lack of success to obtain genuine induced pluripotent stem cells (iPSCs) in some species like farm animals, we decided to focus our study in the reprogramming process itself using a well characterized model as murine cells. It is well known that the remodeling of the chromatin and the expression of the pluripotency transcription factors (TFs) Oct4, Sox2 and Nanog, are key elements during cell reprogramming. For this purpose, we generated doxycycline-inducible ("Tet-on") stable cell lines, over-expressing Oct4 or Sox2 C-terminally fused to a yellow fluorescent (YPet) by lentiviral transduction of the cell lines NIH3T3 (mouse fibroblasts), W4 (mouse embryonic stem cells) and IPS17 (mouse iPSCs generated in our lab). We analyzed different individual clones by studying the morphology and cell cycle with propidium iodide and cytometry, and selected those which presented normal phenotype in the absence of doxycycline, using as control the parental cell lines. To test the "Tet-on" system we tried a dose response curve of doxycycline in the generated cell lines, and established a 2 ug/ul concentration to perform further experiments. Additionally, we analyzed the time required to shut down the expression of YPet-TF, established in 48 hrs. Moreover, we studied the functionality of the transgenes, after induction, by immunofluorescence and RT-qPCR examining the expression of Oct4, Sox2 and Nanog under the different scenarios. We are currently setting the conditions to study the obtained cell lines using confocal microscopy in fluorescence correlation spectroscopy (FCS) experiments, which will reveal the distinct dynamics of interactions between the TFs and the chromatin, visualized by the expression of the chromatin reporter fusion protein H2B-RFP. Overall, we obtained a powerful tool to study the complex process of cell reprogramming by different approaches and to contribute to elucidate the intricate dynamics that could be taking place in refractory species.

Keywords. Pluripotent stem cells, cell reprogrammming, Fluorescence correlation spectroscopy

(1306) **HEME OXYGENASE-1 IS EXPRESSED IN EMBRYONIC STEM CELLS AND INCREASES DURING DIFFERENTIATION**

María Victoria Petrone Parcero (1), Ayelén Rayén Toro (1), Claudia María Solari (1), Camila Vázquez Echegaray (1), María Soledad Cosentino (1), Marcos Gabriel Francia (1), Elba Vázquez (1), Alejandra Sonia Guberman (2)
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Embryonic stem cells (ESCs) are pluripotent, which means that they have the ability to differentiate into cells of all three germ layers, and they possess several mechanisms that ensure the genome sta-

bility avoiding the propagation of genetic damage. Heme oxygenase (HO) is the limiting enzyme in the oxidative catabolism of the heme group, and particularly, the inducible isoform HO-1 is known by its antioxidant and antiapoptotic activities. Some reports have studied HO-1 action in multipotent stem cells. However, little is known about HO-1 role in pluripotent stem cells. Based on these evidences, we aimed to study if HO-1 is involved either in ESCs survival or in pluripotency maintenance. In order to achieve this, we first differentiated W4 ESCs with a non-directed differentiation protocol, culturing them in absence of the cytokine LIF for 4 days. RNA and proteins were extracted from undifferentiated cells, as a control, and from distinct time points along the differentiation protocol (days 1, 2, 3 and 4). HO-1 expression profile was analyzed by RT-qPCR and Western blot (WB). We found that HO-1 mRNA and protein levels increased during differentiation. Furthermore, we analyzed HO-1 protein by immunofluorescence in order to study HO-1 subcellular localization throughout the differentiation process. We found that this protein was ubiquitously expressed, both in cytoplasm and in the nucleus, in ESCs in undifferentiated state, and that its levels were upregulated during differentiation, along with the decreased expression of Oct4 and Nanog, transcriptional factors that are crucial to pluripotency. RT-qPCR and WB results were analyzed using randomized block design ANOVA and Tukey test. These findings show that HO-1 expression is modulated along the differentiation suggesting that this enzyme could be relevant to leave behind the undifferentiated state or to the differentiation process and could be associated with Oct4 and Nanog regulation.

Keywords: pluripotent stem cells, HO-1, gene expression, transcriptional regulation.

(1549) **IDENTIFICATION OF ISOMIRS DURING CARDIAC DIFFERENTIATION: A POTENTIALLY SIGNIFICANT AMPLIFICATION SIGNAL OF THE MIRNOMA**

Ximena Garate, Alejandro La Greca, Gabriel Neiman, Lucia Moro, Natalia Santín Velazque, Agustina Scarafia, Alan Mobbs, Carlos Luzzani, Gustavo Sevlever, Leonardo Romorini, Santiago Miriuka

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MicroRNAs work by fine tuning the expression of key developmental genes. Deeper analyses of microRNA expression revealed that many of them are expressed with small changes in their sequences, termed isomiRs, what possibly have a huge repercussion on the pool of mRNA targets. MicroRNAs belong to different families and clusters, when they share the seed sequence and when they are proximate in the genome, respectively. In this work, we study microRNAs and isomiRs expression changes during cardiac differentiation from pluripotent stem cells by high throughput RNA sequencing and analyze the families and clusters to establish the miRnome identity based on them.

We studied pluripotent stem cells (PSC), an early mesoderm progenitor (MPC) and isolated cardiomyocytes (CM), both derived from PSC. Approximately 700 microRNAs are expressed in the three groups. Unsupervised soft clustering revealed five general patterns of microRNA differential expression. Interestingly, the isomiR analysis showed a widely disperse expression of the isoforms on the 3' extreme (iso3) (55.9% for PSC, 56.5% for MPC, and 50% for CM), on the contrary changes on the 5' extreme (iso5) are less abundant than the iso3 for the three populations (16.1% for PSC, 15.5% for MPC, and 16.9% for CM). Moreover, we identified several iso5 significantly expressed corresponding to pluripotent-related microRNAs (i.e. mir-302 family) broadening their specificity for target mRNAs. Finally, further analysis of the microRNAs families and clusters expression profiles revealed that both groups are quite similar. PCA and SD analysis showed a slight improvement in the discrimination between populations when analyzing expression profiles of clusters.

In conclusion, a wider analysis of microRNAs expression exposes an extended network of them and their respective isomiRs and analyzing families and cluster expression profiles allows to identify new insights into the miRnome identity during cardiac differentiation.

Keywords: MICRORNAS, ISOMIRS, CARDIAC_DIFFERENTIATION, HUMAN_PLURIPOTENT_STEM_CELLS.

(1245) **INHIBITION OF INTEGRIN A5 THROUGH A CONDITIONAL KNOCKDOWN IMPAIRS HUMAN PLURIPOTENT STEM CELL SELF-RENEWAL.**

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Abstract: Human pluripotent stem cells (hPSCs), with their ability to differentiate into mature cell types, represent a strong system to study human development and disease, and efficacy of drugs before clinical trials. Also, these cells provide an unlimited source of 'raw material' for regenerative medicine therapies. However, details of the molecular mechanisms interconnecting the extracellular matrix through integrins of hPSC remain unclear and how this influence on self-renewal and stem cell fate. Our main goal is finding out the role of integrin a5 by disrupting alpha5-fibronectin interaction, in hPSC and during cardiac differentiation. To investigate the role of this protein, the knockdown of integrin a5 was induced in the hES3-hESC line with a DOX inducible KRAB repressor through a variant of crispr-cas9 system. hESC line showed a strong down-regulation of integrin a5 by flow cytometry, reducing the protein expression in an 80% of cells (n=3) after 72h of 500ng/ml DOX treatment. mRNA level was also checked by qRT-PCR and it was 4 times less expression (n=3) than without treatment. As we noticed that cells had a slower proliferation when integrin a5 was inhibited, a XTT cell viability assay was done after 72h of treatment and interestingly cell proliferation was decreased in a 20% (n=4). Since self-renewal is impaired, we are now interested to see how this affects the cell-cycle by using an EDU Proliferation Assay. As well, during a cardiac differentiation protocol (Lian, et al.), we observed that α5 has its mRNA and protein expression peak at d3.5 (early mesoderm) and it is 10 times higher than hPSC (n=3). Owing to this, we are now working on the inhibition of integrin a5 in order to find out if it is also involved during the early mesoderm differentiation.

Keywords: pluripotent, mesoderm, integrin, crispr-cas9, stem cells.

(899) **TRANSCRIPTION FACTORS ESSENTIAL FOR PLURIPOTENCY MODULATE SUPEROXIDE DISMUTASE 1 AND GLUTATHIONE REDUCTASE GENE EXPRESSION IN MOUSE EMBRYONIC STEM CELLS**

Claudia Solari (1), María Victoria Petrone (1), Camila Vázquez Echegaray (1), Soledad Cosentino (1), Ariel Waisman (1), Marcos Francia (1), Timoteo Marchini (2), Pablo Evelson (2), Gabriela Chaufan (1), María del Carmen Ríos de Molina (1), Lino Barañao (3), Santiago Miriuka (4), Alejandra Guberman (1)

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Our aim was to study Superoxide dismutase 1 (Sod1) and Glutathione reductase (Gsr) gene expression in embryonic stem cells (ESCs) in order to unravel the relationship between redox homeostasis and pluripotency. For this purpose, R1 ESCs were differentiated with a non-directing differentiation protocol, culturing them in absence of the cytokine LIF for 4 days. RNA was extracted from undifferentiated and differentiated cells, and the expression profile of Sod1 and Gsr genes was analyzed by RT-qPCR. We found that these genes were expressed in the undifferentiated state and they were modulated in opposite ways when ESCs were induced to differentiate. While Sod1 was downregulated during differentiation, Gsr expression was upregulated (randomized block design ANOVA and Tukey test). Next, we performed a shRNA approach to downregulate Oct4, Nanog and Sox2. Sod1 and Gsr mRNA levels were analyzed in ESCs transfected with vectors encoding each shRNA. In accordance with the expression profile found along differentiation,

we found a reduction in Sod1 mRNA levels when Oct4, Nanog and Sox2 were downreguated. Moreover, Gsr expression was increased when Nanog was downregulated (randomized block design ANOVA and Tukey test). We also performed a trans-activation assay with reporter vectors to study Sod1 and Gsr promoter responsiveness. We found that Oct4, Sox2 and Nanog induced luciferase expression in Sod1 reporter vector, but we did not find a clear response with the Gsr reporter vector studied (randomized block design ANOVA and Tukey test). Finally, we analyzed Sod activity and we found that it decreased along differentiation (Student test).

Our results suggest that Sod1 gene expression is induced by the transcription factors Oct4, Nanog and Sox2, and that Gsr gene expression could be repressed by Nanog in ESCs. These results evidence a role of the crucial pluripotency transcription factors in preservation of redox homeostasis in stem cells.

Keywords: Redox homeostasis; Sod1; Gsr; transcriptional regulation; gene expression

(1202) **USE OF DIFFERENT CRISPR ACTIVATION SYSTEMS TO ACTIVATE HUMAN BETA PANCREATIC GENES**

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CRISPR transcription activation systems (CRISPR/dCas9-VP160, CRISPR/dCas9-TET1 and CRISPR/dCas9p300) are novel RNA-guided system that can turn on specific endogenous gene expression by epigenetic activation. However, it remains unclear how these systems can be used to turn multiple genes on at once. Therefore, we aimed to compare gene activation using as example beta pancreatic TF (*PDX1,NGN3*) and genes (*PAX4,INS*) in HEK293 human cell line. To compare these different CRISPR activation systems we transfected five sgRNAs designed to target proximal promoter for activation of each gene using CRISPR Design Tool (Zhang Lab,-MIT). The sgRNAs target sequences were cloned on the sgRNA expression plasmid (Addgene#47108). dCas9-VP160 plasmid (transcription activation domain-Addgene#48226), dCas9-TET1 plasmid (DNA demethylation domain-Addgene#82559) and dCas9-P300 plasmid (histone acetylation domain-Addgene#61357) were transfected separate at a mass ratio of 1:1 to a mixture of equal amounts of the different sgRNAs plasmids for all genes. Control groups cells were non-transfected. By RT-qPCR at day 4 we observed that dCas9-P300 activated transcription more efficiently that the others systems in *INS, PDX1* and *PAX4 (p<0.05).* Next, we studied how to simplify the dCas9-p300 system by searching for the most efficient guides per gene. Opposite to dCas9-VP160system, using one single guide in dCas9-P300 group was enough to have similar activation efficiency compared to all sgRNAs guide together (analyzed by RT-qPCR at day 4; p>0.05). Consequently, when we used the most efficient guides instead to all guides together in the multiplex gene activation experiments we could equal (*INS*,*PDX1*,*NGN3*) or improve activation efficiency (*PAX4*: 6,26±1.5-fold;X±SD). These data suggest that dCas9-P300 is one of the most powerful CRISPR activation systems and preliminary individual sgRNA validation efficiency may be carried out in order to activate more efficiently multiplex genes at the same time.

Keywords: CRISPR, gene activation, cellular differentiation, diabetes, beta pancreatic cell.

REPRODUCTION AND FERTILITY 7

(1731) **INCORPORATION OF STEROLS INTO RAM SPERM REDUCES THE PERCENTAGE OF ACROSOME REACTED SPERMATOZOA AFTER CRYOPRESERVATION**

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Cryopreservation of sperm is a useful biotechnological tool for artificial insemination. However, ram sperm is particularly sensitive to this process, limiting its use for this species. Previously, we found a significant sterol loss in cryopreserved ram sperm and a concomitant decrease in membrane lipid order. Recently, we established controlled conditions to increase sperm sterol content using methyl-β-cyclodextrin (MβCD). The objective of this study was to evaluate the impact of increasing cholesterol (Chol) and desmosterol (Des) content of ram sperm (2.5, 10, 25mM MβCD-sterol) on: 1) membrane lipid order and level of the putative raft marker, ganglioside GM_1, and 2) sperm quality parameters after thawing. Treatment of semen with 10 and 25mM MβCD-Chol or -Des increased membrane lipid order determined by fluorescence spectroscopy (Laurdan). The presence of GM_1 was detected in living sperm using the fluorescent-labeled cholera toxin B subunit. GM_1-related fluorescence was mainly associated to the post-acrosomal region and was reduced when both sterols were incorporated into the sperm at 25mM MβCD, suggesting a membrane disorganization effect induced by sterol incorporation ($p<0.05$). Therefore, 10mM concentration was selected to incorporate sterols into ram sperm prior to cryopreservation. As to the sperm quality parameters analyzed, no significant differences were found in percentages of total motility, membrane integrity (eosin/nigrosin) and osmotic tolerance (HOS test) between control and treated cells, irrespective of the class of sterol. However, the percentage of acrosome reacted sperm evaluated by concanavalin A staining after cryopreservation was reduced by sterol incorporation ($p<0.05$). We conclude that treatment of ram sperm with 10mM MβCD, complexed either with Chol or Des, increases membrane lipid order without affecting GM_1-related raft organization and exerts a stabilizing effect of the acrosome reducing acrosome reaction induced by cryopreservation.

Keywords: sperm cryopreservation, cholesterol, desmosterol, GM_1

(674) **GENERATION OF MULTIPLE KNOCKOUTS FOR CYSTEIN-RICH SECRETORY PROTEINS USING CRISPR/CAS9 TECHNOLOGY**

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Cysteine-Rich Secretory Protein (CRISP) 1, 2, 3 and 4 are mainly expressed in the reproductive tract and have key roles in mammalian fertilization. In spite of this, mutant mice lacking either CRISP1, 2 or 4 are fertile. To investigate the functional relevance of CRISP3, we developed *Crisp3* knockout mice using the novel CRISPR/Cas9 technique. With the idea of also generating double *Crisp1*$^{-/-}$/*Crisp3*$^{-/-}$ mice, sgRNAs targeting exon 2 of *Crisp1* and *Crisp3* were selected by bioinformatic analysis. These sgRNAs were microinjected together with Cas9 mRNA into C57BL/6 mouse zygotes and then transferred to pseudopregnant females. Mice carrying the targeted mutations were identified by PCR of genomic DNA and SDS-PAGE. Germline transmission of the mutations was confirmed by DNA sequencing and mice carrying heterozygous null-allele mutations in *Crisp3* or in *Crisp1* and *Crisp3* were selected as breeders to obtain homozygous mutants. Expression analysis of CRISP proteins in mutant mice performed by Western blot revealed that both *Crisp3*$^{-/-}$ and *Crisp1*$^{-/-}$/*Crisp3*$^{-/-}$ animals lack CRISP1 whereas CRISP2 and CRISP4 were not affected in either colony. Fertility rates evaluated by natural mating were significantly reduced in male and female mice from both colonies, suggesting that the normal fertility observed in single knockouts involves compensatory mechanisms between homologous CRISP proteins. To generate mice simultaneously lacking the four CRISP family members, we microinjected sgRNAs targeting *Crisp1* and *Crisp3* into *Crisp2*$^{-/-}$/*Crisp4*$^{+/-}$ zygotes. Recent results confirmed the successful generation of mice with null alleles of the four *Crisp* genes. We believe these studies will contribute to a better understanding of the relevance of this important family for animal fertility.

Keywords: CRISPR/Cas9, CRISP, fertility.

(133) **ROLE OF HYPERPOLARIZATION ON HUMAN SPERM ACROSOMAL EXOCYTOSIS**

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Sperm capacitation in the female tract is essential to gain fertilization ability. During this processes sperm undergo a series of physiological modifications that lead to two main events: acquisition of hyperactivated motility and the ability to undergo acrosomal reaction (AR). It has been reported either pharmacologically or genetically (potassium channel Slo3 Knockout mice) that hyperpolarization of the plasma membrane (Em) is necessary and sufficient for sperm acrosomal responsiveness. This change in Em is driven by the cAMP/PKA signaling pathway associated to capacitation. Both, PKA and Slo3 are located in the flagellum and little is known about how this change is transduced to the head where AR takes place and how this plays out in human sperm. In this work we aimed to study the role of cAMP/PKA pathway on Em and its impact on acrosomal responsiveness in human sperm.

Human sperm was incubated with CFTR, NBC, Ouabain and PKA inhibitors, with or without valinomycin to induce hyperpolarization and A23187 to induce AR. Changes in Em were assessed by using Disc(3)5 and flow cytometry. To assess the acrosomal status, human sperm were stained with *Pisum sativum* and observed under an epifluorescence microscope. In the present work we found that, similar to what occurs in mice, activation of the cAMP/PKA pathway is essential for AR responsiveness and that changes in this pathway are involved in the Em. We also found that in conditions where hyperpolarization does not occur inhibitors of (CFTR, NBC or sodium-potassium ATPase), sperm cannot undergo AR in response to Calcium ionophore. By inducing hyperpolarization using valinomycin, we have observed that sperm can undergo AR even in conditions that do not support capacitation. All together, these results suggest a pivotal role of cAMP/PKA in Em changes in human sperm and that Em is essential to prepare sperm to undergo AR.

(712) **INVOLVEMENT OF TYROSINE PHOSPHORYLATION IN CAPACITATION-INDUCED CALCIUM INCREASE IN HUMAN SPERMATOZOA**

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Sperm capacitation involves an increase in protein tyrosine phosphorylation (pTyr) and intracellular Ca^{2+} levels. However, the cross-talk between these two signaling cascades is poorly understood. Based on this and considering our previous results showing that human sperm pTyr is blocked by inhibiting proline-rich tyrosine kinase 2 (PYK2), we investigated the effect of the PYK2 inhibitor PF431396 (PF) on the intracellular Ca^{2+} increase during human sperm capacitation. Using the Ca^{2+} indicator Fluo-4 AM, we analyzed Ca^{2+} levels in sperm incubated for 6 h in capacitating media with or without PF. Interestingly, fluorescence intensityevaluated by both flow cytometry and single cell imaging was significantly reduced at 10 μM PF, a concentration that also affects pTyr. Although PYK2 was reported to play a role in Store Operated Calcium Entry (SOCE) in other cell types, thapsigargin (10 μM), a compound that induces release of Ca^{2+} from intracellular stores and activates SOCE, produced similar effect in PF-treated sperm compared to controls. Another explanation for the PF-induced decrease in Ca^{2+} is that PYK2 is involved in the activation of CatSper, the main sperm Ca^{2+} channel essential for male fertility. In this regard, our observations showing that sperm treated with the CatSper inhibitor HC-056456 (10 μM) prevented Ca^{2+} increase, confirm the key role of CatSper in the Ca^{2+} cascade leading to capacitation. Based on this, we analyzed the effect of PF on cytoplasm alkalinization and membrane hyperpolarization, two events required for CatSper activation. Flow cytometry results showed a reduced alkalinization in PF-treated sperm which could be reverted by NH_4Cl, suggesting that pTyr plays a role in Ca^{2+} signaling through regulation of cytoplasmic pH. Together, these observations reveal a role for PYK2 in the regulation of intracellular Ca^{2+}, contributing to a better understanding of the cross-talk between pTyr and Ca^{2+} signaling pathways during human sperm capacitation.

Keywords: sperm, calcium, capacitation, tyrosine phosphorylation

(1446) **LYSINE ACETYLATION AS A MODULATOR OF SPERM CAPACITATION**

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Mammalian sperm are unable to fertilize the egg before undergoing a series of biochemical and physiological changes in the female reproductive tract, collectively known as capacitation. Functionally, capacitation is associated with changes in the sperm motility (hyperactivation) and with their ability to undergo the acrosome reaction. At the molecular level, capacitation correlates with activation of the cAMP-PKA pathway, increase in intracellular pH and Ca^{2+} concentration, hyperpolarization of the plasma membrane potential and increase in protein tyrosine phosphorylation. How these signaling pathways interact to induce hiperactivation and acrosomal responsiveness, is not well understood. Since mature sperm are transcriptionally and translationally silent, they rely on PTM of proteins more than any other cell type. Therefore, it is an exceptional model for the study of signaling pathways based on PTM. The importance of phosphorylation, an essential PTM in sperm physiology has been well established. Acetylation as a broad and abundant PTM comparable with phosphorylation, however, has not been well analyzed. Recently, two groups identified 576 and 456 acetylated proteins in capacitated and non-capacitated human sperm respectively, of which 250 were present in both conditions. In the present work, we studied the role of acetylation in mouse sperm capacitation. WB and immune-localization analysis with anti-acetyl lysine antibodies showed lysine acetylation of several proteins spanning a wide mass range, both in the sperm head and tail. A significant increase in the fluorescence was detected in capacitated sperms. Pharmacological hyperacetylation was associated with increased PKA activity and, an increase in intracellular Ca2+, even in the absence of HCO3- and BSA. In addition, this PMT regulates hyperpolarization of the plasma membrane and hyperactivation. These results point towards a key role of lysine acetylation in sperm capacitation.

(140) **ONLY A SUBPOPULATION OF MOUSE SPERM DISPLAYS A RAPID INCREASE IN INTRACELLULAR CALCIUM DURING CAPACITATION**

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Freshly ejaculated mammalian sperm do not have the ability to fertilize oocytes. They must undergo a functionally defined process called capacitation. Sperm become capacitated *in vivo* by interacting with the female reproductive tract or *in vitro* in a defined capacitation media that contains bovine serum albumin (BSA), calcium (Ca^{2+}) and bicarbonate (HCO_3^-). In this work, we used flow cytometry to qualitatively analyze changes in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$). For this purpose, sperm were double stained with Propidium iodide and the Ca^{2+} dye Fluo-4 AM to analyze these changes in individual live sperm. An increase in $[Ca^{2+}]_i$ was observed in capacitated live sperm when compared with non-capacitated ones, being able to distinguished one subpopulation of sperm more responsive than the other. These rise in $[Ca^{2+}]_i$ after 90 min of incubation in capacitated media was evidenced by an increase in the normalized median fluorescence ($p<0.0001$) but also in the percentage of sperm that

responded increasing $[Ca^{2+}]_i$ ($p<0.0001$); and depended on the presence of extracellular Ca^{2+}. When sperm were incubated with only BSA or HCO_3^-, we observed that both compounds stimulated $[Ca^{2+}]_i$ increase individually, being BSA stimulation much stronger ($p<0.05$). In addition, we determined that the capacitation-associated $[Ca^{2+}]_i$ increase at 90 min, was blocked by both CatSper inhibitors in wild-type sperm ($p<0.01$) as well as in CatSper knockout sperm ($p<0.0001$). On the other hand, sperm exposed to capacitated medium displayed a rapid increase in $[Ca^{2+}]_i$ within 1 min of incubation, which was not altered in CatSper knockout sperm, but depended on the presence of extracellular Ca^{2+} in the medium. All together, these results suggest that there is a subpopulation of sperm that increases $[Ca^{2+}]_i$ very rapidly during capacitation due to an influx from extracellular Ca^{2+}. These initial rise is not driven by CatSper, whose role is essential for the sustained $[Ca^{2+}]_i$ that occurs at later times.

Keywords: Sperm; Calcium; Capacitation; CatSper.

(896) **ROLE OF MULTIDRUG RESISTANCE PROTEIN 4 (MRP4) AND THE MECHANISMS INVOLVED IN THE REGULATION OF ITS ACTIVITY DURING MOUSE SPERM CAPACITATION**

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In mammals, sperm capacitation correlates with $HCO3^-$ influx, activation of soluble adenylyl cyclase (sAC), cAMP increase, PKA activation (pPKA), and increase in tyrosine phosphorylation (pY). cAMP levels are mainly regulated by its synthesis and degradation, but its extrusion through multidrug resistance protein 4 (MRP4) transporter is also involved in this modulation. Previously, we found that cAMP efflux by MRP4 is critical for pY occurrence in mouse SPZ. Moreover, our results indicated that ~50% of SPZ that undergo capacitation, lose MRP4. Here, we aim to deepen into the role of MRP4 during sperm capacitation. To support the involvement of MRP4 in capacitation, we performed in vitro fertilization (IVF) assays, incubating SPZ in capacitating conditions (CC) in the presence or not of MK571 (MK), a specific MRP4 inhibitor. Results showed a decrease in the % of IVF when SPZ were incubated with MK ($p<0,05$). Then, we investigated the role of MRP4 over the time. Sperm were incubated in CC with or without MK and the evaluation of pPKA, pY, and MRP4 abundance was performed at different incubation times. Results showed that while pPKA was higher at 1 min in SPZ incubated with MK, lesser levels of pPKA and pY were found at 90 min incubation compared to control. To test which component of the capacitation media might be conditioning the presence of MRP4, we incubated SPZ in CC with increasing concentrations of either HCO3- or BSA. While the presence of HCO3- reduced the abundance of MRP4 in a dependent concentration manner, BSA did not have any effect. We also examined the participation of PKA in the MRP4 processing by incubating SPZ in CC or Non-CC with or without H89, a PKA inhibitor. Results showed that the incubation with H89 prevented the MRP4 decrease. All results suggest that MRP4 activity is needed to allow a proper PKA regulation, which in turn might participate in the processing of MRP4 promoting pY and sperm fertilizing ability.

Keywords: Mouse sperm, capacitation, MRP4, PKA

(186) **FIBROBLAST GROWTH FACTOR 2 (FGF2) IS INVOLVED IN THE REGULATION OF SPERMATOGENESIS. STUDIES WITH THE KNOCK-OUT MODEL**

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In previous studies from our laboratory we have described the presence of Fibroblast Growth Factor 2 (FGF2) and its receptors (FGFRs) in human testis and sperm, and the results suggest that this system is involved in the regulation of human spermatogenesis and sperm motility (Saucedo et al, 2015; Garbarino Azúa et al, 2017). The aim of the present study was to further analyze the role of FGF2 in the maintenance of sperm physiology using FGF2 knock-out (KO) mice. Our results have shown that in wild type (WT) animals, FGF2 is expressed in all germ cells of the seminiferous epithelium, with higher expression in spermatids and sperm. In epididymal sperm, the protein was immunolocalized in the flagellum and acrosomal region. The FGF2 KO males display no apparent fertility defects, since their mating with WT females showed no differences in the time to delivery, litter size and pups weight in comparison to WT males. Analysis of cauda epididymal sperm from KO animals indicated no differences in sperm motility, but a significant increase ($p<0.01$) in sperm concentration and in sperm head abnormalities in comparison to the WT. Moreover, sperm from KO mice depicted similar spontaneous acrosomal exocytosis, but increased ($p<0.01$) acrosomal loss after incubation under capacitating conditions compared to the WT. The assessment of spermatogenesis through staging of seminiferous tubules revealed a deregulation of spermatogenesis kinetics in the KO animals. Additionally, the analysis in FGF2 KO males showed higher ($p<0.05$) daily sperm production and number of spermatids per testis. Overall, the results indicate the relevance of FGF2 in the regulation of mammalian sperm production.

Keywords: FGF2, fibroblast growth factor, sperm cells, testis

(1889) **CALCIUM STORES INVOLVED IN CALCIUM SIGNALING ACTIVATED BY PROGESTERONE IN HUMAN SPERM.**

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Calcium signaling is a key regulatory mechanism in sperm functions such as capacitation, motility, hyperactivation, chemotaxis and acrosome reaction. Progesterone (Pg) produced by the oocyte cumulus has been associated with several processes of sperm physiology, since it directly activates plasma membrane Ca2+ channels. Pg open the channels triggering a rapid increase in intracellular calcium coming from the extracellular medium. It's been described that some sperm organelles can operate as Ca2+ stores with functional importance in sperm function, like the acrosome vesicle, redundant nuclear envelope and mitochondria located in sperm's middle piece. Our group in previous work have observed that Pg induces an intracellular calcium increase in media with different [Ca2+], yet is unclear if this calcium increase is due to calcium release from intracellular stores or other source. The aim of this study was to investigate the calcium stores and calcium channels involved in the Pg pathway in human spermatozoa. We used real time dynamic assays with high speed and spatial resolution – in single cells and population – and fluorescent calcium sensors. To meet this goal we loaded capacitated human sperm with Fluo3-AM or Fura2-AM, then incubated in medium containing different $[Ca^{2+}]$ and treated with progesterone in absence or presence of intracellular calcium modulators or mitochondrial modulators. We observed that media with different $[Ca^{2+}]$ generated increases of intracellular Ca^{2+} with particular kinetics and patterns in response to Pg. We also noticed that calcium modulators altered the Ca^{2+} patterns and kinetics previously observed. These results suggest that Pg can activate the exit of Ca^{2+} from intracellular stores in conditions of low extracellular $[Ca^{2+}]$.

Keywords: Calcium signaling, calcium reservoirs, human sperm.

(568) **THE SIGNALING MODULE RAB27-RABPHILIN-3A-GRAB-RAB3 IN SPERM EXOCYTOSIS**

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Exocytosis is a fundamental cellular process used by eukaryotic cells to release biological compounds such as peptide hormones and neurotransmitters and to insert specific lipids and proteins in the plasma membrane. Mammalian sperm exocytose their acrosome (the acrosome reaction or AR) at fertilization. The AR depends on members of the standard fusion machinery, including small GTPases and SNAREs; two such GTPases, Rabs27 and 3, are organized in a Rab-GEF cascade. Western blot and indirect immunofluorescence were used to detect previously undescribed proteins and their localization in sperm. Antibodies and a variety of recombinant proteins were applied in functional assays in intact and streptolysin O-permeabilized sperm to investigate the role of members of the fusion cascade in the AR. The gene encoding Rab27A was subcloned downstream the TAT sequence of the HIV virus to express permeable Rab27A in *Escherichia coli*. Immobilized Rabs loaded with GDP or GTP and Rabphilin-3a were used to pull down interacting proteins from human sperm extracts. Pull down was also the method of choice to determine Rab3-GEF activity. Here we show that a permeable, recombinant version of human Rab27A elicits the AR when introduced into capacitated human sperm. Rab27A promotes the exchange of GDP for GTP on Rab3 in the acrosomal region of intact sperm, so does the catalytic domain of GRAB, a GEF for Rab3, when introduced into permeabilized sperm. Rab27 accomplishes these functions by recruiting its effector Rabphilin-3a, which, in turn, interacts with GRAB.

Keywords: exocytosis, Rab27, Rab3, GEF, sperm

ENDOCRINOLOGY 7

(960) DEVELOPMENT OF A HIGH SENSITIVITY IMMUNO-PCR ASSAY TO DETECT AND QUANTIFY LOW LEVELS OF THYROID STIMULATING HORMONE (TSH) IN HUMAN SERUM SAMPLES.

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Thyroid gland function is regulated by the TSH, a glycoprotein hormone secreted by the pituitary gland. Measurement of TSH in human fluids is utilized as a "first line" thyroid test, in order to assist clinical decision-making. Most of the current TSH methods used in clinical laboratories are two-site "sandwich" heterogeneous immunoassays. Some differences in terms of analytical performance and measured TSH concentrations still exist among the commercially available methods. The between-methods variability is the largest at low TSH concentrations (below 0.4 μIU/ml), due to limit of detection and lower analytical sensitivity of some immunoassay methods. We developed a highly sensitive immuno-PCR (IPCR) assay specific for detection of TSH in human serum samples. First, several anti-TSH monoclonal antibodies (MAbs) were generated using hybridoma technology. Pairs of MAbs were rationally selected and the performance characteristic (specificity, sensitivity, precision and accuracy) were established by sandwich ELISA. Next, we develop a TSH-IPCR assay applying a "Universal-IPCR" format in standard PCR tubes. The signal amplification was achieved through the interaction between the biotinylated detection MAb and mono-biotinylated DNA probe pre-self-assembled with neutravidin. The IPCR prototype was evaluated with standards calibrated with WHO 2nd International Reference Preparation for TSH, with human serum samples and in comparison with a commercial ELISA Kit. The TSH-IPCR assay showed a significant increase in terms of the slope definition of sensitivity in low levels range, providing better quantitative resolution for a given amount of measurement error or, conversely, higher sensitivities can tolerate larger measurement errors for a given amount of quantitative resolution. The sensitivity (m = 1.25), LOD (0.01 ng/ml), and other aspects of our results support the potential of IPCR technique for being applied in clinical diagnosis of thyroid states.

Keywords: TSH, immuno-PCR, IPCR, monoclonal antibodies, high sensitivity.

(1726) EFFECTS OF THYROID HORMONES ON OXIDATIVE PARAMETERS IN MURINE LYMPHOCYTIC CELLS

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BACKGROUND: Thyroid hormones exert actions on growth, metabolism, cellular differentiation and immune functionality. **OBJECTIVE:** To study the effects of hyperthyroidism on oxidative parameters in murine lymphocytes. **METHODS:** Female BALB/c mice were treated with T4 (12mg/l) in drinking water for 30 days (hyperthyroid animals) or with placebo (euthyroid animals). Serum hormone levels were quantified by radioimmunoassay. Reactive oxygen species (ROS) and apoptosis were evaluated by flow cytometry using DCFH-DA and Annexin-V/PI. The expression of antioxidant enzymes and iNOS were analyzed by real-time PCR and western blot. Enzymatic activity was quantified by spectrophotometry. Nitrite production was evaluated by the Griess technique. **RESULTS:** We observed an increase of ROS in lymphocytes purified from lymph node tissue (LN) and spleen (S) of hyperthyroid animals with respect to controls (29.1±2.6 % in LN and 26.2±2.1 in S, $p<0.005$) that was correlated with the increase in the activity and genomic expression of Catalase and Glutathione Peroxidase-1. Hyperthyroid animals showed higher iNOS protein expression (% increase: 37.2±3.0, $p<0.01$) and nitrite production (% increase: 23.1±2.0, $p<0.005$). *In vitro* treatment of lymphoid cells from euthyroid animals with an oxidative stress inducer (H_2O_2) increased the genomic expression of antioxidant enzymes (% increase for Catalase 38.0±3.1 in LN and 32.0±2.9 in S, $p<0.005$). Lymphocytes from hyperthyroid animals treated with H_2O_2 showed a greater capacity to detoxification. Apoptosis levels were similar in both study groups. **CONCLUSIONS:** Hyperthyroidism induces the expression of antioxidant enzymes in lymphoid cells through the increase of ROS. The enzymatic antioxidant system protects cells from oxidative damage and ROS-mediated apoptosis.

Keywords: Hyperthyroidism, lymphocytes, oxidative stress, antioxidant enzymes.

(1847) EVALUATING ZINC IMPORTANCE ON LYMPHOCYTE ACTIVITY IN HORMONE HYPOTHYROID CONDITIONS

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Immunity is modulated by a complex net of immune and neuroendocrine factors, among them, hypothyroidism leading to immunosuppression. Additionally, Zinc (Zn) is a trace element which deficit induces a decrease in lymphocyte number and activity as well as adversely affects the synthesis, metabolism and action of thyroid hormones (TH)**.** Our aim was to study if hypothyroid conditions may alter zinc metabolism and if this is related to the impairment in T lymphocyte activity.

We evaluated the impact of hypothyroidism on Zn metabolism and its possible relationship with T cell activity. Hypothyroid mice showed lower levels of zinc in femur and lymph nodes than controls. T3 reversion of the hypothyroid status, as well as Zn supplementation revert these effects ($p<0.05$). Conversely, hormone hypothyroid conditions were not altered by Zn administration. In vitro, both TH and Zn depletion in culture (by Zn chelators) lead to a decreased response to Con A stimulus, effect selectively reverted by TH or Zn addition, respectively. Zn, but not TH deficit was able to induce lymphocyte apoptosis. In vivo Zn deficiency resembles hypothyroidism as it leads to an impaired proliferation of T cells by selective mitogens. The role of intracellular signals activated by the mitogen stimulus was evaluated in T lymphocytes from hypothyroid mice before and after T3 or Zn replacement. We found decreased translocation of PKC to cell membranes respect to euthyroid mice and lower levels of p-ERK after Con A stimulus reverted by both

T3 replacement and Zn supplementation (p<0.01). Also, Zn supplementation was able to re-establish proliferative responses in a similar manner than T3 replacement in lymphocytes from hypothyroid mice.

Our results showed an important relationship between Zn deficiency and hypothyroidism mediated immunosuppression, thus strengthening the relevance of Zn levels in hypothyroid mice.

Keywords: Hypothyroidism, thyroid hormones, lymphocyte, Zn

(1660) **HYPOTHYROIDISM ALTERS PI3K/AKT SIGNALING DURING LACTATION**

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Lactation is key in mammary differentiation and is regulated by prolactin and the JAK2/STAT5 and AKT signaling pathways. It is known that hypothyroidism (hypoT) has deleterious effects on the JAK2/STAT5 pathway. In this work, we evaluated the impact of PTU-induced hypoT on lactating rat mammary AKT pathway, which has been intimately linked with mammary cellular differentiation, transcriptional and translational cell performance and mammary pathologies like cancer. We evaluated the expression level and phosphorylation of the three AKT isoforms and the two PI3K sub-units by western blot in the mammary glands (n = 6) from rats on lactation days 2, 7 and 14 (L2, L7 and L14). Results were analyzed by two-way ANOVA and Bonferroni post-test. We found that on L7 hypoT decreased the expression level of the p85 subunit of PI3K (p<0.05) but increased its phosphorylation level, proving that at this stage, the hypothyroid mammary gland is able to compensate the activation of the pathway. At the same time, hypoT decreased (p<0.05) the AKT1 protein level (isoform linked to lactation differentiation), and although phosphorylation in threonine residues was increased (p<0.05), serine phosphorylation was not modified, proving that activation of this isoform was not totally achieved. HypoT also decreased the protein level of the AKT2 and AKT3 isoforms in L7 (p<0.05) but increased their phosphorylation level in serine and threonine residues (p<0.05), indicating total activation of these isoforms. We did not observe significant effects of hypoT on L2 and L14. Since AKT2 has been associated with inhibition of lactogenic differentiation and that hypoT caused its full activation in mammary gland with decreased AKT1 activity, these results could indicate that although the mammary cell is able to partially compensate the activity of AKT signaling pathway, this compensation is not complete and therefore hypoT could decrease mammary cell differentiation level already on early lactation.

Keywords: Lactation, AKT signaling, mammary cell differentiation.

(1908) **HYPOTHYROIDISM IN BOTH HIV-INFECTED AND UNINFECTED MALE AND FEMALE ADULT SUBJECTS**

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The prevalence of functional diseases of the thyroid gland is high in the general population. It is more common in women than in men. International studies show a prevalence of hypothyroidism in adults in a range of 4% to 17%. Human immunodeficiency virus (HIV) infection is a disease that affects endocrine function. Thyroid dysfunction is common in HIV-infected patients. The purpose of this study was to compare the frequency of hypothyroidism among male and female in both HIV positive and negative adults patients treated in a public hospital in La Plata. A total of 147 HIV positive patients (HIVp), 62 women and 85 men with a mean age of 41 years, and 280 HIV negative (HIVn), 124 men and 156 women with an average age of 46 years, were studied. Serum's Thyrotrophin (TSH), free thyroxine (T4L), antithyroglobulin (a-TG) and anti-thyroperoxidase (a-TPO) antibodies were measured by chemiluminescence in an ARCHITECTí System (Abbott Diagnostics). The results showed that the frequency of hypothyroidism in HIVp was 29% (43/147) and 9.6% (27/280) in HIVn. Hypothyroidism was detected in 15 women and 28 HIVp men, and in 17 women and 10 HIVn men. TSH levels in HIVp women were (X ± SD) 2.98 ± 2.61, and 3.73 ± 3.04 µIU / mL in men. In HIVn were 2.98 ± 4.28 in women and 2.93 ± 5.84 µIU / mL in men. In HIVn women, serum T4L was higher than in men (p<0.05); Similar values of this hormone were observed in HIVp subjects. In HIVn, thyroid autoimmunity (a-TG and / or a-TPO) was observed in 6/10 men and 10/17 women, whereas in HIVp, autoimmunity was much lower, only 1/43. It is concluded that hypothyroidism in HIVn subjects was higher in women than in men, as expected, however the observed frequency in men in this study was higher than expected, with a high presence of antithyroid antibodies. Whereas in HIVp patients, a higher proportion of hypothyroidism was observed in men than in women.

Keywords: Hypothyroidism; HIV infections; Thyrotropin; Thyroid autoimmunity

(1724) **MODULATION OF OXIDATIVE STRESS IN PATIENTS WITH GRAVES´ HYPERTIROIDISM. IMPLICATIONS ON IMMUNE FUNCTIONALITY.**

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BACKGROUND: Graves´ pathology is an autoimmune disease characterized by humoral hyperreactivity against thyroid autoantigens leading to the state of hyperthyroidism. OBJECTIVE: To study the modulation of oxidative stress in peripheral blood mononuclear cells (PBMC) of patients with Graves' disease, analyzing its impact on lymphocyte functionality. METHODS: PBMC of patients with Graves´ disease (n=9) and healthy controls (n=10) were purified from peripheral blood by Ficoll-Hypaque. Reactive oxygen species (ROS) and apoptosis were evaluated by flow cytometry using DCFH-DA and Annexin-V/PI. The expression of antioxidant enzymes was analyzed by real-time PCR and western blot. Enzymatic activity was quantified by spectrophotometry. Malondialdehyde (MDA) levels were assessed by TBARs method. Proliferation was evaluated by [^{3}H]-thymidine incorporation. RESULTS: We observed a significant increase of ROS in PBMC of patients with Graves´ hyperthyroidism (% 21.2±2.5, p<0.005) that decreased by preincubation of the cells with the antioxidant N-acetylcysteine. Patients who received metimazole to restore their thyroid status to normal values showed levels of ROS similar controls. Hyperthyroid patients showed a higher activity and genomic expression of Catalase and Glutathione Peroxidase (% activity increase: CAT 42.0±3.1 and GPX 28.1±3.1, p<0.005; % expression increase: CAT 46.9±5.1 and GPX 34.2±3.1, p<0.005). The increase in ROS was correlated with an increase in the lipid peroxidation (% MDA increase 18.2±3.1, p<0.005), however the levels of apoptosis were similar to control. In addition, lymphocytes showed a greater proliferative response to mitogenic stimulation than cells from healthy individuals (% increase 38.1±2.9, p<0.005). CONCLUSIONS: Hyperthyroidism modulates the oxidative balance through the induction of ROS and the expression of antioxidant enzymes. ROS causes oxidative damage in PBMC, but this is not enough to affect the viability and functionality of lymphocytes.

Keywords: Graves´ disease, peripheral blood mononuclear cells, oxidative stress, immune functionality.

(1617) **MOLECULAR DIAGNOSIS OF NEONATAL HYPERTHYROIDISM. A CONTRIBUTION TO TRANSLATIONAL MEDICINE.**

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Neonatal hyperthyroidism occurs in 1:25,000 to 1:45,000 births. Primary nonautoimmune hyperthyroidism is a rare cause of neonatal hyperthyroidism. This results from an activating mutation in the thyrotropin-receptor (TSHR). Germline mutations, inherited in an autosomal dominant pattern, cause familial nonautoimmune hyperthyroidism, whereas de novo mutations cause sporadic congenital non-autoimmune hyperthyroidism. Sporadic cases tend to have a more severe course. To date, approximately 21 familial and 12 sporadic gain-of-function mutations have been described. The TSHR gene resides on the chromosome 14 and carries 10 exons.

We report a case of severe congenital hyperthyroidism without family history of thyroid disease. At 21 days of life the thyroid function test of the female patient, confirmed the diagnosis of hyperthyroidism. TSHR and TPO antibodies were not detected. She was treated with *methimazole* and propranolol with poor response. Currently, she is two years old. Then a TSHR mutation was suspected.

Genomic DNA was isolated from blood cells of the patient, parents and her brother and all exons of the TSHR gene, including the flanking intronic regions, were amplified by PCR. Each amplified fragment were sequenced with the Taq polymerase-based chain terminator method. Sequence analysis revealed a de novo, heterozygous and germinal activating mutation: c.1897G>C; p.Asp633His. In silico studies were performed to elucidate a correlation between structural disturbances and putative functional commitment.

The p.Asp633His mutation has been identified previously in the heterozygous state in thyroid nodules (somatic mutation) from two children with benign folicular adenoma and an adult woman with thyroid insular carcinoma. Since this mutation in children could be a higher risk factor for malignancy in adulthood, a thyroidectomy has been performed on our patient.

Molecular techniques lead to an early diagnosis and adequate treatment of this pathology.

Keywords: neonatal hyperthyroidism, TSHR, activating mutations, molecular diagnosis.

(1621) **MOLECULAR DIAGNOSIS OF PARTIAL THYROXINE-BINDING GLOBULIN DEFIENCY: A CONTRIBUTION TO TRANSLATIONAL MEDICINE.**

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Thyroxine-binding globulin (TBG) is the main transporter of thyroid hormones in serum. TBG gene is located on the X-chromosome (Xq21-q22) and contains 4 coding exons spanning 7.5 kb in the genome. TBG defects lead to three phenotypes according to serum TBG concentrations, complete TBG deficiency (TBG-CD), partial TBG deficiency (TBG-PD) and TBG excess (TBG-E). Women with homozygous inactivating mutations and men hemizygous for a deleterious mutation usually manifest as TBG-CD, and heterozygous women as TBG-PD. Gen duplication or triplications have been associated with TBG-E. 45 distinct mutations have been identified and characterized in the human TBG. Prevalence of TBG-PD is 1:4000.

Three unrelated Argentinean families with clinical-biochemical diagnosis of TBG-PD were analyzed. In order to identify mutations causing this pathology, genomic DNA was isolated from blood cells and the exons 0-5 of the TBG gene, including the flanking intronic regions, were amplified by PCR. DNA sequences from each amplified fragment were performed with the Taq polymerase-based chain terminator method and using the specific TBG forward and reverse primers. Direct sequence analysis revealed 3 novel mutations, two nonsense mutations and a missense mutation. Patient 1 showed an hemizygous mutation in exon 2: c.826C>T transition that results in a p.Q276* substitution which was inherited from his mother. Patient 2 carries an hemizygous c.783C>A transvertion in exon 2 which results in a p.Y261* and was inherited from his mother. Patient 3 showed a c.622G>A transition; p.A208T in exon 1. In silico studies were performed to elucidate a correlation between structural disturbances and putative functional commitment, achieving a possible explanation of the pathogenic mechanism of the novel missense mutation identified. This work contributes to elucidate the molecular basis of the defects of thyroid hormone transport in serum and the improvement of the diagnosis avoiding unnecessary therapy.

Keywords: Partial Thyroxine-binding Globulin Defiency, inactivating mutations, molecular diagnosis.

(1429) **NOX4 IS INVOLVED IN THE REPRESSION OF SPECIFIC THYROID GENES**

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Different factors have been proposed to be involved in thyroid function and proliferation such as thyrotropin (TSH), growth factors, iodide, radiation, etc. Insulin and IGF-I are additional important factors for thyroid function that collaborate with TSH in the regulation of thyroid proliferation and differentiation. The phosphoinositol-3-kinase (PI3K) pathway is the most studied mediator of insulin / IGF-1 signalling, whereas TSH receptor activation leads to cAMP synthesis. Although the insulin/IGF-I signalling pathways involved in the regulation of thyroid-specific genes remain unknown, several reports have shown their importance in regulating thyroid genes transcription. Objective: The aim of this study was to further explore the role of NOX4 on thyroid specific genes expression in a thyroid cell line (FRTL-5). Methodology and Results: FRTL-5 cells were incubated in the presence or absence of TSH and/or Insulin and NOX4, TGF-β1, FOXO1/3, catalase and the thyroid specific genes mRNA levels were examined by Real Time PCR. The absence of TSH and Insulin, increased mRNA levels of NOX4 (5 fold, $p<0.01$), TGF-β1 (4 fold, $p<0.01$), catalase (0.5 fold, $p<0.05$), FOXO1 (5 fold, $p<0.01$) and FOXO3 (3.5 fold, $p<0.01$) while NIS ($p<0.001$) TPO ($p< 0.01$), PAX8 ($p<0.01$) and TTF-2 ($p<0.01$) were decreased. To analyse the contribution of NOX4 on thyroid specific genes expression, interference RNA experiments were performed. SiRNA targeted knock-down of NOX4 increased mRNA levels of catalase (5 fold, $p<0.01$), NIS (0.5 fold, $p<0.01$), TPO (5 fold, $p<0.05$), PAX8 (3 fold, $p<0.01$), TTF-2 (1.3 fold, $p<0.05$), FOXO1 (2 fold, $p<0.05$) and FOXO3 (2,5 fold, $P<0.05$). When cells were treated with a PI3K inhibitor (LY294002), it decreased the expression of NOX4 ($p<0.05$), TGF-β1($p<0.01$), and FOXO3 ($p<0.01$) while TTF-2 ($p<0.01$) and FOXO1($p<0.01$) expression was increased. CONCLUSION: These results demonstrate a central role of NOX4 in the repression of specific thyroid genes.

(1822) **APPLICATION OF TARGETED NEXT-GENERATION SEQUENCING AND SINGLE-GENE ANALYSIS TO THE MOLECULAR DIAGNOSTICS OF CONGENITAL HYPOTHYROIDISM**

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rez, Buenos Aires, Argentina.

Congenital hypothyroidism (CH) is the most frequent endocrine disorder in pediatric patients with an incidence of 1:2,000-4,000 newborns. Thirty monogenic forms of CH have been reported, highlighting the genetic heterogeneity of the disease. A meta-analysis demonstrated that only 5-10% of patients with thyroid dysgenesis (TDG) and 45-88% of patients with thyroid dyshormonogenesis (TDH) are diagnosed using single-gene analysis (SGA). Here, we used targeted next-generation sequencing (TNGS) and SGA to investigate the etiology of CH.

TNGS was used to analyze the coding sequence of 17 candidate genes involved in the pathogenesis of CH in patients with TDG (n=2) or TDH (n=10). Additionally, we evaluated *slc5a5* (NIS) coding sequence using Sanger sequencing in patients (n=3) with a clinical phenotype of iodide transport defect (ITD), a cause of TDH.

Among patients studied by TNGS, 7 (58%) presented simple or compound heterozygous variants in genes involved in TDG or TDH. No homozygous variants or small gene deletion/insertions were evidenced. One patient with thyroid dysgenesis showed a heterozygous FOXE1 variant (p.P203R). In addition, one patient with thyroid dyshormonogenesis showed compound heterozygous TG variants (p.D29X/c.177-2A>C). The remaining patients with thyroid dyshormonogenesis showed simple heterozygous TG (p.F1542V*fs**20; p.T2563C; p.S523P) or DUOX2 (p.E1496D*fs**51; p.W178L) variants. None of the patients under analysis presented variations in more than one gene involved in TDH. Of note, all 3 patients with ITD showed compound heterozygous NIS variants (p.Q136L/ p.D369; p.L562M/p.G543K; p.D331N/p.S547R). All identified variants were predicted pathogenic or reported as pathogenic in the literature.

TGNS constitutes an attractive alternative to systematically explore and diagnose CH, particularly when the clinical and biochemical phenotype is unclear for SGA. However, we evidenced that a considerable proportion of patients (42%) remain genetically undiagnosed.

Keywords: Congenital hypothyroidism, Thyroid dysgenesis, Thyroid dyshormonogenesis, Iodide transport defect.

METABOLISM AND NUTRITION 3

(1270) **A FUNCTIONAL MILK FAT CONSUMPTION IMPROVED THE GLUCOSE UTILIZATION IN SKELETAL MUSCLE OF WISTAR RATS FED HIGH FAT DIETS**

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High fat diets (HFD) induce several disorders related to metabolic syndrome, including steatosis, hepatic lipid alterations and insulin resistance. These alterations might be attenuated by bioactive compounds, like rumenic acid (RA). Milk fat (MF) is the major source of RA, and its level can be increased by lipid supplementation of dairy cow diets obtaining a functional milk fat (FMF). The aim of the present study was to investigate parameters related to glucose and triacylglycerol (TAG) metabolism in rats fed HFD containing a FMF. Male Wistar rats were fed (60-d) with diets containing 7% or 30% of soybean oil (S7 and S30) and 30% of milk fat (MF30) or functional milk fat (FMF30). The following determinations were assessed: biochemical parameters on serum; TAG levels in liver and skeletal muscle; carnitine palmitoyl transferase I (CPT-I) expression in liver; hepatic TAG secretion rate; oral glucose tolerance test; metabolites from glycolysis and some key enzyme activities in skeletal muscle; and expression of adiponectin in adipose tissue. Statistical differences ($p<0.05$) were tested by T test (S7 vs. S30) and One Way ANOVA. Compared to S7, S30 diet induced TAG accretion in liver (+36%) and skeletal muscle (+61%), decreased the flux through hexokinase (-53%) and 6-phosphofructokinase (PFK1, -49%) in skeletal muscle and rats showed hyperinsulinemia (+48%). Also, the expression of adiponectin in adipose tissue was reduced (-98%). The FMF30 group showed lower levels of TAG in liver (-26%) associated with an enhanced CPT-I gene expression (+122%) and a higher TAG secretion rate (+31%), compared to S30. In muscle, FMF30 reduced the TAG levels (-49%) and increased the flux through the PFK1 (+116%) improving the glucose utilization that could be related to an enhanced adiponectin expression (20 times) compared to S30. In conclusion, the FMF prevented the TAG accretion induced by HFD and improved the glucose utilization in skeletal muscle.

Keywords: rumenic acid, functional milk fat, glucose metabolism, triacyglyceride regulation

(1607) **DIETARY SUPPLEMENTATION WITH SMALLANTHUS SONCHIFOLIUS (YACON) FLOUR IMPROVES VISCERAL ADIPOSITY AND METABOLIC PARAMETERS IN HIGH-FATDIET FED RATS**

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Smallanthus sonchifolius (yacon) is a native plant of South America. Its tubers contain oligofructans (FOS) as the main saccharides and phenolic compounds. The present study was conducted to determine the mechanisms by which yacon roots, ameliorates metabolic dysfunction in obese rats. Male Wistar rats were fed a standard diet (SD) or high-fat diet (HFD) for 12 wk and then divided into three groups: SD, HFD, and HFD plus yacon roots flour (680 mg FOS/kg body weight: HFD Y). After 8 weeks of treatment, anthropometric, feeding and biochemical parameters were measured. Insulin resistance, glucose tolerance and adipogenesis-related genes were also determined. A second experimental design included SD, HFD, HFD reversed to standard diet (HFD SD) and HFD reversed to standard diet plus yacon roots flour (680 mg FOS/kg body weight: HFD SY). Oral administration of yacon flour significantly reduced HFD-induced body weight gain food intake, feed efficiency and body mass index of the HFD rats. In addition, fat pad masses were reduced in yacon-supplemented rats, as evidenced by reduced adipocyte size. Yacon treatment enhanced plasma adiponectin and decreased plasma leptin concentrations in HFD rats, improved lipid profile, hyperglycemia and hyperinsulinemia and decreases MCP-1 expression in visceral adipose tissue of HFD fed rats improving glucose tolerance and insulin sensitivity. Such effects were associated with the modulated expression of the peroxisome proliferator-activated receptor-γ (PPAR-γ), its target genes aP2, C-EBPa and with an up-regulation of the phospho-Protein kinase B (p-Akt) in visceral adipose tissue. The beneficial effects in lipids metabolism were more evident when yacon supplementation was accompanied by a standard diet. These findings suggest that yacon roots administration suppresses HFD-dietinduced obesity and it can be developed as a potential candidate for the treatment of this disease and its associated complications.

Keywords: yacon roots, obesity, insulinresistence, adipose tissue, peroxisome proliferator-activated receptors.

(33) **EFFECT ON CALCIUM RETENTION OF MALTED FLOUR RYE IN AN EXPERIMENTAL MODEL IN GROWING WISTAR RATS.**

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Abstract: Rye is the second most important winter forage cereal in Argentina. The whole grain can be malted and the flour obtained from malting rye increases the amount of soluble fibers that have functional properties. The aim of this study was to evaluate the effect of malted flour rye intake on calcium bioavailability during 60 days, in a model rat. A total of 16 male Wistar rats recently weaned (8/group) were fed with a control diet prepared according American Institute of Nutrition Diet (C), and a semisynthetic diet prepared with malted flour rye (MR). At the end of the study rats were anesthetized and

changes in total skeleton bone mineral content (BMCT), was determined (mg/cm^2). After sacrifice of rats, the cecum from each animal was excised, split open, and the pH of the cecal content was measured. Also the right femur was excised, dried and fat was extracted to obtain ashes at 700 ºC, then Ca and P analysis were performed. The results showed that MR had a higher BMCT than C (g)(4.411±0.729 vs 2.490±231 ; p<0.0001). The cecal content of MR was acidified significantly greater extent than the cecal content of C showing a lower cecal pH (6.29± 0.16 vs 7.47±0.17; p< 0.0001). MR group had a higher Ca and P femur content than C (mg/100 g) (21.1± 0.9 vs 14.9 ±1.1; p<0.0001 and 13.9±2.1 vs 9.11± 0.36; p<0.001).

The soluble fiber from malted flour rye diet showed a prebiotic effect increasing calcium bioavailability and deposition in bones rats, comparing with control diet. The observed beneficial health effects allow us to consider the design of beaked goods healthier than those made only with wheat flour. Financed by UBACyT Nº 20020130200028BA.

Keywords: calcium retention, malted flour rye, prebiotic effect

(574) **IMMUNOLOGIC ALTERATIONS INDUCED BY A HIGH FAT DIET IN C57BL/6J MICE. EFFECT OF TREATMENT WITH METFORMIN**

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Calories-rich diets in conjuction with genetical factors are key in obesity development. This disease, in addition, is a risk factor for several immunologic and metabolic alterations. Metformin - a drug used as treatment for type 2 diabetes – is recently used to treat prediabetic obesity. In a previous study we've gathered evidence showing that in C57BL/6J mice, a high fat diet (HFD) increases body weight and desregulates glucidic metabolism. Metformin (MET) was able to reduce the body weight gain and normalize sugar metabolism. The objective of this study is to analize the effect of both HFD and MET in the immunologic response and cytokine expression. For this purpose female C57BL/6J mice were randomly divided in two groups receiving either HFD (35% fat w/w) or standard diet (SD, 5% fat w/w) during 6 months. 3 months after initiated the diet, both groups were subdivided into 2 subgroups: one received MET (250 mg/kg*day) during the remaining 3 months and the other was kept as control. Immunity response was quantified by [3H]-thymidine incorporation in a mitogen-induced proliferation assay of B and T lymphocytes. Cytokines mRNA levels were determined in spleen by real-time RT-PCR, using GAPDH as housekeeper.HFD lowered proliferation of T lymphocytes (p<0.05 vs SD) and mRNA levels of IFN gamma (p<0.05 vs SD). HFD+MET did not differ from HFD in any of the analyzed parameters. Interestingly SD+MET reduced proliferation in both types of lymphocytes (p<0.05 vs SD) while diminishing IFN gamma expression in a similar fashion as HFD (p<0.05 vs SD,p=0.22 vs HFD). No significant differences were found in Th2 cytokines mRNA levels (p=0.29). Inflammatory cytokine TNF alpha presented a non-significant decrease in all treatments (p=0.13). These results evidence that HFD reduces lymphocyte function which is not normalized by MET. The fact that MET per se alters immune response alike HFD, is to be considered when treating obese patients with this drug.

Keywords: Obesity, High fat diet, Metformin, Immune response

(939) **INFLUENCE OF DIETS SUPPLEMENTED WITH ω3 PUFA, ON SERUM LIPIDS AND FATTY ACID PROFILES OF GROWING RATS.**

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Abstract: Dietary lipid profile is important to prevent chronic diseases and improve the quality of individual's life. Objetive: The aim of this work was to analyze the effect of diets supplemented with different sources of EPA and DHA, on serum lipids and fatty acids profile of growing rats. Materials and Methods: Weanling Wistar rats were fed during 10 days with normocaloric diets, according to AIN´93, supplemented with: 1- fish oil (F), 2- eggs with high levels of ω3 fatty acid (Eω3), 3- conventional eggs (E). Control group (C) received normocaloric diet (AIN´93).

Serum levels of triglycerides(TG), total cholesterol(TC), noHDL-cholesterol were determined by enzymatic-colorimetric method. Serum fatty acids profile was determined by gas chromatography. Statistical analysis used ANOVA and Turkey as post-test.

Results: Serum (mean±SD, mg/dL) TG: F:55,57±15.28, Eω3:49,13±15.63, E:45,63±14.39, C:62.71±18.79; TC: F:73,94±8.73^a, Eω3:95,27±7.26^b, E:89,82±12.08^b, C:65.73±13.74^a; noHDL-cholesterol: F:48,36±14,17^a, Eω3:67,76±9,73^b, E:65,21±14,95^b, C:44,49±18,35^a.

Results of fatty acids profile expressed as area%±SD (*p<0.01) was: EPA: F:1,77±0,52^b , Eω3:0,62±0,23^a, E: 0,33±0,05^a, C: 0,90±0,38^a; DHA: F:5,75±0,58^b, Eω3:5,58±1,00^b, E:4,43±0,52^b, C:1,25±0,24^a.

The medias that didn´t present the same letter (^{a,b}), were different at p>0.01.

The other fatty acids didn´t present significant difference when compared to C.

Eω3 and E groups, showed higher levels of TC and noHDL-cholesterol. This would be a consequence of the type of lipid received in the supplementation.

The experimental groups showed higher DHA sera levels and only F increased EPA values.

Conclusion: These facts suggest that sera overtake in EPA and DHA as a result of the supplementation, being fish oil supplementation better than eggs.

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Keywords: fatty acids, lipid profile, EPA, DHA.

(1860) **EFFECT OF ANDROGEN DEPRIVATION ON LUNG STROMAL INTEGRITY OF MALE RAT**

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Even though androgens are fundamental in lung function otherwise the castration is the standard therapeutic treatment for advanced prostate cancer. Additionally, epithelial cells produce a big amount of transforming growth factor-β1 (TGF-β1) and inflammatory cells during lung injury. TGFβ1 plays a crucial role in differentiation, proliferation, and apoptosis process, including apoptosis of airway epithelial cells.

Objetive: to explore epithelium integrity in the course of androgen deprivation.

Male Wistar rats (200 ± 20 g) were separated in 3 groups: controls (Co), castrated (Ca), and castrated supplemented with testosterone (Ca+T) for five days. After 60 days rats were killed and lungs were obtained. RNA was extracted by the method of TRIzol. mRNA levels of Bax, Bcl-2, cyclooxygenase-2 (COX-2), vascular cell adhesion protein-1 (VCAM-1), TGFβ, androgen receptor (AR) and nuclear factor-erythroid 2-related factor 2 (Nrf2) were quantified by RT-PCR, using s28 as control. TBAR'S and testosterone level were measured. Lungs were examined for evidence of injury. ANOVA was used for statistical analysis.

Testosterone decrease (p<0,001); TBARS (p<0,05) and AR increased (p<0,005) in Ca. NRf2 increased (p<0,05) in Ca+T. Castration produce an imbalance of Bax/Bcl-2 ratio where there are no significantly changes in Ca, however this ratio increased significantly when testosterone was added (p<0,05). COX-2 mRNA levels increased in Ca (p<0,05) and decreased significantly in Ca+T (p<0,01). TGFβ diminished in Ca (p<0,05) showing no changes with testosterone supplementation. VCAM manifested a significant decreased in Ca+T group compared with Co and Ca groups (p<0,05). In Ca group, light microscopy revealed structural changes, and testosterone does not revert this situation. Our results demonstrate that

androgen deprivation disrupts the oxidant-antioxidant balance, produce pro-inflammatory changes and alters the extracellular matrix. This situation makes the lung more vulnerable to damage.

(1444) **EFFECTS OF AQUEOUS EXTRACTS OF MEDICINAL PLANTS ON HYPERCHOLESTEROLEMIC ApoE-DEFICIENT MICE (APOE-/-)**

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The use of herbals in the treatment of disease is a wellestablished practice in traditional medicine. *Tessaria absinthioides* (*Ta*) and *Prosopis strombulifera* (*Ps*) are native plants from South-America with reported ethnopharmacological uses. Despite recent scientific reports about plant derived compounds identification and activities, there is not a well conducted research about its effects on cardiovascular disease. The present study evaluates the efficacy of aqueous extract of *Ta* or *Ps* on genetically hypercholesterolemic ApoE-deficient mice (ApoE -/-), an experimental atherosclerosis animal model. ApoE-/- mice were treated with *Ta* (300mg/day/animal) and *Ps* (150mg/day/animal) in their drinking water for eight weeks and compared to age-matched controls, (C). Blood samples were collected by cardiac puncture to determine, cholesterol, triglycerides (TG), and blood glucose. Atheroma plaques in aortas were measured with Oil Red staining. The administration of *Ta* or *Ps* in ApoE-/- mice significantly reduced TG levels compared to control mice ($p < 0.05$), and *Ta* treatment significantly reduced blood glucose levels when compared to controls (247.3± 17.6 vs 150.6 ± 17.5 $p < 0.02$). None of the herbal extract studied had significant effect on the levels of cholesterol. Histopathological findings revealed that atheromatous plaques was significantly diminished in ApoE -/- mice by the treatment with both *Ta* or *Ps*. In conclusion, our results show that consumption of aqueous extracts of *Ta* and *Ps*, may have beneficial effects on metabolic parameters and atherogenesis. Further studies are needed to understand the molecular mechanisms of action of these medicinal plants.

Keywords: hypercholesterolemia, atherosclerosis, herbal medicine

(207) **EFFECTS OF INTERFERON ALPHA 2B (IFN-α2B) COMBINED WITH VITAMIN K2 (VK2) ON ALTERED HEPATIC FOCI (AHF) IN RATS**

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IFN-α is the primary choice for viral hepatitis treatment and a promising therapy for hepatocellular carcinoma (HCC). VK2 exerts growth-inhibitory effects in several human cancer cells, including HCC. Objective: to evaluate if the combined therapy of IFN-α2b with VK2 has a synergistic inhibitory action on the development of liver cancer, cell proliferation, apoptosis and fibrosis. Methods and Results: Adult male Wistar rats were subjected to a 2-phase model of liver cancer. Rats received 2 ip doses of diethylnitrosamine (150 mg/kg) 2 weeks apart and, one week after, they received 20 mg/kg of 2-acetylaminofluorene by gavage 3 days/week for 3 weeks (IP group). Animals were divided into 4 groups: IP; IFN: IP rats that received IFN-α2b 6.5x105 U/kg ip 3 times/week/3 weeks; VK2: IP rats that received VK2 8 mg/kg ip 3 times/week/3 weeks; and IFN+-VK2: IP rats which received both drugs. Animals were euthanized after treatments and livers were obtained. AHF were determined by immunohistochemistry using anti rGST-P and number of AHF/liver and % AHF/liver were calculated. As expected IP rats showed a decrease in number (-39%*) and % (-65 %*) of AHF/liver upon IFN treatment. VK2 and IFN+VK2 groups did not show differences respect to IP. Analysis of liver fibrosis (Direct Red 80/picric acid staining) showed a reduction in IFN and IFN+VK2 groups (-32%* and -31%*, respectively). Immunoblot analysis showed an increase in mitochondrial proapoptotic Bax protein in IFN and IFN+VK2 groups (+219%* and +97%* respectively), and in antiapoptotic Bcl-2 (+94%*) in VK2 group. In addition, Bax/Bcl-2 ratios were calculated, IP: 1, IFN: 3.2±0.2*, VK2:0.5±0.1, IFN+VK2:1.4±0.07 (*$p<0.05$vsIP). Conclusion: obtained results do not show any beneficial effects on the reduction of AHF in IP rats treated with IFN+VK2. Interestingly, VK2 seems to block the proapoptotic effect of IFN. Although it needs to be evaluated, we believe that VK2 affects lipid metabolism enzymes that participate in IFN signals.

Keywords: Liver, Hepatocellular carcinoma, chemical carcinogens, lipids.

(1537) **METABOLIC EFFECTS OF ORAL ADMINISTRATION OF SIMVASTATIN IN DIET-INDUCED HYPERCHOLESTEROLEMIA RATS WITH PERIODONTITIS. A PRELIMINARY STUDY**

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Simvastatin (SMV) can modulate cholesterol metabolism. Previously, we had demonstrated the negative effect of cholesterol-rich diets on bone health. Recently, statins have been postulated to affect bone metabolism. As periodontitis (P) is a bone-destructive disease, statins may be effective for trabecular and cortical bone recovery. This study evaluated the cholesterolemia (chol), periodontal bone support (PBS) and biomechanical competence in response to SMV administration in hypercholesterolemic rats with P. **Methods**: rats were assigned to 1 of 3 groups: 1.control (C): fed pellets, 2.high-cholesterol diet (HCD), 3.HCD+SMV (5mg/day by gavage). After 3 weeks, rats were anesthetized to induce P by ligature of the 1° molars. 2 weeks later, rats were euthanized, blood was drawn for chol (mg/dL). Structural properties of the femur (load at fracture(Wf), load at yield(Wy), diaphyseal stiffness(Wydy)), were determined by using a three-point bending test (Instrom 4442). X-rays were used for PBS measurements. **Results** (mean±SD, ANOVA-SNK): SMV did not decrease chol (HCD+SMV: 209±34 = HCD:197±29 > C: 63±02 mg/dl; $p< 0.0001$). However, SMV could revert deleterious effects of HCD in the structural properties of the femoral diaphysis, Wf, Wy and Wy/dy, $p<0.001$, $p<0.05$ and $p<0.01$, respectively. PBS was higher in HCD+SMV than HCD, showing a recovery of alveolar bone (HCD+SMV: 47,6±0,9 = C:47,7 ± 2, 7 > HCD: 42,4±1,3%; $p<0.001$). **Conclusion**: Due to that five week treatment with SMV was not able to decrease diet-induced hypercholesterolemia in animals without genetic background of hypercholesterolemia but enough to improve alveolar and cortical bone in rat with P, this could be related to time –effect necessary for pleiotropic actions of statins.

Keywords: diet-induced hypercholesterolemia, bone, simvastatin.

(965) **METFORMIN AND LOSARTAN MODIFY PROSTANOID RELEASE IN THE MESENTERIC VASCULAR BED OF HIGH-FAT DIET RATS**

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Metabolic diseases are linked to hypertension. High-fat (HF) diet

in rats is an experimental model that resemble the human metabolic syndrome. The mechanisms that trigger the blood pressure (BP) increase in this model are not fully understood. Prostanoids (PR) play an important role in vasomotor tone regulation. The aim of this study was to analyze metformin (M) and losartan (L) effects on mesenteric vascular bed (MVB) PR release, adiposity index and its relation to BP.

Six groups (n=6) of male Sprague-Dawley rats were studied during 8 weeks: Control (C), standard diet (SD) and tap water (W) to drink; HF diet (HF), 50% (w/w) bovine fat added to SD and W; C + M (CM), SD + 500 mg/Kg/day M in W to drink; C + L (CL), SD + 30 mg/Kg/day L in W to drink; HF + M (HFM) 500 mg/Kg/day M in W to drink; HF + L (HFL) 30 mg/Kg/day L in W to drink. MVBs were removed and incubated and the released PRs measured by HPLC.

HF diet increased systolic BP (SBP, mmHg, HF: 145±5 vs. C: 118±2, p<0.01); MVB adiposity index (%, HF: 1.7±0.1 vs C: 0.9±0.04, p<0.01); and the release of vasoconstrictor PR such as thromboxane (TX) B_2 (ng PR/mg of tissue, HF: 117±6 vs C: 66±2, p<0.001) and prostaglandin (PG) $F_{2\alpha}$ (ng/mg, HF: 153±9 vs C: 88±3, p<0.001). In HFM and HFL groups, M and L treatment prevented the increases of SBP (HFM: 127±2, HFL: 111±3 vs. HF, p<0.001 and p<0.01), TXB_2 release (ng PR/mg of tissue, HFM: 65±12, HFL: 66±7 vs. HF, p<0.05 and p<0.01); and PGF2alpha (ng PR/mg of tissue, HFM: 99±13, HFL: 90±7 vs. HF, p<0.01 and p<0.05). Meanwhile M also prevented the increase of MVB adiposity index (%, HFM: 1.3±0.2 vs HF, p<0.05).

Treatments with M and L could exert beneficial effects on the vascular system improving endothelial dysfunction by preventing the increase of vasoconstrictor PR in MVB. In addition, M prevents adiposity increase.

Metformin, losartan, high-fat diet, prostanoids, hypertension

FARMACO 8 / PHARMACOLOGY 8

(512) **ANTITUMORAL ROLE FOR NANOHYDROXYAPATITE IN GLIOMA CELLS**

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Gliomas are brain and spinal cord tumors originated from glial cells. Brain tumors treatment is difficult since the blood brain barrier prevents the uptake of most pharmaceuticals. Thus, the challenge remains to develop new therapies with the potential to counteract this devastating disease. Hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$; HAP) is an essential component of the human bone inorganic phase. Nanoscaled HAP (nHAP) presents emergent properties in comparison to its bulk counterpart, including an inhibitory effect on the proliferation of some types of tumor cells. In this study, we aimed to determine the possible role of nHAP as an anticancer agent employing C6 glioma cells. Exposure to nHAP (24-72h, 50-500μg/ml) induced cell death measured by MTT assay (72h- 50μg/ml: 21±2%; 100μg/ml: 31±1%; 200 μg/ml: 28±2%; 500μg/ml: 29±1%; p<0.001). nHAP increased reactive oxygen species (ROS) production in the concentration range tested (p<0.001, spectrofluorometry). Furthermore, an increment in the percentage of cells presenting a rise in the size/number of lysosomes was measured by Lysotracker Red DND-99 staining (48h- 50μg/ml: 54±11%; 100μg/ml: 59±3%; 200 μg/ml: 32±14%; 500μg/ml: 68±22%; p<0.01). However, no lysosomal membrane permeabilization was detected. Then, we studied the occurrence of DNA damage. Exposure to nHAP (24-48h; 50-100μg/ml) augmented histone 2AX phosphorylation (γ-H2AX)(western blot). On the other hand, no alterations in nuclear morphology by staining with Hoechst 33258 were observed. We did not detect any changes in PARP cleavage or p53 expression levels (western blot). Last, we tested the effect of nHAP on relevant processes for tumor progression. Exposure to nHAp decreased cell proliferative capacity (clonogenic assay) as well as cell migration (wound assay). These approaches are promising to support an antitumor effect of nHAp on glioma cells.

Keywords: cancer, glioma, nanohydroxyapatite, nanomedicine.

(1209) **DEVELOPMENT OF OXALIPLATIN-LOADED PEGYLATED LIPOSOMES USING MICROFLUIDIC TECHNOLOGY FOR COLORECTAL CANCER TREATMENT**

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Chemoresistance and metastatic recurrence are the main barriers for the effective treatment of cancer. Targeting drugs to resistant tumor cells increases the therapeutic effectiveness because it is possible to increase the dose in the pathological sites and to decrease secondary effects in healthy tissues. PEGylated liposomes with size in the order of 100 nm are regulatory well-accepted vehicles for use in patients. This liposomes have the advantageous to accumulate preferably in tumor tissue by passive targeting due to the enhancement permeably retention effect, reducing peripheral toxicity. However, conventional liposome synthesis technologies are inefficient with variations in the batch-to-batch procedures, which make it difficult for a rapid translation to patients. The objective of this work is the development of PEGylated liposomes containing oxaliplatin, a high efficiency chemotherapeutic drug but with severe adverse neurotoxic effects, by conventional and non-conventional methods such as microfluidic technology, in order to obtain an efficient method for encapsulation oncological drugs. Thin film hydration method with extrusion using polycarbonate membrane filters with decreasing pore size was used as conventional method and microfluidic technology was used as non-conventional method. We developed a *chip* for the encapsulation of oxaliplatin in liposomes using a microfluidic platform previously validated by our group. Both methods were compared, and significant improvements in size of liposomes and % of encapsulation of oxaliplatin were obtained using the microfluidic method respect the traditional technology. Both types of liposomes were active in *in vitro* assays. Additionally, the microfluidic technology allowed to reduce the times of synthesis and to decrease the costs of production. These results could have a high impact on the synthesis and the encapsulation of oncologic drugs in liposomes in the national and regional pharmaceutical industry.

Keywords: Oxaliplatin, liposome, colorectal cancer, microfluidics

(910) **LINALOOL AS AN ANTICANCER-AGENT: MOLECULAR MECHANISMS OF ACTION AND ITS ENCAPSULATION IN NANODELIVERY SYSTEMS FOR CANCER THERAPY**

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Linalool (LN) is a monoterpene found in essential oils of many plants with multiple pharmacological effects including anticancer activity. We have previously shown the ability of LN to impair cancer cells viability, however, to be considered as a potential anticancer-drug, it is still necessary to elucidate the specific mechanisms of action involved and improve its administration and bioavailability. For this purpose we first analyzed the antiproliferative mechanisms

of action of linalool in HepG2 cells. Cells were treated with LN at different times and concentrations and cell proliferation (BrdU incorporation), cell cycle progression (flow cytometry -FC-, western blot -WB-) mitochondrial membrane potential (MMP) and reactive oxygen species (ROS) (fluorescence microscopy -FM- and FC), and MAPK and Akt/mTOR activities (WB) were evaluated. LN inhibited CP by G0/G1 arrest through downregulation of Cdk4, cyclin A and cyclin E and increasing p27 inhibitor, and at longer times, it also induced apoptosis. LN stimulated ROS generation which were, at least in part, responsible for the cytotoxic effects since the antioxidant N-acetyl-L-cysteine (NAC) significantly prevented cell death. LN also promoted Akt inhibition, MAPKs activation (ERK, JNK and p38) and MMP depolarization. NAC prevented JNK activation and MMP depolarization, both of them associated with apoptosis, suggesting a direct role of ROS in LN-induced apoptosis. Thereafter, we developed a novel delivery system based in LN-loaded solid lipid nanoparticles (LN-SLN) of different composition, whose anticancer activities and cellular uptake were assessed. Higher inhibitory effects were found for LN-SLN in comparison with free LN (MTT assay). Additionally, the cellular uptake of SLN was proved by FM, enhancing the ability of SLN to deliver LN into the cells. Our results suggest that LN should be considered as a potential anticancer agent and its loading into SLN would improve its bioavailability and efficiency.

Keywords: linalool – MAPKs – oxidative stress – nanodelivery – cancer therapy

(103) **FUSION PROCESSES OF LIPOSOMES MEDIATED BY MAGNETIC NANOPARTICLES AND REGULATED BY EXTERNAL MAGNETIC FIELD**

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Magnetic iron oxide nanoparticles (NP) are one of the best choices for biological and biomedical applications, because of their biocompatibility, superparamagnetic behavior as well as for their chemical stability. Oleic acid (OA), a surfactant commonly used to stabilize NPs, have the ability to intervene and regulate vital mechanisms of great implication in many cellular processes such as the membrane fusion, a phenomenon that continues to be the object of innumerable investigations today. Liposomes represent a widely used model of biomembranes to provide key insights into the characteristics and dynamics of substrate-membrane interactions.

In this work, we have synthesized NP coated by OA (NPs@OA) through the co-precipitation method and evaluated their potential to induce the fusion of liposomes (LUVs) composed of POPC. The fusion process was studied by dynamic light scattering (DLS) and a fluorescence assay commonly employed to evidence fusion of liposomes which is based on the fluorescent complex formation between Terbio and dipicolinic acid (Tb/DPA). With the purpose of access information regarding the morphology of the NP-LUVs TEM experiments was performed.

We monitored, along time, the increment on fluorescence intensity (If) after adding to a mixture containing both LUVs population (filled with Tb or DPA) different amounts of MNPs@OA. The maximum emission intensity (I_{max}) was obtained by preparing vesicles loaded with the pre-formed Tb^{3+}/DPA complex and the percentage of fusion induced by the MNP was calculated (Biochemistry, 1980, 19, 6011).

In addition, we have evaluated the effect of applying variable magnetic fields in the order of 0.1-0.5 mTesla to the MNPs-LUVs system. It was found that the percentages of fusion considerably increase in the presence of the magnetic field.

From these studies, it is concluded that MNP stabilized by OA represent a potent tool to induce and manipulate fusion processes in the presence of low-intensity magnetic fields.

Keywords: membrane fusion, fluorescent complex, magnetic field, magnetic nanoparticles, liposome.

(1582) **NEW APPROACHES OF PHOTODYNAMIC THERAPY AGAINST GLIOBLASTOMA: ASSAYING METALLETED PORPHYRIN DOPED CONJUGATED POLYMER NANOPARTICLES AND CELLULAR DELIVERY IN 3D CULTURE MODEL**

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A new field of application of Photodynamic Therapy (PDT) is the treatment of brain tumors, such as Glioblastoma. Conjugated polymer nanoparticles (CPNs) have excellent properties that make them suitable as photosensitizers (PS) against cancerous diseases.

In this work, we propose to study a therapeutic strategy of cell vectorization of CPNs into Glioblastoma spheroids in an attempt to improve PDF efficacy. This novel strategy employs macrophages recruited in the tumor, which previously are intended to be preloaded with CPNs based on Poly(9,9-dioctylfluorenealtbenzothiadiazole) (F8BT) and platinum octaethylporphyrin. First, we evaluated the biocompatibility of CPNs and load ability in 2D culture of mononuclear cells: RAW 264.7 macrophages (Ma) and THP-1 monocytes. Both cell lines incorporated CPNs efficiently (more than 80% of cell population by flow cytometry) without affecting cell viability (MTT, Guava ViaCount Assay flow cytometry). On the other hand, the subcellular localization of CPNs (confocal microscopy) within cellular charges (Ma) was studied. Colocalization analysis with late endosomes (DQ-BSA, anti-LBPA) yielded a Pearson correlation coefficient of 0.7. The localization of CPNs in late endosomes could be related to the release of CPNs within exosomes. Afterward, the chemotaxis ability of loaded Ma with CPNps was evaluated using Boyden chamber assay, U-87 MG (glioblastoma) spheroids and its conditioned medium (CM). Loaded Ma migrated through the membrane following soluble factors of CM and infiltrated spheroids (80% migration compared positive control with SFB). Finally, we compared PDT-CPNs and PDT-loaded Ma in U-87 MG spheroids co-cultivated achieving a better PDT killing effect with PDT-loaded Ma. In conclusion, loaded Ma with CPNps represents a feasible PDT treatment for Glioblastoma.

Keywords: glioblastoma, conjugated polymer nanoparticles, photodynamic therapy, macrophages, Trojan horse therapy

(647) **NOVEL HYBRID NANO-TRACER, FOR DUAL-MODALITY IMAGING WITH ^{99m}TC AND INDOCYANINE GREEN, FOR BIOPSY OF THE SENTINEL LYMPH NODE: PRELIMINARY STUDIES**

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Precise examination of the sentinel lymph node (SLN) can accurately predict the lymph node status of the entire lymph basin at early stages of cancer progression. The aim of our work is to develop a hybrid tracer based on a 99mTc-radiocolloid and fluorescent indocyanine green (ICG) for mapping SNL in an attempt to combine radioactive and optical signals simultaneously, for direct integration of pre and intraoperative stages of the procedure. For this purpose, ICG stock solution was prepared using saline (2.5 mg/mL) and then working solutions of 1/10, 1/100, 1/1000 y 1/10000 concentrations were prepared to find optimal dilution for labeling and *in vivo* detection. 99mTc-radiocolloid precursor was prepared using bovine gelatin collagen, BGC (7.5 mg) and saline (1.88 mg/mL). Then, 1 mL of each working solution was mixed with 1 mL of BGC solution and finally 1 mL of 99mTc (821 MBq) was used to radiolabel each of the preparations by direct method. After 10 minutes, quality controls were perfomed and 0.1 mL (82 MBq) were intradermally administered to female Sprague Dawley rats. Pre-operatory radioactive static images using a gamma camera and intra-operatory fluorescent images using a portable fluorescence detector (λexc 745 nm λem 820 nm) were acquired after 24 hs of the injection. Lymph nodes were excised using optical images guidance and were kept for hys-

topathological analysis. Relevant results showed that the tracer is a true hybrid as co-localization of both labels was confirmed. Lymph node identification and localization were successful, using preoperatory radioactive images and intraoperatory fluorescent images. Then, images acquired were merged showing the hybrid nature of the tracer *in vivo*. Both SLN and second echelon were identified because of tracer migration after 24 hs. In conclusion, although further studies are needed the results of the study revealed that the novel hybrid tracer allowed node detection by a dual-modality approach for SLN biopsy.

Keywords: sentinel lymph node, indocyanine green, 99mTc-radiocolloid, hybrid

(1091) **NOVEL RADIOINMUNOTHERAPY FOR METASTATIC COLORECTAL CANCER**

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Radioimmunotherapy (RIT) is a type of cancer cell targeting therapy which uses monoclonal antibodies against tumor-associated antigens labeled with radionuclide. The epidermal growth factor receptor (EGFR) is expressed in 80% of advanced colorectal metastatic cancer (CCR) patients for witch it is a good antigen to be used in RIT. In the other hand, the Lutetium-177 (Lu-177) has a half-life of 6,7 days and a maximum negative beta emission of 497 KeV, for which also is ideal for the development of therapeutic radiopharmaceuticals. In this work, we proposed to develop novels EGFR-based radiopharmaceuticals for metastatic colorectal cancer treatment, labeling the anti-monoclonal antibody Cetuximab and camelid nanobodies to EGFR (Nb-EGFR) with Lu-177. At this stage, we advanced with Cetuximab labeling and with the obtaining of Nb-EGFR. Lu-177 was generated in the Centro Atómico Ezeiza. Cetuximab was labeled by Lu-177 using pSCN-Bn-DTPA as a bifunctional chelating agent. The specific activity of 177Lu-Cetuximab radiopharmaceutical was of 1.3 mCi / mg and the radiochemical purity was of 99,9% determined by HPLC. The HEK-293 cell line (EGFR+) was used for specificity assay, blocking for 1-2 h with cold Cetuximab as negative control. After that, cells were exposed to radiopharmaceutical for 24h at 37 °C. Fractions of non-internalized (supernatant and washed with PBS) and cell-associated (washed with NaOH) were collected. The fractions were manually measured in a well-glass radiometric detector. The percentage of activity bound to cells in a specific form (internalized) was between 68-70%, with 2% of non-specific activity. These results demonstrate that 177Lu was achieved with high specific activity and Cetuximab was efficiently labeled by achieving a radiopharmaceutical capable of selectively binding to cells expressing EGFR. In future experiments, we will advance labeling of Nb-EGFR and evaluating the therapeutic activity of these radiopharmaceuticals CCR *in* vivo models.

(1515) **SYNTHESIS, CHARACTERIZATION AND RADIOSENSITIZING EFFECT OF MAGNETIC NANOPARTICLES IN MELANOMA CELLS**

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Malignant melanoma (MM) is the most deadly skin cancer, is highly metastatic and resistant to conventional therapies. Advances in nanomaterials contributed in recent years to develop new strategies for cancer treatment. In this context, magnetic nanoparticles (MNPs) exhibit great potential for diagnosis and therapy. MNPs are highly biocompatible, stable and can be directed under an external magnetic field. Moreover, incubation of cells with MNPs can increase intracellular levels of reactive oxygen species (ROS). Based on the high endogenous ROS levels of melanoma cells, we propose that combined treatment with MNPs and ionizing radiation (IR) could induce a cytotoxic oxidative stress by surpassing antioxidant capacity and sensitizing radioresistant melanoma cells. To test this hypothesis, the aim of this study was to synthesize, characterize and evaluate the radiosensitizing effect of MNPs in A375 human melanoma cells.

Iron oxide MNPs were synthesized by the coprecipitation of Fe^{2+} and Fe^{3+} method and stabilized by methyl-poly(ethylene glycol). MNPs characterization by transmision electron microscopy, X-ray diffraction, magnetometry and Mössbauer spectroscopy showed superparamagnetic behavior and low dispersion in shape and sizes (8-17 nm). No cytotoxicity was observed by MTT assay in A375 cells exposed up to 250 μg/ml for 24 h. DCFH assay revealed higher ROS levels in A375 cells after MNPs treatment ($p<0.05$). A375 cells pre-treated with MNPs 50 μg/ml for 24 h were irradiated with a ^{137}Cs gamma source (0-5 Gy). Survival curves were obtained by clonogenic assay and fitted to the linear-quadratic model. A significant increase in radiation effect in MNPs-IR treated cells was found ($p<0.05$), with surviving fraction at 2 Gy of 0.51 and 0.28 for IR and MNPs-IR treated cells, respectively. Increased DNA damage by MNPs-IR vs IR was detected in A375 cells by immunofluorescence of γH2AX ($p<0.05$). In conclusion, MNPs proved to be effective radiosensitizers of melanoma cells.

Keywords: Magnetic Nanoparticles, Radiotherapy, Melanoma, Reactive Oxygen Species, Radioresistance

(1437) **TWO IN ONE: MULTIFUNCTIONAL NANOPARTICLES FOR BREAST CANCER MANAGEMENT UNDER HORMONAL TREATMENT RESISTANCE**

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Our group has been studying endocrine resistance mechanisms with the aim of developing new therapeutic strategies for breast cancer treatment. In this context, we showed that fibronectin, through its interaction with beta 1 integrin, confers endocrine resistance. We hypothesize that a therapeutic strategy based in the use of Tamoxifen carried in NPs coated with the tumor penetrating peptide iRGD, would be more effective than free Tamoxifen, and reduce recurrence.

Polyethyleneglycol and polycaprolactone nanoparticles were coated with the iRGD peptide and a FAM fluorophore in order to track the NPs. To evaluate cell uptake, we incubated MCF7 or T47D cells for 5 h with iRGD-NP or FAM-NP as a control. We detected increased NP uptake when they were coated with iRGD, both in 2D and in 3D culture.

Cell viability studies demonstrated that that Tamoxifen encapsulated in NPs was more effective than the free drug ($p<0,05$), and even more toxic when the NPs were coated with iRGD ($p<0,01$).

Previously we demonstrated that treatment with Tamoxifen enhances the stem cell compartment. In this regard, we found that Tamoxifen in NPs with iRGD reduced the mamospheres number and size.

Ultimately, we studied tumor homing. iRGD-NP or FAM-NP were injected by the tail vein of Balb/c mice carrying MCF-7 tumors. iRGD coated NPs had better homing to the tumor than FAM-NP, and very little accumulation in other organs.

(1941) **SOLID LIPID MICROPARTICLES (SLM) AS NATURAL ANTIOXIDANT TRANSPORTERS.**

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The use of natural antioxidants has increased lately in the food industry. However, the properties of the extracts can be affected by the storage and production conditions of these, which led to the proposal of a lipid protection system.

In this work two natural sources of antioxidant agents were used: aguaribay berries oleoresin (*Schinus molle*), and blueberry extract (*Vaccinium myrtillus*). The extracts were obtained by means of solid-liquid extraction. Oleoresin and blueberry extract presented antioxidant activity, as determined by DPPH method. Oleoresin has more antioxidant power than synthetic antioxidants, increasing with temperature (65 ºC). Blueberries are equally efficient as antioxidant than oleoresins but at higher concentrations. The objective of this work was to obtain solid lipid microparticles (SLM) from the extracts to be used as transporters of naturally occurring antioxidants.

SLMs were obtained by means of a lipid emulsion by stirring at 65 °C and 15000 rpm for 10', using an Ultra-Turrax T25 (Janhke & Kunkel, Germany). The lipid phase was obtained by blending soy lecithin and cocoa butter in equal parts. Tween20 was used as surfactant, incorporating 40 mg of oleoresin or 320 mg of blueberry extract.

Formulations were characterized by size and morphology. The size stability was determined by a laser particle analyzer (Mastersizer 2000E, UK). The microparticles had a constant average size of 15.13 µm (oleoresin) and 13.18 µm (cranberry extract) for 2 months of storage at 4 °C.

Morphology was determined by optical microscopy (Olympus Q color U-CMAD3). Both formulations were observed to preserve population homogeneity during storage. We can conclude that the SLM of both extracts could be used as carriers of natural antioxidants and also contribute to the health Ω3 and Ω6 fatty acids of lecithin. The use of natural extracts from regional crops would allow replacing synthetic antioxidants by forming a new additive with nutraceutical characteristics.

(1707) **VALIDATION OF SILICA NANOPARTICLES FOR CONTINUOUS GH DELIVERY IN A TRANSGENIC MOUSE MODEL**

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Pharmacological treatments with GH in short stature syndromes require daily injections and therefore new formulations are needed. In the present work we sought to validate a silica nanoparticles (SiNP) formulation for continuous GH delivery. To that end we first established a mouse model in which continuous GH secretion feminizes hepatic gene expression, and secondly we analyzed how surface chemical modifications of SiNP allow loading of GH to be used in this model. Determination of GH adsorption isotherm was performed by exposing each SiNP to different GH concentrations and monitoring the remaining hormone concentration by RIA after 24 h incubation. GH secretion is sexually dimorphic in many species, and impacts on sexual dimorphism of hepatic gene expression. Central disruption of the Dopamine D2 Receptor (D2R) in neuroDrd2KO mice feminizes the growth axis (continuous secretion). We therefore used neuroDrd2KO mice to establish a model which will enable us to test the action of GH-loaded SiNP formulation. We first studied the impact of the feminized GH axis on hepatic gene expression in males by Real Time PCR, and found that mRNA expression of the male predominant genes *Cyp7b1, Igf1, Cy2d9* and *Mup1/2/6/8* was feminized (decreased) in transgenic mice; while the female predominant genes *Cyp2b9, Adh1, Cyp2a4* and *Prlr* were demasculinized or increased. In a second step, 200nm-diameter SiNP were prepared following the Stöber method and their surface was chemically modified with 3 different functional groups (–NH_2, –SH, isobutyl) or kept unmodified (SiOH). The maximum adsorption capacity of SiNP Q (ng GH/ng SiNP) indicated the following order: –NH2>SiOH>–SH>isobutyl (12.5, 6.5, 4.9, 2.7, respectively). All these results indicate that a –NH2 surface modification is more suitable for GH adsorption on SiNP and that, once continuous GH release is determined, the nanoformulation can be successfully tested on the neuroDrd2KO mouse model by analyzing hepatic gene expression.

Keywords: Silica nanoparticles, GH deficiency treatment, hepatic gene feminized expression

INFECTOLOGY 7

(1198) **COMBINATION THERAPY OF LOW DOSES OF BENZNIDAZOLE AND ALLOPURINOL ON EXPERIMENTAL CHRONIC *Trypanosoma cruzi* INFECTION.**

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The aim of this study was to evaluate the effectiveness of low doses of benznidazole with continuous administration (BNZc), combinated with allopurinol (ALO), in two mouse models (C3H/HeN and C57BL/6J) infected with *T. cruzi* Nicaragua (*Tc*N) and *T. cruzi* clone Sylvio-X10/4. In C57BL/6J we also evaluated treatments with intermittent administration of BNZ, (BNZit) (1 dose every 7 days for 13 times). Drug administration was performed at 3 months post infection (pi) in chronic infection of mice with progression of heart disease, followed-up by euthanasia at 6 months pi. A group of C57BL/6J BNZc was monitored for serology and electrocardiograms (ECG) at 12 months pi. The bioethical management of the animals was approved by a corresponding Committee. The treatment efficacy was evaluated by serology by ELISA, qPCR in blood, inflammation and fibrosis by histopathology of hearts and electrical alterations by electrocardiograms (ECG). Significant reductions in antibody titers, parasitic loads and cardiac abnormalities were observed after treatments. The addition of ALO to the lowest dose of BNZc, caused negative IgG titers in serum and chronic myocarditis only in C57BL/6J mouse model. At one year of follow-up the IgG titers of mice treated with low doses of BNZc (50+ALO, BNZ75 and 75+ALO) were negative, while BNZc 50 induced only 40 % of negative titers. The ECGs showed severe electrical abnormalities in infected mice and its reversion with either BNZc or BNZit treatment. There were no significant differences between the results of treatments with continuous and intermittent administration of the BNZ. Overall BNZc and BNZit at low doses, suggesting a lower accumulation of drug, seems to be useful approaches to treat *T. cruzi* chronic infection in mice. Relevance of these preclinical models is discussed.

Keywords: Benznidazole, Allopurinol, Chagas´ chronic disease, Preclinical model

(1431) **BENZNIDAZOLE SOLID DISPERSIONS: A NEW STRATEGY TO IMPROVE TREATMENT OF *TRYPANOSOMA CRUZI* INFECTION**

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Benznidazol (BZL) and Nifurtimox are the only drugs approved to specifically treat *T. cruzi* infection. Both are orally administered, and can present adverse side effects or treatment failure. BZL, the first-line treatment in Argentina, is a compound with low solubility in water, limited bioavailability, and is administered for long periods of time at high doses. One possibility to improve treatment lies on pharmaceutical formulations designed to increase water solubility of poorly soluble molecules. For that reason, a new liquid formulation based on an amphiphilic polymer (P) acting as a carrier of BZL was tested on mice models of acute and chronic *T. cruzi* infection.

Female Swiss mice (N=57) were infected with Tulahuén strain (300 parasites/mouse for the acute phase model, and 50 parasites/mice for the chronic phase model). Treatments were given orally once a day, every day for 2 months, starting at 5 days post-infection (dpi) in the acute phase model, and 150 dpi in the chronic phase. Doses of BZL-P were: 15 mg/Kg/day, 60 mg/Kg/day, and 60 mg/Kg/day twice per week. Controls: placebo, and the comercial formulation BZL-C at 50 mg/Kg/day. Results showed that the effect of BZL was dose-dependent: higher doses (60 and 50 mg/Kg/day) were more effective in clearing parasites from blood and tissue than lower doses (15 mg/Kg/day). Intermittent administration of BZL (twice a week) was effective in reducing parasitaemia in blood, but it did not prevent the establishment of parasites in muscle or heart tissue. The new formulation was as effective as BZL-C at equivalent doses. Adverse effects were not detected. Being liquid, the new formulation tested in these experiments may provide an alternative for better dosing, since commercial tablets must be divided, mashed and weighted to achieve a given dose. A liquid formulation of BZL could represent an improvement for treatment in children and adults.

(871) **DRUG REPOSITIONING FOR THE DISCOVERY OF NEW TREATMENTS AGAINST CHAGAS DISEASE. OXIDATIVE DAMAGE REPAIR ENZYMES FROM *Trypanosoma cruzi* AS NEW THERAPEUTIC TARGETS**

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Methionine is an amino acid susceptible to be oxidized to methionine sulfoxide (MetSO). The reduction of MetSO to methionine is catalyzed by methionine sulfoxide reductases (MSR), enzymes present in almost all organisms. Recently, we characterized MSR proteins from *Trypanosoma cruzi,* which would be relevant for the survival of these pathogens in the various stages of their life cycle. Chagas is a neglected disease caused by the parasite *T. cruzi*, which affects underdeveloped countries. The current drugs are nifurtimox and benznidazole, but both have severe adverse effects and less effectivity in chronic infections; therefore, the need to discover new drugs is essential. Drug repositioning is an interesting option within the international drug development community. Through molecular modeling and virtual screening using the commercially available approved drugs extracted from ZINC database (2924), we identified drugs with potential binding capacity to *T. cruzi* MSR. From a preliminary molecular docking analysis, a set of ten compounds with the best binding energies were selected and tested by *in vitro* and *in vivo* assays. Epimastigote and metacyclic trypomastigote cells were used to test the trypanocidal effect of ten selected compounds. Among these drugs, flunarizine, trifluoperazine, estradiol-benzoate, domperidone and itraconazole showed better or similar trypanocidal effects (IC_{50} range 1 to 50 μM) in comparison with nifurtimox or benznidazole (IC_{50} of 6 and 20 μM, respectively). *In vitro* enzymatic assays confirmed the inhibitory effect of these drugs over methionine sulfoxide reductase activity of *T. cruzi* MSRs, which exhibited similar inhibitory potency. This work suggests that five known drugs could be used to design new therapy strategies against Chagas disease. Granted by ANPCyT (PICT2014-2103) and Fundación Bunge y Born (subsidio para investigación de la enfermedad de Chagas - 2016).

Keywords: *Trypansoma cruzi*, drug repositioning, methionine sulfoxide reductase

(310) **EFFECT OF FENOFIBRATE ON THE PROMOTION OF A REGULATORY RESPONSE IN AN ACUTE MODEL OF *Trypanosoma cruzi* INFECTION**

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Development of *T. cruzi* infection is conditioned by events that occur early in secondary lymphoid organs, in which naïve cells are activated. These events imply the development of a proinflammatory response, the intensity of which may be decisive for the control of infection or the evolution to severe forms of the disease. Given that PPARα ligands modulate inflammation, we decided to evaluate the effect of Fenofibrate, a synthetic PPARα ligand, on the modulation of the immune response in an experimental murine model of acute *T. cruzi* infection.

To this aim, 8-week old BALB/c mice were infected by intraperitoneal route with 1.10^5 bloodstream trypomastigotes of the non-lethal CA-I strain (clone K98, DTU TcI). Infected mice were treated with 100 mg/Kg/day Fenofibrate for 3 weeks by oral gavage, since day 1 post-infection. Then, mice were euthanized and the expression of several genes involved in the modulation of the immune response to *T. cruzi* was analyzed in spleen cells by qRT-PCR.

Three weeks after infection, we observed a trend towards higher levels of expression of FoxP3 mRNA, a marker of T regulatory (Treg) cells in *T. cruzi*-infected mice treated with Fenofibrate in comparison with untreated infected mice. A similar trend was observed with regards to IL-10 and its receptor (IL-10R). Furthermore, a trend to increased expression of IL-2 and TGF-β (cytokines involved in Treg cell differentiation) was also observed.

These results suggest that treatment with Fenofibrate early during infection would induce a favorable microenvironment for the development of a regulatory response, in the context of *T. cruzi* infection, promoted by the expression of factors inducing Treg cell differentiation.

Keywords: *T.cruzi*, FOXP3, IL-10, Fenofibrate, qRT-PCR.

(1183) **NANOFORMULATIONS OF BENZNIDAZOLE IN THE TREATMENT OF CHRONIC MURINE INFECTION BY *Trypanosoma cruzi* Nicaragua**

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We evaluated the effect of new Benznidazole nanoformulations (BNZ-nps) in mice with chronic infection by *T. cruzi* Nicaragua (*Tc*N) with the aim of reduce the progression of cardiac pathology with low doses of administered drug and avoiding the adverse effects of BNZ evidenced in patients. In previous work, acute *Tc*N-infected C3H/HeN mice treated with 30 doses of BNZ-nps at concentrations of 10, 25 or 50 mg/kg/day, survived a 100%. The specific antibody titers for *T. cruzi* were negative at 6 months post infection with 25 and 50 BNZ-nps. Parasitemia by PCR showed that 80% of the mice treated with BNZ-nps 25 mg/kg/day were negative for *Tc*N and 50% with BNZ-nps 50 mg/kg/day. The hearts of the untreated infected mice showed multiple and extensive inflammatory foci that with BNZ-nps treatments are reversed. In this study, *Tc*N-infected C57BL/6J mice were treated in the chronic phase of infection with 30 daily doses of BNZ-nps at concentrations of 25 or 50 mg/kg/day or 13 intermittent doses (1 dose each 7 days) of 50 or 75 mg/kg/day. The different treatments effectiveness was evaluated measuring parasitemia by qPCR, serology by ELISA, inflammation and fibrosis by histopathology of hearts and electrical alterations by electrocardiograms. The bioethical management of the animals was approved by a corresponding Committee. With all treatments: 100% of the qPCR were negative, inflammation and fibrosis significantly decreased as well as antibody titers specific for *T. cruzi*. Electrocardiograms showed the normalization of the decrease of the heart rate and the PR inter-

val. For this, we can choose the intermittent treatment of 50 mg/kg/day, because with a lower total dose of BNZ (650 mg/kg) we obtain similar results (without significant differences) that whit high doses of the daily treatment. BNZ-nps treatment may be effective in successfully treat Chagas' disease in experimental models of *T. cruzi* infection by decreasing the amount of BNZ administered without loss the efficacy.

Keywords: Nanoformulations, Benznidazole, Chagas' disease, *Trypanosoma cruzi*

(483) **DEATH PHENOTYPES INDUCED BY O-NAPHTOQUINONES IN *Trypanosoma cruzi*. ROLE OF THE ALDO-KETO REDUCTASE IN β-LAPACHONE MECHANISM OF ACTION**

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Several *ortho*-naphthoquinones (o-NQs) have shown important trypanocidal activity against *Trypanosoma cruzi*, the ethiological agent of Chagas´ disease. We have previously demonstrated that the aldo-keto reductase from this parasite (*Tc*AKR) reduces o-NQs, such as b-lapachone (b-lap) and 9,10-phenanthrenquinone (9,10-PQ), with concomitant reactive oxygen species (ROS) production. *Tc*AKR activity and expression has been recently characterized in two *T. cruzi* strains, CL and Nicaragua, showing that CL has 2-fold higher *Tc*AKR expression than Nicaragua. Here, we studied the trypanocidal effect and the induction of several death phenotypes by b-lap and 9,10-PQ in epimastigotes of these two *T. cruzi* strains.

CL was more resistant to both o-NQs than Nicaragua with IC_{50}s of 4.05 and 1.17 μM, respectively, suggesting *Tc*AKR activity may not be relevant in o-NQs activation *in vivo*. b-lap induced ROS, evaluated with H_2DCF-DA probe, in both strains while 9,10-PQ only did it in CL. Staining with annexin V and propidium iodide (A/PI) showed that both drugs induced an increase of A+/IP- (early apoptotic), A+/IP+ (late apoptotic or necrotic) and A-/IP+ (necrotic) cells only in CL. However, 4-fold monodansyl cadaverine (MDC) labelling was observed after treatment in CL and Nicaragua suggesting autophagy may be a common mechanism induced by these drugs. Altogether, these results highlight that death mechanisms used by these o-NQs differ depending on the combination of drug and *T. cruzi* strain evaluated.

To study whether *Tc*AKR participates in o-NQs activation, b-lap and 9,10-PQ trypanocidal effect was evaluated in *Tc*AKR-overexpressing parasites. Only b-lap induced a greater ROS production and showed a lower IC_{50} value in *Tc*AKR-overexpressing epimastigotes than in controls (5.1 μM and 7.9 μM, respectively). We conclude that *Tc*AKR may be involved in b-lap activation through ROS generation, although *Tc*AKR activity is not a determinant of susceptibility in wild type *T. cruzi* strains.

Keywords: Chagas´disease, drug, oxido-reductase, autophagy, apoptosis

(421) **DISCOVERY OF A NOVEL *Trypanosoma cruzi* BROMODOMAIN INHIBITOR USING PROTEIN-DIRECTED DYNAMIC COVALENT CHEMISTRY**

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Bromodomains are the only protein domains capable of recognizing and interacting with acetylated lysines. *Trypanosoma cruzi*, the causal agent of Chagas disease, presents an atypical bromodomain (*Tc*BDF3) that interacts with acetylated α-tubulin present in the cytoskeleton and flagella of this parasite. *Tc*BDF3 is an interesting target for the development of new trypanocidal drugs that interrupt the Bromodomain-acetylated ligand interaction due to its essentiality in the differentiation of *T. cruzi*.

Protein-directed dynamic combinatorial chemistry has emerged as a powerful strategy to identify ligands for biological targets. This method relies on a library of small molecules that react reversibly with each other to generate a dynamic combinatorial library (DCL). When a protein is added to the equilibrium mixture, and if the protein interacts with any components of the combinatorial library, the position of the equilibrium will shift and those components that interact with the protein will be amplified. We have set up a DCL of acylhydrazones that would display a variety of functional groups potentially capable of interact with the hydrophobic pocket of *Tc*BDF3. For this purpose, four acylhydrazides and six aldehydes were selected as building blocks for the DCL, giving rise to potentially 26 acylhydrazones. We observed the amplification of one member of the library in the presence of recombinant *Tc*BDF3 using HPLC-DAD and HPLC-MS. This compound binds specifically to recombinant *Tc*BDF3, with a dissociation constant of 1.7 μM, measured by fluorescence quenching. *Trypanosoma cruzi* has a complex life cycle with two hosts and four different developmental forms. We also observed a cytotoxic effect in all life cycle stages similar to the drug Benznidazol, currently use as therapy but with numerous and severe side effects.

These results validate this strategy to search for novel candidates against Chagas disease.

Keywords: Trypanosoma cruzi, Bromodomain inhibitors, dynamic covalent chemistry, mass spectrometry, Chagas disease

(1868) **DESIGN OF GENETICALLY ENCODED PROBES TO DETECT PEPTIDASE ACTIVITY *IN VIVO*.**

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Proteases play a major role in regulating a wide range of physiological and pathological processes. Unregulated protease activation or inhibition could eventually result in a number of important diseases including cancer, atherosclerosis, and neurodegenerative diseases. Currently, protease activity can be determined by several methods. Western blot analysis and quantitative real-time polymerase chain reaction can estimate the expression levels of the enzymes and their inhibitors although limited information for understanding the dynamic activity of the proteases *in vivo* is provided. One of the most commonly applied methods is the activity measurement in cell lysates using small chromogenic or fluorogenic synthetic peptides that are, in many cases, commercially available. Although some probes are able to penetrate cells they cannot be delivered into specific subcellular compartments. Moreover, they are not ideal for continuous measurements due to their limited lifetime, specificity and stability.

In this work, we report a strategy to create protease sensors by grafting an enzymatic cleavage linker into a sensitive location for changing chromophore properties of enhanced green fluorescent protein (EGFP) following protease cleavage. We first validated the system in *Escherichia coli* by expressing and purifying the protease and FRET sensors, using the TEV protease as the model enzyme. Once the cleavage was confirmed by Western blot analysis and by changes in the emission spectra, we moved to *in vivo* studies in *Saccharomyces cerevisiae* were the same analyses were performed.

This strategy opens a new avenue for developing specific protease sensors to investigate enzymatic activity in real time, to probe enzymatic relevance corresponding to proteases *in vitro* and *in vivo*, and to screen protease inhibitors with therapeutic effects.

Keywords: proteases, substrate, FRET

(1723) **THE C4 STEROL DEMETHYLATION STEP OF *Trypanosoma cruzi*: ACTIVITY OF NEW CANDIDATE IN-**

HIBITORS ON PARASITE GROWTH

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In *Trypanosoma cruzi* the sterol biosynthesis pathways is a validated target for chemotherapeutic intervention. Humans and trypanosomes share many of the enzymes leading to essential isoprenoid and sterol precursors. However, there are also key differences in these pathways. Whereas the main sterol in mammals is cholesterol, *T. cruzi* (and yeast/fungi) have ergosterol as their main sterol. Hence, while similar, the pathways also show a number of key steps that differ and that could be exploited to selectively affect parasite enzymes (Cosentino RO et al, 2014). During their synthesis, sterol precursors become functional only after removal of the two methyl groups at C4 by a membrane-bound multienzymatic complex. Intriguingly, enzymes and inhibitors of the C4 demethylation step have remained poorly characterized. In this study, we report the identification of a number of candidate inhibitors of steroid C4-demethylation, based on computational and literature searches. As part of these searches, we have identified 5 compounds associated with inhibition of C4-sterol demethylation at either of these enzymatic steps in different organisms. These are: APB (2-(pentylsulfanyl)-1,3-benzothiazol-6-amine); Fenhexamid; Totarol; NCE-893, and FR171456. We tested them for inhibition of intracellular amastigote replication using transgenic *T. cruzi* parasites expressing a β-galactosidase reporter gene, and benznidazole as a positive control. Briefly, we treated infected cells and measured β-galactosidase activity with CPRG after 72hs. Except for totarol (which was toxic), all other drugs were active in the assay, with EC50s ranging from 30 μM (fenhexamid) to 0.1 μM (FR171456). Using FR171456, we observed an altered morphology of amastigotes by SEM. In conclusion, these data suggest that the C4-demethylation step of the ergosterol biosynthetic pathway of *T. cruzi* is also a valid target for development of new drugs for Chagas Disease. Supported by PICTO-Glaxo-2013-0067.

Keywords: Trypanosoma cruzi, C4 sterol demethylation step, drug development, growth inhibitors.

(1839) **EFFECT OF METRONIDAZOLE ON IN VITRO ISOLATES OF *Tritrichomonas foetus***

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"Bovine Trichomonosis" (BT), caused by infection with the flagellated parasite *Tritrichomonas foetus*, is a sexually transmitted disease limited to the urogenital tract of cattle. In Argentina, BT is endemic and a relevant health problem as it causes reproductive inefficiency in cattle, which is accompanied by large economic losses. For decades, Metronidazole has been the drug of choice in the treatment of BT. However, more recently both aerobic and anaerobic resistance have been reported, *in vivo* and *in vitro*, respectively. Presently, little is known as to Metronidazole values for minimal lethal (MLC) and minimum inhibitory (MIC) concentrations against these parasites. Thus, the goal of the present study was to explore the susceptibility of six different isolates of *T. foetus* under aerobic (AC) and anaerobic (ANC) conditions. Isolates were obtained from three Argentine provinces, Buenos Aires (1), Salta (4) and Santa Fe (1). The 24-h exposure times of isolates to the drug rendered MLC values in the range of 2.5 - 5 µg/mL and ≥5 µg/mL for ANC and AC conditions, respectively, while the values after 48-h were 1.25 – 2.5 µg/mL and 2.5 and 5 µg/mL, for ANC and AC conditions, respectively. MIC values after 24 h were between 1.25 - 2.5 µg/mL and 2.5 and >5 µg/mL for ANC and AC, respectively, while the 48 h values were between 0.625 and 1.25 µg/mL and 1.25 and 2.5 µg/mL for ANC and AC, respectively.

The present data provide updated information on *T. foetus* susceptibility to Metronidazole, which reveal both MLC and MIC higher values than those previously reported in available bibliographic records.

Keywords: *Tritrichomonas foetus*, Bovine Trichomonosis, susceptibility *in vitro*, Metronidazole

TOXICOLOGY 5

(321) **ALTERNATIVE METHODS TO ANIMAL EXPERIMENTATION FOR EVALUATION OF OCULAR IRRITATION**

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The principles of the 3Rs (Replacement, Reduction and Refinement) for laboratory animals use in regulatory toxicity assays began to be implemented decades ago in Europe and was accompanied by the development of alternative methods. Our objective was to incorporate some of the currently validated methods and in this instance we focused on tests to determine eye damage. The methods that we used were **(1)** Cytotoxicity assays in cultures of different cell lines i) HaCaT (human keratinocytes) and ii) 3T3 (mouse fibroblasts); **(2)** HET-CAM (chorioallantoic membrane of hen's eggs) which has a highly vascularized structure similar to the conjunctive and that is capable of responding to irritant products.

We have carried out the method **(1)** in the 3T3 and HaCaT cells lines comparing two protocols, one guided by ICCVAM and ECVAM and another one recommended by the OECD. We evaluated the neutral red uptake (NRU) as a marker of cell viability after 24 hours (according to ICCVAM and ECVAM protocols) and 48 hours (according to TG 432 OECD) exposure to two known cytotoxic agents: SDS and triton. Applying non-linear Hill regression to the dose-response data, we calculated the IC_{50} and then we estimated the LD_{50} (mg/kg) for the tested substances using a prediction model. On the other hand, we performed the method **(2)** as established by the ECVAM protocol testing the following substances: imidazole, benzalkonium chloride and SDS in different concentrations. We quantified hyperhemia, hemorrhage and coagulation in the CAM as irritation parameters during 300 seconds and then we calculated an ocular irritation index.

The results obtained by the two methods used were compared with the results reported by reference laboratories and showed that the set-up was successfully developed. This is the first step for the incorporation of alternative methods to laboratory animals use for ocular toxicity evaluation in our laboratory.

Keywords: alternative methods, citotoxicity, NRU,HET-CAM.

(1261) **BIOCHEMICAL AND TOXICOLOGICAL CHARACTERIZATION OF DUVERNOY'S GLAND SECRETION FROM THE FALSE CORAL SNAKE *ERYTHROLAMPRUS AESCULAPII***

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Erythrolamprus aesculapii is a colorful colubrid snake widespread in South America, and commonly known as a false coral snake due to its mimicry with some *Micrurus* sp. snakes. This feature constitutes the main reason of its demand in the illegal pet trade; however, people are not aware of its dangerousness. Herein, we aimed to study the biochemical composition and some biological activities related to the toxicity of its Duvernoy's gland secretion (DGS). We performed one-dimensional SDS-PAGE (12%) of this secretion and we evaluated its ability to hydrolyze molecules such as azocollagen, azocasein, acetylthiocholine, lecithin, hyaluronic acid, and human fibrinogen. In order to understand its biological role, the effect of DGS on mouse skin was tested and the Minimal Hemorrhage Dose

(MHD) was determined. SDS-PAGE showed - under non-reducing conditions – a profile with protein bands ranging from 15 to 75 kDa. In relation to the enzymatic activities, hydrolysis of azocollagen was significantly high (229.9 U/min/mg), and a little lower with azocasein (41.45 U/min/mg). Although it was not capable to hydrolyze acetylthiocholine and lecithin, DGS exhibited a slightly activity toward hyaluronic acid. Furthermore, in presence of Ca^{++}, the secretion rapidly hydrolyzed the Aα-chain of fibrinogen, leaving the γ-chain unaffected. When DGS was injected into the skin, the histopathology analysis showed intense hemorrhage (MHD= 18.76 μg) and inflammatory reaction with an important detachment of epidermis and hair follicles, but slight myonecrosis. These findings support the fact that *E. aesculapii* may cause human envenomation with mild to severe effects (especially in children), and give insight into future directions for the isolation and characterization of key components present in its "venom".

Keywords: Hemorrhage, Collagen, Fibrinogen, Colubridae, Venom.

(1910) ***Bothrops alternatus*** **VENOM INDUCES THE EXPRESSION OF $cPLA_{2\alpha}$, COX-2 AND RELEASE ARACHIDONIC ACID IN MACROPHAGES**

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Bothropic intoxications are characterized by the development of local inflammatory symptoms that are poorly controlled by commercial antivenom. Thus, it is interesting to understand the activated mechanisms in the envenomation in order to find new pharmacological targets. So the aim of this study was evaluate the expression of enzymes involved in lipid mediators biosynthesis. Additionally, a lipidomic analysis (LA) of macrophages stimulated by *B.a.* venom was performed.

Considering that macrophage plays a key role in the coordination of inflammatory events, RAW 264.7 cells were cultured and exposed to the action of B.a. venom for 6 h. The enzymes genes expression (cPLA2α and COX-2) was assessed by qPCR. To detect both proteins and 505-Ser $cPLA_{2\alpha}$, Western blott test was made. Additionally, MS lipidomic techniques were assayed. Total lipids were extracted and separated into classes (PL, DAG, FFA, TG and CE) by TLC. Then, fatty acids belonging to the phospholipids fraction were derivatized and then analyzed by GC-MS.

After 6 h of exposure to *B.a.* venom, it was observed that the venom induce the mRNA expression of enzymes related to lipid signaling, cPLA2α and COX-2. Besides, an increment in the total expression of enzymes and also in its activity (measured by phosphorylation in Ser 505 $cPLA_{2\alpha}$) was detected. The LA revealed a moderate reduction of cellular fatty acids, particularly those present in membrane phospholipids, like of arachidonic, oleic and stearic acids. Simultaneously, a marked increase in the Free Fatty Acid fraction demonstrates an evident action of venom PLAs on the macrophage membrane.

LA of macrophages stimulated with venom evidenced that the main FFA are implicated in the inflammatory response, and also demonstrated that this venom, is able to activate lipid metabolism. Thus, deeper lipidomic studies, poorly developed up to now in ophidian toxicology, could reveal novel pharmacological targets for the treatment of bothropic intoxications.

Keywords: inflammation, lipidomic analysis, macrophage

(686) **DEVELOPMENT AND EVALUATION OF AN IMAGING PROTOCOL TO SHOW DOXORUBICIN ADVERSE RENAL EFFECTS IN SMALL ANIMAL MODELS**

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The ability of small animal imaging to show renal side effects of a doxorubicin treatment in animal models (AM) was studied. A previously proven effective treatment in a breast cancer AM was assayed (ip doxorubicin, 2mg/Kg, 6 doses in 2 weeks) in two healthy AM: male Sprague Dawley rats (n=10) and Balb-c mice (n=10). Two radiopharmaceuticals (RF) were used: ^{99m}Tc-DMSA that shows renal function and structure by gamma camera imaging; and ^{99m}Tc-DTPA, which shows glomerular filtration (GF) by RF retention curves. Studies were performed prior (Control) and post treatment (Dox) by intravenous injection of each RF. Serum samples were taken to measure urea and creatinine levels, urine samples were taken (24h urine collected in metabolic cages) to evaluate proteinuria and kidneys were excised for histopathological evaluation. Qualitative visual analysis of ^{99m}Tc-DMSA images showed no differences between groups in a species independent way. However, quantitative analysis showed a significant increase in kidneys, liver and joints uptake for Dox groups in rats ($P<0.01$) but just in kidneys in mice ($P<0.05$), suggesting an impaired elimination. GF, urea and creatinine plasma levels showed non- significant differences for Control and Dox groups in both species. Urinary protein was found significantly increased in Dox rats ($p=0.017$, paired) but highly variable results were obtained for mice samples.

Preliminary histological analysis showed presence of tubular protein casts, vasocongestion and mild to severe tubular damage with no glomerular changes. In conclusion we found imagenological parameters associated to renal function, which could be used to follow up doxorubicin renal side effects and thus to further test potential protective agents in AM. Additional research is needed to elucidate the mechanism of altered pharmacokinetic of ^{99m}Tc-DMSA.

Keywords: small animal imaging, ^{99m}Tc-DMSA, ^{99m}Tc-DTPA, kidney, doxorubicin

(1898) **MUSCLE REGENERATION AFTER HIGHLY MYOTOXIC VENOM FROM *Bothrops diporus***

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Due to its wide geographical distribution and aggressive behavior, *Bothrops diporus* (Bd) is a major source of snakebites in Argentina. Bd venom is highly proteolytic, hemorrhagic and myotoxic as a result of PI- and PIII- SVMPs and PLA_2 proteins. Most of these myotoxins, proteolytically active D49 class, induced marked local muscular necrosis. However, there is no information about the muscle regeneration following necrosis. Herein we present local lesions in muscle induced by Bd venom and main events in myogenesis subsequent to myofiber necrosis. Groups of six CF-1 mice were injected in the right gastrocnemius with 60 µg of Bd venom dissolved in 0.1 mL of PBS pH 7.2. After 1, 3, 7, 14 and 28 days mice were sacrificed and muscle tissues processed for histological analyses. Samples were stained with hematoxylin and eosin (H&E) for examination by light microscopy at low and high magnification. Localization of cell proliferation in the muscle venom-injected was investigated by indirect immunohistochemical technique using anti-proliferating cell nuclear antigen (PCNA) monoclonal antibodies. Twenty-four hours after envenomation, abundant necrotic fibers associated with inflammatory infiltrate and scarce hemorrhagic sites were evident. Also, many PCNA-positive cells were observed in the whole sample. On day 3, necrotic areas are accompanied by intense inflammatory infiltrate; although splinde-shape cells still displayed intense staining. On days 7 and 14, many newly myotubes and proliferating cells were PCNA

positive. Phagocytosis by infiltrating neutrophil remains increased. Muscle fibers in varied stages of regeneration are widely extended despite; necrosis still appeared on day 28. We demonstrated that Bd venom exerts basically myotoxic activity but, positive PCNA cells were present in fields at the early stage of regeneration showing favorable regenerative microenvironments in fields of tissue injury. A successful regeneration of injured skeletal muscles is expecting.

Keywords: BOTHROPS DIPORUS – MYOTOXICITY- PROLIFERATING CELLS- MYOGENESIS- SNAKE VENOM.

(144) **RADIOPROTECTIVE EFFECT OF VEGETABLE OILS IN AN EXPERIMENTAL MODEL OF ACUTE RADIATION SYNDROME**

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In the present study, the radioprotective effect of several vegetable oils containing different amounts of polyunsaturated fatty acids, tocopherols and tocotrienols were tested using an experimental model in Sprague-Dawley rats (both sexes) exposed to X radiation (2 Gy, whole body). Groups of 8 animals were exposed at a dose of 2 Gy. Blood samles were obtained after 48 hours by caudal puncture, followed by sampling at 7, 14, 21, 28 and 60 days. The erythrocyte, leukocyte and leukocyte formula were counted. Survival curves up to 60 days were also performed. Genotoxic effects in leukocytes were assessed by the Comet assay one hour post-irradiation. Oils (wheat germ, rice seed, palm, grape seed, chia, avocado and olive) were administered subcutaneously two hours before irradiation at a dose of 1 g/kg. For comparison, the effect of alpha-tocopherol was tested at a dose of 570 mg/kg (i.p.) administered 4 hours prior to irradiation, and also for its acetate (given at a dose of 750 mg/kg (i.p.) 24 hours before irradiation). In irradiated animals erythrocytes were significantly depleted (females, $p<0.01$) and the white blood cell count was drastically reduced (both sexes, $p<0.01$), also presenting an altered formula. In addition, Comet assay showed an important damage on DNA ($p<0,01$ compared to control). All of the treatments with the exception of avocado oil resulted in significant protection against DNA damage in both sexes (Comet, $p<0.05$). Although the protective effect on the hematological parameters measured was complete in the survivors of both sexes (from day 30), no significant protective effect was observed with any of the treatments regarding survival. These oils showed ability to protect leukocyte DNA significantly but less than that of a radioprotective agent such as the toxic amifostine (WR-2721). They could be useful to prevent or mitigate the acute harmful effects of ionizing radiation as coajuvants of amifostine.

Keywords: ionizing radiation; radioprotection; acute radiation syndrome; oxidative stress

(1687) **EVALUATION OF THE ACUTE TOXICITY PRODUCED BY THE DECOCTION OF *PHLEGMARIURUS SAURURUS***

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The promissory results obtained in previous investigations of *Phlegmariurus saururus* (Lam.) B. Øllg. show that it is imperative to assess their toxicity in view of a possible therapeutic application. Consequently, it was the objective of this study to determine if the decoction of *P. saururus* administered acutely produces toxic effects. Following international guidelines, we worked with 4 groups of Wistar rats, 2 consisting of females of 10 weeks and another 2, made up of males of 20 weeks. The animals of the control group received distilled water and those of the treatment group, 2000 mg/kg of the decoction of *P. saururus* in a single intake. After oral administration by cannula, the animals were observed for 15 days, during the first 6 hours uninterruptedly and daily on the remaining days. At the beginning and at the end of the study, the animals were weighed and their blood was collected on both occasions in order to perform hematological and biochemical tests. The amount of water and food consumed was also determined. At the end of the study, the animals were sacrificed and their organs extracted for histopathological examination. In relation to the behavior, there was a decrease in the degree of exploration and an intensification of the gregarious behavior. All this simultaneously with the appearance of tremor and abdominal contractions (10 minutes after administration of the decoction), reaching its maximum expression 30 minutes after the experiment beginning. These signs slowly decayed, reversing completely at 6 h after the study began. There was no mortality in any group. Analyzing the results of the different studies and tests it was shown that there were no significant differences between the treatment groups and the controls. The results obtained here allow us to infer that decoction would be safe at the effective dose, since the therapeutic range (1 to 30 mg/kg in sexual behavior experiments) is 67 times lower than the toxic range (2000 mg / kg).

Keywords: *Phlegmariurus saururus*, acute toxicity, Wistar rats

(529) **HISTO-MORFOLOGICAL AND MOLECULAR CHANGES ON GONADAL DIFFERENTIATION IN *Caiman latirostris* MALES. EFFECT OF THE EXPOSURE *in ovo* TO ATRAZINA**

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Atrazine (ATZ), a widely used herbicide is an endocrine disruptor compound (EDC) that alters male reproductive tissues during development. *Caiman latirostris* is a species with temperature dependent sex determination (TSD), thus, egg incubation temperature is the main factor that determines the sex during a thermo-sensitive period (TSP). Caimans have eco-physiological features that make them particularly vulnerable to EDC exposure. The aim of this study was to describe the effects of an environmentally relevant dose of ATZ on gonad histo-morphology and the expression patterns of key molecules involved in embryonic testis development. Caiman eggs were incubated at male producing temperature, at stage 20, prior to TSP, vehicle (TSD-males) or 0.2 ppm of ATZ (ATZ-males), were administered. Embryos were obtained in stages 22, 24 and 27. Gonad histo-morphology was studied using trichromic stained sections and the expression of estrogen receptor alfa (ERα), progesterone receptor (PR), proliferating cell nuclear antigen (PCNA) and TAp63 isoform (p53 family member) were assessed by IHC. ATZ-males showed morphological changes in the gonad at stage 27. ERα expression showed a decline towards the end of development both in TSD and in ATZ-males ($p<0.05$). At stage 24, ERα protein levels were higher in TSD-males ($p=0.02$). PR showed an increase from stage 22 to the end of the development in both groups ($p<0.05$). The expression of PCNA in TSD-males remained low throughout development. At stage 22, PCNA was significantly higher in ATZ than TSD-males ($p=0.01$). High PCNA expression observed at stage 22 preceded a significant fall at stages 24 and 27($p<0.05$). Specific TAp63 immunoreactivity in caiman embryos germ cells was detected being higher in the gonads of ATZ-males at stage 24 ($p=0.04$). The exposure to low ATZ dose disrupted embryonic male gonad histo-morphological features. These could have detrimental effects on male sexual maturation and/or on caiman reproduction success.

Keywords: ATRAZINE; REPTILE; ENDOCRINE DISRUPTOR; GONADAL DIFFERENCIATION

(888) **IN VITRO MUSCLE REGENERATION AFTER LOCAL TREATMENT IN BOTROPIC INTOXICATION**

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Viperid snakebite envenoming is characterized by prominent local

tissue damage, including muscle necrosis. The specific treatment of ophidian envenomation is serotherapy and consists in the empirical administration of antivenoms administered intravenously. Although the vascular therapy ensures a rapid distribution of antibodies and controls the systemic alterations caused by the venom, the same thing does not always happen in the bite site where traces of venom are capable to preclude a successful regenerative response.

In this work, we evaluated the local administration of antivenom and its effectiveness on in vitro miogénesis. Groups of CF-1 mice (18–22 g) were injected intramuscularly in the right gastrocnemius with 50 μg of *B. alternatus* venom. After 1h, half of the intoxicated mice were injected in the same muscle with 20 μL of commercial bivalent antivenom (INPB Anlis "Carlos G. Malbran"). This relationship was established by total neutralization of the hemorrhagic and hemolytic activities of venom. Control mice received only the antivenom under identical conditions. At various time intervals after injection (3, 6, and 24h), animals with or without treatment were sacrificed, muscles dissected out and homogenates prepared. Myoblast cells (C2C12 cell line) were exposed to these muscle homogenates for myogenesis evaluation. Results evidenced differences between treated and non-treated mice 6 and 24h post intoxication. At these times, assays with treated muscle homogenates showed mature myotubes formation. In contrast, a complete lack of myoblast fusion occurred when myogenic cells were incubated with muscle homogenates from mice injected only with venom. No significant differences were observed between mice with or without treatment after 3h of intoxication. These findings suggest that a local treatment with antivenoms, complementary to the serotherapy, could improve the snakebite envenomings and accelerate the processes of muscle regeneration.

Keywords: Bothrops alternatus, myogenesis, antivenom, regeneration

(867) LOSARTAN PREVENTS THE VASCULAR DISFUNCTION ALTERATIONS CAUSED BY HEXACLOROBENZENE

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In previous work we showed that environmental dioxine-type hexachlorobenzene (HCB) increases blood pressure (BP) in rats, causing alterations in arterial structure and function. Thyroid hormone, angiotensin II receptor type 1 (AT1) and endothelial nitric oxide synthase (eNOS) are involved in the toxic effect.

Here we study whether the AT1 receptor antagonist Losartan (L) can prevent alterations caused by HCB intoxication.

Male Wistar rats treated with HCB (500 mg/kg bw) by gastric intubation, 45 days and treated or not with L (30 mg/Kg/day in drinking water). BP was measured during all intoxication period. It was assessed aortic morphology by analysis of its thickness and cells number and vascular physiology by arterial contractility in aortic rings contracted with Phenylephrine (P), and relaxed with Acetylcholine (Ach) and Nitroprusside (N) (isometric tension). Molecular markers potentially involved in the mechanism of toxic action were analyzed by western blot.

HCB treated rats showed an increase in BP (145.5±5.1 mmHg) which was prevented by simultaneous administration of L (110.5±8.2 mmHg, $p<0.01$). HCB decreased the aorta cells number and increase aortic thickness, both effects were partially impaired with L. The maximum contraction by P (% K^+ 60mM) decreased which was not altered in L+HCB treated rats (control: 143±5%, HCB: 123±5%, HCB+L: 122±5%, $p<0.05$). However, aortas of HCB-treated rats showed less relaxation to Ach stimulus, which was prevented in L+HCB- treated rats. Arteries from HCB treated rats does not show changes in the N-response curve respect to C group. HCB decreased PCNA levels ($p <0.05$) and eNOS ($p <0.01$) and increased AT1 ($p <0.05$). L+HCB treatment maintained expression levels of the three parameters similar to control.

Conclusion: L prevents the increase of BP produced by HCB intoxication in rats and also the HCB induced alterations in arterial relaxation endothelium dependent and molecular markers.

Keywords: Hexachlorobenzene, hypertension, Losartan, aorta, AT1 and
dioxin-like

CELL SIGNALING 7

(1302) RACK1 PROMOTES PROLIFERATION AND MOTILITY OF MELANOMA CELL LINES.

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RACK1 (Receptor for Activated C kinase 1) is a scaffold protein that coordinates the interaction of key signaling molecules implicated in both physiological cellular functions and tumorigenesis. RACK1 has been shown to be aberrantly expressed in cancer where it has either pro- or anti-oncogenic effects.

Melanoma is the deadliest skin cancer and its incidence has increased over the past three decades. The role of RACK1 in melanoma has been poorly studied. Recently, it was shown that RACK1 cooperates with NRASQ61K to promote melanoma in vivo. The aim of this study is to investigate the effect of RACK1 overexpression in melanoma. To this end we stably transfected RACK1-HA into the melanoma cell lines SK-Mel28 and A375. To determinate the effect of RACK1 in cell proliferation we performed Crystal Violet assays. We found that RACK1 overexpression significantly increased the proliferation of SK-Mel28-RACK1 and A375-RACK1 cell lines ($p<0.05$). To identify possible underlying mechanisms we performed Western blots. We found that RACK1 overexpression significantly increased Akt phosphorylation (T308 and S473) and Cyclin D1 levels ($p<0.05$).To study the effect of RACK1 in cell motility we performed Wound Healing assays. These experiments showed an increase in cell motility in SK-Mel28-RACK1 and A375-RACK1 ($p<0.05$). The increase in cell motility was in agreement with the elevated expression of the mesenchymal markers, N-cadherin and Vimentin. These results suggest that RACK1 promotes proliferation and migration in melanoma and could play an important role in the tumorigenesis and aggressiveness of this disease.

Keywords: Melanoma, Cell Proliferation, Cell Motility, Akt, N-cadherin.

(1056) SMAUG2/SAMD4B REGULATES MESENQUIMAL DEVELOPMENT

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Our laboratory described that Smaug1/2, a translational repressor that binds specific motifs termed *Smaug recognition elements* (SREs), forms cytoplasmic silencing bodies similar to Processing Bodies. Rodent Smaug1/Samd4a modulates synaptic plasticity (Baez et al., J Cell Biol 2011) and other authors reported that Smaug2/Samd4b directs early neuronal differentiation (Amadei et al., J Neurosci 2015). In addition, Smaug1/Samd4a KO mouse show strong developmental defects of mesenquimal tissues (bone, cartilage, muscle and fat). Our objective is to investigate how Smaug1 / 2 regulate mesenquimal differentiation. We used mesenquimal cell lines and specific antibodies and found that Smaug proteins forms mRNA silencing bodies, which are different from PBs. Strickingly, Smaug silencing inhibits cell differentiation. We designed an algorithm to predict SREs and found several potential targets relevant to this phenotype that are conserved in mouse and human. Quantitative PCR indicated that Smaug KD prevents the up-regulation of key transcription factors that govern cell differentiation ($p<0,05$). In particular, western blot analysis indicates that the early pro-developmental transcription factor C/EBP-β are downregulated upon Smaug KD. We are currently analyzing the contribution of Smaug1 and Smaug2 and which specific targets are under their respective control.

Keyword: SMAUG2, mesenquimal differentiation

(331) **A TANKYRASE INHIBITOR IMPAIRS TUMOUR GROWTH AND ANGIOGENESIS THROUGH THE WNT/β-CATENIN PATHWAY ATTENUATION**

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Aberrant regulation of Wnt signaling pathway is a prevalent theme in cancer biology. While it has been demonstrated to be involved in many types of tumours it has been poorly studied in ovarian cancer. We analyzed the effect of inhibiting Wnt/β-catenin in a xenograft model of human ovarian cancer. A human ovarian adenocarcinoma cell line (IGROV-1) was subcutaneously injected in 6-8 weeks-old female nude mice. Once the tumour was palpable, we injected a tankyrase inhibitor, which attenuates Wnt/β-catenin signalling (XAV939: 2.5 and 5 mg/kg) every two days three times. Mice were euthanized 3 days after the last injection. The involvement of Wnt/β-catenin pathway in tumour growth, morphology and angiogenesis was evaluated.

Our results showed a significant decrease in tumour size when mice were treated with XAV939, which strikingly, was higher at the 2.5 mg/kg dose than the 5 mg/kg dose (day 7: no treated animals vs. 2.5 mg/kg group, $p<0.001$; no treated animals vs. 5 mg/kg group: $p< 0.05$). No significant differences were appreciated in mice weight between groups. There was also a significant decrease in β-Catenin and Cyclin D1 levels measured by western blot at both doses used. On the other hand, we observed a decrease in the endothelial cell area stained with CD31 marker. Additionally, we detected a decrease in the periendothelial cell area, measured using α-Smooth-muscle-actin, compared with tumours from animals with no treatment. In hematoxilin-eosin stained sections of tumours we distinguished that the morphology notably changed after treatment with XAV939. A tumour loss of integrity and accumulation of interstitial fluid was evident and dose dependent.

In conclusion, we demonstrate a clear involvement of Wnt/β-catenin in ovarian tumour growth and we suggest and implication of this pathway in tumour angiogenesis.

Keywords: Wnt signaling, ovarian cancer, angiogenesis, tumour growth

(1634) **WNT5A REGULATES N-CADHERIN SHEDDING IN MELANOMA**

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The loss of E-cadherin and the gain of N-cadherin expression, usually known as “cadherin switching” is a crucial process in melanoma progression. The loss of E-cadherin expression has been correlated with advanced melanoma stages and poor prognosis, whereas the upregulation of N-cadherin associates with increased cell motility and metastasis. N-cadherin function is also regulated by shedding by cellular proteases that cleave the 135 KDa full-length protein (p135) in its N-Terminal Fragment of 95 KDa (ectodomain or p95) and the C-Terminal Fragment (CTF, 40 KDa).

Wnt5a is responsible for the activation of several Wnt-no canonical signals that have been implicated in tumor progression. In a previous work we demonstrated that Wnt5a induces cell motility and migration by inducing the expression of N-cadherin in melanoma. In the present work we studied the regulation of N-cadherin shedding by Wnt5a. Treatment of three melanoma cell lines (A375, Lu1205 and Mewo) with Wnt5a conditioned media significantly increased the amount of cellular p95 and the ratio p95/p135 ($p <0.05$). In line with these results, silencing Wnt5a expression in WM9 cells (a cell line with high Wnt5a expression) reduced N-cadherin shedding ($p <0.05$). The cleavage of N-cadherin was further confirmed by detecting the release of the ectodomain to the culture media and the release of the CTF to the cytosol.

N-cadherin shedding induced by Wnt5a was reduced to different extent in different cell lines by the pharmacological inhibitors LY294002 and Go6976, suggesting the participation of Akt and PKC pathways, respectively. In line with these data, P-Akt levels were increased ($p <0.05$) or reduced ($p <0.05$) upon Wnt5a treatment and Wnt5a silencing, respectively. In agreement Treatment of cells with TPA or Ionomycin induced N-cadherin shedding confirming the participation of PKC in this process. In summary, our results indicate that Wnt5a regulates N-cadherin shedding via PI3K/Akt and PKC pathways.

Keywords: Melanoma, N-Cadherin, Shedding, Wnt5a

(1400) **COMPARATIVE METASTATIC AND NONMETASTATIC TUMOR RESPONSE TO HYALURONIC ACID TREATMENT. ROLE OF BETA-CATENIN AS A POSSIBLE MEDIATOR**

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Tumor microenvironment has a significant role in tumor malignancy, modulating several functions as angiogenesis. In this work we studied the relation of the extracellular matrix component, Hyaluronic acid(HA) and the protumoral signaling pathway Beta-Catenin and the effect of that interaction in angiogenic processes in tumors of different behavior. We used the metastatic breast cancer MDA-MB-231 cells and the nonmetastatic prolactinoma MMQ cells. Cells were treated with HA (20 mg/ml) or the Wnt synthesis inhibitor, IWP-2 (5uM) for 48hs. Protein levels were analyzed by WB or FC and VEGF biosynthesis measured by ELISA. We found that the HA-receptor CD44 has low expression in MMQ cells (3%) while it was high in MDA cells (98%). HA induced Beta-Catenin expression in MDA cells ($p<0,05$; N=3) and produced a trend of increase of the protein in MMQ cells. HA treatment was unable to modulate VEGF release in any of the cell lines (ns; N=3), although MDA supernatants of HA treated cells increased HMEC endothelial cell migratory capacity evaluated by wound healing assay ($p<0,001$; N=3). In order to determine whether Wnt/Beta-Catenin pathway affected tumor angiogenesis, we treated both cell lines with IWP-2 and observed a trend of reduction of Beta-Catenin levels and of increase of pGSK3 active form in MMQ cells; however, no effect was found in MDA cells (ns; N=3) suggesting that Beta-Catenin expression under HA treatment could be independent of Wnt ligands. Regarding VEGF secretion, IWP-2 reduced VEGF levels ($p<0,05$; N=3) in MMQ cells but not in MDA (ns; N=2). In accordance, IWP-2 treated MMQ supernatants showed a trend of reduction of HMEC cell migration. Our results suggest that the influence of HA on angiogenic responses could differ according to the aggressiveness of the tumor studied, which can be explained by a different sensitivity to HA in relation to CD44 expression and different mechanisms of regulation of Beta-Catenin.

Keywords: METASTASIS, ANGIOGENESIS, HYALURONIC ACID, BETA-CATENIN

(734) **AN AUTOCRINE WNT5A LOOP IS A MAIN MECHANISM OF CONSTITUTIVE NF-κB ACTIVATION IN MELANOMA**

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Melanoma is the most deadly type of skin cancer and has a poor prognosis when not diagnosed at early stages. Wnt5a is a secretory glycoprotein involved in the non-canonical Wnt signaling pathway that plays an important role in melanoma by increasing motility, invasion, proliferation and resistance to apoptosis. Wnt5a expression positively correlates with melanoma progression, tumor grade and patient outcome. Constitutive activation of NF-κB is a hallmark of several types of tumors including melanoma. The aim of this work was to study a possible role of Wnt5a in regulating inflammatory responses in melanoma by activating the NF-κB pathway. To this end, we stimulated 1205Lu melanoma cells with both Wnt5a conditioned media and purified recombinant Wnt5a. A kinetic analysis showed that phosphorylation of the critical S536 residue in p65 is induced by 5 min and reaches a maximal 8-fold increase at 30 min after Wnt5a treatment ($P<0.005$) concomitant with a 2-fold increase

in IKKαβ phosphorylation and IκBα degradation (P<0.005). These results were confirmed in five additional melanoma cell lines. Interestingly, cell lines expressing high levels of Wnt5a also displayed very high basal levels of P-p65 suggesting an active functioning Wnt5a/ NF-κB autocrine loop. This possibility was confirmed since inhibition of Wnt5a pathway by IWP-2 (a Wnt5a inhibitor) or a Wnt5a shRNA reduced endogenous P-p65 by 40% and 60% respectively (P<0.005).

Wnt5a stimulation induced P-p65 translocation to the nucleus as shown by immunofluorescence and Western blot of subcellular fractions. Wnt5a fully stimulated the transcriptional capacity of NF-κB as demonstrated by a 7-fold increase in the Luciferase activity of a NF-κB reporter (P<0.005). Moreover, Wnt5a induced a time-dependent increase on protein levels of known NF-κB targets including RelB, PKCδ, BCL2 and Fas. In summary, these results demonstrate that autocrine Wnt5a stimulation is partly responsible for NF-κB activity in melanoma.

Keywords: Melanoma, Wnt5a, NF-κB, signaling, crosstalk

(700) **THE WNT5A RECEPTORS ROR1 AND ROR2 EXERT OPPOSITE EFFECTS ON THE PROLIFERATION OF MELANOMA CELLS**

Maria Victoria Castro

Abstract: Melanoma is the deadliest form of skin cancer and its incidence continues to increase, making it the fifth most frequent type of tumor. In the past years, several molecular pathways involved in melanoma development and progression have been uncovered. Wnt5a is a secreted glycoprotein that activates the non-canonical Wnt pathway and has been implicated in progression of several tumor types including melanoma. Wnt5a activates various downstream signaling pathways upon binding of Fzd, Ryk or ROR1/2 receptors. Antibodies targeting ROR1 have been proposed as a new therapeutical approach to inhibit Wnt signaling. The tyrosine kinase receptors ROR1 and ROR2 play a key role in embryonic development but their expression becomes undetectable in adulthood. However, the aberrant expression of these receptors has been linked to the progression of various types of cancer.

The aim of the present project is to determine the role of ROR1 and ROR2 on melanoma proliferation and the underlying molecular mechanisms. Overexpression of ROR1 was shown to increase proliferation by 44,7% ± 6,6% (p <0,01). This effect was consistent with a positive regulation of Akt, p65 and Cyclin D1. Western blot experiments determined an increase of 62,46% (p <0,01), 296,18% (p <0,01) and 23,87% (p <0,05) in Akt, P-p65 and Cyclin D1 levels, respectively.

On the other hand, ROR2 overexpression decreased proliferation by 22,38% ± 9,68% (p <0,01), which was accompanied by a negative regulation of Akt, STAT3 and Cdk4. Western blot experiments determined a decrease of 62,46% (p <0,01), 55,46% (p <0,01), 33,67% (p <0,01) in P-Akt, P-STAT3 and Cdk4 levels, respectively. In addition, we observed an increase of 149% (p <0,01) in p21 levels. In summary, these results suggest an opposite role for these receptors. While ROR1 would favor proliferation of melanoma, ROR2 would slow it down. This result has important implicancies for the development and application of therapies against the Wnt pathway in melanoma.

Keywords: Melanoma, Wnt5a, ROR1/2, proliferation.

BIOPHISICS 7

(1804) **OSCILLATORY CURRENTS GENERATED BY BUNDLES OF BRAIN MICROTUBULES**

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Microtubules (MTs) are unique components of the cytoskeleton formed by hollow cylindrical structures of ab tubulin dimeric units. A variety of other microtubular organizations are often observed, including two-dimensional sheets and bundles. The structural wall of the MT is interspersed by nanopores formed by the lateral arrangement of its subunits. Two-dimensional arrangements of tubulins display spontaneous oscillatory electrical behavior elicited by voltage and/or ionic gradients. Because of the potentially relevant implications of these electrical oscillations in cellular physiology, we explored this phenomenon in bundles of microtubules, heretofore known as macrotubules. Voltage-clamped MT macrotubules were studied by the patch clamp technique. For this study macrotubule bundles obtained from rat brain were utilized. Despite the fact that these structures did not render high resistance seals, as recently reported for two-dimensional MT sheets (Cantero et al, Sci Rep, 2016), we detected and preliminarily characterized highly regular electrical oscillations in this preparation. Thus, we obtained direct evidence for the presence of cation-selective oscillatory electrical currents. The oscillations progressed through various periodic regimes, being prominent a fundamental frequency at 39 Hz. In physiological K^+ (140 mM), the electrical oscillations represented, in average a 625 ± 21 % change in conductance (p<0.001, n=4). The preliminary findings support the contention that electrical oscillations are an intrinsic behavior of these components of the brain cytoskeleton, which may be important in the control of higher cognitive functions. **Keywords**: microtubules, electrical oscillations, tubulins

(748) **FLUORESCENCE MICROSCOPY STUDY OF THE LATERAL STRUCTURE OF GIANT VESICLES COMPOSED OF PSEUDO- BINARY MIXTURES OF SULFATIDE, ASIALO-GM1 AND GM1 WITH POPC**

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We comparatively studied the lateral structure of giant unilamellar vesicles (GUVs) composed of three pseudo binary mixtures of different glycosphingolipid (GSL), i.e. sulfatide, asialo-GM1 or GM1, with POPC. These sphingolipids possess similar hydrophobic residues but differ in the size and the net charge of their polar head group. Fluorescence microscopy experiments using LAURDAN and DiIC18 show coexistence of micron sized domains in a molar fraction range that depends on the chemical nature of the GSLs present in the mixture. In all cases, experiments with LAURDAN show that the membrane lateral structure observed in the three different mixtures resembles the coexistence of solid ordered and liquid disordered phases. Notably, the overall extent of hydration measured by LAURDAN between the solid ordered and liquid disordered membrane regions show marked similarities and are independent of the size of the GSL polar head group. In addition, the maximum amount of GSL incorporated in the POPC bilayer exhibits a strong dependence on the size of the GSL polar head group following the order sulfatide>asialo-GM1>GM1. This observation is in full harmony with previous experiments and theoretical predictions for mixtures of these GSL with glycerophospholipids. Finally, compared with previous results reported in GUVs composed of mixtures of POPC with the sphingolipids cerebroside and ceramide, we observed distinctive curvature effects at particular molar fraction regimes in the different mixtures. This suggests a pronounced effect of these GSL on the spontaneous curvature of the bilayer. This observation may be relevant in a biological context, particularly in connection with the highly curved structures found in neural cells.

Keywords: Glycosphingolipids; giant unilamellar vesicles; LAURDAN GP; membrane domains; membrane hydration.

(1307) **QUANTITATIVE IMAGING OF OCT4 AND HP1 IN EMBRYONIC STEM CELLS**

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The dynamical interactions of transcription factors (TFs) and DNA targets play a fundamental role in different stages involved in gene expression. Knowing the transcription machinery distributes among different sub-nuclear domains, we seek to understand how the architecture of the nucleus modulates the transcriptional response.

Embryonic stem cells (ESC) have the possibility of self-renewal and are pluripotent, i.e. they can give rise to all adult cell types. These properties make ESC studies relevant for understanding embryo development. Pluripotency depends on specific TFs such as Oct4, Sox2 and Nanog, which induce genes necessary to preserve an undifferentiated state and repress genes related to differentiation.

In this work, we used fluorescence correlation spectroscopy (FCS) analyses to quantitatively explore the dynamical organization of TFs in the nucleus of ESC. We transfected the cells with vectors encoding TFs or chromatin-associated proteins fused to enhanced green fluorescent protein (GFP), and measured fluorescence fluctuations as a function of time using confocal microscopy. We focused our studies on the dynamics of Oct4 in undifferentiated ESC and analyzed the FCS data with a model that considers fast and slow interactions with DNA targets. We verified that Oct4 partitions between fast and slow sites in undifferentiated ESC and that this equilibrium changes when ESC are subjected to a differentiation protocol. Moreover, the knock-out of a chromatin remodeler (KAT6B -/-) also modulates the dynamical distribution of the transcription factor and of the heterocromatin-associated protein HP1, showing differences respect to the wild type ESC line.

These results show the fine modulation of TF-DNA interactions in the cell nucleus and thus these studies may provide important clues for understanding the transcriptional response, especially at a not so well studied system such as ESC.

Keywords: Embryonic stem cells, transcription factors, chromatin, fluorescence correlation spectroscopy.

(1248) **REAL-TIME TOPOLOGICAL CHANGES AND SIMULTANEOUS ELECTRICAL RECORDINGS OF POLYCYSTIN-2 (TRPP2) WITH A COMBINED LIPID BILAYER-ATOMIC FORCE MICROSCOPY PLATFORM.**

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Ion channels are transmembrane proteins that mediate ion transport across biological membranes. Ion channels are traditionally characterized by electrical parameters acquired with techniques such as patch-clamping and reconstitution in lipid bilayer membranes (BLM). High resolution structural information of ion channels requires independent technologies, of which atomic force microscopy (AFM) is the only one that provides topological information on functional channel proteins in their native environments. To date, however, no direct correlations exist between electrical features and identifiable structural parameters from functional single channel complexes. The present study reports on preliminary data of polycystin-2 (TRPP2, PC2) channel function and topological features from single channel complexes studied in a platform containing a BLM reconstitution microchamber and an AFM imaging unit. To correlate single channel currents with the topological features of identifiable channel complexes, we simultaneously collected AFM scans and BLM electrical recordings in identifiable single channel complexes. Simultaneous single channel currents and AFM images were matched for the same collection times. Matched data showed an inverse linear correlation between the relative height of the channel complex, and the single channel currents, with a slope of -7.63 ± 1.2 pA/nm, n = 5, (r = 0.9862). The observations were confirmed with a second approach where the PC2 single channel currents collected in the BLM system were directly fed into an I/O analog port of the AFM controller. Again we observed an inverse linear correlation between the two features with a slope of -8.07 ± 0.35 pA/nm, n = 3 (r = 0.9642). The present study provides direct structural-functional correlations from single PC2 channel complexes disclosing topological changes between the open and closed states of the functional channel.

Keywords: Ion channels, atomic force microscopy, polycystin-2

(158) **EARLY ACTIVATION OF FAS RECEPTOR IS LIMITED AND LOCALIZED TO THE CYTOTOXIC SYNAPSE**

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The cytotoxic synapse formed between cytotoxic T lymphocytes or Natural Killer cells expressing Fas ligand and target cells with Fas receptor on their surface is a key pathway for apoptosis induction by the immune system. Despite similarities with the immune synapse in antigen presenting cells, little is known about the role of the spatiotemporal organization of ligand/receptor interactions for downstream Fas signaling leading to cell death. Here we have developed an artificial cytotoxic synapse to examine how mobility and geometry of Fas ligand affect receptor activation. By measuring the distribution, diffusion coefficient and fraction of immobile Fas receptor in living cells, we show that at short times, the activation of Fas receptor occurs locally and is limited to the contact region of the cytotoxic synapse. This anisotropic activation of apoptotic signaling supports a role for confined interactions on the efficiency of signal transduction that may have implications for biomedical applications of extrinsic apoptosis induction.

Keywords: Fas/CD95/Apo1, apoptosis, cytotoxic synapse, microcontact printing, fluorescence correlation spectroscopy

(1783) **ANALYSIS OF MOLECULAR TRAJECTORIES OBTAINED BY STORM SUPERRESOLUTION IMAGING OF THE ACETYLCHOLINE RECEPTOR**

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Various biophysical mechanisms are involved in the very broad deviations from Brownian motion observed in cell membrane proteins. From molecular crowding and motor-driven diffusion to interactions with the lipid microenvironment and cytoskeletal proteins, these anomalous diffusive processes play distinctive roles in cell physiology. We are currently using single-particle tracking (SPT) methods to study the mobility of a neurotransmitter receptor, the nicotinic acetylcholine receptor. This protein is robustly expressed at the surface of CHO-K1/A5, a mammalian clonal cell line developed in our laboratory. Receptors tagged with fluorescent (Alexa-Fluor^{532})-labeled α-bungarotoxin were imaged with STORM, a form of single-molecule localization microscopy suitable to study the dynamics of membrane proteins in living cells. We applied a wide palette of analytical and statistical tools to characterize the underlying single-molecule trajectories' physical properties. This led to the identification of various motional modalities associated with the experimentally determined translational diffusional parameters. We also employed tools to determine whether the trajectories were ergodic or not. The emerging picture is that the α-bungarotoxin-labeled acetylcholine receptor protein displays a highly heterogeneous complex of motional regimes, with predominance of anomalous subdiffusive components of ergodic nature.

(758) **MECHANICAL COMMUNICATION BETWEEN MICROTUBULE MOTORS**

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Molecular motors transport a wide variety of cellular cargoes positioning them in the cytoplasm with high spatial–temporal precision. In particular, microtubule-dependent-motors, dynein and kinesin, drive organelles bi-directionally; dynein transports cargoes toward the minus end of the microtubule whereas kinesin moves in the opposed direction. Therefore, these opposed-polarity motors compete with each other to determine the final direction of motion. In this work, we explore the interplay of the opposed polarity motors kinesin-1 and cytoplasmic dynein during peroxisome transport along microtubules in *Drosophila* S2 cells. Using single particle tracking, we registered fluorescent peroxisomes trajectories with nanometer accuracy and millisecond time resolution to extract quantitative information on the bidirectional motion of organelles. We computed the distributions of run length and speeds. These last data were statistically analyzed with a Gaussian functions mixture model following the Akaike information criterion. Transport performance was studied in cells expressing a slow chimeric plus-end directed motor or the kinesin heavy chain. We also analyzed the influence of peroxisomes membrane fluidity in methyl-β-ciclodextrin treated cells. The experimental data was confronted with numerical simulations of two well-established tug of war scenarios. Our results support the existence of a cross-talk between opposed-polarity motor teams. Moreover, the slowest teams seem to mechanically communicate with each other through the membrane to trigger transport.

Keywords: single particle tracking, molecular motors, intracellular transport

(340) **REGULATION OF PHOSPHOLIPID TRANSLOCATION IN THE PLASMA MEMBRANE BY TUBULIN**

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The asymmetrical distribution of phospholipids in biological membranes plays major physiological roles at the cellular level. This asymmetry is generated and kept by transporters. Among them, the lipid flippases belongs to the family of P4-ATPases. Some P-ATPases, such as NKA and PMCA, are regulated by tubulin. In erythrocytes from hypertensive and diabetic subjects, tubulin is increased in the plasma membrane (PM) and as a consequence, the NKA and PMCA activity results partially inhibited. The objective of this work was to evaluate the putative inhibition of flippases by tubulin, both *in vitro* and *in vivo*. Here, we used Inside-Out vesicles (IOVs) from erythrocyte ghosts and the NBD labeled phosphathydilserine analog to test our hypothesis. When IOVs were incubated with purified tubulin, the NBD-PS translocation rate was decreased by 40% when compared with controls without tubulin, suggesting that tubulin inhibit the flippase activity *in vitro*. For the *in vivo* assay, erythrocytes were loaded with NBD-PS and the rate of NBD-PS translocation was assayed by flow cytometry. The results showed that the NBD-PS translocation rate is 20% slower in hypertensive and diabetic erythrocytes when compared with the control (erythrocytes from normal subjects). In addition, when tubulin levels are pharmacologically modified in the PM of the erythropoietic K562 cells or erythrocytes, the percentage of NBD-PS translocated into the cell decreases in taxol-treated and in starved cells (conditions that promotes the association of tubulin to the PM) and the opposite effect was observed in cells treated with nocodazol (a condition that removes tubulin from the PM). These results are consistent with those obtained in *in vitro* experiments. Finally, we are currently performing pull down experiments in order to test the putative interaction between ATP11C, the main flippase in erythrocytes and tubulin. These experiments will bring some light to understand this inhibition mechanism.

Keywords: Phosphatidylserine, tubulin, flippases, plasma membrane

(846) **CYTOSKELETON STRUCTURE AND ROLE IN STEM CELLS**

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The cytoskeleton is a complex network of interlinking polymeric filaments, which is fundamental in cellular mechanics. It has been suggested that active cytoskeleton forces could regulate chromatin organization and dynamics, and indirectly gene expression. We propose to study how these networks can respond and transmit mechanical signals to the cell nuclei during cellular differentiation and early embryo development. As a first step, we studied cytoskeleton organization in mouse embryonic stem cells (mESC).

W4 mESCs were transfected with GFP-tagged actin, α-tubulin or vimentin and H2B-mCherry to label the chromatin. We obtained 3D confocal images to get insight into the organization of the different networks and combined fluorescence correlation spectroscopy (FCS) and fluorescence recovery after photobleaching (FRAP) experiments to study their dynamics. In contrast to the typical star-like ordered networks in differentiated, adherent cells, in mESCs the cytoskeleton filaments do not seem to form a well-defined network, instead it is much more relaxed, affecting the viscoelastic properties of the cytoplasm (1). Complementary, FCS and FRAP experiments showed that the dynamics of GFP-tagged tubulin and vimentin follows an anomalous diffusion model in μs - ms time scale.

These results show that cytoskeleton has different structures in stem and differentiated cells, and open the possibility to study its evolution during cell differentiation process.

Keywords: stem cells, cytoskeleton organization, cellular mechanics.

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(1044) **MECHANICAL CHARACTERIZATION OF INTERMEDIATE FILAMENTS NETWORK IN BHK CELLS**

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Together with microtubules and actin filaments, intermediate filaments (IFs) are one of the main components of the eukaryotic cytoskeleton. IFs are crosslinked with the stiffer microtubules and actin networks, and contribute to the viscoelasticity of the cytoplasm and cell mechanical integrity. IFs have been studied in in-vitro assays, however, we still do not know key aspects of the organization of these filaments in the intracellular environment. IFs are composed of several members of a large family of cytoskeletal proteins, including nuclear lamins, which contributes to the structural integrity of the nucleus. In order to explore the mechanical properties of IFs in living cells, we transfected BHK cells with a vimentin-GFP containing plasmid and obtained images of the IFs network using confocal microscopy. From these images, we recovered the coordinates of individual fluorescent filaments with sub-pixel precision using an algorithm developed in our lab. By performing a Fourier analysis of the IFs shapes and a curvature analysis, we determined the persistence length of these filaments (a measure of the distance over which a filament appears approximately straight) and found a value of ~1.5 um which is in the order of the values determined in in-vitro conditions. We also analyzed the effect of microtubules depolymerization on the stiffness of IFs. This work contributes to the comprehension of the mechanical behavior of the cytoskeletal filaments to get a better insight into cell mechanics and organization.

Keywords: Cytoskeleton mechanics, vimentin, persistence length.

STRUCTURAL AND FUNCTIONAL BIOCHEMISTRY 7

(1771) EXPRESSION ANALYSIS OF *ubaI* and *ublA* GENES IN *Streptococus uberis* STRAINS

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Bovine mastitis is a disease that causes great economic losses. *Streptococcus uberis* is the main environmental pathogen. Different procedures are utilized to prevent and treat the disease. One of the biggest challenges of the dairy industry is to reduce the use of antibiotics, and bacteriocins offer an alternative as potential antibacterial agents for the treatment of mastitis. It is known that *S. uberis* produces different types of bacteriocins. Previously, we determined the presence of bacteriocin associated genes in 59 *S. uberis* isolates by PCR assays. *ubaI* (ubericin A putative immunity protein) and *ublA* (uberolysin precursor) genes were the most common bacteriocin associated genes in the examined isolates. The aim of the present work was to investigate the expression level of *ubaI* and *ublA* genes in an *S. uberis* strain at different times. qRT-PCR was normalized to *gapdh* gene. Changes in transcriptional expression between 4, 6 and 10 hours were calculated by the comparative threshold cycle 2-ΔΔCT method and plotted as the fold change in mRNA expression levels. The results indicated a significant increase in *ubaI* and *ublA* genes at 6 and 10 hours, respectively. The results suggest that the putative immunity protein and the uberolysin precursor are produced between 6 and 10 hs, during the stationary phase. The study contributes to a better understanding of the gene expression of bacteriocin genes in *S. uberis* strains.

Keywords: Bovine mastitis, bacteriocin associated genes, expression level

(1834) POTASSIUM MODULATES THE METABOLIC REGULATION OF PHYTOPLASMA MALIC ENZYME

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Phytoplasmas ("*Candidatus* Phytoplasma") are insect-vectored plant-pathogens in which malic enzyme (AYWB-ME) fulfils a critical role for ATP generation. Previous results have shown that AYWB-ME is selectively regulated by several different metabolites, which agrees with its metabolic proposed role. In this work, we further characterize the kinetic behavior of AYWB-ME in the presence of potassium as modulator of this metabolic regulation. In the presence of potassium AYWB-ME resulted highly activated, reaching around two- and fifteen-times of activation in the presence of 0.2 and 5 mM respectively. When 2 mM ADP was added in the presence of 5 mM potassium, an additional 10% AYWB-ME activation was observed. Besides, 2 mM addition of ATP, in the presence of 5 mM KCl, resulted in a negative effect over AYWB-ME activity reaching around 25% inhibition. On the other hand, the addition of 0.2 and 2 mM glutamine increases AYWB-ME activity around 40% and 98% respectively. In this case, potassium superimposes the activation of glutamine, with no additive effect between these two modulators. This is the first study that shows a modulation effect produced by potassium over metabolic effectors of ME activity which could be relevant *in vivo*, considering the physiological roles that potassium levels fulfil in bacteria, e.g. modulating ion transport. Furthermore, phylogenetic analysis of several different MEs suggests that AYWB-ME is a member of a ME group, which is considered as the smallest scaffold known from which other MEs may have evolved. This work suggests that this group of MEs may display a fine regulation despite presenting a small structure, with a synergistic modulation by potassium and metabolites.

Keywords: Phytoplasma, malic enzyme, potassium regulation

(1321) THE THERMOSENSITIVE PHENOTYPE OF AN *Escherichia coli sbmA tolC* DOUBLE MUTANT RESULTS FROM A HIGHER SENSITIVITY TO EXOGEN HYDROGEN PEROXIDE

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Microorganisms live in environments where they are sometimes exposed to different types of stress (high temperatures, oxidative damage, etc) which can be lethal. Therefore bacteria have evolved diverse mechanisms to overcome these adverse conditions. We have previously reported that an *Escherichia coli sbmA tolC* double mutant is unable to form colonies at 42°C in LB plates. The molecular bases of this phenotype remain unknown. In the present study we demonstrate that the growth defect may be corrected by the addition of either iron, enterobactin or its degradation products to the medium, suggesting that iron starvation is the cause of the phenotype. To test this possibility, we used a reporter plasmid carrying a transcriptional fusion between *lacZ* and the *ryhB* promoter, which is induced under iron deficiency. Results showed that the double mutant was not under iron starvation and it was capable of incorporating iron. We also found that the addition of exogenous catalase to the medium suppressed the thermosensitive phenotype. There is evidence indicating that hydrogen peroxide is generated abiotically in LB medium via autoxidation. Moreover, relieving of the phenotype by iron could be due to direct scavenging of hydrogen peroxide by the metal. Taken together, our results indicate that the thermosensitive phenotype of the double mutant *sbmA tolC* may result from an increased sensitivity to hydrogen peroxide when the cells grow at low densities.

Keywords*:* *Escherichia coli*, *sbmA tolC* double mutant, thermosensitivity, oxidative stress, hydrogen peroxide

(744) NATIVE BACTERIUM WITH BIOCONTROL ACTIVITY AGAINST *Xanthomonas* spp

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Plant diseases caused by phytopathogenic species of *Xanthomonas* are currently controlled using copper compounds and resistant host varieties. However, the use of chemical products has failed to control plant diseases due to the appearance of resistant bacterial strains. Moreover, these compounds pollute the environment and can affect human and animal health. For these reasons, it is important to find alternative strategies to manage diseases caused by *Xanthomonas*. Nowadays, the use of beneficial microorganism for phytopathogens control is becoming common. Our aim is to find novel native bacteria with antagonistic activity against different species of *Xanthomonas*: *X. citri* subsp *citri*, *X. campestris* pv. *campestris*, *X. vesicatoria*, *X. albilineans* and *X. sacchari*. Until now, we isolated from tomato rhizosphere a bacterium (named TVM05) that antagonizes all the different strains of *Xanthomonas* considered in this study. TVM05 is a Gram-positive spore-forming bacillus. The antagonistic activity of TVM05 against *Xanthomonas* spp. was analyzed plating the phytopathogenic bacteria on tryptic soy agar and 30 µl of TVM05 free-cell supernatant (FCS) into 5 mm-wells. After incubation for 48 h at 28 ºC, inhibition growth area was analyzed using Image J32 program. Optimal activity of FCS was detected at early stationary phase. Besides, results showed that FCS was stable after heat treatment at 50 and 100 °C during 15 minutes ($p<0.05$) and conserved its activity when storage at 4 and -20 ºC for a month ($p<0.05$). These findings suggest that TVM05 is a good candidate for biological control of *Xanthomonas* spp. Our next step is to purify the compound(s) responsible(s) for the antagonistic activity against

Xanthomonas spp. and to carry out *in plant* assays to analyzed the effect of the purified antagonistic compound(s) on *Xanthomonas* spp. virulence.

Keywords: *Xanthomonas*, biological control, beneficial microorganism, native bacterium

(746) **EFFECT OF GRAPEFRUIT LEAVES EXTRACT IN XANTHAN STRUCTURE AND COMPOSITION**

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Xanthomonas citri subsp. *citri* (*Xcc*) is the causal agent of citrus canker, a disease that affects different species of citrus. *Xcc*, as well as other *Xanthomonas* spp., produces xanthan, a polyanionic exopolysaccharide (EPS), composed by a cellulose backbone with a side chain containing a glucuronic acid residue linked to a mannose that connects to the backbone and a second terminal mannose. Mannose residues can be substituted by nonglycosidic groups (acetyl or pyruvate). Xanthan is an important virulence factor that affects bacterial growth *in planta* and biofilm formation. We previously demonstrated an association between xanthan structure/composition and its role as virulence factor. Considering this background, we aimed to analyze whether xanthan structure/composition changed in presence of citrus leaf extract. For this work, we cultured *Xcc* in grapefruit leaves aqueous extract (GLE). First, we observed that *Xcc* grew better in GLE than in peptone-yeast extract-malt extract medium (PYM). Xanthan synthetized by Xcc growing in GLE (Xan-GLE) showed different macroscopic qualities compared to xanthan produced by *Xcc* growing in PYM (Xan-PYM). Using scanning electronic microscopy, we observed that Xan-GLE presented a more heterogenous and disordered structure than Xan-PYM. The atomic force microscopy analysis revealed Xan-PYM molecules as long strands or double strands, while Xan-GLE molecules appeared to be shorter than the Xan-PYM ones and with a different arrangement (looking like spiders). On the other hand, analytical determinations reveled that Xan-GLE has higher content of pyruvate groups than Xan-PYM. These results could explain the behavior of the xanthan molecules in presence of GLE. A greater number of pyruvate groups causes an increase in intramolecular electrostatic repulsions between negative charges of xanthan molecules, affecting the ability of this EPS to hydrate and to interact with other molecules in the media.

Keywords: *Xanthomonas*, xanthan, citrus

(864) **GLUCAN BIOSYNTHESIS IN *Xanthomonas citri* subsp. *citri* REQUIRES OF THE GLUCOSYLTRANSFERASES HrpM AND NdvB AS CONCERTED COMPLEX AND GalU, AN UTP-GLUCOSE-1-PHOSPHATE URIDYLTRANSFERASE**

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*Xanthomonas citr*i subsp. *Citri* (*Xcc*) is the causal agent of citrus canker disease in citrus plant. One of the virulence factor produces by the bacteria is a β-1, 2 cyclic glucans. In previous work of our laboratory, two *Xcc* mutants impaired in glucan biosynthesis were obtained one of them by marker exchange mutagenesis (*ndvB*) and the other (*hrpM*) was identified in an *Xcc* EZ-Tn5 <R6Kγori/KAN-2> Tnp transposon library. Also, from the library, a tn*5* insertion in *galU*, a gene encoding an enzyme that is supposed to be involved in the formation of uridine diphosphate (UDP)-glucose from UTP and glucose-1-phosphate, was identified. Previous study showed that *galU* is required for biosynthesis of extracellular polysaccharides xanthan gum. Our aim is to study the synthesis of cyclic glucans as a possible target for the control of citrus canker. We show here, for the first time, that *galU* mutant is also affected in the cyclic β-1,2 glucan *in vivo*. Instead, *in vitro*, using total membrane preparation from *Xcc* and incubated with UDP-[^{14}C]Glucose and in the presence of Cl_2Mg, the *galU* mutant was able to synthesis β-1,2 glucan, demonstrating that GalU is a UTP-glucose-1-phosphate uridylyltransferase in which product is required for β-1,2 glucan synthesis as a precursor. On the other hand, while the membrane preparations from the *ndvB* and *hrpM Xcc* mutants were unable of β-1,2 glucan production both *in vivo* and *in vitro*, a mixture of membranes of both mutants restored the glucan synthesis if they were previously sonicated and preincubated in buffer Tris-HCl pH8 in the presence of Cl_2Mg and then and UDP (^{14}C)-Glc, suggesting an interaction between NdvB and HrpM could be essential in the process. An *in silico* model, by homology, shows a putative complex which includes HrpM as an integral inner membrane protein and NdvB as a peripheral inner membrane protein facing the periplasmic space. These results present new insights in the biosynthesis mechanism of β-1, 2 cyclic glucan in *Xcc*.

Keyword: β-1,2 glucan, Xanthomonas citri, canker disease, citrus plant.

(1048) **HPLC SEPARATION AND ANALYSIS METHOD TO EVALUATE MOLECULES INVOLVED IN THE ARGININE – ORNITHINE CYCLE IN *Phytomonas***

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Phytomonas spp. are trypanosomatid parasites that infect a variety of plants and can cause diseases that affect regional economies, mainly in South America. Little is known about *Phytomonas* biology; however, recently published genomic data provide new venues for research. We are interested in the study of arginine – ornithine cycle with special focus on the enzymes involved in the conversion of arginine-citrulline-ornithine.

To confirm our previous evidences about the presence of NOS and ODC and the absence of ADC and AGMATINASE enzymes in this cycle, we set up a method for separation and analysis of products and intermediates using high-performance liquid chromatography (HPLC) that involves precolumn derivatization with o-phthaldialdehyde, C18 analytical column and fluorescence detection. We tested two mobile phase solutions (MP): 1- MPA: 0.1M sodium acetate, pH7.2/MPB: 100% methanol and 2- MPA: 25mM phosphate buffer pH7.5/MPB: acetonitrile:methanol:water 45:45:10, both at three different temperatures (15, 25 and 40ºC). Under these different conditions we separated a mixture of standard aminoacids: arginine, ornithine, citrulline, agmatine, lysine, glycine and threonine. We found that mobile phase 2 at 40ºC was the best condition to obtain individual peaks for each aminoacid with a detection limit of at least 1uM. As a first *in vivo* approach, *Phytomonas Jma* was cultured in a semi-defined media (SDM-79) and extracts obtained from 2 and $10x10^6$ cells were deproteinized and neutralized. The HPLC chromatogram, under the selected conditions, showed the seven aminoacid peaks well resolved and a low background signal. Further analysis will be made to analyze the aminoacid profile under different culture conditions.

In conclusion, we have set up a HPLC protocol that will be useful to analyze *Phytomonas* metabolism. This method could also be used to analyze aminoacids in a variety of biological samples such as physiological fluids, tissue extracts and different cell types.

Keywords: *Phytomonas*, ARGININE-ORNITHINE CYCLE, HPLC

(246) **TCHTE MODULATES HEME TRANSPORT IN *TRYPANOSOMA CRUZI***

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Trypanosoma cruzi, the parasite responsible of Chagas disease, presents auxotrophy for heme. It contains several heme-proteins involved in essential metabolic pathway such us mitochondrial respiratory complexes, unsaturated fatty acid and ergosterol biosynthesis. It must take heme from the different hosts to supply this requirement, being heme transport and distribution essential for the parasite survival. We are interested in elucidating how heme is acquired and used by *T. cruzi* and to accomplish this, different strategies are utilized.

T. cruzi presents a dedicated system to transport heme that can discriminate between structurally related compounds, as it was observed by confocal microscopy and fluorescence measurements using fluorescent heme analogs (HAs). Also, we have shown that heme (HAs) was selectively taken by the parasite replicative stages where the protein we named TcHTE (*T. cruzi* Heme Transport Enhancer protein) plays an essential role. TcHTE belongs to HRG family proteins described in *C. elegans* (Heme Response Genes) and also conserved in trypanosomatids. These proteins show four putative transmembrane domains and no homology to other reported transporters. Our results demonstrate that TcHTE protein level changes according to heme availability, being almost no detected when epimastigotes were maintained in a media with adequate heme source (hemin). Surprisingly, the recombinant TcHTE-GFP was clearly located in the flagellar pocket region of the parasite, where the metabolites transport seems to proceed in trypanosomatids, with its C-terminal domain facing the cytoplasm of the cell. The expression of the rTcHTE-GFP in HECK293 cells allowed the analysis of oligomers formation by TIRF microscopy, where our preliminary results showed that TcHTE could form trimers.

In summary, our results indicate that TcHTE plays an essential role modulating the heme transport activity in *T. cruzi*, presumably being part of a sophisticated complex transporter system.

Keywords: Heme, transport, *T. cruzi*

(682) **A MULTIDOMIAN GLUTATHIONE-S-TRANSFERASE OF *Trypanosoma cruzi*: BIOCHEMICAL AND CELLULAR CHARACTERIZATION**

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Abstract*:* Glutathione-S-transferase (GST), a member of the Trx family, has the ability to conjugate glutathione (GSH) to different molecules (such as pharmaceuticals) to make them easily excreted. According with this, it has been previously reported that GST in *Plasmodium falciparum* is important for antiparasitic neutralization. Therefore, the study of this enzyme could be interesting in anti-Chagas disease drugs design. This disease is caused by the protozoan parasite *Trypanosoma cruzi*. In this work, we present an isoform of GST identified in the genome of *T. cruzi*, which has a signal peptide, a glutaredoxin (Grx) domain and a prostaglandin E synthase domain. The recombinant enzyme was obtained and GST activity was measured by following the increase of absorbance at 340 nm, that result from the conjugation between GSH and the 1chloro2,4dinitrobenzene. The reaction was optimal at pH range from 7 to 9 and followed non Michaelis-Menten kinetics. However, no Grx function was detected for the enzyme. On the other hand, by a western blotting assay a higher cellular abundance of the protein was detected in vector life stages (metacyclic trypomastigote and epimastigote) than in the mammalian host. By cell fractionation with $CaCl_2$ and $NaCO_3$ (pH 11.0), it was demonstrated that the enzyme is a membrane protein and has no mitochondrial localization. The association to membrane of *Tc*GST could be promoted by a mechanism of palmitoylation. This mechanism is a reversible lipid modification in which one or more cysteine thiols on a protein are modified to form a thioester with a palmitoyl group. Through an *in silico* assay we identified that the protein has a high probability to be modified with a palmitoyl group. This induces us to continue studying this protein that apparently perform extensive functions in the cellular metabolism of *T. cruzi*.

Keywords*:* Glutathione S-transferase, *Trypanosoma cruzi, redox metabolism.*

(1015) **ROLE OF THE *Trypanosoma cruzi* PANTOTHENATE TRANSPORTER (*Tc*PPT1) IN SURVIVAL UNDER STRESS CONDITIONS**

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Chagas disease is an endemic parasitosis originally from Latin America, caused by the protozoan *Trypanosoma cruzi*. The current therapies are limited in efficacy and show multiple side effects based on their poor specificity. Thus, there is a need to identify new targets to develop novel tripanocidal strategies.

Vitamins are essential micronutrients for all living cells; in particular B vitamins are relevant in the biology of *T. cruzi*. Previously, we have shown that pantothenate, the precursor of Coenzyme A, has a key role in *T. cruzi* metabolism. *In silico* studies suggested that *T. cruzi* is auxotrophous for this vitamin, and we identified a putative pantothenate transporter (*Tc*PPT1). The aim of this work is to study *Tc*PPT1 role in *T. cruzi* proliferation under stress conditions.

To approach this aim, we constructed mutant Y-GFP strain epimastigotes that overexpress *Tc*PPT1 fused to a mCherry tag. Its presence and expression were assessed by PCR, RT-PCR and fluorescence microscopy.

To evaluate possible differential proliferative responses we performed cultures of wild type (mCherry) and transgenic (*Tc*PPT1-mCherry) epimastigotes under different stressing conditions: nutrient deprivation (20nM pantothenate), oxidative stress (H_2O_2 0-150uM) and tripanocidal drug (Nfx 0-100uM). Proliferation rate of *Tc*PPT1-mCherry epimastigotes was significantly higher than mCherry epimastigotes in low pantothenate conditions ($p<0.05$) (35.0±2.3 million/ml vs 21.7±2.9 at 5th day, respectively). Additionally, *Tc*PPT1-mCherry cultured 24 and 48 h in media with H_2O_2 and Nfx showed increased proliferation, evaluated by growth curves and MTT assay, compared with the control counterpart.

The findings presented herein suggest that *Tc*PPT1 is a relevant permease for *T. cruzi* epimastigotes growth especially under stressing conditions.

Keywords: Chagas, *Trypanosoma Cruzi*, Permeases, Pantothenate

ONCOLOGY-ONCOIMMUNOLOGY 15

(1929) **INTEGRIN-MECHANOSIGNALING ROLE IN SMALL GTPASES ACTIVATION AND CANCER**

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The ability of cells to adhere and simultaneously probe their mechanical environment is central to many developmental, homeostatic but also pathological processes. Yet, the molecular mechanisms that govern mechanotransduction during cell adhesion and invasion are complex and remain incompletely understood. We determined the importance of studying integrins in genetically modified cell models that only express either family of fibronectin (FN)-binding integrins. Using biochemical assays in combination with mass spectroscopy, traction force microscopy and micropatterns, we observed that α5β1-integrins (pKO-β1) promote the formation of small nascent adhesions with high turnover, low RhoA activation and high force, while αVβ3 (pKO-αV) promotes adhesion maturation leading to large focal adhesions connected to contractile stress fibers resulting in high RhoA and low force, while cells expressing both integrins contain small and large adhesion structures and intermediated GTPases activites. Therefore, we observed that the levels of integrins

expression and subtype in different cells lines affect G-actin polymerization, MRTF-A/SRF activation and the ubiquitin-like modifier interferon-stimulated gene 15 (ISG15) expression that promotes cell migration and invasion. Interestingly, the malignant breast cancer cell line MDA-MB-231 expressing high levels of β1 integrins and the β1-class expressing pKO-β1 cells showed high ISG15 expression and high amounts of ISGylated proteins, which we could show are directly responsible for cell invasion. In contrast, the non-invasive tumor cell lines MDA-MB-468 or MCF-7 and the αV-class integrin expressing pKO-αV cells, which display low levels of β1 integrins, ISG15 and ISGylated proteins failed to invade a 3D matrix. The future findings have important implications for our understanding of cancer progression and will help identifying new targets for future therapies.

Keywords: Breast Cancer, Integrins, Rho-GTPases, mechanosignaling

(1138) **ANTI-TUMORAL PROPERTIES OF CHLOROGENIC ACID: MAIN BIOACTIVE COMPONENT OF YERBA MATE**

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Abstract: Yerba Mate (*Ilex paraguariensis*) is a native South American tree but Yerba Mate tea is growing in popularity around the world by biological properties. This plant contains several active phytochemicals which are responsible for its health benefits. One of the most abundant polyphenol compounds in Yerba Mate is chlorogenic acid. It has been reported many biological properties for this polyphenol, including antioxidant, anti-inflammatory, antiviral, and anticancer activities, and may be responsible for the reduced risk of some chronic disease. We previously showed the anti-tumoral properties of Yerba Mate extract in several experimental systems. The aim of this study was to explore whether chlorogenic acid is the molecule responsible of biological effects observed in Yerba Mate extract. Total polyphenol concentration was measured using Folin-Ciocalteau method, with a mean of 0.03 gallic acid equivalents/mg Yerba Mate. Several classes of chemical constituents as caffeoyl derivatives (chlorogenic acid and caffeic acid) and flavonoids (rutin, kempherol and quercetin) were quantified by HPLC. *In vitro* assays were performed using tumor cell lines from different localization (CT26, MDA-MB-231, H125, Colo205, SN12C, PC3). Both, Yerba Mate extract and chlorogenic acid inhibited cell proliferation in a dose-dependent manner. However, Yerba Mate extract was the most potent inhibitor of cell proliferation at a concentration lower than the concentrations used of chlorogenic acid (ranged IC_{50}= 0.027-0.1 mM for YM and 0.312-0.75 mM for chlorogenic acid). On the other hand, we demonstrated that both Yerba Mate extract and chlorogenic acid modulate cell adhesion, migration and the invasive capacity of tumor cells. Our results suggest that the *in vitro* biological effects of Yerba Mate extract are not only due to chlorogenic acid but also could be additive and synergistic effects among the different bioactive components present in Yerba Mate.

Keywords: chlorogenic acid, Yerba Mate, tumor cell, phytochemical, antioxidant

(650) **COMPARATIVE PROTEOMICS OF SOLUBLE FACTORS SECRETED BY HUMAN BREAST ADIPOSE TISSUE FROM TUMOR AND NORMAL BREASTS**

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Epithelial-stromal cell interaction is a crucial factor in cancer progression. Adipose tissue is the main stromal component of breasts. We have previously demonstrated that conditioned media (CMs) from human adipose tissue explants of tumor breasts (hATT) differentially regulate proliferation, adhesion and migration of breast cancer epithelial cells compared with CMs from human adipose tissue explants of normal breasts (hATN). Now, we intend to identify the proteins present in those CMs. For this, we separated in polyacrylamide gels proteins in CMs –hATT (n=6) and –hATN (n=3). Aliquots from these CMs were analyzed by means of 2D nano-LC-MS/MS (mass spectrometry). The data was analyzed using ProteoIQ (Premier Biosoft) and FunRich softwares. In addition, CMs –hATT (n=6) and -hATN (n=5) were assayed using a 42 Cytokine Antibody Array. We found that CMs-hATT present more protein diversity than CMs-hATN. Moreover, CMs-hATT expressed greater amount of proteins involved in biological processes such as signal transduction and cell communication; energy metabolism; cell growth; and immune response. Specifically, levels of apolipoprotein AI and AII (lipid metabolic processes), C3 complement factor (immune system) and vimentin and desmin (mesenchymal cells glycoprotein related to an invasive breast cancer phenotype) were significantly increased in CMs-hATT vs. CMs-hATN (cut-off 5-fold change). Furthermore, a multivariate discriminant analysis of the cytokines detected by the array showed that IL-6, MCP-2 and GRO cytokines are sufficient and necessary to differentiate CMs-hATT from CMs-hATN. In addition, this analysis showed that the levels of these three cytokines taken together correlate with tumor stage of CMs-hATT and with BMI of CMs-hATN. These results allowed us to identify proteins potentially responsible for the observed effects, and let us proposed stromal IL-6, MCP-2 and GRO as potential markers of the stage of the disease.

Keywords: breast cancer, adipose tissue, epithelial-stromal interaction, proteomics analysis.

(1547) **DIFFERENTIAL ANTITUMORAL EFFECTS BETWEEN THE ANALOGUES OF CALCITRIOL SG1 AND EM1.**

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1α,25-dihydroxyvitamin D_3 (calcitriol) shows potent growth-inhibitory properties on different cancer cell lines although its hypercalcemic effects have severely hampered its therapeutic application. Therefore, it is important to develop synthetic analogues that retain or even increase the antitumoral effects without causing hypercalcemia. Based on the previous evidence of the potent antitumor effects of the synthetic alkynylphosphonate analogue EM1, we have now synthesized a novel analogue called SG1, which bears a vinylphosphonate in its side chain. The aim of the present work was to evaluate the calcemic activity in mice and the antitumor effect of SG1 on different cancer cell lines, comparing them with that exerted by calcitriol and by EM1. In addition, we performed computational modeling studies in order to analyze and compare the affinity of the compounds to the vitamin D receptor (VDR). By manual cell count we observed that SG1 exerted a slight decrease in the viability of the HCT116 (IC_{50}: 3,13 nM; $p< 0.05$) and LM3 (IC_{50}: 0,19 nM; $p< 0.001$) cell lines whereas it did not affect the viability of HN12, T47D, U251 and T98G cells. By wound healing assays, we observed reductions in the migration rates of the LM3 ($p< 0.001$) and T98G ($p< 0.05$) cell lines, whereas it did not affect the migration of the HCT116, U251, GL26, HN12, T47D. Calcemic assays performed in CF1 mice showed that, similarly to EM1, the new analogue SG1 did not cause

hypercalcemia (at 5 μg/kg) or toxic effects. Computational studies were performed using as reference the crystallographic structure of the calcitriol-VDR complex (PDB code: 1DB1) and conclude that SG1 binds with lower affinity to VDR than the other two compounds. In conclusion, these results suggest that the modifications in the lateral side chain of analogue SG1 (vinylphosphonate instead of alkynylphosphonate) affect VDR binding affinity and the antitumoral effects previously observed for EM1, while not changing the calcemic activity.

Keywords: Analogues, Antitumor agents, Calcitriol, Cancer, Cell lines.

(553) **DUAL APOTOTIC/NECROTIC RESPONSE INDUCED BY PHOTODYNAMIC TREATMENT WITH A Zn(II) CATIONIC PHTHALOCYANINE IN MELANOMA CELLS**

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IQUIFIB, UBA-CONICET

Melanoma is an aggressive form of skin carcinoma, highly resistant to traditional therapies. Photodynamic therapy (PDT) is an alternative form of treatment, which combines a photosensitizer, visible light and molecular oxygen to produce reactive oxygen species (ROS) that selectively destroy target cells.

In order to find an efficient photosensitizer to be used in melanoma treatment, we evaluated the effect of a sulfur-linked cationic zinc(II) phthalocyanine (Pc13) on a panel of melanoma cells (B16F0, B16F10, WM35, M1/15, A375). Incubation with Pc13 and irradiation, diminished cell viability in a concentration and light dose-dependent manner in all the cell lines studied, with IC_{50} values ranging from 0.20±0.03 μM to 3.60±0.23 μM for B16F0 and A375 cells, respectively. The most sensitive melanoma cells B16F0 were further employed for studying the mechanisms of cell death triggered by Pc13. Acridine orange/ethidium bromide dual staining showed morphological changes characteristic of both necrosis (42±4%) and apoptosis (21±2%) 3h post irradiation of cells treated with 0.2 μM Pc13. Under these experimental conditions, a significant decrease in the levels of Bcl-2, Bcl-xL and Bid, and a reduction of pro-caspase-3 were observed by Western Blot, evidencing an apoptotic response. Furthermore, a time dependent increase of hypodiploid cell population and cell cycle arrest in G0/G1 were assessed by propidium iodide staining and flow cytometry analysis. In addition, permeabilization of plasma membrane, as sign of necrosis, was evaluated by measuring the release of lactate dehydrogenase (LDH). A light dose and photosensitizer concentration dependent increase of LDH activity was detected in Pc13 treated-cells culture mediums.

Taken together, these results indicate that a dual apoptotic and necrotic response is triggered by Pc13 photoactivation in melanoma cells, suggesting that combined mechanisms of cell death could result in a promising alternative for melanoma treatment.

Keywords: photodynamic therapy, melanoma, phthalocyanine, antitumoral action

(1701) **IMIQUIMOD-INDUCED INHIBITION OF ANTIOXIDANT ENZYMES AND REACTIVE OXYGEN SPECIES ACCUMULATION IN MURINE HEMANGIOMA CELLS.**

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Infantile hemangiomas (IH) are the most common benign tumours of infancy, however intervention may be required when major complications are developed. Imiquimod (IQ), a TLR7/8 agonist, is a therapeutic alternative and previous *in vitro* studies of our laboratory have shown a cytotoxic selective effect of IQ towards hemangioma cells in terms of viability, migration and apoptosis triggering. The aim of this study was to investigate the ability of IQ to trigger reactive oxygen species (ROS) generation and its influence on antioxidant enzymes, prior to apoptosis of murine hemangioma cells. H5V cell line was treated with IQ (0, 5, 10 and 50 μg/mL) for 2, 4 or 12 hours and analyzed for ROS generation and mitochondrial stability by flow cytometry using fluorescent probes DCF-DA and MitoTracker Red CMXRos, respectively. Antioxidant enzymes catalase (CAT) and superoxide dismutase (SOD) activities were assessed through disappearance of H_2O_2 (μmol H_2O_2/min*mg protein) and inhibition of the epinephrine oxidation (USOD/mg protein) respectively. Early after treatment with IQ (2 hs) there was a steep drop (70±12%; $p<0.05$) in the specific activity of CAT, accompanied by a 40%-increase in ROS levels ($p<0.05$). When treating H5V cells for 4 hs, inhibition of the activity was about 40% for both CAT and SOD. ROS increased from 40 to 100% for 5-50 μg/mL ($p<0.05$) along with (50±15)% loss of mitochondrial membrane potential. After 12 hs treatment, there was a restoration of CAT activity at IQ concentrations ≤ 10 μg/mL and induction at 50 μg/mL. SOD showed a 25% increased activity and mitochondrial stability remained impaired (40±10)% for ≥ 5 μg/mL IQ. In conclusion, IQ treatment of H5V cells would induce ROS accumulation, mitochondrial dysregulation and inhibition of antioxidant enzymes. These early modifications of the oxidative status may contribute with previously reported IQ-induced apoptosis in H5V cells.

Keywords: imiquimod, hemangioma, ROS, antioxidant enzymes.

(1325) **N-TERMINAL PORTION OF C-FOS AS A NEW THERAPUETIC STRATEGY FOR THE TREATMENT OF GLIOBLASTOMA MULTIFORME TUMORS**

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CIQUIBIC-UNC

The survival time for patients with Glioblastoma multiforme has not improved significantly over the last ten years with an average survival period for these patients of ~1 year after diagnosis, thus representing the most aggressive and lethal type of tumors of the central nervous system (CNS). We found that in addition to its role as an AP1 transcription factor, c-Fos activates the rate of synthesis of phospholipids, key components for membrane biogenesis, at the endoplasmic reticulum. Furthermore, we determined that the regulation of this metabolism is implicated in tumor biology, sustaining the exacerbated growth characteristic of brain tumor cells. We also found c-Fos overexpressed in brain tumors co-localizing with components of the endoplasmic reticulum contrasting with the lack of detectable expression of c-Fos in normal CNS. These results point to c-Fos as a potential new target for glioblastoma treatment. Consequently, the aim of the present work was to test N-terminal deletion mutants of c-Fos as possible negative dominants of the lipid synthesis activation capacity of c-Fos. Using several *in vitro* approaches, (transfection, profection of recombinant proteins and generation of stable cell lines) we identified negative dominants whose overexpression inhibits proliferation of T98G cells, we evaluate the induction of cell death and we dissect the domains of N-terminal portion of c-Fos (NA) involved in the physical interaction of c–Fos with enzymes that it activates such as phosphatidylinositol 4 kinase IIα (PI4KIIα) using FRET microscopy. Moreover, the negative dominance of NA resulted effective in an *in vivo* model of CNS tumors using T98G xenografts on immunodeficient NOD-SCID mice. Taken together, our results point to specific domains of NA as possible new therapeutic strategies for the treatment of glioblastoma multiforme.

Keywords: Cancer- c-Fos- Glioblastoma - Phospholipids

(352) **PERSONALIZED TEST FOR HIGH SENSITIVE DETECTION OF RESIDUAL PRIMITIVE LEUKEMIC CELLS BY DIRECT NESTED-PCR OF *BCR-ABL1* REARRANGEMENT AT DNA LEVEL**

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Chronic myeloid leukemia (CML) patients who achieve a stable undetectable minimal residual disease (UMRD) by RT-qPCR are candidate for tyrosine kinase inhibitors (TKIs) suspension. In TKI-discontinuation trials, around 60% of patients loose UMRD and immediate TKI reinitiation is necessary. These results suggest that the risk of molecular relapse might be related to the achievement of deeper molecular response, below the detection limit of RT-qPCR. Molecular relapse has been attributed to the persistence of leukemic stem cells (LSCs). Thus, strategies that increase PCR sensitivity for LSCs detection may be relevant to better select patients with the highest likelihoods of maintaining treatment-free remission (TFR). Breakpoints that lead to *BCR-ABL1* chimeric gene are highly dispersed over a ~150kbp region. Therefore, high quality DNA from newly diagnosed CML patients was necessary and amplified by a multiplex long-range PCR approach. The amplicons were sequenced using the Ion Torrent PGM platform. After alignment, the fusion point sequences were localized using CREST, and used for personalized PCR primers design (Primer3Plus). For LSCs isolation, 6-weeks-long term culture initiating cell (LTC-IC) assays were performed using $CD34^+$ cells from fresh samples. Individual colonies plucked from methylcellulose were directly used for nested-PCR (dnPCR) without DNA extraction. We characterized the specific fusion breakpoint at DNA level in 4 out of 8 CML patients. For one of them, attaining $MR^{3.0}$ at 18 months of Imatinib treatment, $CD34^+$ cells were isolated for a LTC-IC assay; the proportion of residual LSCs (5 out of 20; 25%) was determined by personalized dnPCR. This study addressed two problems to improve deep monitoring of CML patients: determining the BCR-ABL1 breakpoint sequence and the detection of residual LSCs. Due to its complexity, the initial clinical role of DNA-PCR will be confined to monitor patients once they achieve RT-qPCR negativity and desire to enter TFR.

Keywords: Chronic myeloid leukemia, next-generation sequencing, leukemic stem cells.

(636) **PRECLINICAL EVALUATION OF THE NOVEL VASOPRESSIN PEPTIDIC ANALOG [V⁴Q⁵]dDAVP WITH ANTITUMOR ACTIVITY IN COLORECTAL CANCER**

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Abstract: Colorectal cancer (CRC) represents a major public health concern. In CRC patients, liver and lung metastases are the main cause of cancer-related mortality. Additionally, despite novel combinations of therapeutic strategies, efficacy is rather limited due to drug induced toxicity and resistance.

[V⁴Q⁵]dDAVP is a novel vasopressin analog with reported antimetastatic activity in breast and lung cancer, which acts as a specific vasopressin type-2 receptor (V2r) agonist in tumor and microvascular cells. The aim of the present work was to explore the antitumor activity of [V⁴Q⁵]dDAVP, alone or in combination with standard-of-care therapy, on highly aggressive human COLO-205 and murine CT-26 V2r-expressing CRC models. In vitro, [V⁴Q⁵]dDAVP (1µM) impaired clonogenic growth and proliferation of CRC cells, resulting in a synergic growth-inhibitory effect after combination with sub-IC50 concentrations of cytotoxic drug 5-FU (≤5µM). Using syngeneic CT-26 models of experimental cancer cell colonization of lung and liver we demonstrated that sustained i.v. treatment with [V⁴Q⁵]dDAVP (0,3µg/kg, thrice weekly) interferes with CRC metastatic spread causing a dramatic decrease of intra-pulmonary and –hepatic nodules, respectively. Moreover, on COLO-205 xenografts, [V⁴Q⁵]dDAVP was also able to reduce tumor progression, exhibiting modulation of local tumor aggressiveness and reduction of tumor-associated angiogenesis. Finally, in animals bearing log-phase growing subcutaneous CT-26 tumors, addition of [V⁴Q⁵]dDAVP to weekly cycles of 5-FU (50 mg/kg i.p) displayed beneficial therapeutic effects, reducing tumor growth rate by 47% (T-test or ANOVA, $p<0.05$, GraphPad Prism 6.0 or Compusyn software).

As a conclusion, taking into account the urgent need for novel CRC therapies with high efficacy and low toxicity, and the preliminary antitumor effects and biological activity of [V⁴Q⁵]dDAVP, this novel peptide is a promising candidate for further preclinical testing in this disease setting.

Keywords: colorectal cancer, vasopressin analog, [V⁴Q⁵]dDAVP, antimetastatic, adjuvant therapy.

(1192) **STUDY AND INTERFERENCE OF THE TUMOR MICROENVIRONMENT OF THE HNSCC**

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Head and neck squamous cell carcinoma (HNSCC) is a malignancy of the oral cavity that often progress from a premalignant oral lession (POL). The tumor immune status and the profile of tumor-infiltrating lymphocytes (TILs) may be directly involved in the transition. POL often have increased levels of Th17 cells, which are replaced with Treg as lesions become HNSCC. The aim of this work is to investigate if tumor microenvironment of malignant lessions can skew immune cells toward a Treg-like phenotype and if POL can induce a Th17-like phenotype. Supernatants from single cell suspensions of primary HNSCC tumors were collected at different time points to stimulate Jurkat T cell line for 6 days. Th17/Treg profile was assessed by measuring RORgT and FoxP3 levels by qRT-PCR. We found increased FoxP3 RNA levels and concomitant decreased RORgT RNA levels, compared to the non-stimulated control.

In parallel, we are developing a T-cell targeted gene therapy strategy to interfere with Th17/ Treg balance, based on lentiviral vectors with tropism to TILs. We pseudotyped HIV-based lentiviral vectors with glycoproteins from human endogenous retrovirus-w (HERV-w) which recognize ligands enriched in hematopoietic cells including T cells. Preliminary results showed that HERW-w-pseudotyped LV transduced Jurkat cells efficiently as revealed by Flow Cytometry. These vectors will be tested on naive T cells, in order to deliver molecules that can interfere with the transition Th17/Treg exerted by tumor microenvironment. More thorough understanding of the roles of the immunological status in premalignant lesion and HNSCC environments will allow targeted modulation of immune response and sustain the attempt to eradicate tumor development.

Keywords: HNSCC, POL, Th17, Treg, Lentiviral vector

ENDOCRINE SOCIETY AWARD

(100) **ROLE OF GONADAL ADIPOSE TISSUE FROM METABOLIC SYNDROME MICE IN PROSTATE CANCER DEVELOPMENT**

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Metabolic syndrome (MeS) increases prostate cancer (PCa) risk and aggressiveness. C-terminal binding protein 1 (CtBP1) is a transcriptional co-repressor of tumor suppressor genes that is activated by low NAD+/NADH ratio.

Previously our group established a MeS and PCa mice model that identified to CtBP1 as a novel link associating both diseases. Moreover, we found that CtBP1 represses aromatase transcription, an enzyme that converts androgens to estrogens. Our hypothesis is that gonadal adipose tissue (gWAT) from MeS mice is a biologically active organ that contributes to PCa development by affecting

the circulating levels of pro-inflammatory cytokines, estrogens and miRNAs. To address this hypothesis, MeS was induced in C57BL/6 male mice by chronically feeding with high fat diet (HFD). MeS increased 30% mice body weight compared to CD fed animals. After 15 weeks of diet, PCa TRAMP-C1 murine cells were injected s.c. on MeS and control mice. 12 weeks after cell inoculation mice were sacrificed and tumors, testes and gWAT were collected for RNA isolation and *ex vivo* co-cultures. Peripheral blood was obtained for biochemical and hormone levels determinations. MeS significantly increased serum estradiol levels. Moreover estradiol (10 nM, 96 h) induced TRAMP-C1 proliferation in vitro. Given that the major sources of estradiol in mice are adipose tissue and testes, we analyzed the expression of aromatase and other genes in these tissues by RT-qPCR. In gWAT we found that MeS dramatically decreased CtBP1 and Cyclin D1 expression, without aromatase expression levels changes. Notably, we found that MeS mice had twice as much gWAT as control animals. Furthermore, we developed an *ex vivo* co-culture system using gWAT obtained from CD or HFD fed mice and TRAMP-C1 cells. gWAT from MeS mice induced the number of tumor cells compared to the co-culture from CD fed animals. Altogether, these results define a key role for gWAT in MeS mice that impact in PCa development.

Keywords: Metabolic syndrome, prostate cancer, gonadal adipose tissue, estrogens

(201) **CAFETERIA DIET ALTERS THE EXPRESSION OF KEY GENES OF THE BRAIN REWARD SYSTEM OVER TIME**

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We aim to determine the effects of Cafeteria diet (CAF), rich in palatable and energy dense foods, on the expression of key genes of the brain reward system (RW) in the short and long term. Female Wistar rats were fed chow or CAF for 4 or 11 weeks. Animals were sacrificed and 2 regions of the Accumbens Nucleus (NA – Core, NAC; and shell, NAS), Ventral Pallidum (VP) and Ventral Tegmental Area (VTA) were dissected. Serum leptin was assessed by RIA. mRNA expression of genes of the dopaminergic and GABAergic pathway, and the leptin receptor (ObRb) was evaluated by qPCR in the nuclei. Data was statistically analyzed by two-way ANOVA followed by Tukey post-test. Four weeks of CAF increased energy intake and adiposity, not affecting circulating leptin or body weight. In VTA, 4 weeks of CAF increased the expression of the dopamine active transporter (DAT) and decreased both isoforms of the enzyme involved in the synthesis of GABA (glutamate decarboxylase, GAD 1 and 2), without altering tyrosine hydroxylase (TH) expression. CAF decreased dopamine receptor (DR) 2 expression in NAS and increased DR1 levels in NAC. Also, CAF increased GAD2 levels in VP. After 11 weeks of CAF, animals sustained the hyperenergetic intake and further increased adiposity, leading to hyperleptinemia and higher body weight, only concomitant to an increased expression of ObRb in VTA. Our results indicate that the higher energy intake of CAF animals in the short-term would respond to hedonic mechanisms, given by molecular deregulations in the RW. The palatability of the diet could lead to a hypodopaminergic state, as DAT expression increase in VTA and DR2 decrease in NAS. Besides, the increment in GAD2 expression in VP indicates an inhibitory GABAergic input to dopaminergic and GABAergic VTA neurons that may, inhibit dopamine and GABA release, in line with the low expression levels of GAD1 and GAD2. Conversely, in the long-term the hypercaloric intake could respond to an altered homeostatic control.

Keywords: Cafeteria diet – obesity – mesolimbic system – energy intake

(269) **AN *IN VIVO* ZEBRAFISH FUNCTIONAL ASSAY TO CHARACTERIZE *IGFALS* GENE VARIANTS**

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ALS is essential for the stabilization of IGF-I and IGFBP-3 as ternary complexes in the vascular system. ALS deficient (ACLSD) patients, homozygous or compound heterozygous for *IGFALS* gene mutations present severe IGF-I, IGFBP-3 deficiencies and a variable degree of growth retardation. The aim of this study was to evaluate the consequences of ACLSD in a zebrafish model to establish an *in vivo* assay for determining the pathogenic effect of *IGFALS* gene variants found in ACLSD patients. To do this we performed knockdown of zebrafish *igfals* using morpholinos to evaluate the absence of ALS in the context of a whole organism and designed a rescue assay using wildtype human *IGFALS* mRNA and a frameshift variant (p.E35Gfs*17) identified in an ACLSD patient. We also characterized the expression pattern of *igfals* in the zebrafish by RT-PCR and in situ hybridization at different stages of development and in adult tissues for the first time.

Igfals is expressed throughout development in zebrafish embryos by RT-PCR and is mainly restricted to the kidney, heart, liver, muscle, and ovary during adulthood. In situ hybridization of zebrafish embryos shows *igfals* expression only in the brain and liver. Knockdown of zebrafish *igfals* using 6 ng of morpholinos shows severe dorsalization in 185/214 embryos, $p<0.0001$. Co-injection of 600 pg of wildtype human *IGFALS* mRNA rescues this phenotype in 152/196 of embryos, $p<0.0001$. Preliminary results show that p.E35Gfs*17 mRNA fails to rescue the dorsalization phenotype in 28/48 embryos, $p<0.0001$.

In conclusion, we were able to develop an *in vivo* rescue assay to evaluate the pathogenicity of a frameshift *IGFALS* variant found in an ACLSD patient. The zebrafish, and its conserved GH-IGF-I axis, constitutes an ideal *in vivo* model for characterizing the functional effect of genetic variants in ortholog human genes.

Keywords: Zebrafish, Growth, Mutations, IGFALS, IGF-I

(346) **FUNCTIONAL CHARACTERIZATION OF TWO NOVEL STAT3 MUTATIONS ASSOCIATED TO PARTIAL GH INSENSITIVITY.**

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Abstract: Germinal heterozygous activating *STAT3* mutations represent a novel monogenic defect associated with multi-organ autoimmune disease and severe growth retardation. Here, we aimed to characterize two *novel STAT3* variants, p.E616del and p.C426R, identified by whole-exome sequencing in two unrelated pediatric patients with insulin like growth factor (IGF)-1 deficiency and immune dysregulation. *STAT3* gene variants were generated by site-directed mutagenesis and transfected into HEK293T cells. The impact of these variants on STAT3 expression, phosphorylation and activity was study using a luciferase reporter system and by Western Immunoblot. The functional analyses showed that both variants were *gain-of-function* (GOF), although they were not constitutively phosphorylated. While both variants increased their transcriptional activities in response to GH ($p<0.01$), only p.C426R depicted an increased transcriptional activity in response to IL-6 ($p<0.05$). WT-STAT3 and the two variants were phosphorylated in response to GH and IL-6, but p.C426R exhibited delayed dephosphorylation under GH treatment, while p.E616del, only under IL-6-stimulation. Because phosphorylation of STAT5 can be negatively regulated by SOCS3, which is one of the main targets of STAT3, we explored STAT5 phosphorylation in HEK293T cells expressing the STAT3 GOF variants. STAT3 GOF variants did not suppress STAT5 phosphorylation in response to GH. Nonetheless, STAT5b transcriptional activity was diminished in the presence of STAT3 GOF variants ($p<0.05$). Our results suggest that these STAT3 activating variants may drive increased transcriptional activity by slightly different mechanisms and decrease STAT5b function by a mechanism that differs from the suppression of tyrosine phosphorylation, leading to partial GH insensitivity.

Keywords: STAT3 mutations, STAT5b, GH insensitivity, *in vitro* studies.

(372) **NEXT GENERATION TECHNOLOGIES FOR MOLECULAR CHARACTERIZATION OF PATIENTS WITH 46,XX TESTICULAR AND OVOTESTICULAR DISORDERS OF SEX DEVELOPMENT**

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Disorders of sex development (DSD) are those congenital conditions in which development of chromosomal, gonadal, or anatomical sex is atypical.The aim of this study is to characterize a cohort of 46,XX-*SRY* negative (in lymphocyte and gonad) testicular and ovotesticular DSD patients. We studied 17 patients who were divided in two groups:isolated DSD (n=13) and syndromic DSD (n=4), mainly based on the presence of additional anomalies, dysmorphic features and intellectual disability. Copy number variations were studied by whole genome CGH+SNP microarray in patients with syndromic DSD. Direct sequencing of candidate genes and CNVs in *SRY*, *SOX9*, *NROB1*, *NR5A1* and *WNT4* were assayed by MLPA in all patients.Genetic variants were searched by whole exome sequencing (WES). Deleterious variants were established by *in silico* tools. In gonadal tissues, *RSPO1* genetic variants were studied by Sanger and CNVs by same MLPA assay as in genomic DNA. Expression of HSD3B2 and CYP17A1 was analyzed by IHC staining in gonadal tissues.

Clinically significant chromosomal alterations were detected in 2/4 with syndromic DSD. A complex deletion/duplication rearrangement involving the 12p,a loss in copy number in the 9q31.2 region as well as multiple contiguous stretches of homozygosity were identified in the child of related parents (coefficient of consanguinity of 1/8). The second patient was found to carry an 11.2 Mb deletion in 1p chromosome,containing multiple genes implicated into ovarian development. WES analysis revealed 2 mutations in *WT1* in 3 patients. IHC in gonad tissues showed atypical expression of CYP17A1 and HSD3B2 in interstitial and Sertoli cells.

It has been recently reported that the alteration in *WT1* might lead to an upregulation of testis determining genes and a reduced activation of *FOXL2* promoter. We propose that the atypical expression of steroidogenic enzymes may be due to a switch in cell fate lineage in the bipotential gonad resulting in abnormal gonadal differentiation.

Keywords: disorders of sex development, genetic variations, gonad

(590) **LH CONTROLS BREAST CANCER CELL MIGRATION AND INVASION VIA PAXILLIN/CORTACTIN/N-WASP**

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Reproductive hormones influence breast cancer development and progression. While the actions of sex steroids in this setting are established, tentative evidence suggests that luteinizing hormone (LH) may also play a role, yet this remains elusive. We identify that T-47D breast cancer cells express functional receptor for LH, and this hormone regulate breast cancer cell motility and invasion. This occurs through the control of the actin cytoskeleton and the formation of cortical actin aggregates and focal adhesion complexes. Such actions are mediated by the cytoskeletal controllers like Paxillin, Cortactin and N-WASP. N-WASP phosphorylation is found to be triggered by a rapid extragonadal signaling of LH receptor (LHR) to c-Src, Focal Adhesion kinase (FAK) and Paxillin. After N-WASP phosphorylation by LH, the Arp-2/3 complex concentrates at actin nucleation sites, where it triggers the local reorganization of actin fibers, promoting the breast cancer cell movement. These results contribute to the emerging area of investigation on the extra-gonadal actions of gonadotrophins suggesting potential implications of the changing levels of these hormones throughout life for the development or progression of breast cancer. Plus, they may have clinical implications for the use of drugs that modulate gonadotrophins in breast cancer patients.

Keywords: Gonadotrophins, LH, kinases, breast cancer, migration and invasion

(1191) **GONADOTROPIN INDEPENDENT STEROIDOGENESIS IN THE HUMAN PREPUBERTAL TESTIS**

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Although controversial, gonadotropin independent intratesticular testosterone (T) production has been reported in children. The insulin-like growth factor (IGF) family is essential for testis development. We reported induction of T secretion by IGF1 in human prepubertal (Pp) testis cultures.

Aim: To evaluate the expression of steroidogenic enzymes in human Pp testes, and a possible relation with the IGFs system.

Testes were collected at necropsy from 18 subjects without endocrine diseases and divided in Infancy n=8 (median age 1.3 mo, range 2 days-7 mo) and Childhood n=10 (6 years, 1-9 y). Expression of the front and backdoor androgenic pathway enzymes and of IGFs and its receptors was studied by IHC, WB and qRT-PCR.

IHC and WB: Mature Leydig cells (LC) in Infant samples expressed LHCGR, SRD5A1, HSD3B2, CYP11A1 and CYP17A1. A small number of peritubular (PTM) and interstitial cells (IC) in all samples expressed SRD5A1, CYP11A1 and HSD3B2. IGF2 was expressed only by mature LC in Infant samples while INSR was expressed in Sertoli cells, LC, IC and PTM of all samples.

qRT-PCR: CYP17A1 mRNA expression was significantly higher in Infancy than in Childhood, while IGF1R, SRD5A1 and AKR1C3 were higher in Childhood. In Infancy, a strong negative correlation between IGF1 and CYP17A1 expression and a positive correlation between alternative pathway enzymes (AKR1C3, AKR1C1 and AKR1C2) was found. In Childhood, CYP17A1 and AKR1C3 increased significantly with age; a positive correlation between enzymes (SRD5A1, AKR1C1, AKR1C3 and AKR1C2) and a negative correlation between IGF1R and INSR was found; IGF1R correlated positively with CYP17A1 while INSR negatively with CYP17A1 and AKR1C3.

This is the first report of the Pp testicular expression of genes involved in both androgen synthesis pathways and its gonadotropin independent increase throughout childhood. Also, our results hint to a possible role of the IGFs in the testis steroidogenic maturation previous to central puberty onset.

Keywords: Testis, Human, IGFs, Steroidogenesis, Prepuberty

(1274) **CONSTITUTIVE GHRELIN RECEPTOR SIGNALING MODULATES THE MAGNITUDE OF THE COMPENSATORY HYPERPHAGIA TRIGGERED BY AN EVENT OF FASTING**

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Ghrelin is the only peptide hormone known to stimulate food intake. Ghrelin acts through the growth hormone secretagogue receptor (GHSR), which is a G protein coupled receptor highly expressed in the central nervous system. Notably, GHSR displays the highest known constitutive activity. The physiological relevance of

the constitutive GHSR signaling is uncertain. Our goal here was to study if the ghrelin/GHSR system modulates the magnitude of the hyperphagia that follows an event of fasting. First, we characterized the food intake and body weight responses of wild-type (WT) mice that have been exposed to a 48-h fasting event and then refed. We found that refed WT mice display a robust hyperphagia after fasting that continues for 5 days after refeeding and changes its food intake daily pattern. Fasted WT mice show an increase of plasma ghrelin levels as well as the GHSR levels in the hypothalamic arcuate nucleus (ARC), indicated by both a ghrelin binding assay and gene expression analysis. Then, we compared the fast-refeeding response of WT, ghrelin-KO and GHSR-deficient mice in our protocol. In contrast to ghrelin-KO mice, only GHSR-deficient mice showed a significantly smaller compensatory hyperphagia than the observed in WT (14.4±3.5%, unpaired t test). Then, we tested the compensatory hyperphagia of WT mice intracerebroventricularly-treated during the fasting period with either a GHSR antagonist (D-Lys_3-GHRP-6) or a GHSR inverse agonist (K-(D-1-Nal)-FwLL-NH_2). The compensatory hyperphagia was significantly smaller only in the inverse agonist-treated group (14.8±3.8%, unpaired t test) as compared to vehicle- and antagonist-treated mice. Thus, the constitutive GHSR signaling modulates the magnitude of the compensatory hyperphagia triggered by an event of fasting.

Keywords: Ghrelin, GHSR constitutive activity, arcuate nucleus, fasting

(1276) **THE HYPOTHALAMIC ACTION OF GHRELIN IS REQUIRED FOR A FULL RESPONSE OF THE HYPOTHALAMIC–PITUITARY–ADRENAL (HPA) AXIS TO FASTING**

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Ghrelin is a stomach-derived hormone that regulates food intake and neuroendocrine axis via its action on the GHSR (*growth hormone secretagogue receptor*). The administration of ghrelin actives corticotropin-releasing factor (CRF) neurons of the hypothalamic paraventricular nucleus (PVN) and, as a consequence, the HPA axis via inhibition of the local GABA tone and independently of the neuropeptide Y (NPY)/GABA neurons of the arcuate nucleus (ARC). Under fasting ghrelin levels increase; however, the impact of endogenous increments of plasma ghrelin on the HPA axis is currently unknown. Here we studied the response of the HPA axis to fasting of wild-type (wt) mice, GHSR-deficient mice and mice expressing GHSR only in GABA neurons. In addition, we quantified if fasting affects the NPY and GABA inputs to the PVN in an *in vitro* setting and in mice expressing tdTomato fluorescent protein in GABA neurons. Results: As compared to wt mice, GHSR-deficient mice showed an impaired fasting-induced increase of both plasma corticosterone and the marker of neuronal activation, c-Fos, in the PVN (corticosterone: 204±30 vs. 113±30 ng/ml; c-Fos: 44±9 vs. 13±6 cells/side, respectively; p≤0.05, 2-way ANOVA). In contrast, mice expressing GHSR only in GABA neurons displayed a full response to fasting. As compared to *ad libitum* fed mice, fasted mice showed an increase of the NPY-fiber density and an increase of the area of tdTomato positive fibers in the PVN (NPY-fibers: 0.09±0.02 vs. 0.17±0.02 OD; td-Tomato:28.0±3.1 vs. 38.1±1.5 % of total area; respectively; p≤0.05, T-test). As compared to PVN explants of *ad libitum* fed mice, PVN explants of fasted mice showed a reduction of basal and KCl-stimulated GABA release (basal:4.5±0.3 and 3.1±0.5; KCl:6.1±0.3 and 4.3±0.8 % of total incorporated tracer, respectively; p≤ 0.05, two-way ANOVA). Thus, these data indicate that ghrelin signaling in GABA neurons is relevant for the normal response of the HPA axis to fasting.

Keywords: GHSR, GABA, NPY,CRF

(1316) **CSD-C2, A COLD SHOCK DOMAIN RNA BINDING PROTEIN AND ITS ROLE IN DECIDUALIZATION**

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Abstract: RNA binding proteins (RBPs) have been described in cancer cell progression and differentiation, although there is still much to learn about their mechanisms. Here we describe the role of the RBP CSD-C2 in differentiation using *in vivo* decidualization as a model. Kinetic analysis of *Csd-c2* mRNA expression during rat pregnancy in uterine horns (0, 2, 4 days post-coito (dpc)) and implantation sites (ISs) (6, 7, 8, 9, 10 dpc) by qPCR showed that it increased at 7 dpc, reaching its maximum mRNA expression at 8 dpc (P<0.001). The *Csd-c2* mRNA expression correlated with CSD-C2 protein increment at 8 dpc (8 vs 0 dpc, P<.001; 8 vs 6 dpc, 8 vs 4 dpc P<0.05). To localize the specific decidual area for *Csd-c2* expression we performed a laser capture extraction for slices from 8 dpc ISs. The content of *Csd-c2* mRNA determined by qPCR was significantly higher in antimesometrim (AM), followed by its expression in the Junctional Zone and Underneath myometrium (JZ+UM) and barely noticeable in the mesometrium (M) (AM vs JZ+UM, P<0.05; AM vs M, P<0.01). These results show that *Csd-c2* mRNA expression was differentially regulated depending on time and areas of decidua development, with the most variation in antimesometrium and, to a lesser degree, in the junctional zone. Inmunohistochemistry of CSD-C2 showed a preferentially cytoplasmic localization at antimesometrium and junctional zone, and nuclear localization in underneath myometrium and mesometrium. Cytoplasmic localization coincides with differentiated areas, marked by Desmin, while nuclear localization coincides with proliferative zones marked by Ki67. Uterine suppression of CSD-C2 using intrauterine injected specific siRNA led to abnormal decidualization in early pregnancy, with more extended antimesometrial area and with poor mesometrium development if compared to control siRNA-injected animals (Notched box-plot). These results suggest that CSD-C2 could be a master regulator of differentiation during decidua development.

Keywords: CSD-C2, endometrium, differentiation, RNA Binding Proteins

GENETICS AND MOLECULAR BIOLOGY 15

(1151) **CHARACTERIZING THE ACTIVITY OF THE SPLICING FACTOR SRSF1 IN THE SUMO CONJUGATION PATHWAY**

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The SR family of proteins, widely characterized as regulators of the splicing process, share a conserved molecular structure comprising one or two RNA recognition motifs (RRMs) and a serine-arginine rich domain. Some members of this family, and in particular SRSF1, are involved in a wide variety of regulatory functions at different levels of gene expression, being considered multifaceted proteins. Our work is focused on characterizing the E3 SUMO ligase-like activity of SRSF1 that was previously described by our laboratory. In addition to its interaction with enzymes of the SUMO machinery, we identified different substrates whose SUMOylation is regulated by SRSF1, including proteins involved in different aspects of pre-mRNA processing and metabolism. By comparing the SUMOylation-enhancing activity of various members of the SR family, as well as analyzing a variety of deletion mutants, our laboratory postulated the involvement of SRSF1 RMM2 in the E3 ligase-like activity of this protein. By generating a battery of SRSF1 mutants, we have demonstrated that mutating a particular single residue within the RRM2 domain abolishes the SUMOylation-enhacing activity of this protein while mutating two specific residues within the RRM1 domain exacerbates this function. Interestingly, it has been reported that these two SRSF1 mutants disrupt the RNA binding capacity of

this protein by the corresponding domains. These findings suggest a correlation between the SUMOylation E3 ligase-like activity of this splicing factor and its RNA binding ability. As SRSF1-RNA interaction can also mediate the nucleo-citoplasmic shuttling of this protein, it would be interesting to define whether the E3 ligase-like activity of this factor depends on its subcellular/subnuclear localization. Moreover, we are currently characterizing both cellular components as well as cellular conditions that modulate SRSF1 activity along the SUMO pathway.

Keywords: Splicing factors, SUMO conjugation, SUMO E3 ligase, RNA binding proteins

(1158) **LIGHT-MEDIATED REGULATION OF ALTERNATIVE SPLICING AND TRANSCRIPTION ELONGATION IN PLANTS**

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Light is an environmental stimulus that regulates several biological processes in plants. Through the chloroplast, light regulates alternative splicing of a subset of genes. In this work, we aim to study the mechanism of light regulation of alternative splicing. We chose the Ser-Arg-rich splicing factor RS31 as a model. Treatment of plants with trichostatin A, a drug that suppresses histone deacetylase activity and therefore increases histone acetylation, mimics the effect of light on RS31 alternative splicing. TSA and light regulate alternative splicing of a subset of Arabidopsis transcripts, as shown in a high-resolution RT-PCR panel. The effect of light on alternative splicing involves changes in transcription elongation: treatment of plants with camptothecin, a drug that inhibits transcription elongation, mimics the effect of darkness on alternative splicing. In a mutant plant defective in the elongation factor TFIIS, the light/dark effect on alternative splicing is completely abolished. Finally, we performed measurements of transcription elongation rate and found that in light-treated plants transcription elongation is faster than in dark-treated plants along the RS31 gene. These results show that the effect of light on alternative splicing involves changes in transcription elongation.

Keywords: alternative splicing – light – transcription - Arabidopsis

(1310) **CHROMATIN AND TRANSCRIPTION ELONGATION CONTROL SMN2 EXON 7 ALTERNATIVE SPLICING**

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Spinal muscular atrophy (SMA) is caused by mutations on the *SMN*1 gene causing the loss of function of the protein it encodes. Humans have a paralog of this gene named SMN2, but it cannot compensate for the deficiency in the SMN protein because exon 7 (E7) is poorly included in its mature mRNA.

At present, one of the most promising therapies for SMA is to restore normal levels of SMN expression by the use of antisense oligonucleotides (ASOs) designed to increase E7 inclusion in the *SMN2* transcript. One of the most outstanding aspects of the ASO therapies assayed is the perdurability of the effect. One possible explanation to it is that ASOs induce changes in chromatin structure, which in turn affect transcriptional elongation and modulate alternative splicing decisions.

In this line of thought, we are studying if modulating chromatin structure as well as transcriptional elongation have an effect on SMN2 E7 inclusion. For this we treated the cells with the histone deacetylation inhibitor trichostatin A (TSA), which, as predicted, caused an increase in E7 inclusion. Furthermore, we found that the combined treatment of HEK293 cells with TSA and an anti-sense oligonucleotide (ASO) with similar effects in promoting E7 inclusion as Nusinersen, promotes a much greater enhancement of E7 inclusion than treatment with each reagent separately. The main conclusions of our preliminary results are that alternative splicing of *SMN2* E7 is subjected to the control of transcriptional elongation and histone acetylation, and that the combined used of histone deacetylation inhibitors and antisense oligonucleotides may yield a more effective treatment of SMA. These results strongly support our aim of investigating the roles of histone marks and chromatin structure in the control of *SMN2* alternative splicing.

Keyword: alternative splicing; gene therapy, spinal muscular atrophy; chromatin, histone deacetylation

(1314) **GENOMICS DATA INTEGRATION REVEALS A ROLE OF INTRON ARCHITECTURE IN THE ALTERNATIVE SPLICING RESPONSE TO DNA DAMAGE**

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DNA damage caused by conditions such us UV irradiation affects gene expression patterns by different mechanisms, also including switching between isoforms of the same genes by means of alternative splicing (AS) regulation. Previous work uncovered a pathway that modulate AS patterns in UV-treated human keratynocites, involving the generation of pyrimidine dimers, the kinase ATR and the phosphorylation of RNA pol II, a major mediator of the response. Here we analyze alternative splicing regulation in response to DNA damage genome wide, integrating transcriptomics, proteomics and RNA binding protein target scanning. We have taken advantage of global characterization of AS patterns through RNA-seq in three conditions: untreated cells, UV-treated and UV-treated reverted by photolyase-mediated repair of damaged DNA. Analysis of the general architecture of the gene suggested that the length of the flanking introns is well correlated with the direction of the AS effect: exon cassettes showing higher inclusion after DNA-damage have shorter introns than expected, while those showing lower inclusion have longer introns. Hypothesis testing using a logistic regression model confirmed the influence of intron length and highlighted differences between both modes of regulation: only upstream intron was important for exon up-regulation, while both flanking introns influenced exon down-regulation. Our working model is that up-regulation is mainly mediated by a phosporylation-induced change in RNA pol II elongation rate, as previously reported, while down-regulation is related to splicing regulators binding both flanking introns. Target scanning revealed that indeed longer introns tend to have a higher ratio between negative (hnRNP proteins) and positive (SR proteins) regulators. Finally, quantification of protein abundance in normal vs. UV-treated cells confirmed that hnRNP proteins but not SR proteins increase their abundance in this condition, further supporting our model.

Keywords: alternative splicing, DNA damage, functional genomics, RNA pol II, splicing regulators

(1136) **MIRNAS CONTRIBUTE TO THE GENE REGULATORY NETWORK RESPONSIBLE FOR ZEBRAFISH MELANOCYTE DIFFERENTIATION BY REGULATING THE EXPRESSION OF *SOX10* AND *MITFA***

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IBR-Conicet

Melanocytes derive from neural crest cells and are genetically well characterised, making them an excellent model for understanding how regulation of gene expression enables cell differentiation. A core gene regulatory network (GRN) has been reported for zebrafish melanocyte differentiation. In this network, the expression of SRY-box containing gene 10 (*sox10*) is essential to promote cell differentiation since it activates the master gene microphthalmia-associated transcription factor a (*mitfa*). However, *sox10* levels need subsequently to be reduced because it inhibits the expression of the enzymes responsible for melanin biosynthesis. In mouse and human Mitf regulates DICER expression, leading us to hypothesise that miRNA expression contributes to down-regulation of *sox10*. Using RT-qPCR and whole-mount *in situ* hybridization on zebrafish embryos mutant for *dicer1*, *mitfa*, and *sox10*, we demonstrated the participation of *dicer1* in melanocyte differentiation. Zebrafish *dicer1* mutants showed low levels of melanin, a reduced number of pigment cells, and an aberrant expression of *sox10* during embryo development, while *mitfa* mutants showed low levels of *dicer1* mRNA.

Furthermore, *in silico* analysis of the *dicer1* promoter region revealed the presence of putative binding sites for Mitfa, and the presence of complementary miRNAs seed-regions in both *mitfa* and *sox10* 3'UTRs. EGFP reporter gene experiments showed the functional relevance of these 3'UTRs in melanophore differentiation in zebrafish embryos. We suggest that *dicer1* and some miRNAs are important components of the melanocyte GRN, controlling *sox10* and *mitfa* levels during cell differentiation.

Melanocyte differentiation, *dicer*, miRNAs, *sox10*, zebrafish

(1034) **FROM IN VITRO HIGH CONTENT SCREENINGS TO MAMMALIAN IN VIVO ASSAYS: THE CASE OF miR-34/449's ROLE DURING CEREBROCORTICAL DEVELOPMENT**

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Abstract: During mammalian cerebral cortex development, spindle orientation defects result in severe neurodevelopmental disorders. Proper orientation of the mitotic spindle determines the plane of cellular cleavage in neural progenitors. However, the precise mechanisms that control this important event are not fully understood. Using a combination of high-content screening and mouse genetics, we identified miR-34/449 family as key regulator of mitotic spindle orientation in the developing cerebral cortex. By screening through all cortically expressed microRNAs *in vitro*, we show that several members of the miR-34/449 family control mitotic duration and spindle rotation. Analyzing miR-34/449 knockout (KO) mouse embryos, we found significant spindle misorientation phenotypes in cortical progenitors, resulting in an excess of radial glia cells at the expense of intermediate progenitors and a significant delay in neurogenesis. Additionally, we identify the junction adhesion molecule-A (JAM-A) as a key target for miR-34/449 in the developing cortex that might be responsible for those defects. Our data indicate that miR-34/449-dependent regulation of mitotic spindle orientation is crucial for cell fate specification during mammalian neurogenesis in the developing cortex. Finally, using a similar high-content screening pipeline, we also identified novel cortical microRNA candidates regulating cell migration, which are being validated *in vivo*.

Keywords: Cortex Development, miR-34/449, Neurogenesis, Radial Glia, Spindle Orientation

(1565) **MIR-274 IS A NEW PLAYER IN THE REGULATION OF HIF-DEPENDENT HYPOXIA RESPONSE IN *DROSOPHILA***

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Fundación Instituto Leloir

When oxygen levels descend, metazoans generate a complex physiological response in order to restore homeostasis. In mammals, the main regulator of this response is the heterodimeric transcription factor HIF-1 (Hypoxia Inducible Factor 1). At normoxia, the HIF-1α subunit is hydroxylated by specific prolyl hydroxylase domain proteins (PHDs), which use oxygen as substrate. The hydroxylation leads to ubiquitination and proteasomal degradation of HIF-1α. In hypoxia, the activity of the PHDs is inhibited and HIF-1α is not hydroxylated. This leads to stabilization and accumulation of HIF-1α, dimerization with HIF-1β, and translocation of the dimer to the nucleus where induces the transcription of its target genes. Given this adaptive response is extremely well conserved, we use *Drosophila* to uncover new regulatory mechanisms. We have established that the microRNA processing machinery plays a central role in the regulation of HIF-dependent response, and we identified four miRNAs whose overexpression increases the transcription of a HIF-dependent reporter. We are characterizing the function of one of those microRNAs, mir-274, in relation with the adaptation to hypoxia and its possible regulatory function of HIF. We have observed that both the overexpression and loss of function of miR-274 is sufficient to induce changes in the expression of a HIF transcriptional reporter, compared to control embryos ($p<0.05$). When wild type larvae are exposed to low oxygen levels, they display a very characteristic response that includes an increase in the number of ramifications of the tracheal terminal cells. This response depends on HIF-1α and we have observed that is blocked in miR-274 mutant larvae ($p<0.05$). Finally, real-time PCR experiments will be carried out in order to quantify the levels of HIF targets mRNA, both in conditions of over-expression and loss of function of miR-274. These results suggest that miR-274 plays a central role in the regulation of the hypoxia response.

Keywords: HIF, hypoxia, microRNAs, *Drosophila*

(1211) **DIFFERENTIAL ROLE OF CIRCULAR AND LINEAR TARGET RNAS ON MICRORNA STABILITY.**

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Abstract: Non-coding RNAs (ncRNAs) have emerged as key regulators of gene expression. Their functions range from scaffolds for the assembly of macromolecular complexes to catalytic activities. In neurons, which rely not only on the activity but also on the three dimensional localization of complexes, non-coding RNAs present a great potential to exert relevant functions and regulations. Circular RNAs (circRNAs) are a diverse class of ncRNAs shown to be particularly abundant in neurons and, although their function/s remain/s largely unknown, some of them bind and interfere with microRNA (miRNA) activity. However, it remains unclear whether this "miRNA sponge" effect involves mechanisms that affect activity and/or stability of miRNAs. The latter is conceivable considering that under certain conditions, high affinity target RNAs can trigger miRNA degradation, reverting their canonical function normally leading to specific target RNA degradation. This so-called target RNA-directed miRNA degradation (TDMD) is highly effective in neurons. Our preliminary data suggest the intriguing possibility that circRNAs binding tightly to miRNAs might bypass TDMD and stabilize miRNAs, as opposed to linear targets which trigger TDMD leading to miRNA degradation. Through bioinformatic predictions and experimental approaches we aim at characterizing how circular RNA species affect miRNA stability and activity. In particular, we are exploring the role of an abundant circRNA (ciRS7) in controlling the stability/activity of a specific miRNA (miR-7) that specifically binds to it and which was shown to be downregulated in sporadic Alzheimer's disease (AD). Because miR-7 has several predicted targets of central importance to the pathogenesis of AD, understanding the consequences of miR-7 stability changes upon ciRS7 level variations will increase our knowledge of both circRNA funtions and the biological mechanisms of AD.

Keywords: Circular RNAs, microRNAs, post-transcripcional regulation

(589) **GENOME-WIDE IDENTIFICATION AND EXPERIMENTAL VALIDATION OF 3'UTRS AND MICRORNA TARGETS IN THE NEGLECTED DISEASE PATHOGENS OF THE GENUS *ECHINOCOCCUS***

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MicroRNAs (miRNAs) are key regulators of gene expression at the post-transcriptional level and play essential roles in different biological processes. MiRNAs silence target mRNAs by binding to complementary sequences in the 3′untranslated regions (3′UTRs). The parasitic helminths of the genus *Echinococcus* are the causative agents of echinococcosis, or hydatidosis, a zoonotic neglected disease. Here, we aimed to define a set of 3'UTRs and to predict miRNA targets sites in *Echinococcus*. We performed RNA-seq

and developed a pipeline that integrates the transcriptomic data with available genomic data. We defined a confidence set of 4375 3′UTRs in *E. canadensis* and validated the expression and the length of a random subset by RT-PCR. We performed an *in silico* prediction of miRNA target sites and found 941 potential sites distributed in 724 3′UTRs. MiR-71 and miR-2 had the higher number of target genes, accounting for ~30% of all target genes. Evolutionary conservation analysis of miRNA target sites in a set of orthologous 3′UTRs of *Echinococcus* and *Taenia* showed a significant decrease in the average number of nucleotide substitutions at miRNA target sites compared with random regions within those 3′UTRs ($p<0.05$) suggesting that the predicted sites may be functional and under selective pressure. In addition, functional analysis of miRNA targets in *E. canadensis* showed that the MAPK signaling pathway was significantly enriched (padj<0.05) suggesting miRNA roles in parasite growth and development. MiRNA targets were selected according to secondary structure to perform experimental validation of miRNA target interactions by reporter assay. This study provides a valuable resource to guide functional experimental studies such as reporter assays or miRNA silencing and/or overexpression. Parasite specific miRNAs involved in essential functions might be considered as novel therapeutic targets for echinococcosis control.

Keywords: Hydatidosis, Parasite, 3′UTRs, MicroRNA, Target

GENETICS AND MOLECULAR BIOLOGY 16

(1798) DNA-DAMAGE INDUCIBLE PROTEIN 1 IS A CONSERVED METACASPASE SUBSTRATE THAT IS CLEAVED AND FURTHER DESTABILIZED UNDER SPECIFIC METABOLIC CONDITIONS.

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Metacaspases, distant relatives of metazoan caspases, have been shown to participate in programmed cell death, progression of the cell cycle and removal of protein aggregates in unicellular eukaryotes. However, since natural proteolytic substrates have scarcely been identified to date, their roles in these processes remain unclear. Here, we report that the DNA-damage inducible protein 1 (Ddi1) represents a conserved protein substrate for metacaspases belonging to divergent unicellular eukaryotes (trypanosomes and yeasts). We show that although the recognized cleavage sequence is not identical among the different model organisms tested, in all of them the proteolysis consequence is the removal of the ubiquitin-associated domain (UBA) present in the protein. We also demonstrate that Ddi1 cleavage is tightly regulated *in vivo* as it only takes place in yeast when calcium increases but under specific metabolic conditions. Finally, we show that metacaspase-mediated Ddi1 cleavage reduces the stability of this protein which can certainly impact on the many functions ascribed for it, including shuttle to the proteasome, cell cycle control, late secretory pathway regulation, among others.

Keywords: metacaspase, DNA-damage inducible protein 1, protease substrate, trypanosomatids, yeast.

(1691) CHARACTERIZATION OF THE UNFOLDED PROTEIN RESPONSE THROUGH THE USE OF FLUORESCENT REPORTERS IN CULTURED SINGLE CELLS AND IN DISEASE MODELS

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The Unfolded Protein Response (UPR) is a cellular stress signaling cascade essentially triggered by the accumulation of misfolded proteins in the Endoplasmic Reticulum (ER). Three mechanistically distinct pathways (IRE1, PERK and ATF6) make up this collective response aimed at restoring homeostasis. However, if this objective is not met within a logical time span, the UPR triggers apoptosis. This dichotomy of outcomes makes determining the cell's fate a real enigma which could be solved through a clear characterization of the UPR dynamics.

In order to do this, we employed a set of fluorescent reporters that allows us to monitor the activation of the UPR in human single cells and in real time. We have previously characterized novel reporters for the ATF6 and IRE1 pathways, and we describe here the design of new reporters for the PERK pathway. We have also developed a protocol for automated imaging and segmentation of cells as well as for quantitative analysis of UPR activation. We aim to study the activation dynamics of the three UPR pathways in single cells in order to shed light onto the decision-making mechanisms involved in cell death and survival.

Interestingly, some evidences show that the UPR might be associated with neurodegenerative diseases: activation of UPR in patients suffering from frontotemporal lobar degeneration (FTLD) and amyotrophic lateral sclerosis (ALS) has been linked to the toxicity of Tar DNA binding Protein-43 (TDP-43), the main component of intracellular inclusions related to these diseases. Confirmation of this integrated network would open the possibility of designing new therapies targeted to patients suffering from neurodegeneration.

(104) MOLECULAR DISECTION OF BIOLOGICALLY RELEVANT PATHWAYS AFTER INHIBITION OF CHECKPOINT KINASE 1

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Checkpoint signalling allows tumour cells to cope with high rates of replication stress, a hallmark of cancer. Specifically, Chk1 delays the progression of S phase, thereby increasing the chances to repair damaged DNA before using it as a replication template. Therefore, the inhibition of checkpoint kinase 1 (Chk1) has emerged as a promising anti-cancer therapy. However, in the clinic, treatments with Chk1 inhibitors fail with unexpected frequency, highlighting the need to further understand how cells adapt to such therapy. It is very well established that Chk1 inhibition alters the replication choreography, increasing origin firing while slowing down replication fork progression. In turn, such defective replication choreography generates increased levels of double strand break (DSB), compromising the genomic stability of cells to a degree that triggers cell death. The current model proposes that such events are part of a linear pathway, so that cell death is intimately linked to genomic instability in Chk1-inhibited cells. However, the molecular signals that link those phenotypes are unknown. We have undertaken a systematic analysis involving the elimination of factors which are crucial for each of the processes modulated by Chk1 inhibition. In such experimental settings, we have examined the replication choreography (DNA fiber assay which allows to measure fork rate and percentage of origin firing). We have also quantified DSB accumulation using neutral COMET assay and genomic instability using the micronuclei assay. We will present preliminary data demonstrating that the phenotypes caused by Chk1 deficiency, i.e. altered replication choreography, genomic instability, and cell death can be dissected at the molecular levels. We believe that such information could be used to improve anti-cancer therapies based on Chk1 inhibition.

Keywords: Genomic instability; Checkpoint Kinase 1; Replication choreography; Cancer cell death

(141) INHIBITION OF TRANSLESION DNA SYNTHESIS AS A STRATEGY TO SENSITIZE CANCER CELLS TO DNA-DAMAGING AGENTS

María Belén De La Vega, Sabrina Florencia Mansilla, Vanesa Gottifredi
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Cancer cells proliferate more rapidly than normal cells and are thus sensitive to DNA-damaging agents used in traditional chemo-

therapy. These agents target cells with high proliferation rate by producing DNA damage that impairs replication and causes cell death. The efficacy of anticancer treatments is, however, highly influenced by the cellular capacity to respond to DNA damage. One central mechanism that can enable cancer cells to survive is Translesion DNA Synthesis (TLS), where specialized DNA polymerases bypass lesions encountered during DNA replication, a situation where replicative DNA polymerases normally stall. Therefore, inhibiting TLS would be deleterious to these cells when used in combination with DNA-damaging agents. We have previously identified p21, the cyclin-CDK inhibitor, as the first global inhibitor of TLS. A stabilized version of p21 can inhibit the recruitment of TLS polymerases to replication factories after DNA damage and this is dependent on its PCNA-binding domain. We have recently found a smaller version of p21 which is sufficient to robustly inhibit the recruitment of TLS polymerases to replication factories post ultraviolet (UV) radiation. By using a non-replicative lentivirus system as a tool to overexpress such small version of p21, we want to test the effect of TLS inhibition on genome stability and cell survival in the context of DNA-damage inductors used in chemotherapy, like platinum compounds. A second objective is to evaluate whether co-inhibition of TLS and other proteins involved in mechanisms that can serve as a back-up in a TLS deficient context can efficiently synergize with anticancer drugs to enhance cell death.

Keywords: p21, Translesion DNA Synthesis, cell death, DNA-damage, PCNA

(121) **EVALUATION OF THE RELATIVE CONTRIBUTION OF ALTERNATIVE DNA POLYMERASES TO THE DNA DAMAGE RESPONSE (DDR)**

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The DNA damage response (DDR) is a multifaceted network of signals which is activated by structural and chemical alterations of the DNA. One key aspect of DDR is DNA damage tolerance by alternative DNA polymerases which facilitates DNA replication across DNA lesions. It is unclear if all alternative DNA polymerases (Alt. Pols) have complete overlapped functions in DDR. By using siRNA technology we depleted the expression of 6 different alternative DNA polymerases, either individually or in combination, and evaluated their relevant contribution to the accumulation of DDR markers after the exposure to DNA damaging agents such as cisplatin. We analysed the induction of known replication stress markers such as the phosphorylation of H2AX (γH2AX) and the formation of 53BP1 foci. As expected, the loss of most Alt. Pols caused the accumulation of increased levels of such DDR markers, as a consequence of a suboptimal response of cells depleted from Alt. pols to cisplatin. In contrast to other Alt. Pols, the loss of polymerase iota (Pol ι) caused a reduction in the induction of γH2AX and the formation of 53BP1 foci. Hence, the function of Pol ι might not be restricted to the DNA synthesis across DNA lesions as reported for the rest of the Alt pols. By means of studying DNA replication parameters such as origin firing and the rate of nascent DNA elongation we will present evidence suggesting that, in fact, the contribution of pol ι to DNA replication is different from that of other Alt. pols. We will also present evidence demonstrating that the contribution to DDR of pol ι is relevant for the survival and the genomic stability of cells. Together our data demonstrates that pol ι has a contribution to DDR that is not shared by other Alt. pols hence suggesting that the loss of its function may be more difficult to compensate in cancer cells.

Keywords: pol iota, DNA damage response, γH2AX, 53BP1, DNA replication

(1086) **IDENTIFICATION AND VALIDATION OF A NOVEL TRANSLESION DNA SYNTHESIS INHIBITOR THROUGH A WESTERN-BLOT BASED SCREENING PLATFORM**

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Translesion DNA synthesis (TLS) is a DNA damage tolerance process that employs specialized polymerases to bypass DNA damage during replication. Several lines of evidence suggest that TLS inhibition would be crucial in the homologous recombination (HR) deficient context of certain types of cancers (i.e. BRCA-/- breast and ovarian tumors). However, the limitation to explore such type of therapeutic strategy is the lack of chemical inhibitors to target TLS.

The main goal of this project is to identify specific inhibitors of TLS that can be used as “a proof of concept” to induce selective toxicity in BRCA-deficient cells. Our rational is that since TLS polymerases recruitment to sites of DNA damage is a key step for TLS success, we can indirectly monitor TLS efficiency by studying two key markers: 1) The mono-ubiquitylation of PCNA and 2) the accumulation of a TLS polymerase into replication foci. Herein, we developed a screening platform to identify inhibitors of PCNA mono-ubiquitylation through a Western-Blot based method.

From our first screening with an open source library of kinase inhibitors from GlaxoSmithKline we identified a number of hits that inhibit mono-ubiquitylation PCNA. Here we describe the validation of these hits using commercial inhibitors, cell cycle analysis, characterization of DNA damage markers and DNA combing assays to select targets that impair replication processivity after UV irradiation. Moreover, with the validated hits we performed proof of concept experiments in HR-deficient cells, unveiling a link between these pathways in the promotion of cell survival after UV.

Keywords: Translesion DNA Synthesis, DNA Repair

(1813) DNA DAMAGE EVALUATION IN *RHINELLA ARENARUM* (ANURA: BUFONIDAE) TADPOLES EXPOSED TO MIXTURES OF HERBICIDES

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Glyphosate (GLY) and imazethapyr (IMZT) represent key pesticides in modern agricultural management. It has been claimed that the decline in amphibian is predominantly related to pesticide pollution in natural and agricultural areas. In this assay, the acute toxicity of two herbicide formulations, the 48% glyphosate (GLY)-based Credit® and the 10.59% Imazethapyr (IMZT)-based Pivot® H alone as well as the binary mixture of these herbicides was evaluated on *Rhinella arenarum* late-stage larvae, exposed under laboratory conditions. Acute lethal effects was determined using mortality as endpoint, whereas sublethal effects were determined employing the single-cell gel electrophoresis (SCGE) assay. Individuals were exposed to 0.05 and 0.10 mg/L Pivot® H and 3.91 and 7.82 mg/L Credit®, representing the concentrations 5 and 10% of the $LC50_{96h}$ of each herbicide, respectively. Negative (tap water); and positive controls (40 mg/L CP) were performed simultaneously with Credit®- and Pivot® H-exposed tadpoles. Lethality results showed $LC50_{96\ h}$ values of 78.18 mg/L GLY and 0.99 mg/L IMZT and for Credit® and Pivot® H, respectively. SCGE assay demonstrated, after exposure to 5% and 10% of either the Credit® $LC50_{96\ h}$ concentration or the Pivot® H $LC50_{96\ h}$ concentration, a significant dose-dependent increase of the genetic damage index (GDI). The binary mixtures of 5% Credit® plus 5% Pivot® H $LC50_{96\ h}$ concentrations and 10% Credit® plus 10% Pivot® H $LC50_{96\ h}$ concentrations induced equivalent significant increases in the GDI in regard to GDI values from late-stage larvae exposed only to Credit® or Pivot® H. This study represents the first experimental evidence of a synergistic effect of the mixture of GLY and IMZT on the induction of primary DNA breaks on circulating blood cells in *R. arenarum* late-stage larvae is demonstrated.

Keywords: Amphibians, Genotoxicity, Glyphosate, Imazethapyr

(1827) **GENOTOXIC AND CYTOTOXIC EFFECTS INDUCED BY A NOVEL COMMERCIAL FORMULATION OF THE HERBICIDE 2,4 D IN *IN VITRO CELLS***

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The last two decades have witnessed significant progress in nanotechnology and its application in diverse anthropogenic activities, including agriculture. Several novel nanoparticulated commercial formulations of pesticides are available in Argentina, including formulations of 2,4 D. The aim of the present study is to evaluate the genotoxic and cytotoxic effects exerted by a technical formulation namely Dedalo Elite® (30% 2,4 D, Red Surcos, Argentina). The alkaline single cell gel electrophoresis (comet) assay was employed as endpoint for genotoxicity in 90 min exposed-CHO-K1 cells whereas the neutral red uptake assay was employed after 24 h of exposure as cytotoxic endpoint. Cells were exposed within the 1-100 μg/ml of 2,4 D concentration-range and bleomycin (1 μg/ml) and ethanol (5% v/v) were employed as positive control. Results demonstrated a significant concentration-dependent increase of the genetic damage index evaluated by the comet assay within the 4-10 μg/ml concentration range ($P<0.001$). Besides, results revealed a concentration-dependent decrease in the lysosomal activity within 4-100 μg/ml concentration range ($P<0.05$). Our findings rather than revealing the induction of genotoxic and cytotoxic effects by the nanoparticulated herbicide-based formulation reveals the ability to jeopardize nontarget biotic systems exposed to the xenobiotic.

Keywords: Cytotoxicity, CHO-K1 cells, Genotoxicity, 2,4 D

(1812) GENOTOXICITY EXERTED BY THE AUXINIC HERBICIDE 2,4 D IN *RHINELLA ARENARUM (ANURA, BUFONIDAE)*

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Auxinic herbicides are used to selectively control broadleaf weeds in grass crops such as cereal grains and turfgrass swards. Among these, 2,4 D is the second herbicide commonly employed in Argentina, alone or in combination with other herbicides. The aim of the present study is to evaluate the acute sublethal genotoxic effects exerted by the auxinic technical formulation commonly used in Argentina, namely 2,4 D DMA® (58.4% 2,4 D-based formulation, Dow AgroSciences) on circulating blood cells *in vivo*. The alkaline single cell gel electrophoresis (comet) and micronuclei (MN) assays were employed as endpoints for genotoxicity in specimens of *R. arenarum* exposed under laboratory conditions. Tadpoles were exposed during 48 and 96 h within the 62.76-188.30 mg/L of 2,4 D concentration-range. Negative (dechlorinated tap water) and positive (40 mg/L cyclophosphamide) controls were conducted and run simultaneously. Results demonstrated a significant increase of the genetic damage index evaluated by the comet assay in all treatments lasting for both 48 and 96 h ($P < 0.001$). When the frequency of MNs was analyzed, an increased frequency of micronucleated erythrocytes was only observed in tadpoles exposed to 188.30 mg/L of 2,4-D during 48 h ($P < 0.01$). Rather than demonstrating the induction of DNA damage by the herbicide, this study represents the first evidence of sublethal effects exerted by 2,4 D DMA® on an anuran species native to Argentina. Finally, our findings highlight the properties of this auxinic herbicide-based formulation that jeopardize nontarget living species exposed to these agrochemicals.

Keywords: Amphibians, Genotoxicity, 2,4 D

(230) **CHECKPOINT KINASE 1 IS A MASTER REGULATOR OF THE DNA REPLICATION PROGRAM IN CANCER CELLS**

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The encounter of replication forks with DNA lesions activates Checkpoint Kinase 1 (Chk1), which impedes replication fork breakage and inhibits firing of replication origins, reducing in turn the global rate of DNA replication. Such modification of the replication program by the so called intra-S phase checkpoint allows cells to repair damaged DNA, promoting cell survival. Cancer cells, which are inherently challenged with high doses of replication stress, rely on Chk1 for survival. Thus, Chk1 inhibition has emerged as a promising anti-cancer therapy.

Nevertheless, little is known about the molecular mechanisms that follow the inhibition of Chk1. The current model proposes that Chk1 inhibition initially unleashes origin firing, which in turn negatively affects the progression of active replication forks. This model implies that both variables are linked in a linear pathway and that fork progression in Chk1-inhibited cells is regulated exclusively by a global, nucleoplasmic signal, rather than by local signals at the fork. We will present data that challenges such model, demonstrating that Chk1 inhibition generates a local signal at replication forks that impairs their elongation rate. Therefore, we have unraveled a novel mechanism that underpins the impaired fork progression phenotype of Chk1-inhibited cells, while it has no effect on origin firing. Our results thus add a layer of unprecedented complexity to our current understanding of how cancer cells deal with Chk1 deficiency.

Keywords: Chk1; fork elongation; origin firing; intra-Sphase-checkpoint; DNA replication

IMMUNOLOGY (INNATE IMMUNITY) 5

(143) **ISONIAZID - MAIN ANTI-TUBERCULOUS DRUG-ALTERS NEUTROPHIL EXTRACELLULAR TRAPS STRUCTURE AND TURNOVER.**

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Abstract: Drug induced lupus-like reactions are characterized by the presence of anti-nuclear antibodies (ANA) without hypersensitivity to the drug itself. One of such drugs is the isoniazid (INH), an effective antimicrobial worldwide used to treat tuberculosis (TB). INH is a prodrug that needs to be activated by the mycobacterial peroxidase/catalase *KatG* (*Rv1908c*) to give the INH• toxic radical. Considering that Neutrophil Extracellular Traps (NETs) formation requires enzymatically active myeloperoxidase (MPO), we asked if the extensive neutrophil (PMN) activation typically observed during active TB may lead to undesirable autoimmune reactions when it is combined with an INH-based therapy. When primary granules -containing MPO- isolated from normal human blood PMN were incubated with INH or rifampicin -another anti-TB compound-, we found that only the first drug was a good substrate for human MPO at therapeutic serum concentrations (K_m=30±5μM; C_{max}=40±15μM) giving an active product with oxidant properties ($p<0.01$). Moreover, in the presence of INH, *in vitro* induced NETs adopted a differential conformation ($p<0.005$) with several unique features including reduced DNAse 1 sensitivity ($p<0.01$) and diminished phagocytic removal by autologous macrophages ($p<0.05$). As a clinical correlate, we observe that prevalence of ANA/ANCA+ sera were higher in a subset of TB patients under prolonged INH-combined chemotherapy ($p<0.05$) despite lack of lupus related symptoms. Therefore, our results suggest a possible mechanism for a drug-induced autoimmune reaction in which MPO-oxidized INH alters somehow the NET structure and its later processing, leading to pathogenic accumulation of complexed self-antigens (i.e: DNA+ Core Histones+ primary granules enzymes).

Keywords: Tuberculosis, Drug induced lupus, Isoniazid, Myeloperoxidase, NETosis.

(205) **LIPIDS FROM *TRYPANOSOMA CRUZI* AMASTIGOTES OF RA AND K98 STRAINS GENERATE A PRO-INFLAMMATORY RESPONSE VIA TLR2/6**

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It is well known that lipids from microorganisms are ligands of Toll like receptors (TLRs) and are able to modulate the innate immune response. Previously we demonstrated that total lipids from *Trypanosoma cruzi* amastigotes of RA and K98 strains, with polar biological behavior, induced in murine macrophages lipid bodies' formation, COX-2 expression and TNF-α and nitric oxide release (Bott E et al., SAP 2016).

Herein, we analyze TLR involvement in T. cruzi lipid recognition and the consequent induction of pro-inflammatory parameters in HEK cells transfected with TLRs constructs (TLR2-6, TLR2-1 and TLR4) after stimulation with total lipids from RA and K98 strains (RA, K98). Besides, in order to mimic the enzymatic processes that occur during amastigote nest degeneration, where released enzymes could contribute to the generation of bioactive lipids (Wainzelbaum M et al., 2001), we also evaluated the effect of total lipids obtained from parasites incubated 16 h at 37ºC, after disruption (RAinc, K98inc). Results showed that all the lipid stimuli induced NF-κB activation (Luciferase assay) and IL-8 release (ELISA) through a TLR2-6 dependent pathway and in a dose dependence manner.

PPARs are a unique set of fatty acid regulated transcription factors that control lipid metabolism and inflammation. To study if PPARγ was involved in NF-κB trans-repression after T. cruzi lipid stimulation (RA, K98, RAinc, K98inc) we analyzed NF-κB activation as well as IL-8 secretion in HEK cells co-transfected with TLR2-6 and PPARγ. A significant decrease in NF-κB activation and IL-8 secretion in response to T. cruzi lipids was observed, being dependent on PPARγ activation.

Altogether, these findings indicate a relevant role of TLR2-6 pathway in the induction of a pro-inflammatory response induced by *T. cruzi* lipids that could contribute to the control of infection and host survival. Our results also suggest that PPARγ participates in the regulation of this response. Supported by UBA/Conicet.

(323) ***L.CASEI CRL 431*** **AND ITS CELLULAR WALL HAS EFFECT ON THE THYMUS FUNCTIONALLITY IN A MICE OBESE MODEL**

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Aim: to study the effect of *L. casei 431* and/or its cell wall on the thymus restoration in obese mice.

Five weeks old BALB/c mice were divided into 2 groups: Normal Control (NC) that received a conventional balanced diet and water, Obese Control (OC) fed a high fat diet (HFD). NC and OC were divided into sub-groups: N+Probiotic (NP) and O+Probiotic (OP) who received probiotic strain suspension for 12 weeks.

Body weight was determined 3 times per week. Animals were sacrificed at 12 weeks. The samples were thymus (T) for histology and T cells analyses, small intestinal fluid (IF) for cytokines determination (IL-6, IFNγ and IL-10), large intestine for microbiota studies. Cytokines were also determined in the supernatant of Thymus, peritoneal macrophages (PM) and spleen (SM) cultures treated with whole bacteria (B) or its cell wall (W).

Results: OP reached peak body weight at week 9 and decreased at week 10 reaching to NC values and was maintained until end of the study. In relation body weight/thymus OC showed significantly higher values, probiotic supplementation decreases this relation. OC showed enterobacteria and total anaerobes population increased, and lactobacilli decreased. Higher values of lactobacilli were showed in OP. The immune cells in thymus, CD8+ and CD4+/CD8+ population in OL increased significantly ($p<0.05$) respect to OC (CD8+: OC=13,5% ±1,5 OL=21,5%±12,8; CD4+/CD8+: OC=8,6%±2,9 OL=12,6% ±4,9). Cytokine in cellular cultures and IF showed IFNγ and IL-10 decrease in OC respect to NC, probiotic treatment increases significantly these values. IL-6 values increased in the supernatant of MP and Thymus of OC and in the IF. These values decreased with probiotic or cell wall addition.

Both probiotic and its cell wall treatment were effective on the thymus weight and on T cells recovery during HFD, regulating the cytokine levels. At the intestinal levels probiotic supplementation ameliorated the inflammatory effect observed in obese mice.

Keywords: Thymus, Obesity, Probiotic strain, Probiotic cell wall

(427) **M2 MACROPHAGES GENERATE HYPORESPONSIVE NK CELLS THROUGH OVEREXPRESSION OF THE INHIBITORY RECEPTOR CD85j.**

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Upon activation, macrophages can become pro-inflammatory (M1) or anti-inflammatory (M2) cells. Moreover, NK cells are critical players during immunity against tumors. Previously, we interrogated the consequences of the crosstalk between M2 and NK cells and demonstrated that M2 macrophages inhibit IFN-γ secretion through TGF-β secretion and NK cell degranulation and cytotoxicity against tumor cells in a contact dependent manner but independent of TGF-β. To further get insight into the negative regulation of NK cell-mediated cytotoxicity by M2, here we investigated the underlying mechanisms involved in this inhibition. Accordingly, human monocytes were differentiated to unpolarized macrophages (M0) with M-CSF for 6 days and then exposed overnight to LPS and IFN-γ or IL-4 to obtain M1 and M2, respectively. These cells were then co-cultured overnight with isolated NK cells. A phenotypic characterization of NK cells cultured with M1 or M2 revealed no differences in the expression of the activating receptors CD16, DNAM-1, NKp46, NKp44, NKp30, NKG2D, NKp80 and NKG2C ($n>7$) but we observed an overexpression of the inhibitory receptor CD85j (ILT-2) in NK cells co-cultured with M2 compared to M1 ($n=6$, $p<0.05$) and blockade of this receptor increased NK cell degranulation against K562 cells ($n=8$, $p<0.01$). This effect was likely triggered by interaction between CD85j expressed on NK cells and its cognate ligand HLA-G which was overexpressed in M2 compared with M1 ($n=3$, $p<0.05$). Additionally, blockade of CD85j also increased IFN-γ production regardless of TGF-β blockade ($n=4$, $p<0.0001$). Moreover, impaired degranulation of NK cells exposed to M2 persisted for several hours even in the absence of M2 ($n=6$, $p<0.001$). Therefore, we conclude that M2 negatively regulate NK cell effector functions through CD85j overexpression and such crosstalk generates transiently hyporesponsive NK cells.

Keywords: M2 macrophages, NK cells, TGF-β, ILT-2, HLA-G.

(448) **LIPOPOLYSACCHARIDE OF THE HYPEREPIDEMIC CLONE CARBAPENEM RESISTANT *Klebsiella pneumoniae* IMPAIRS THE RESPIRATORY BURST INDUCED BY FMLP IN HUMAN NEUTROPHILS**

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Klebsiella pneumoniae (Kp) is a widespread opportunistic pathogen associated with high mortality rates in immunocompromised patients, attributed to the production of Kp-Carbapanemase (KPC), an enzyme that mediates resistance to carbapenems. Previously, we have demonstrated that Kp-KPC sequence type 258, a multi-resistant hyperepidemic clone, induced poorly bactericidal responses in PMN and Kp bacterial extracts (KpBE) were able to subvert reactive oxygen species (ROS) production induced by fMLP. In this work, our aim was to study the mechanisms and bacterial components involved in this ROS inhibition. Using isolated human PMN we found that KpBE also reduced ROS production induced by another seven trans-membrane agonist, Leukotiene B_4 (LB_4), measured with

dihydrorhodamine 123 (DHR) by FACS (%DHR+ PMN: LB_4=41,4±4; KpBE+LB_4=23,9±5, p<0.01). To elucidate if KpBE affects other PMN functions, IL-8 production was measured by ELISA. KpBE reduced the secretion of IL-8 induced by fMLP [IL-8 (ng/mL): fMLP=1143±179; KpBE+fMLP=531±130, p<0.05]. Formerly, we have proved that the phenomenon was mediated by a saccharide. As LPS is the most important saccharide component of the Gram-negative membrane, we used a polymyxin B-agarose column to deplete LPS from KpBE, and found that these depleted extracts were no longer inhibitory. Conversely, the column-retained LPS (Kp-LPS) was eluted using deoxycolate and tested for its inhibitory activity. Kp-LPS (100 ng/ml) impaired ROS production induced by fMLP (%DHR+ PMN: fMLP=60±2; Kp-LPS+fMLP=43±3, p<0.01). A similar amount of commercial LPS from Kp ATCC 15380 was not able to reduce ROS induced by fMLP (%DHR+ PMN: cKp-LPS+fMLP=58±4). Our results revealed that KpBE is able to reduce ROS by different seven trans-membrane agonists and also other functions triggered by fMLP in human PMN. The impaired ROS production is mediated by LPS from Kp-KPC. This mechanism could be a potential advantage for the establishment/persistence of Kp-KPC infections.

Keywords: LPS, *Klebsiella pneumoniae*, ROS, PMN.

(659) ***Lactobacillus rhamnosus*** **CRL1505 IMPROVES EMERGENCY MYELOPOIESIS AGAINST RESPIRATORY INFECTION IN IMMUNOSUPPRESSED HOST BY CYCLOPHOSPHAMIDE**

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Emergency myelopoiesis is critical to control the infection with pathogens. Patients undergoing chemotherapy have a damage in hematopoiesis associated with an ineffective immune response against infections. *Lactobacillus rhamnosus* CRL1505 diet supplementation has proved to be an interesting alternative to improves steady-state and emergency myelopoiesis. Although the viability of lactic acid bacteria is an important factor, it is possible to stimulate immunity by using the non-viable microorganism. In this work we evaluated whether *L. rhamnosus* CRL1505 was able to improve emergency myelopoiesis induced by *Streptococcus pneumoniae* (Sp) infection. Adult Swiss-mice were orally treated with viable or non-viable *L. rhamnosus* CRL1505 (Lr05V or Lr05NV, respectively) during five consecutive days. On day 6, lactobacilli-treated and untreated control mice received one intraperitoneal dose of cyclophosphamide (Cy 150 mg/kg). On day 3 post-Cy injection, mice were nasally challenged with Sp (10^7 UFC/mice). Innate and myelopoietic responses were evaluated after the pneumococcal challenge. The Cy group showed a high susceptibility to pneumococcal infection, an impaired innate immune response and a decrease of hematopoietic stem cells (HSCs) ($Lin^-Sca\text{-}1^+c\text{-}Kit^+$) and myeloid multipotent precursors (MMPs) ($Gr\text{-}1^+Ly6G^+Ly6C^-$) in bone marrow (BM). However, lactobacilli treatments were able to significantly increase blood neutrophils and peroxidase (Px)+ cells, and improve cytokine production and phagocytic capacity of alveolar macrophages. This, in turn, led to an early Sp lung clearance compared with the Cy group (p<0.05). Besides, the treatment with Lr05V was more effective than Lr05NV to increase growth factors in BM, which allowed an early HSCs and MMPs recovery with respect to the Cy group (p<0.05). In conclusion, both viable and non-viable *L. rhamnosus* CRL1505 are able to improve BM emergency myelopiesis and protection against respiratory pathogens in mice undergoing chemotherapy.

Keywords: Myelopoiesis; Lactobacilli; immunobiotic; cyclophosphamide

(826) **INTERLEUKIN 17-PRODUCING EPIDERMAL CELL SUBSETS IN EXPERIMENTAL *MICROSPORUM CANIS* DERMATOPHYTOSIS**

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Microsporum canis is a dermatophyte fungus that causes highly prevalent skin infections in immunocompetent children. We previously demonstrated that a Th17 response is induced after epicutaneous infection of C57BL/6 mice with *M. canis* and that IL-17-mediated immunity prevents fungal proliferation in skin. However, the mechanisms of dermatophyte recognition and skin cell populations involved in IL-17-driven host defense remain unknown.

The aim of this study was to determine skin cell sources of IL-17A and the role of TLR-2 and Dectin-1 receptors in the protective immunity against *M. canis* infection.

Wild type (WT), IL-17GFP reporter, IL-17RAKO, TLR-2KO and Dectin-1KO C57BL/6 mice were epicutaneously infected with *M. canis* hyphae or treated with saline. After 8 days of infection, skin fungal burden was determined and IL-17A-producing epidermal cell populations were evaluated by FACS with anti-CD45, CD11b, Ly6G, TCRβ, TCRγδ, CD3, CD4 or CD8 antibodies. Cytokine production by epidermal sheets explants was quantified in supernatants (ELISA) after 48h of culture with or without *M. canis*.

We observed that, in *M canis*-infected IL-17GFP reporter mice, CD45+TCRβ+ and TCRγδ+ epidermal cells were the main IL-17A producing-cells. No IL-17 expression was observed in keratinocytes, neutrophils or macrophages. In addition, after culture with epidermal sheets explants, *M. canis* significantly increased IL-6 and IL-1β (IL-17-type response cytokines) respect to controls in medium alone. Furthermore, infected IL-17RA KO mice showed a significant increase in fungal burden respect to WT mice (38200 ± 13980 vs 867 ± 392 CFU/g, p < 0.05). TLR-2 or dectin-1 deficient mice showed a similar fungal burden and IL-17-producing T cell frequency compared to infected WT mice.

This study is the first characterization of skin IL-17-producing cells in dermatophytosis and it strongly suggests that TLR-2 or Dectin-1 receptors are not involved in the IL-17-driven host protection against *M. canis*.

Keywords: Skin immunity, fungi, IL-17, Tγδ

(972) **JUNÍN Z PROTEIN OF VIRUS-LIKE-PARTICLES (Z-VLPS) INDUCES MATURATION OF MICE DENDRITIC CELLS.**

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Arenavirus matrix Junín Z protein is important in virus budding and is able to generate enveloped virus-like-particles (VLPs) in absence of any other viral proteins. These genome-free Z-VLPs are often morphologically similar to the live virus from which they are derived without genome. Its describe that different VLPs are capable of activating cells involved in both innate and adaptive immunity, ultimately generating both humoral and cell-mediated immunity. Mature dendritic cells (DCs) are antigen-presenting cells that transports antigen to the peripheral lymph nodes to initiate CD4 and CD8 T cell responses. VLPs appear to be good tools to deliver different antigens. We hypothesized that these Z-VLP should be a good immunogen and would be useful for vaccine purposes. Because DCs represent a critical link between innate and adaptive immune responses and play a crucial role in vaccine immunogenicity, we tested the effect of Z-VLPs on DCs maturation. In this work we evaluate the immunogenicity of Junín Z protein to produce VLPs carrying the green fluorescent protein (Z-VLP), as a model antigen. We generated DCs from murine bone marrow cells and exposed them to Z-VLP or LPS for 24 h. Z-VLP-exposed DCs showed (by flow cytometer) an increase of maturation markers similar to responses induced by LPS. Cell-surface expression of CD86 (p<0.05) and MHC class II marker IA^d (p<0.05) were up-regulated in DCs incubated with Z-VLP. Then we analyzed the level of proinflammatory chemokines and cytokines in cell-free supernatants by ELISA. Incubation of DCs with Z-VLP induced secretion of IL-10 (p<0.05) and TNF-α (p<0.01) in a similar

pattern to LPS. DCs are also critically involved in generating antibodies, and VLP vaccinations can protect against different diseases. Therefore, VLPs would be useful as a tool to deliver different antigens to vaccinate purposes.

Keywords: VLP, dendritic cells, CD86, IL-10, TNFα

(1216) **MICROBIOTA, IGG AND CD64⁺ MACROPHAGES: THEIR INTERACTIONS IN THE COLON LAMINA PROPRIA**

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Intestinal macrophages (MΦs) identified by CD11b, F4/80 and CD64 markers play homeostatic roles in the colonic environment. To evaluate frequency and localization of these cells, whole mount colon sections were stained with fluorescent anti-CD64 and anti-CD11b antibodies. As these MΦs can uptake immune complexes (ICs), we used ex vivo and in vitro strategies to evaluate the uptake of IgG-ICs and stimulatory activity during inflammatory conditions. Ex vivo, we obtained colonic lamina propria MΦs from control and colitis mice by enzymatic digestion and cell sorting using the F4/80 marker. MΦs were stimulated 1 h at 37°C with ICs and the internalized IgG was evaluated by Western blot. In vitro, bone marrow-derived MΦs were cultured with conditioned medium obtained out of colonic explant cultures from control or colitis mice; in the later condition a significant increment in Ly6c and F4/80 expression was found ($p<0.05$), possibly due to the inflammatory mediators released by colitis tissue. When these MΦs were stimulated with IgG-ICs, we found a significant decrease in geometric mean fluorescence intensity for CD64 ($p<0.05$), Ly6c ($p<0.05$) and F4/80 ($p=0.008$) either with conditioned medium from control or colitis mice. As we previously found that the opsonization of fecal bacteria increases significantly during colitis development, bone marrow-derived MΦs were stimulated for 48 h with fecal bacteria opsonized with IgG and IgA and sorted from control or colitis mice to evaluate the proinflammatory potential of luminal ICs; NO, IL-6 and IL-10 were evaluated in supernatants by colorimetric assays. Our results show that the CD64⁺ CD11b⁺ MΦs represent an abundant population in the colonic mucosa, localized around the cripts in narrow contact with the epithelial layer. Inflammatory environment modulated the phenotype of MΦs meanwhile the ICs down-modulated mainly CD64 and F4/80, suggesting a crosstalk between these molecules.

Keywords: MΦs, colonic mucosa, IgG-ICs, microbiota.

(1395) **INTERACTION OF BACULOVIRUS WITH MAMMALIAN FIBROBLASTIC CELLS**

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The baculovirus Autographa californica nuclear polyhedrosis virus (AcNPV) is a dsDNA virus that is pathogenic to insects. Baculoviruses were shown to be capable of entering into various mammalian cells without completing their full infectious cycle. Some research groups have described the immunomodulation and adjuvant properties of baculoviruses in mammals and poultry. In view of the fact that they induce the expression of type I IFNs through a TLR independent pathway in non-immune mammalian cells, we propose to study the role of cytoplasmic sensing of baculoviral nucleic acids in these cells. Understanding these mechanisms would help us to design strategies that impact in this response and then evaluate its use as nonspecific antiviral. First, to elucidate the main baculoviral genomic destination, recombinant baculovirus were generated and the expression of different report genes in nucleus and cytoplasm were analyzed. Our results showed that in MEF, L929, NHI 3T3 and BHK-21 cells the main viral genomic destination was the cytoplasm. Then, RT-qPCR analyses revealed that infection with AcNPV at 4 hpi produced substantial amounts of IFN-β mRNA expression in MEF and NHI 3T3 (400 and 3200 fold change, respectively; $p<0.05$) and resulted in 60% of protection of VSV cytopatic effect. Cytoplasmic RNA polymerase III (Pol III) transcribes AT-rich dsDNA into RIG-I and MDA5 ligands that stimulate type I IFN responses. When cells were treated with a Pol III inhibitor, the level of IFN-β mRNA and the antiviral activity were not modified. The cGAS-STING pathway triggers the transcription of IFNs in response to dsDNA. Baculovirus infection of HEK293T cells, a cell line that do not express cGAS-STING molecules, did not influence VSV replication. In conclusion, the baculoviral genome, mostly available in the cytoplasm of non-immune cells, produces an increase in the expression of the INF-β gene, independently of the Pol III pathway and dependently of STING sensor.

ONCOLOGY-ONCOIMMUNOLOGY 16

(469) **ROLE OF ANTITUMOR IMMUNITY ON CHRONIC STRESS ENHANCEMENT OF SPONTANEOUS METASTASIS OF EL4 LYMPHOMA IN MICE. EFFECT OF FLUOXETINE AND SERTRALINE TREATMENT.**

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Many clinical studies have demonstrated that stress can promote the dissemination of tumor cells. Moreover, antidepressants such as fluoxetine (F) and sertraline (S) are able to modulate immune system and thus, tumor prognosis. We have previously reported that both F and S are able to prevent chronic stress enhancement of spontaneous metastasis in a murine model of lymphoma. In this context, our aim was to study the effects of chronic stress and of antidepressant treatment on tumor invasion taking into account the role of anti-tumor immunity. For this purpose, female C57BL/6 mice were treated with F or S and subjected (E) or not (C) to a heterotrophic chronic stress for five week and subcutaneously injected with EL4 cells to generate a solid tumor. Specific cytotoxic activity against tumor cells was evaluated co-culturing spleen cells suspensions from tumor bearing mice with labeled EL4 cells. The percentage of EL4 cell lysis was significantly lower when incubated with splenocytes of E mice compared to C mice ($P<0.01$). F and S treatment prevented this effect. To further asses the involvement of immune system in tumor invasion induced by chronic stress, irradiated animals were transferred with lymphoid cells of C and E animals treated or not with F and S and then inoculated with EL4 cells. The assessment of spontaneous metastasis indicated that animals transferred with lymphoid cells of E animals have a higher incidence of liver metastasis ($P=0.023$) and a major number of liver metastatic nodules respect to animals transferred with cell from C animals ($P=0.027$). In addition, tumor in animals transferred with cell from E mice that received F or S administration have similar biological behavior than those of C animals. Our results indicate the important role of immune system in the stress induced stronger tumor invasion.

Chronic Stress – Tumor Invasion – Fluoxetine – Sertraline – Antitumor Immunity

(979) **SUPERANTIGEN INDUCE EXTRINSIC AND INTRINSIC APOPTOSIS PATHWAY IN HUMAN NEOPLASTIC T CELLS**

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Superantigens (Sags) are bacterial and virus protein that share the ability to activate large number of T-cells. Sags bind to major histocompatibility complex (MHC) class II molecules as unprocessed proteins and interact with T cells expressing particular T-cell receptor (TCR) Vβ chains. Sags activated T-cells after several rounds of proliferation. We previously demonstrated that T-cell Sags induced apoptosis of cognate malignant T cells. We have shown that both bacterial- and mouse mammary tumour virus (MMTV)-encoded Sags induce apoptosis of different murine-cognate lymphoma T cells *in vitro* and *in vivo*, being the Fas/Fas-L pathway involved. *In vivo* exposure to bacterial T Sags increased the survival

of lymphoma-bearing mice. We also observed that Sags induce apoptosis in cells of the Jurkat-established human cell line from an acute T-carrier leukemia of the Vβ8 region in TCR. We proposed to analyze those mechanisms involved in apoptosis of Jurkat cells induced by Sag. We co-cultivated Jurkat cells with THP1 cell line (as antigen presenting cells). Then we treated them with or without different Sags (specific or not for the TCR Vβ chain) at different time points. We observed an increase in Fas-L expression analyzed by PCR and flow cytometer ($p<0.001$) suggesting that Sags activate the extrinsic pathway of apoptosis. Moreover, an increase in mitochondrial membrane potential (ΔΨm) (measured by the percentage of $DiOC_2(3)^{low}$ Jurkat cells exposed to a specific Sag) revealed the involvement of the intrinsic pathway ($p<0.001$). Also we observed an increase of truncated Bid protein (Bid-t) by western blot in Jurkat cells treated with specific Sag ($p<0.05$). This protein linked the extrinsic with intrinsic apoptosis pathway. Together, these data suggest that these two pathways are involved in Sag-induced apoptosis in Jurkat cells. The possibility of a therapeutic use of Sags in lymphoma/leukemia T cell malignancies is discussed.

Keywords: Superantigens, Apoptosis, leukemia, Jurkat

(1696) **TUMOR-EXPERIENCED NK CELLS INHIBIT T CELL PROLIFERATION AND ACTIVATION THROUGH PD-L1**

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Classical functions of Natural Killer (NK) cells include the elimination of tumor and virus-infected cells, however, novel reports show a regulatory role for NK cells in different models of autoimmunity and viral infections. Our previous results demonstrated that NK cells from tumor bearing mice express the inhibitory molecule PD-L1 and control the magnitude of $CD8^+$ T cell priming to tumor antigens *in vivo*. Moreover, we also observed that PD-L1 expression is up-regulated on human NK cells upon tumor recognition, but little is known about the function of human PD-$L1^{hi}$ NK cells. Thus, the objective of this work was to further study the phenotype and activity of tumor-experienced human PD-$L1^{hi}$ NK cells and their possible immunoregulatory role. To this end, we cultured peripheral blood mononuclear cells (PBMC) from healthy human donors with K562 tumor cells for 48 h and we evaluated the expression of the activation markers CD25 and CD69, and the effector molecules TRAIL, FasL, CD107a and IFN-γ on PD-$L1^{hi}$ and PD-$L1^{-/low}$ NK cells ($CD56^+CD3^-$) by flow cytometry. We observed that all markers were preferentially expressed in PD-$L1^{hi}$ NK cells ($p<0.05$ for all markers). To evaluate the immunosuppressive potential of these NK cells, CFSE-labeled autologous T cells were stimulated with anti-CD3/anti-CD28 antibodies (Ab) and cultured in the absence or in the presence of sorted tumor-experienced NK cells or control NK cells, and in the absence or in the presence of anti-PD-L1 blocking Ab. After 5 days, T cell proliferation and activation (evaluated as CFSE dilution and CD25 expression by flow cytometry) were diminished in the presence of tumor-experienced NK cells compared to control NK cells, and this inhibition was reverted by PD-L1 blockade. These results show that tumor-induced PD-$L1^{hi}$ NK cells exhibit an activated phenotype and increased effector functions in response to tumor cells, however these NK cells can inhibit autologous T cell proliferation and activation trough PD-L1.

Keywords: PD-L1, NK cells, T cells, tumor

(845) **THE THYROID STATUS MODULATES THE COMPOSITION OF TUMOR MICROENVIRONMENT AND THE ANTITUMOR IMMUNE RESPONSE IN A 4T1 MURINE BREAST CANCER MODEL**

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Several findings suggest that the patient's hormonal context plays a crucial role in determining the outcome of cancer. However, very little is known about the nature of thyroid hormone action on tumor growth. Our aim was to evaluate the effect of thyroid status on the immunity and microenvironment of breast cancer. For this, Balb/c mice were orthotopically inoculated with 4T1 breast carcinoma cells after the treatment with thyroxine (12mg/l) for 30 days or propylthiouracil (500mg/l) for 15 days in the drinking water to obtain hyperthyroid (hyper) or hypothyroid (hypo) mice, respectively. An increased tumor growth rate was seen in hyper mice compared to controls ($p<0.05$), while hypo mice bared tumors with reduced volume ($p<0.05$), but developed a greater number of lung metastases ($p<0.05$). The analysis of immune subsets indicated that hyper tumors presented a decreased immune cell infiltration ($p<0.05$), with a reduced percentage of activated $CD4^+$ and $CD8^+$ T cells ($p<0.05$). Tumor draining lymph nodes (TDLN) of hyper mice also showed a reduced number of activated $CD8^+$ T cells ($p<0.01$) and NK cells ($p<0.01$), but increased percentage of activated $CD4^+$ T cells ($p<0.001$). Additionally, a decreased number ($p<0.01$) and activity ($p<0.05$) of NK cells was detected in hyper spleens, accompanied by increased percentages of MDSC ($p<0.05$). On the other hand, hypo mice showed a higher percentage of activated $CD8^+$ tumor infiltrating T cells ($p<0.05$), but a decreased number of these cells in TDLN ($p<0.05$). To evaluate the effect of thyroid status on the migration of mesenchymal stromal cells (MSCs) to 4T1 tumors, MSC were inoculated in the tail vein of tumor-bearing mice and analyzed by *in vivo* imaging. Hyper tumors and lungs showed a decreased presence of MSC compared to control and hypo mice ($p<0.05$). Our results suggest that the thyroid status modulates the antitumor immune responses and the migration of MSC to the 4T1 tumors, thus modifying tumor growth and metastasis formation.

Keywords: hyperthyroidism, hypothyroidism, breast cancer, mesenchymal stromal cells, antitumor immunity.

(1362) **THERAPEUTIC BLOCKADE OF FOXP3 USING GENE THERAPY VECTORS**

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Our previous results indicate that systemic administration of a cell penetrating peptide (P60) that inhibits Foxp3, a transcription factor required for Treg function, improves the efficacy of antitumor vaccines in experimental breast cancer. Although there is controversy over the role of Foxp3 in tumor cells, we found that P60 inhibits survival and release of IL-10 in $Foxp3^+$ breast tumor cells. Here we aimed to evaluate the regulatory pathways that control Foxp3 expression in LM3 breast tumor cells and to develop gene therapy vectors encoding P60. We assessed the effect of recombinant TGF-β, mTOR inhibitor rapamycin and COX-2 inhibitor indomethacin, all of which modulate Foxp3 expression in Tregs. Stimulation of LM3 cells with TGF-β and rapamicyn upregulated Foxp3 expression ($p<0.05$) as assessed by flow cytometry, whereas indomethacin inhibited Foxp3 expression ($p<0.05$), suggesting that the regulatory mechanisms of Foxp3 expression are similar in tumor cells and Tregs. We next developed a plasmid that encodes P60 linked to dTomato as a reporter gene (pCMV.P60.dTomato) and a control plasmid. Transduction efficiency of these plasmids was evaluated in 4T1 cells, which exhibit low expression of Foxp3 and thus are not affected by P60. Expression of d.Tomato was readily detected by fluorescence microscopy. Conditioned media from pCMV.P60.dTomato-transfected 4T1 cells

reduced the proliferation and the secretion of IL-10 in Foxp3$^+$ LM3 cells when compared to control plasmid-transfected cells ($p<0.05$). In order to develop a gene therapeutic strategy to deliver P60 *in vivo*, we developed an adenoviral vector encoding the therapeutic cassette (Ad.P60.dTomato), as well as a control vector (Ad.dTomato). These vectors successfully transduced 4T1 cells, as evaluated by dTomato expression. Our findings indicate that P60 could be delivered using gene therapy vectors, which could be useful to improve the survival of experimental breast cancer models.

Keywords: Foxp3, IL-10, breast cancer, gene therapy.

(540) **FUCOSYLATED CLUSTERIN PRESENT ON HUMAN BREAST CANCER MODULATES THE FUNCTION OF DC-SIGN EXPRESSING MACROPHAGES**

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Clusterin (CLU) is a ubiquitous glycoprotein up regulated in many types of cancer. We have previously described a novel fucosilated clusterin glycoform, present in semen, able to bind to DC-SIGN, a C type lectin receptor present on dendritic cells (DCs) and macrophages. The interaction of fucosylated clusterin with dendritic cells promotes the endocytosis of misfolded extracellular proteins and modulates the function of DCs favoring the acquisition of a tolerogenic profile. Numerous studies have shown that glycosylation changes are associated with the development of cancer. The aim of this study was to analyze properties of clusterin produced by breast cancer. We hypothesize that breast cancer clusterin might bear fucosylated glycans and bind to DC-SIGN, inhibiting the immune response.

The presence and properties of CLU were analyzed on human luminal A breast cancer samples, and non-invaded breast tissue was used as control. The concentration of total CLU didn´t show significant differences between tumor and "healthy" tissues (n=21, p=0.3105). The presence of fucosylated clusterin was analyzed by an ELISA based assay, using the fucose binding lectin ulex europeaus. The amount of fucosylated clusterin was higher on tumor than helthy-tissue (n=21 p=0.0071). Moreover, tumor-CLU was shown to bind DC-SIGN when analyzed by western-blot while CLU from healthy tissue did not (n=5). We also demonstrate the presence of DC-SIGN+ macrophages on tumor samples by either RNAseq and flow cytometry (n=9-5). Furthermore, we show that fucosylated clusterin binds to monocyte derived macrophages and inhibits the upregulation of activation markers upon LPS stimulation (HLA-DR n=5 $p<0.05$, CD86 n=4 $p<0.05$, CD40 n=3 $p<0.05$). These results show that breast tumor-CLU bears fucosylated glycans and binds to DC-SIGN, inhibiting macrophage activation.

Keywords: Breast Cancer Clusterin Macrophage

(59) **REVISITING THE ROLE OF INTERLEUKIN-8 IN CHRONIC LYMPHOCYTIC LEUKEMIA**

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Abstract: The proliferation and survival of malignant B cells in chronic lymphocytic leukemia (CLL) depend on signals from the microenvironment in lymphoid tissues. Among a plethora of soluble factors, IL-8 has been considered one of the most relevant to support CLL B cell progression in an autocrine fashion, even though the expression of IL-8 receptors, CXCR1 and CXCR2, on leukemic B cells has not been reported. The purpose of this work was to re-examine the role of IL-8 in CLL evaluating the expression of IL-8 receptors in leukemic cells and their capacity to produce IL-8. To this aim we studied samples from patients at different stages of the disease and belonging to different risk groups according to IgVH mutational status, CD38 and CD49d expression levels. Our results show that CLL B cells, resting or activated through their B cell receptor, do not express CXCR1 or CXCR2 (analysis was performed by flow cytometry using neutrophils as positive control, n=39). In accordance to these, CLL cells did not show increased cell survival in response to exogenous IL-8 when cultured *in vitro* alone or in the presence of monocytes/nurse like cells (n=9). We next determined if CLL B cells were able to produce IL-8. To this end, peripheral blood mononuclear cells (PBMC) were activated with anti-IgM plus CD40 ligand for 24-72 hs and IL-8 production was assessed by flow cytometry (n=17). We found that CLL B cells (CD19$^+$) do not produce IL-8 spontaneously or upon activation, while monocytes (CD14$^+$ cells) did. Therefore, we compared by ELISA, the capacity of PBMC and highly purified CLL cells to release IL-8. We found that a minor proportion of monocytes (PBMC: 0.82% versus purified CLL-B cells: 0.03%) was responsible for IL-8 levels in supernatants (1.178 ± 544 pg/ml versus 42 ± 20 pg/ml, n=13 $p<0.001$). Altogether our results indicate that CLL B cells are not able to secrete or respond directly to IL-8 and highlight the importance of methodological details in *in vitro* experiments.

Keywords: IL-8, chronic lymphocytic leukemia, CXCR1, CXCR2, monocytes, microenvironment

(451) **ABSCOPAL EFFECT OF BORON NEUTRON CAPTURE THERAPY (BNCT) COMBINED WITH BCG AS IMMUNOTHERAPY**

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The abscopal effect refers to the inhibitory action of radiotherapy on tumor growth at a site distant from the area of irradiation, mediated by radiation-induced immune responses. We demonstrated the abscopal effect of BNCT that combines selective tumor uptake of ^{10}B compounds and neutron irradiation. However, coupling irradiation with immunotherapy could amplify the radiation-induced immune response to elicit a robust abscopal effect. The aim of the present study was to evaluate the abscopal effect of BNCT combined with BCG (Bacillus Calmette-Guerin) as immunotherapy. BDIX rats were inoculated sc with $1x10^6$ DHD/K12/TRb syngeneic colon cancer cells in the right hind flank. Four weeks post-inoculation, tumor-bearing rats were injected with borono-phenyl-alanine (BPA) iv and the right leg bearing the tumor nodule was locally irradiated at RA-3 at an absorbed dose of 7.8 Gy to skin (BNCT-group). Tumor-bearing rats were treated similarly but injected with three intratumoral doses of BCG (0.2 mg/0.1 ml, viability $6x10^5$ CFU) 1, 7, and 14 days post BNCT (BNCT+BCG-group). Tumor bearing rats were treated with BCG only (BCG-group). The Sham-group consisted of untreated tumor bearing animals exposed. Two weeks post-BNCT, $1x10^6$ DHD/K12/TRb cells were injected sc in the contralateral left hind flank in all BDIX rats. Inhibition of tumor growth in the untreated left leg vs Sham-group was considered indicative of an abscopal effect. Once weekly for 7 weeks post BNCT the tumor volume (TV) was measured in both legs. Animal experiments were approved by IACUC. The BCG-group and the BNCT-group exhibited an abscopal effect that did not reach statistical significance. However, when both treatments were combined in the BNCT+BCG-group, a robust abscopal effect was observed vs the Sham-group (p=0.007), i.e. left leg TV 41 ± 58 mm^3 vs 156 ± 52 mm^3 respectively. The present study would demonstrate an abscopal effect induced by BNCT combined with BCG.

Keywords: BNCT; BCG; Abscopal Effect; Immunotherapy; DHD/K12/TRb cells

(629) **SEC22B-MEDIATED CROSS-PRESENTATION IS REQUIRED FOR ANTITUMOR IMMUNITY AND POSITIVE IMMUNOTHERAPY OUTCOME**

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CD8⁺ T cells mediate antigen-specific immune responses that can induce rejection of solid tumors. In this process, dendritic cells (DCs) are thought to take up tumor antigens, which are processed into peptides and loaded onto MHC-I molecules, a process called "cross-presentation". Neither the actual contribution of cross-presentation to anti-tumor immune responses, nor the intracellular pathways involved *in vivo*, are clearly established due to lack of experimental tools to manipulate this process. We aimed to develop such tools, by generating mice bearing a conditional DC-specific mutation in the *sec22b* gene, a critical regulator of ER-phagosome traffic required for cross-presentation *in vitro*. Bone Marrow DCs generated from these mice show impaired cross-presentation *in vitro*, but unaltered antigen presentation by the classical MHC-I and MHC-II pathways. In addition, the cross-presentation capacity of DCs isolated from spleen is reduced *ex vivo* and, as a consequence, defective cross-priming of CD8⁺ T cell responses *in vivo* is evident when immunizing with dying cells. These mice are also defective for anti-EG7 tumor immune responses, and resistant to treatment with anti-PD-1 when using the tumor cell line MCA101. Moreover, CD8⁺ T cells are equally functional in wild-type and silenced mice. These findings suggest that Sec22b-dependent cross-presentation in DCs is required to initiate CD8⁺ T cell responses to dead cells and to induce effective anti-tumor immune responses during anti-PD-1 treatment in mice.

Keywords: Dendritic cells, Immunotherapy, CD8⁺ T cells, Cross-presentation

PLANT BIOLOGY 4

(783) DECIPHERING THE MOLECULAR ROLES OF IQD28 AND IQD30 PROTEINS OF *ARABIDOPSIS THALIANA.*

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Plant-specific IQ67 domain (IQD) members are part of an emerging family of calmodulin binding proteins. Although systematic characterization of this family has been carried out in various plant species, little is known about their biological roles. There are 33 IQD members distributed in four different classes in *Arabidopsis thaliana*. We have shown that IQD28 and -30 *in vitro* interact with calmodulins in a calcium dependent and independent form. In addition, we have observed that their transcriptional levels are increased in roots in response to osmotic stress and drought. In this study, we have advanced in the characterization of two class IV proteins of Arabidopsis, IQD28 and -30. In order to identify specific ligands of these proteins, we carried out pull down assays followed by mass spectrometry. For this purpose both recombinant proteins were immobilized on affinity Ni^{2+}-agarose columns, due to the presence of the histidine tag in its amino acid sequence. Subsequently, protein extracts from adult roots of Arabidopsis plants were loaded to these columns. After several washes, bound proteins were eluted, precipitated with trichloroacetic and analyzed by MALDI TOF TOF. Identified proteins are predicted to be located at different cellular compartments, such as, membrane, mitochondria, chloroplast, reticular and cytoskeletal components. Interestingly, the most enriched cellular functions among the identified ligands are related to structural components, trafficking and cellular transport. These evidences might suggest a putative role of the IQDs as scaffold proteins. According to this, IQDs might be able to interact with multiple members of signaling pathways and recruit them to the components of the cytoskeleton. Thus, they could regulate the transduction of signals and assist the localization of cellular components in specific subcellular areas in response to various stimuli.

Keywords: IQD proteins, scaffold functions, *Arabidopsis thaliana.*

(855) DIFFERENT WATER MANAGEMENT STRATEGIES OF TWO *Sorghum bicolor* PARENTAL LINES EXPOSED TO SALT STRESS

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Sorghum [*Sorghum bicolor* (L.) Moench.] is known as a tolerant crop with capacity to grow and sustain high yields in unfavorable environmental conditions. Dissecting the physiological traits involved in its water adjustment capacity in early developmental stages could improve our understanding in water use efficiency. In previous work, we characterized the differential response to water deficit of two parental lines from Argentina (RedLandB2 and IS9530; Sutka *et al*., 2016). Here we extend our study to salt stress to analyze the water adjustment capacity of these two genotypes at the whole plant level. Plants were grown in hydroponic conditions for 7 days and then submitted to NaCl (0 up to 300 mM) for 4 hours. Al the leaf level, relative water content is only affected in IS9530 being statistically different at the maximum salt concentration. Interestingly, the osmotic potential component of leaf water status showed a strong robustness, no differences were observed between genotypes along treatment. In contrast, leaf water potential values differ between genotypes (-0.16±0.016 and -0.28±0.031 MPa for RedLandB2 and IS9530 respectively). Stomatal conductance was coupled to root hydraulic behavior in RedLandB2 where remained invariant along treatments. In IS9530, the profile showed a decreased to 50% of the control value.

At the root level, hydraulic conductivity (L_{pr}) maintains in RedLandB2 independently of the salt treatment while IS9530 showed a different oscillatory pattern, decreased its L_{pr} at 200 mM NaCl but recovered the control value at 300 mM NaCl (14.4±2.8 versus 57.8±7.1 mL g^{-1} h^{-1} Mpa^{-1}).

In the absence of a transpiration force, root exudate fluxes showed similar reduction in both genotypes but only IS9530 showed a reduced osmolarity difference between the medium and the exudate. In conclusion, all water parameters traits showed that these two lines of sorghum are able to cope with salt stress using different strategies to adjust water management.

(1255) *FITNESS* TRANSCRIPTION FACTOR ACTS AS A NODE IN ABIOTIC AND BIOTIC STRESS RELATED PROCESSES

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The transcription factor *FITNESS* is highly repressed by oxidative stress. Knock-out lines (*fitness*) showed higher tolerance to oxidative stress generated in the chloroplast and to salt stress due to decreased levels of ROS. When analyzed at metabolic level *fitness* lines showed an increment of salicylic acid (SA). In plants, the major route of SA synthesis is the isochorismate (IC) synthase pathway. *ICS1* transcripts were significantly increased in *fitness* mutants. SA accumulation is maintained through transcriptional regulation of EDS1 (ENHANCED DISEASE SUSCEPTIBILITY 1) and *PAD4* (PHYTOALEXIN DEFICIENT 4) and also rise of endogenous SA correlates with pathogenesis-related genes induction. Transcriptional reprogramming is controlled mainly by *NPR1* (non expressor of pathogenesis-related gene 1). Monomerization and nuclear accumulation of *NPR1* in the presence of SA results in PR1 increased expression and is therefore considered a marker for activation of salicylate-signalling pathway. Consistent with the increased levels of SA measured in *fitness* mutants we observed an induction of both *NPR1* and *PR1* transcript levels. The finding that *PAD4* and *EDS1* transcripts are both up-regulated in *fitness* mutants confirms that the ROS perturbation leads to modification of hormonal homeostasis. Our data indicate that altered expression of *FITNESS* acts modulating SA-related processes: *fitness* lines show a high transcriptional up-regulation of the SA-responsive marker gene PR1, whose

expression is NPR1-dependent. Also, *fitness* lines resulted less sensitive to *Pseudomona syringae* pv. *tomato* DC 3000 infection. Therefore, we postulate that *FITNESS* acts as a node regulating signal transduction pathways in response to both abiotic and biotic stresses.

ROS- salicylic acid- abiotic stress-biotic stress

(1899) **GERMINATION UNDER STRESS CONDITIONS: THE POTENCIAL ROLE OF BBX24**

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The B-Box family of transcription factors consisted in 32 members (BBX1-BBX32) that play diverse functions in plant development. BBX transcription factors participate in germination, flowering, photomorphogenesis, shade avoidance syndrome, etc. Under stress conditions, BBX21 promotes the germination of *Arabidopsis thaliana* seeds through the abscisic acid (ABA) pathway inhibiting HY5 activity and preventing ABI5 expression (Xu et al., 2014). However, it is hardly known if other BBX proteins are involved in the regulation of germination under suboptimal conditions. The objective of this work is to investigate the role of BBX24 during germination under stress conditions. Wild-type (WT, Columbia) and *bbx24* mutant seeds were sowed in agar medium with ABA, sodium chloride and polyethylene glycol. After chillling for three days, the boxes with seeds were exposed to white light/dark daily cycles of 12/12 h. Germination was recorded every 48 hours. Gene expression was evaluated using QRT-PCR technique in control and ABA-treated seeds exposed for 12 h in white light. However, both genotypes germinated at the same rates in water, *bbx24* seeds germinated significantly lower than WT in ABA, sodium chloride and polyethylene glycol. We found a significant increase in the expression of genes related to ABA signaling pathway in *bbx24* seeds with respect to WT. We conclude that BBX24 can promote germination under stress conditions through ABA signaling pathway.

Keywords: B-box, germination, abscisic acid, stress conditions.

(742) **IDENTIFICATION OF TARGET GENES IN NITROGEN CONDITIONS IN RICE AND ARABIDOPSIS**

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Plants are sessile and therefore they are forced to adapt to environments with different stress such as cold, salinity, heavy metals, drought and nutrient deficiency essential for their growth and development. The genes that are induced in these adverse conditions not only protect the cells by producing metabolic proteins but also regulating the expression of other “master” genes, such as transcription factors, in response to these stresses.

A MYB-like transcription factor (TF) family known as *hypersensitive to low Pi-elicited primary root shortening 1*, has seven members which, two of those were recently described to be involved in nitrogen and/or phosphorous regulation. Interestingly, four members of this TF family were found to be involved in mediated nitrogen regulation when plants were treated for two hours with nitrate and ammonium. In these work, we analyzed the potential share targets under nitrogen conditions of these nitrogen regulated members in *Arabidopsis thaliana* and *Oryza sativa*. We also predicted a conserved motif sequence by statistical analysis of their targets genes through bioinformatic studies. These results will allow us to understand the regulatory mechanisms of nitrogen assimilation in these two model species.

Palabras claves: transcription factor, rice, Arabidopsis, nitrogen

(693) **GROWTH MODIFICATIONS AND OXIDATIVE STRESS IN SEEDLINGS OF DIFFERENT MAIZE HYBRIDS SUBJECTED TO CADMIUM AND COPPER STRESS**

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Maize is one of the most important agronomical crops. It is used in the human diet and it is considered an interesting source for the production of biofuels. Since early seedling growth is critical in plant life cycle, the effect of Cd (a redox inactive metal) and Cu (a redox active metal) on growth parameters of 10 commercial *Zea mays* L. hybrids (labelled H1 to H10) was analyzed at this stage. Maize seeds were germinated on filter paper in plastic dishes containing distilled water. Seedlings of a comparable size with roots of 1-2 cm length were selected and transferred to hydroponic culture without (control, C) or containing an aqueous solution of 100 μM $CdCl_2$ or $CuCl_2$. They were grown in a controlled climate room at 24±2°C in darkness during 72 h. For statistical analysis of growth parameters, a hierarchical model factorial design was applied, taking “H” as factor A, and “treatments (C, Cd, Cu)” as factor B nested in A. Despite metals significantly decreased root and coleoptile length, fresh and dry weight, no statistically significant interaction was detected between genotypes and metals treatment. Metals increased oxidative protein damage in roots and decreased transcript accumulation of the gene family from the enzyme methionine sulfoxide reductase (MSR): ZmMSRA4, ZmMSRA5.1, ZmMSRB1, ZmMSRB5.1 in the root apex. Further analysis in root apex of H10 showed that protein conjugation pattern with SUMO and ubiquitin were modified during metal treatment. In addition to previous data obtained in our laboratory, the present results indicate that the oxidative posttranslational modifications (PTM) produced during abiotic stress could be a nexus between stress recognition and growth reprogramming. Despite further studies are needed, we propose that this could be an interesting mechanism where oxidative PTM regulates another PTMs like sumoylation and ubiquitination as part of the signalling process during abiotic stress.

Keywords: Maize, Abiotic Stress, Metals, Oxidative posttranslational modification

(1369) **EXPLORING THE BALANCE BETWEEN WATER UPTAKE AND TRANSPIRATION IN *ARABIDOPSIS THALIANA* WITH GENETICALLY ALTERED STOMATAL DENSITY**

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A balance between water loss by the shoot through transpiration and the capacity for water transport by the root is essential to the coordinated growth and development of plants and also to the plant´s appropriate response to environmental perturbations. Aquaporins have been suggested as dynamic regulators of root hydraulic conductivity to match the transpirational demand of the leaves. To understand the mechanistic aspects of such regulation we are using *Arabidopsis thaliana* lines which show altered transpiration rates due to genetic manipulation of their stomatal densities, which range from 50% to 200% of the values shown by the wild type line (Col 0) and which result in matching variations in transpiration rates (Dow *et al.* 2013, New Phytol 201: 1218-1226). We developed a growth protocol for these lines consisting in germination in vertical plates of solid 0.5X MS medium and transfer of two-week old seedlings to hydroponic conditions for a further two-week growth in a temperature controlled chamber, with a photoperiod of 12 hours. The *epf1* and *epf1 epf2* lines showed plant morphology, rosette areas and growth rates similar to those of the wild type. The mutant *epf1* presented a stomatal density 52% higher than that of the wild type and a 47% higher whole-rosette transpiration; while the double mutant *epf1 epf2*, presented a stomatal density 84% higher than that of the wild type and a similarly high (82%) whole-rosette transpiration rate (all data $p \leq 0.01$). Our next step includes characterizing the root hydraulics of

the mutant lines with differential stomatal density, with the aim of delineating the aquaporin-dependent and -independent paths of water transport and their modulation by the driving force of transpiration.

(362) **HEAT TREATMENT APPLIED PRIOR TO COLD STORAGE RESTORED CELL WALL METABOLISM IN 'DIXILAND' PEACH FRUITS**

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Abstract: Peaches are highly perishable and deteriorate quickly at ambient temperature. Cold storage is commonly used to prevent fruit decay; however, it affects fruit quality causing physiological disorders collectively termed "chilling injury" (CI). To prevent or ameliorate CI, heat treatment (HT) is often applied prior to cold storage. In this work, we analyzed ethylene production and the expression of enzymes and proteins involved in cell wall metabolism in 'Dixiland' peach fruits exposed to 39 °C, cold stored, or after a combined treatment of heat and cold, and were compared with fruits ripening at 20 °C. For the group of fruit kept at 20 ºC, an increase in ethylene production was observed, meanwhile cell wall-related genes showed different expression patterns. After HT (3 d at 39 ºC), fruit maintained their firmness, exhibiting low *PpACO1* level and significant lower levels of the twelve cell wall-modifying genes. Once fruits were transferred to 20ºC after HT, an increase in gene expression occurred to different extent depending on the transcript analyzed, except in the case of *PpPME1*. Cold storage for 3 and 5 d at 0 ºC (R3 and R5) prevented softening, the increase in the levels of *PpACO1*, and the accumulation of transcripts encoding for cell wall-related genes. Fruits subjected to both heat and cold treatments (HT+R2) showed very low ethylene production and expression of enzymes and proteins involved in cell wall metabolism; however, when fruits were transferred to 20 ºC for 2 d (HT+R2+2), softening and the levels of *PpACO1* and cell wall-modifying transcripts were similar or even higher than fruits of the same post-harvest age maintained at ambient temperature. Overall, our results indicate that when HT is applied prior to cold storage, the expression of enzymes and proteins involved in cell wall metabolism is recovered following return to ambient temperature, in contrast to R3+2 and R5+2 samples, revealing the benefits of applying a HT prior to refrigeration.

Keywords: chilling injury, heat treatment, peach, postharvest, *Prunus persica*

(796) **MITOCHONDRIAL FUNTIONALITY AND ROS PRODUCTION ARE COMPROMISED IN *Arabidopsis thaliana invh* MUTANTS**

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Sucrose (Suc) plays a central role in plant growth and development, and in response to environmental stresses. Alkaline/Neutral-Invertases (A/N-Invs) (localized in the cytosol, mitochondria and/or plastids) are sucrose hydrolyzing enzymes with optimal pH between 6.8 and 8.0. Currently, there are many questions about the role of the organellar isoforms. Previous studies in *A. thaliana* suggested that two mitochondrial A/N-Invs (isoforms A and C) have significant roles in root growth and development, and in abiotic stress adaptation. However, A/N-InvH is a third isoform whose role is still unknown. In the present work, we showed using fluorescence and confocal microscopy that *invh* (a mutant lacking A/N-InvH*)* exhibits lower mitochondrial membrane potential than wild-type plants. Moreover, ROS production in *invh* roots is impaired not only in salt and ABA treated plants but also in control conditions when are compared with wild-type plants. Taking together our data, we conclude that A/N-InvH plays a relevant role in mitochondrial functional status and in ROS production either under control or stress conditions. Supported by CONICET, ANPCyT (PICT 1288), UNMdP (EXA645) and FIBA.

Keywords: Sucrose, Alkaline/Neutral-Invertases, ROS, Mitochondria

(1633) **PLANT GROWTH PROMOTION ASSAYS IN *EUCALYPTUS GRANDIS* MEDIATED BY BENEFICIAL BACTERIA.**

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Eucalyptus grandis is one of the most commercially important forest tree species in Argentina. Outplanting of greenhouse-grown *E. grandis* seedlings to the field is a critical step, since the availability of water and nutrients in the immediate vicinity of the rhizosphere determines the survival of individuals and affects the health and future performance of the plantation. Plant-growth promoting bacteria are used in many crops to improve nutrition and tolerance to abiotic stress, however their use in trees is very limited, in part due to difficulties in application. In this work we have used a superabsorbent hydrophylic polymer (used in forestry to alleviate water stress during seedling establishment) as a carrier for plant growth-promoting bacteria. A collection of bacterial leaf endophytes was screened for their ability to promote *E. grandis* germination and initial seedling growth. Four promising strains were further tested for their capacity to alleviate nutrient deficiency (phosphate deficiency or a sequential decrease in added fertilizer) in two-month old *E. grandis* seedlings grown in environmentally controlled chambers. The seedlings were inoculated by transplanting them to pots containing substrate together with superabsorbent polymer, which had been previously mixed with the selected plant-growth promoting bacterial strains. Preliminary results show that all four strains improved plant height and stem diameter, both with and without nutrient deprivation. One of these strains, which was identified as a *Pseudomonas fluorescens,* significantly reduced leaf stomatal conductance (one-way ANOVA, $p<0,05$) without affecting photosynthesis, which implies that it improves the water use efficiency of the plant. These results show that: 1) The selected plant growth-promoting bacteria can improve seedling growth after transplantation and 2) that the superabsorbent polymer can be used as a vehicle to apply bacteria to tree seedling roots during transplantation.

Keywords: PGP bacteria, superabsorbent polymer, *Eucalyptus grandis*.

NEUROC 11 / NEUROSCIENCE 11

(1130) **OBTENTION OF NATURAL MEMBRANES ENRICHED WITH GABA$_A$ RECEPTOR FROM DISEASE VECTOR INSECTS (*AEDES AEGYPTI* AND *TRIATOMA INFESTANS*)**

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γ-aminobuyric acid (GABA) is the major inhibitory neurotransmitter in the vertebrate CNS. Its specific receptor, the GABA$_A$ receptor (GABA$_A$-R), is constituted by five subunits which compose a chloride channel. In mammalian organisms there is a wide diversity of receptor subunits, while in insects this variety is considerably minor, being the so-called RDL the most representative subunit. Considering that the receptor subunit composition is critical for the pharmacological and biophysical properties of GABA$_A$-Rs, functional and structural differences between receptors from mammals and insects provide multiple opportunities for the design of more selective and non-toxic insecticides. Considering this argument, in the present work we obtained insect natural membranes as a source of GABA$_A$-Rs to be used in the search of new insecticide agents. The membranes were extracted from the head-thorax portion of

A. aegypti larvae (IV stage) or *T. infestans* nymphs (V stage), by differential centrifugation procedures. Protein electrophoretic profiles contained in the purified membranes showed bands with MW similar to those described for $GABA_A$-Rs from other insects. In addition, we also determined the presence of $GABA_A$-Rs in the samples by using radiolabeled ligands which recognize specific sites in the receptor. All these results indicate that, through the procedures used in the work, it is possible to obtain natural membranes enriched in native $GABA_A$-Rs from insects, which could be utilized for the evaluation of compounds able to block the receptor channel, with a consequent potential insecticidal activity.

Keywords: $GABA_A$ receptor, insect membranes, vector insects, insecticides

(454) **N2A IS A VERSATILE MURINE NEUROBLASTOMA CELL LINE USEFULTO EVALUATE THE *IN VITRO* NEUROPROTECTIVE AND NEURONAL PLASTICITY ROLE OF HUMAN ERYTHROPOIETIN (hEPO)**

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Neurodegenerative diseases affect the central nervous system, with an incidence that increases every year. Researchers are involved in the development of neuroprotective agents. In this sense, hEPO has a leading role because its antiapoptotic, antiinflammatory, antioxidant and cell proliferative effects have been observed in neural tissues expressing the EPO receptor. Thus, the recombinant form (rhEPO) results a potential tool for neuroprotection and neurogenesis of brain-damaged areas.

In this work, the mouse neuroblastoma cell line (N2a) was used to evaluate not only the neuroprotective but also the neuronal plasticity role of hEPO.

The neuroprotection role of CHO cell line-produced hEPO was studied by measuring the capacity of cytokine to reverse the staurosporine (STP)-induced apoptosis in N2a cells. Different cellular densities, distinct STP and rhEPO concentrations and incubation times were assayed, demonstrating that rhEPO was capable to reverse significantly the induced apoptosis in a dose-response effect ($p<0.01$ and $p<0.001$).

Simultaneously, neuritogenesis was also studied in N2a cells after treating them with 10, 50, 300 ng/ml of rhEPO. The neuritogenesis was quantified as the number of primary neurites, and the average of the neurite length and the longest neurite per cell. N2a cells treated with 50 and 300 ng/ml of rhEPO showed a significant increase in the neurite length ($p<0.05$ and $p<0.001$) and number ($p<0.05$ and $p<0.01$) compared with the control cells. In order to validate the neuronal plasticity of rhEPO a culture of rat hippocampal neurons was used. As in N2a cells, neurons treated with 50 and 300 ng/ml of rhEPO showed a significant increase in filopodia ($p<0,05$ and $p<0,001$) and synapses ($p<0.01$ and $p<0.01$) formation compared with control cells. In summary, N2a represents a versatile cell line useful to measure both the neuroprotective as the neuronal plasticity role of rhEPO aiming to find new hEPO analogues with those biological properties.

Keywords: N2A cell line, rhEPO, neuroprotective role, neuronal plasticity role, in vitro assays.

(1896) **TRANSLATING BIOLOGICAL MECHANISMS INTO COMPUTATIONAL MODELS OF THE REACTIVE GLIOSIS.**

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We developed a Bayesian Computational Model (BCM) of the reactive gliósis (RA), a typical response of the glial cells after brain injury characterized by a change in the morphology of asctrocytes in a graded maner. The mechanism by it occurred is still unclear. Experimental data showed a controversy about the mechanism of this propagation, whether the initial spatial distribution of molecules associated to damage (DAMP) was determinant or if a secondary secretion of soluble mediators (SM) like interleukins was needed the microglia played a major role.

The BCM is fed with experimental data from the GFAP immunohistochemistry of the brain cortex with a pial disruption vessel and with the prior distribution of the model parameters. It returns their posterior distribution and the model evidence, which allows comparing alternative propagation mechanisms.

The model divided the cortex in segments and in each segment contained a concentration of each kind of cell, a concentration of DAMPs and of SM. Each cell had a number of DAMP and SM-receptors and the molecules could be free or bound. The receptors acted as catalytic centers, each bound molecule would be degraded. We modeled the astrocyte morphological change as a Markovian Process that depended on the proportion of bounded receptors of each kind. The model uses partial differential equations for the diffusion of the molecules, Michaelis Menten the instantaneous proportion of bound molecules and the changes in the morphological states of the astrocytes and microglia. The soluble molecules where liberated by the cells depending on the state of the cell and the proportion of bound receptors. The BMC analysis indicated that there was strong evidence for models where the microglia drives the propagation of the gliosis.

(670) **SYNTHESIS OF NOVEL CHALCONES WITH MONOAMINE OXIDASE INHIBITORY EFFECTS**

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Monoamine oxidase (MAO) is a flavin adenine dinucleotide (FAD) containing enzyme bound to mitochondrial outer membranes of the cells. MAO consists of two isoforms, MAO-A and MAO-B. Due to their fundamental roles in the metabolism of biogenic amines, both MAO isoforms are of considerable pharmacological interest. In the central nervous system (CNS), MAO-A metabolizes serotonin and norepinephrine. Deficiency of these two neurotransmitters is associated with the development of depression. Oxidative deamination catalyzed by MAO-B is one of the major pathways of dopamine degradation in the human brain. MAO-B is therefore a target for the treatment of Parkinson's disease (PD). Our aim is to contribute with novel MAO inhibitory compounds for the treatment of this CNS pathologies.

In this work, we synthetized by aldol condensation, purified, and identified (MS and $^1H/^{13}C$ NMR spectra) a series of 27 chalcones (1,3- diphenyl-2-propen-1-one) based on structure activity relationship (SAR) studies of our previously synthetized chalcones and bibliography.

The inhibitory activity of the compounds was evaluated in a fluorimetric assay by their effects on the production of hydrogen peroxide (H_2O_2) from p-tyramine by recombinant hMAOA and hMAOB. H_2O_2 was detected using Amplex Red reagent in the presence of horseradish peroxidase, where resorufin, a fluorescent product, is produced at stoichiometric amounts.

The series of chalcone derivatives presented herein selectively inhibited hMAO-B (IC_{50} values ranged between 30 nM to 70 μM). 5'-chloro-2´-hydroxy-3-nitrochalcone; 4´,5´-dimethyl-2´-hydroxy-3-nitrochalcone; 5'-bromo-2´-hydroxy-3-nitrochalcone and 5',3-dichloro-2´-hydroxychalcone were the most active ones (IC_{50}= 29.4 ± 4.1 nM; 69.3 ± 11.9 nM; 74.2 ± 8.1 nM and 87.9 ± 6.0 nM, respectively).

Our series of chalcone derivatives represent a promising contribution for the development of novel drugs for the treatment of PD.

Keywords: 2´-hydroxychalcones, monoamine oxidase, Parkinson

disease

(287) **EVALUATION OF CSF NEUROFILAMENT LIGHT CHAIN LEVELS AS A ROUTINE BIOMARKER IN A MEMORY CLINIC**

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The revised diagnostic guidelines for Alzheimer's disease recognize the potential use of biomarkers such amyloid beta, tau and phospho-tau. New biomarkers are currently under development to evaluate their utility in the diagnosis of different neurodegenerative diseases. In this work, we assessed the diagnostic performance of cerebrospinal fluid neurofilament light chain (NfL) in a clinical setting. A total of 62 samples were evaluated; of them, 51 corresponded to patients with neurodegenerative diseases usually found in a memory clinic and 11 cognitively normal individuals. Clinical diagnoses included: Mild cognitive impairment (MCI, n=12), Dementia of Alzheimer's Type (DAT, n=14), Behavioral variant Frontotemporal dementia (bvFTD, n=13), and logopenic (lvAPP, n=6), semantic (sAPP, n=2), and non-fluent (nfAPP, n=4) primary progressive aphasia (PPA). We quantified CSF NfL levels using a commercially available ELISA (IBL International). CSF NfL was significantly increased in MCI, FTD and DTA patients compared to controls (ANNOVA $p<0.0001$). Interestingly, receiver operating characteristic (ROC) curve analysis showed the highest area under the curve (AUC) value when comparing control versus bvFTD patients (AUC= 0.9522). Also, NfL levels differed within the PPA categories, although the low sample number precluded any statistical analysis. Overall, we propose that CSF NfL quantification be used as a primary biomarker in cases of suspected bvFTD in order to rule out a possible psychiatric condition.

Keywords: alzheimer's disease, frontotemporal dementia, neurodegeneration

(387) **CHARACTERIZATION OF THE ANTIPARASITIC BEPHENIUM AS AN AGONIST OF *Caenorhabidits elegans* LEVAMISOLE-SENSITIVE NICOTINIC RECEPTORS**

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Nicotinic acetylcholine receptors (nAChRs) are ligand-gated ion channels that are involved in neuromuscular transmission. Nematode muscle nAChRs are of clinical importance because they are targets of anthelmintic drugs. The muscle nAChRs of nematode parasites fall into three pharmacological classes that are activated by levamisole (L-type), nicotine (N-type) and bephenium (B-type). In *Caenorhabditis elegans* muscle, only the N-AChR and L-AChR types have been described. We here determined the behavioral (by paralysis assays) and molecular effects (by patch clamp recordings) of bephenium, which is used for infections caused by intestinal helminths, on *C. elegans*. As in parasites, bephenium produced spastic paralysis. Strains lacking accessory (LEV-8) or essential (UNC-38) subunits of the L-AChR showed partial or full resistance to bephenium, respectively, thus indicating that the L-AChR is the drug target. To decipher its molecular actions, we performed single-channel recordings from isolated larvae L1 and L3 cells. Bephenium (1-100 μM) activated L-AChRs of both developmental stages, eliciting channel openings of ~3.6 pA (-100 mV) with mean durations of 0.26±0.03 ms. Analysis of the channel properties as a function of drug concentration showed that at higher concentration it also acts as an open-channel blocker. To determine the selectivity of bephenium among muscle nAChRs, we studied its action at the mammalian muscle nAChR. Bephenium (10-100 μM) elicited single-channel currents, indicating that it is also an agonist of the mammalian muscle nAChR. However, when compared to ACh, openings were significantly briefer and activation episodes were not observed, indicating that bephenium is a very low efficacious agonist of this nAChR. Overall, we revealed that bephenium is an agonist of the L-AChR of the free-living nematode *C. elegans*, which is used as a parasite model, and a very low-efficacy agonist of mammalian nAChRs, at which it might mediate adverse effects.

Keywords: nicotinic receptor, ion-channel, patch-clamp, *Caenorhabditis elegans*.

(705) **EFFECT OF PHOSPHOLIPIDS ON NEURAL STEM CELLS PROLIFERATION AND DIFFERENTIATION UNDER OXIDATIVE STRESS**

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After brain damage, neural stem cells (NSCs) migrate to the site of lesion, proliferate and differentiate into neurons, oligodendrocytes and astrocytes in order to repair the damaged tissue. However, their number and regenerative ability are limited because the local microenvironment prevents neuronal regeneration due to the presence of factors that influence survival, self-renewal, migration and neuronal differentiation.

We have previously shown that phosphatidylcholine (PtdCho) enhances neuronal differentiation by decreasing the number of astrocytes and unspecified post-mitotic cells while phosphatidylethanolamine (PtdEtn) promotes glia differentiation due to a reduction of unspecified post-mitotic cells. Approaching different strategies, we concluded that these phospholipids influence the behavior of undifferentiated post-mitotic cells, without affecting the proliferation of NSCs. The fact that PtdCho and PtdEtn can alter the specification of post-mitotic cells makes them a powerful tool for recovery therapy. Specifically, PtdCho and PtdEtn could be effective even in reactive environments of tissue damage (hypoxia, oxidative stress or cellular reactivity).

In order to test this hypothesis, we analyzed different methods to induce oxidative stress in NSCs. Mouse embryonic NSCs were exposed to different concentrations of hydrogen peroxide, ferrous sulfate and cupric sulfate to generate oxidative damage. To determine the appropriate concentration and exposure time for decreased cell survival, the viability of the cultures treated with hydrogen peroxide and metals was assessed using the MTT assay. Moreover, NSCs were cultivated in the presence of the phospholipids after the damage was induced to evaluate their neuroprotective effect.

Finally, by immunofluorescence analysis using specific markers, we studied the effect of PtdCho as inducer of neurogenesis and PtdEtn as inducer of astrogenesis during the differentiation of NSCs in a stress microenvironment.

Keywords: neural stem cells, oxidative damage, phospholipids, neuronal differentiation.

(1335) **A NEUROSPHERE APPROACH TO REVISIT THE CUPRIZONE MODEL**

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Cuprizone (CPZ) is a copper-chelating agent which induces demyelination in mice. Although its neurotoxic mechanism is still unknown, CPZ has been shown to produce astrogliosis, microglial activation and loss of oligodendrocytes *in vivo*, which progressively leads to demyelination and neuronal degeneration. Neural stem and progenitor cells (NSC/NPC) are able to generate all neural cell types and can be cultured as neurospheres (NS). Depending on culture conditions, NS can be maintained in a proliferative and undifferentiated state or alternatively be forced to differentiate into neurons, astrocytes or oligodendrocytes. In the present work we used NS cultures to evaluate CPZ effects on NSC/NPC survival and proliferation, cell migration and cell differentiation.

Although NS generation was not affected when cultures were ini-

tiated in the presence of CPZ, the size of NS slightly decreased at CPZ concentrations above 500μM (P≤0.05), indicating that NSC/NPC proliferation was affected. General migration was not affected in the presence of CPZ. However, we observed differences in migration patterns and in the maximal migration distance reached by cells detached from NS in comparison to controls (P≤0.05).

Treatment of dissociated NS during differentiation did not change mature oligodendrocyte, astrocyte or neuron proportions. However, when dissociated NS were treated with CPZ after cell differentiation had taken place, we observed a decrease in the numbers of MBP-positive cells in a dose-dependent manner (P≤0.05), whereas the number of βTubIII and GFAP-positive cells did not change. The detection of oligodendroglial precursor cells in these conditions suggests that CPZ has a deleterious effect on mature oligodendrocyte cells without affecting their precursors.

Keywords: Neurospheres, Cuprizone, Neural Stem Cells

(348) **DESIGN OF A LENTIVIRAL VECTOR TRANSCRIPTIONALLY REGULATED FOR SPECIFIC NEURON GENE DELIVERY**

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Alzheimer's disease is a neurodegenerative disorder characterized by a progressive loss of cognitive functions. One hallmark is the formation of amyloid plaques, composed mainly by Aß peptide oligomers (AβOs). Neprilysin (NEP) is the main endopeptidase for the degradation and clearance of Aß in the brain. Strategies aiming to increase NEP levels should contribute to decrease the amount of AβOs and could have a therapeutic effect. The aim of this work is to evaluate the performance of the human synapsin promoter to design a neuron-specific lentiviral vector for the delivery of transgenic NEP. The vector should exhibit transcriptional activity only in differentiated neurons and not in progenitors or non-neuronal cells.
We developed LV coding the red fluorescent protein (RFP) downstream the SYN promoter (SYN-RFP) and green fluorescence protein (GFP) downstream the ubiquitous EF1a promoter (EF1a-GFP). LV titers were determined by qPCR and expressed as transduction units per milliliter (TU/mL). Neuronal progenitor cell line SH-SY5Y was transduced with SYN-RFP and EF1a-GFP. 72 hours post transduction, SH-SY5Y cells were put under neuron differentiation conditions (Retinoic Acid 100nM during 3, 5, 7 and 10 days). The time required for the appearance of RFP+cells was monitored by microscopy. The presence of GFP+ cells was taken as a positive control for the whole transduction process.
LV-SYN-RFP and LV-EF1a-GFP non concentrated titers were 1x106 and 1x107 UT/ml respectively, in 293T cells and 1x105 and 1x106 UT/mL, respectively, in SH-SY5Y cells.
RFP was observed in SH-SY5Y cells at 4 days after neuronal differentiation and no RFP was observed in 293T cells. GFP was observed in both cell lines at all time points assessed.
In conclusion, we developed a neuron-specific lentiviral vector, by introducing SYN promoter that shows activity 4 days after neural differentiation in SH-SY5Y cells and could be used to deliver transgenes, like NEP, to hippocampal cells.
Keywords: Alzheimer's disease, NEP, SYN promoter, Lentiviral vectors, transcriptional targeting

BIOTECHNOLOGY BIOINFORMATICS 5

(56) **ANTIMICROBIAL ACTIVITY OF FUNGAL METABOLITES OF *Nomuraea rileyi* ARSEF 1972**

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To date, studies on the biological activity of *Nomuraea rileyi* are mostly directed at their insecticidal activity, so there are very few reports on their antimicrobial activity. However, there is a history of extracts from other species of active entomopathogenic fungi against human pathogenic bacteria. In the present work we propose to find isolated compounds of *Nomuraea rileyi* ARSEF 1972 active against *Staphylococcus aureus* ATCC 6538 (1) and *Pseudomonas aeruginosa* ATCC 27583 (2). Fungal growth was performed on sabouraud-maltose-1% yeast extracts and 1% insect remains, at 25 °C for 15 days at 180 rpm. The supernatant of biomass and insoluble residues were then separated and extracted with ethyl acetate and methanol. The growth (**A**) was performed by microplate technique; the extracts were tested at 50 to 400 µg/mL, and the pure compounds at 5 to 100 µg/mL against both bacterial strains. The effect on biofilm production (**B**) was evaluated by the violet crystal technique. The supernatant extract was selected to perform bio-guided study, isolating 5 pure compounds. Cycle (Pro-Val) was the most active inhibiting **A** of **1** and **2**, a (47.5 ± 1.5) % and (33.2 ± 0.3) % respectively, in addition to inhibiting **B** of **2** a (67.5 ± 0.4) %.Therefore, the antimicrobial activity of *N. rileyi* can be attributed to the isolated diketopiperazines as well as being the first time reported for this fungus.
Keywords: *Nomuraea rileyi, Staphylococcus aureus, Pseudomonas aeruginosa, d*iketopiperazines, biofilm

(177) **MICROVESICLES PRODUCED BY *LACTOBACILLUS CASEI* BL23 CONTAINED PROTEINS ASSOCIATED WITH ITS PROBIOTIC ACTIVITY**

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Extracellular vesicles in Gram positive bacteria they are also known as microvesicles (MVs) and they have been proposed to be involved in signaling between probiotic bacteria and their mammalian hosts. The aim of this study is to investigate the isolation and characterization of MVs released from the probiotic strain *L. casei* BL23 which is a regular resident of the mammalian gastrointestinal (GI) microbiome.
L. casei BL23 was grown in MRS medium at 37°C for 48h. Cells were removed from the culture by centrifugation. Supernatant was then concentrated using a 100 kDa filter membrane. The concentrated supernatant was spun at 110000 g for 2h to pellet MVs. MVs have been characterized by Dynamic Light Scattering (DLS), Atomic Force Microscopy (AFM) and Transmission Electronic Microscopy (TEM). The shedding of MVs was investigated using CFSE staining and further visualization by Confocal Laser Scanning Microscopy (CLSM). Total DNA, RNA and protein were isolated from MVs using QuickZol. Proteins were analyzed by HPLC coupled to mass spectrometry.
CLSM imaging of *L. casei* BL23 showed a protrusion on cells consistent with the shedding of MVs to the extracellular medium. MVs size obtained from DLS data were consistent with measurements from AFM and TEM images (30-50 nm). The negative surface charge (-9±2 mV) could make the MVs stable by virtue of electrostatic repulsion. AFM images suggests that MVs might have a shape ranging from spherical to cup shape morphology. In TEM images we observed MVs with bilayered membranes and electron dense luminal contents; consistent with the presence of DNA, RNA and proteins. Proteomic analysis identified 103 proteins in the MVs including p40 and p75 that have been associated with its probiotic activity.
MVs could play a key role in signaling between GI bacteria and their mammalian hosts. The expression and encapsulation of proteins into MVs could represent a scientific novelty, with applications in food, nutraceuticals and clinical therapies.

(1018) **C-DI-AMP LEVELS IN *LACTOCOCCUS LACTIS*: OVEREXPRESSION OF CDAA AND GDPP CAN SIGNIFICANTLY MODIFY THIS SECOND MESSENGER INTRACELULAR POOL**

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Cyclic di-adenosine monophosphate (c-di-AMP) is a recently discovered second messenger which has been associated to different intracellular processes: cell wall biosynthesis, antibiotic resistance, DNA repair and others. In pathogenic and sporulating bacteria it also plays important roles upon infection and in sporulation respectively. Nevertheless, the main interest relies on its essential role which is currently under investigation.

c-di-AMP is synthesized by specific cyclases and phosphodiesterases and only one gene of each were found in *Lactococcus lactis* genome: *cdaA* and *gdpP* respectively. Studies in Bacillus subtilis have shown that concentrations of this metabolite are tightly regulated since high and low amounts are detrimental for cell viability and recent investigations determined that in this microorganism essentiality is directly related to potassium metabolism.

In this study we constructed different genetic tools in an attempt to modify the intracellular amounts of c-di-AMP in *L. lactis*. Using vectors previously obtained in our laboratory we separately cloned in *L. lactis* IL1403 *cdaA* and *gdpP* from *L. lactis* IL1403 on one hand and from *E. faecalis* JH2-2 on the other hand. Moreover, we also constructed deletion mutants for the gene *gdpP* in *L. lactis* IL1403 which were also analyzed for its c-di-AMP levels.

Concentrations of c-di-AMP could be increased more than 20 times and reduced more than 70% with respect to wild type values. Interestingly, whereas CdaA from *E. faecalis* doesn't modify c-di-AMP levels, GdpP from this same bacteria does. This results suggest that specifity is higher for cyclases while phosphodiesterases could be more promiscuous. Finally, the fact that stable mutants for *gdpP* were obtained and its intracellular c-di-AMP levels are not significantly modified supports the hypothesis that other degradative enzyme(s) can be encoded by *L. lactis* genome.

Keywords: cyclic-di-AMP, lactococcus, di-adenylate cyclase

(1105) **CHARACTERIZATION OF AN ACTIVE FIBROSA FILM WITH MCCJ25 (G12Y) / TWEEN 80**

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In Argentina, the hemolytic uremic syndrome (HUS) is considered endemic, and the principal infective agent is *Escherichia coli* O157:H7. HUS is the main cause of acute renal failure and the second cause of chronic kidney failure and kidney transplantation in children in Argentina. In the meat industry, several episodes of contamination in fermented dry sausages were registered with this pathogen. Active packaging technology employing polymeric materials as support for antimicrobial agents can improve the food hygienic-sanitary quality. The Fibrous film is used for embedding salamis. the obtaining of an active film would be of great importance in the production of artisan salamis since there is no rigorous control of the pH and water activity, which, can lead to a slow acidification, where the growth of pathogenic microorganisms could be favored. Microcin J25(G12Y) [MccJ25 (G12Y)] is an antimicrobial peptide with great potential as an additive in the food industry for its activity on *E. coli* O157: H7 and its sensitivity to digestive tract enzymes. Our results demonstrated that MccJ25(G12Y) did not inhibit the growth of *E. coli* O157: H7 in meat, at least up to 4 mg/ml of microcin concentration. The hypothesis was that the elevated fat content of the meat could be interfering in microcin activity. Tween 80 is a surfactant approved by the United States Food and Drug Administration (FDA) for use in foods, which has the ability to destabilize and emulsify fats. Surprisingly, our results showed that the addition of MccJ25(-G12Y) (4mg/ml) + Tween 80 (0.1%) totally inhibited the growth of *E. coli* O157: H7 in meat. According to this, the objective of this work was the characterization of a fibrous film active with MccJ25(G12Y) +Tween 80 for its potential use in the meat industry. Based on this, the aims were determined the minimum contact time, minimum MccJ25(G12Y) concentration for film activation, stability of active film and microcin migration in food simulants.

Keywords: *Escherichia coli* O157:H7, Active Packaging, Microcin J25 (G12Y).

(1111) ***PSEUDOMONAS PUTIDA* KT2440 AS A PLANT GROWTH PROMOTING RHIZOBACTERIA (PGPR) UNDER SALINITY CONDITIONS**

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The aim of this work was to study the role of *Pseudomonas putida* KT2440 as a PGPR under saline soils. A *P. putida* KT2440 mutant (Mut11), which was affected in the LPS synthesis and characterized in our laboratory as a low salinity tolerance strain, was also evaluated. This analysis could allow us to have an insight in which is the mechanisms used by *P. putida* KT2440 for this salt tolerance. To this aim, plants growth promoting properties such as indol acetic acid production (IAA), phosphate solubilization and siderophore synthesis of both strains were evaluated under saline (0.5 M NaCl) and non saline conditions (control). Additionally, seed germination and growth promotion of soybean and corn were assayed in saline soils.

Both strains exhibited plant growth promoting properties, showing similar activities in phosphate solubilization and IAA production under the analyzed conditions, suggesting that they were not affected by salt ($p>0.05$). Under saline conditions, the siderophore synthesis of both strains was significantly affected: the wild type strain exhibited a smaller halo than in the control, while no activity was detected in the mutant ($p\leq0.05$).

Seeds inoculated with the wild type showed longer roots and stems than those of uninoculated seeds ($p\leq0.05$), demonstrating the plant growth promoting effect of *P. putida* KT2440 under salinity conditions. Meanwhile, seeds inoculated with Mut11 displayed reduced effects on the growth promotion activity than those treated with the wild type ($p\leq0.05$). Based on these results we evidenced that the mutation apparently is not only important for bacterium salinity tolerance, but it is also involved in the PGPR effect under salinity conditions in soybean and corn.

This study showed that *P. putida* KT2440 strain is a useful PGPR in the tested conditions and also suggested that LPS are relevant for the tolerance to the salinity. This data provides new information on the KT2440 strain adaptation in response to saline environment.

Keywords: *Pseudomonas putida* KT2440, PGPR, Salinity stress, Lipopolysaccharides

(1254) **VARIANTS AND RECOMBINOGENIC PROPERTIES OF *attC*s ASSOCIATED TO THE *aacA* ANTIMICROBIAL RESISTANCE GENE CASSETTES IN *Pseudomonas* SPP.**

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Pseudomonas aeruginosa is naturally sensitive to aminoglycosides. In the nosocomial niche, this species rapidly evolves to a resistant phenotype by acquisition of antimicrobial resistance gene cassettes (ARGCs) in class 1 integrons (C1I). The *attC* site is recognized by the integrón integrase IntI1, which lets accumulation of ARGCs in integrons. The aim of this work was to analyze the role of the *attC* variants associated to aminoglycoside resistance (*aacA)* ARGCs, in the dissemination of *aacA* in *Pseudomonas* and

its relation with other *attC*s related to different families of antibiotics. The alleles of the *aacA* ARGCs in the *Pseudomonas* spp. and other ARGCs reference sequences were extracted from the INTEGRALL database. The *attC* sequences and the phylogenetic trees were studied with MEGA 6. A total of 128 C1I from *P. aeruginosa* (117), *P. putida* (9), *P. mendocina* (1) and *P. fluorescens* (1) were downloaded. We identified 157 *aacA* ARGCs, with 90 $attC_{aacA}$ variants. The length of $attC_{aac(6')}$ was 43-114 bp, and $attC_{aacA7}$ 72-113 bp. The most abundant ARGC was *aac(6')-Ib* (98). A variant of $attC_{aac(6')-Ib}$ was found in 13 strains, one of them in a fused *aacC1/aac(6')-Ib* ARGC. Only the *aacA8* ARGC was unique to *Pseudomonas* spp.. The $attC_{aac(6')}$s were the most diverse *attC* sites. Variants of $attC_{aac(6')}$ and $attC_{aacA7}$ were scattered in several clusters. Most of the *attC*s related to other families of antibiotics grouped separated from the *aacA* clusters. Recombination assays mediated by IntI1 evidenced that $attC_{aac(6')-Ib}$ possessed both low insertion (3%) and excision (3%) frequencies suggesting that once inserted in the variable region they are poorly lost. This finding could be related to the high rate of this ARGC in clinical samples. In contrast, *attC* sites from other ARGCs evidenced high rate of excision with a low rate of dissemination. Together with the antibiotic pressure, the properties of *attC* variants could be related to epidemiological distribution of ARGCs.

Keywords: *attC*, cassettes, *Pseudomonas*, antimicrobial resistance

(1257) **THE *INTIPSTQ*-LIKE INTEGRASE RECOMBINATION SITES (*ATTC* SITES) ARE POORLY ASSOCIATED TO *ATTC*S FROM ANTIMICROBIAL RESISTANCE GENE CASSETTES OF CLASS 1 INTEGRONS.**

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Pseudomonas stutzeri is a naturally competent gram-negative species widely distributed in the environment, also described as an opportunistic pathogen and integron carrier. Such characteristics make *P. stutzeri* a promising organism for the study of lateral genetic transfer (LGT) mechanisms. In order to study the diversity of the *attC* recombination sites associated with IntIPstQ-like and its contribution to multidrug resistant integrons we performed a search for sequences with an identity >65% with *intIPstQ* integrase. Sequences were analyzed with INTEGRON FINDER to detect: C-In (complete integrons, Integrase+*attC*), CALINs (*clusters of attC sites lacking integron-integrases)* and *In0* (Integrase only). Sequence and phylogenetic analysis of *intIPstQ* were made to establish its relationship with other integron integrases. We also carried out a phylogenetic analysis of the obtained *attC* sites and the ones of the main antibiotic resistance gene cassettes (ARGC). Twenty sequences belonging to *Pseudomonas*, *Halothiobacillus*, *Azotobacter*, *Methylibium*, *Thioalkalivibrio* were analyzed in which 15 C-In, 25 CALINs and 4 In0 were identified. IntIPstQ was found more related to IntI2, evidencing the tetrad RHRY conserved in most cases, and 50% of the strains showed an ALAR motif. A total of 285 *attC* sites were found, 170 located in C-In and 115 in CALINs. The length of *attC* sites was between 42 and 132bp, being the most frequent of 76bp. Most *attC* sites from ARGCs were found in 3 main subclusters related to *attC*s from *Halothiobacillus* sp., *Azotobacter* and *Methylibium* while only $attC_{qnrVC1}$ was identified among *attC* sites from *Pseudomonas*. Taken together, our results suggest that the genus *Pseudomonas* is not an active reservoir of *attC* sites that circulate by the mechanisms of the LGT in the genetic platform of class 1 integrons, although IntIPstQ-related integrases with *attC*s sites belonging to other clusters are part of the resistome of ARGCs.

Keywords: gene cassettes, antimicrobial resistance, integrons, *Pseudomonas*, *attC* sites

(1308) **MICROBIAL COMMUNITIES ADAPTATION TO ANAEROBIC DIGESTERS**

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Anaerobic digestion is a sustainable process widely used for organic waste management that stabilizes organic matter while generating biogas. Production of biogas depends on the activity of a complex microbial community, in which methanogenic archaea and syntrophic bacteria are the leading actors. It has been argued that for certain substrates a suitable inoculum source may be critical for efficient biogas production. This work aims at understanding the adaptation of inocula arising from different sources to food waste.

Two lab-scale anaerobic reactors (5 L) were operated during 91 days and fed daily with increasing concentrations of food waste. Reactor operational parameters, such as biogas production, volatile fatty acids concentration (VFA), volatile solids (VS) and total alkalinity (TA) were measured on regular basis. Metagenomic DNA was obtained from sludge samples taken weekly. Microbial community analyses were conducted using two different set of primers for next generation sequencing (NGS) analysis: one targeting rRNA16S V3-V4 region of bacteria, another targeting *mcrA* gene that encodes methyl coenzyme-M reductase of methanogenic archaea. Abundances were estimated through qPCR assays.

Cumulative biogas production increased similarly in both reactors, during the acclimation phase, despite differences in the source of the inoculum. Non-Metrical Multidimentional Scaling (NMDS) analyses of the time series of acclimation showed that both bacteria and archaea samples clustered according to the reactor of origin. However, under our experimental set up adaptation to food waste led to convergence in bacterial composition, while methanogenic archaea displayed smaller changes and maintained a similar separation of the distances between samples of both reactors. These results suggest bacteria may play a critical role in the adaptation of the inoculum´s community to the substrate during start up stage of anaerobic digesters.

(1620) **MEDIA PHOSPHATE CONTENT INFLUENCES STRESS TOLERANCE AND BIOFILM FORMATION OF *Lactobacillus paraplantarum* CRL 1905**

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Lactic acid bacteria (LAB) comprise a group of Gram positive microorganisms commonly used in food fermentation industry. They are usually exposed to a range of stressful environmental conditions in both natural and/or industrial habitats, such as high temperature, low pH, nutrient starvation, and oxidation. Previous studies showed that media phosphate (Pi) content modulates polyphosphate (polyP) levels in different bacteria, affecting stress responses, stationary-phase survival, and biofilm formation. The aim of this work was to investigate the effect of Pi concentrations on stress resistance and biofilm formation of *L. paraplantarum* CRL 1905. Intracellular polyP levels of the strain grown in chemically defined media (CDM) containing different Pi concentrations were determined. As control, CRL 1905 was grown in the routinely used rich medium MRS. It was observed that high polyP levels were maintained up to 120 h only when cells were grown in CDM supplied with 60 mM Pi. The polyP accumulation under this condition was correlated with a long-term cell survival, when compared to cells grown in CDM medium supplemented with sufficient Pi; loss of viability over time was even more pronounced in the rich medium. CRL 1905 stationary-phase cells grown in 60 mM Pi were more tolerant to high temperatures than those grown in 2 mM Pi. However, there was no correlation between media Pi concentration and resistance to acidic and oxidant conditions. The biofilm-forming capacity of *L. paraplantarum* under different culture conditions was characterized by microtiter plate biofilm assays. It was observed that a high Pi concentration in the medium impaired biofilm formation; this phenotype could be related to the maintenance of polyP in stationary phase. Taken together, this study contributes to a better understanding of alternative mechanisms by which beneficial bacteria can counteract several stress conditions and colonize different niches.

Keywords: Lactic acid bacteria; polyphosphate; stress tolerance; biofilm formation

(1703) **BACTERIOCIN-PRODUCING *BACILLUS PUMILUS* STRAINS AS A TOOL TO CONTROL ENTERIC CLOSTRIDIAL DISEASES**

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Abstract: *Bacillus* spp. are Gram-positive, rod-shaped, aerobic or facultative anaerobic bacteria. In stressful conditions form central endospores resistant to high temperatures and common chemical disinfectants. Several strains of *Bacillus* spp. have been used as probiotics for the control of pathogens, in part due to their ability to produce bacteriocins. Bacteriocins are defined as biologically active peptides with bactericidal properties.

The objective of the present study was to isolate and characterize bacteriocin-producing *Bacillus* spp. strains with activity against avian *Clostridium perfringens* strains to control necrotic enteritis in broilers.

Bacillus spp. were isolated from commercial poultry litter samples after ethanolic shock. Six isolates were identified as *Bacillus* species based on standardized methods including observation of cell morphology Gram-staining, catalase activity, 50 CH API test and 16S rRNA gene sequence analysis. Inhibition tests were repeated against several *C. perfringens* poultry strains and some strains of *Bacillus* identified as *B. pumilus* were able to inhibit 100% of the challenged strains. The bacteriocin title and percentage of sporulation of the *B. pumilus* strains were further studied. With a title of bacteriocin and a percentage of sporulation from 16, 0.05%; 1, 1.07%; 32, 0.29%; 4, 1.81%; 64, 0.18%; 2, 0.29% to INTA201401, INTA201402, INTA201403, INTA201404, INTA201405, INTA201406 respectively. Furthermore, *in vitro* assays were performed using Caco-2 cell line to study the adhesion of *B. pumilus* strains with a reference strain ATCC 13124. Adhesive properties of selected *B. pumilus* strains to higher percentage of adherence (20% INTA201402, 38.83% INTA201405) with respect to the other *Bacillus* strains and *C. perfringens* (5% ATCC 13124). In conclusion, bacteriocins from several strains of *B. pumilus* as well as bacteria cells could be used as a biotechnological tool for the control of necrotic enteritis in chickens.

Keywords: Bacteriocins, *Bacillus pumilus*, *Clostridium perfringens*.

REGENERATIVE MEDICINE AND CELL THERAPY 3

(479) ANALYSIS OF HOMOTYPIC AND HETEROTYPIC SPHEROIDS CELL CULTURE CONDITIONS AS A PROMISING TOOL FOR HAIR FOLLICLE REGENERATION

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Dermal Papilla Cells (DPC) induction on Hair Follicle Stem Cells (HFSC) is necessary for embryonic hair follicle neogenesis and adult hair cycling. DPC could be used in tissue bioengineering to regenerate hair follicles. However, its inductive ability is lost with successive culture passages. It was shown that culturing DPC as spheroids could restore their inductivity.

Our objectives were to test different culture conditions 1) to generate DPC spheroids in order to improve their inductive signatures, 2) to generate heterotypic spheroids containing adult human DPC and HFSC in order to find histological characteristics similar to hair follicle precursors.

DPC-spheroids were done by seeding cells on Polyvinyl alcohol (PVA)-coated wells. Inductive parameters as alkaline phosphatase activity (APa), the expression of agonist (Wnt10b) and antagonist (Dkk1) Wnt ligands and angiogenic factors were evaluated comparing culture times and conditions. Heterotypic spheroids were done using different ratios of HFSC and DPC on PVA-coated wells. Histological studies were performed.

In DPC-spheroids, APa was 20-fold higher than in monolayer cultures. Moreover, DPC-spheroid showed a 4-fold increase of APa after 72hs in culture, and a 12-fold increase after 96hs, both compared to APa observed after 48hs. After 48 hours spheroids showed a 4-fold increase of Wnt10b and a 4-fold decrease of Dkk1 expression. DPC spheroids showed an 8-fold higher VEGF and Angiogenin expression than DPC monolayer cultures.

In one of the culture conditions, heterotypic spheroids showed a core of DPC expressing α-Smooth Muscle Actin and Vimentin surrounded by an epithelium with a p63 positive basal layer. Protrusions similar to those of embryonic hair follicles precursors were also seen.

These results show the importance of the appropriate culture conditions in order to obtain DPC-spheroids with improved inductivity and heterotypic spheroids with histological features reminiscent of embryonic hair-buds.

Keywords: Dermal papilla cells, Hair follicle stem cells, 3-D cell cultures, hair follicle, inductivity.

(351) COPPER-INDUCED CROSSLINKING OF COLLAGEN BIOMATERIALS

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The interest in collagen biomaterials has increased in the recent years. Among the different collagens present in the extracellular matrix, type I collagen has proved to be biocompatible and easily obtained from different sources. However, given the loss in the mechanical properties after the extraction procedure, commercial collagen matrices are often crosslinked chemically or physically. In the present work, a novel crosslinking of collagen is presented, which employs low concentrations of the reagents in a water based media. For this purpose, type I collagen was extracted with acetic acid from rat tails, gelified under ammonia vapors, rinsed and treated with solutions containing either a buffered copper solution (Cu) or a copper-peroxide solution (CuP). Untreated collagen (Col) and peroxide treated collagen (P) matrices were used as controls during the experiments. FT-IR, SEM and a parallel plate rheometer were employed for the characterization of the resulting materials, while acid dissolution, accelerated collagenase degradation and primary dermal fibroblasts were used to evaluate the material´s degradability and biocompatibility respectively. FT-IR spectra showed an increase in the intensity of the amide I band which originates from C=O vibrations at around 1630 cm^{-1} for CuP and acid insoluble CuP materials, suggesting an increase in the carbonyls present within the scaffolds. Scanning electron microscopy showed no major differences between the treated and untreated materials, indicating that collagen´s open porous structure was maintained throughout the process. Parallel plate rheology showed a 2 fold increase in the rheological properties of CuP materials in relation to Col. Additionally, CuP scaffolds appeared to resist acid dissolution when compared to Col, Cu and P, showed an increased resistance to collagenase degradation and good biocompatibility when exposed to dermal fibroblasts.

Keywords: Collagen crosslinking, Copper, Biomaterials

(171) EFFECT OF SUBMANDIBULECTOMY ON LATE STAGES OF WOUND HEALING POST TOOTH EXTRACTION

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The submandibular and sublingual glands are of great interest given the type and content of the secretion they produce. Previously, we have shown that submandibulectomy (SMx) delays the post tooth socket wound healing at early studied times (72hs). The aim of this work was to study tissue repair in rats subjected to SMx-induced hyposialia, at later stages of post-tooth extraction wound healing.

Male Wistar rats of 70 g weight were randomly assigned to an experimental or a control group. The experimental group underwent submandibulectomy (SMx) of the SMG and SLG. The control group was subjected to a sham operation. One week later, bilateral extraction of the first mandibular molars was performed under i.m. anesthesia. All the animals were euthanized (CO2 chamber) in groups of n=10 at 7, 14, or 30 days post–tooth extraction. The mandibles were resected and processed for biochemical, histological and biomechanical analyses. Results: Histologically, epithelialization was not complete and a lower proportion of woven bone tissue in the socket was found in SMx rats at 7 days compared to controls. At 14 days, both groups of rats showed a greater osteoblastic activity and newly trabecular bone formation. At 30 days, greater volume and density of lamellar bone tissue with smaller medullar spaces into the alveolar bone was seen in SMx comparing to controls. Also, SMx rats exhibited higher bone mineral density measured in the mandibular bone of the tooth than in controls ($p<0.05$). The biomechanical structural properties were significantly increased at the end of the study period (30 days) in the SMx group when compared to controls ($p<0.01$). These changes were associated with altered levels of inflammatory parameters including COX-II expression, TNFα and PGE2 content and EP receptors mRNA expression in the socket tissue of SMx rats compared to controls. Therefore, these findings provide evidence for the importance of submandibular gland to optimal bone socket healing.

Keywords: Submandibulectomy, Socket healing, Osteogenesis, Bone formation

(1768) **EVALUATION OF A NEW BOVINE BIOMATERIAL TO INDUCE MANDIBULAR BONE HEALING IN DENTAL PROCEDURES**

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Bovine bone grafts are biocompatibile, osteoconductive, generate new bone apposition and prevent resorption. Synergy Bone Matrix (SBM) [Odontit Implant System, Argentina] effect was evaluated and compared with a recognized commercial material Bio-Oss (BO) [Geistlich, Switzerland] in rabbits. Bone healing process was evaluated by creating a critical sized bone defect (CSBD) at both sides of mandibles, and filling it with each biomaterial or remained unfilled (C: control). Assessments: clinical daily, systemic toxicity (usPCR and leukocytes) every 4 days, lungs, kidney and liver pathology, bone formation and device resorption evaluation; X-rays and compression and flexural biomechanical tests at sacrifice (4, 8 and 12 weeks). No systemic toxicity signs, fibrosis or adipose tissue development was observed. New bone formation, remaining bone substitute and mandibular radio-opacity were similar in BO and SBM. C presented significantly lower values (*$p<0.001$) than BO and SBM which were similar as follows: elastic modulus (Mpa) [12 weeks: 14.61±3.1 vs. 50.57±15.7* and 49.32±18.9*]; shear modulus (Mpa) [12 weeks: 172.2±13.1 vs. 464.6±26.9* and 473.1±32.4*] and compressive strength (KgF/mm^2) [12 weeks: 0.0694±0.0104 vs. 0.6983±0.0199* and 0.6956±0.178*]. Conclusions: bone regeneration, osteoconduction and newly formed bone quality are similar in SBM and BO. Therefore, SBM can be used as an alternative biomaterial for bone defects healing.

Keywords: bovine bone graft, bone formation, critical sized bone defect, osteoconduction.

(409) **EVALUATION OF BIOENGINEERED ACELLULAR BONE SUBSTITUTES IN THE HEALING OF CRITICAL INJURIES**

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Bioengineering strategies will allow the development of new approaches for the treatment of bone loss. Biocompatible matrices containing bio-modulating molecules, involved in cell signaling and in cell-extracellular matrix interaction, are a promising alternative to treat critical bone injuries.

The aim of this study was to evaluate a bone substitute, constituted by a collagen matrix embedded with BMP-2 or oligonucleotide (ODN) 504, in the healing of critical bone lesions in a rat calvaria model.

Bone substitutes were generated using Hellitape (collagen type I membrane) containing BMP-2 500ng/ml or Biopad (collagen type I sponge) containing oligonucleotide (ODN) 504 1mg/ml. These constructs and their specific controls (matrices) were applied to surgery-generated critical calvaria lesions (10 male Wistar rats per group). An absolute control group (untreated lesion) was also performed. Half of the animals in each group were sacrificed after 10 days and another half after 40 days post-surgery. The analysis of the remaining bone lesion area was performed using Image-J software from X-rays images.

Ten days post-surgery, radiographic studies did not show significant differences in wound closure areas between groups. Even if 40 days post-surgery, all groups showed a significant decrease in the lesion area compared to 10 days post-surgery, only the Hellitape-BMP2 group and its control showed a significantly improved closure of the lesion. In particular, the Hellitape-BMP2 group showed the greatest closure when compared to its control. Our results support the Hellitape-BMP2 construct as a promising bone substitute to treat critical bone lesions. However, a greater number of animals should be analyzed.

Keywords: Bioengineered bone substitutes, BMP-2, Oligonucleotides, bone injury healing.

(769) **MAGNETO-TARGETING OF ADIPOSE-DERIVED STEM CELLS (AdSC) AS A PERIPHERAL NERVE REGENERATION STRATEGY**

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Neuropathies are common problems in public health with high prevalence worldwide. Despite the regenerative capability of the peripheral nervous system (PNS), the poor clinical evolution of patients turns these affections into a crippling disease, which is why the development of new regenerative therapies is of great importance.

Research in stem cells has recently become an important tool to accelerate regeneration in the PNS and adipose-derived stem cells (AdSC) are a promising tool to develop regenerative therapies.

The aim of the present work was to test whether magnetic nanoparticle targeting of AdSC (AdSC-MNP) can enhance the regenerative ability of AdSC upon rat sciatic nerve crush.

To this end, AdSC were harvested from 60-day-old Wistar rats and characterized for multipotent cell marker expression. MNP were synthetized by co-precipitation and characterized by vibrating sample magnetometry, transmission electron microscopy, electron diffraction and nanoparticle tracking analysis. MNP internalization was evaluated through dc magnetometry experiments and AdSC-MNP viability was studied through MTT assays. The effect of transplanted AdSC-MNP was assessed through western blot and immunofluorescence analysis.

AdSC-MNP were positive for CD90 and CD105 and MTT assays showed MNP not to affect cell viability. In addition, VSM and epifluorescence microscopy showed the arrival of transplanted AdSC-MNP exclusively at the injured nerve. Furthermore, Western blot and immunofluorescence for MBP demonstrated AdSC-MNP beneficial effects on nerve regeneration.

Our results prove magneto targeting to facilitate AdSC arrival at the lesion site, probably enhancing nerve regeneration. Further experiments will determine the effect of AdSC-MNP on other aspects of the regeneration process.

Keywords: cell therapy, magnetic nanoparticles, neuroregeneration, magneto targeting.

(907) **MODIFIED POLYELECTROLYTE THIN FILM MULTILAYERS TO MODULATE CELL ADHESION**

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Abstract: The Layer by Layer assembly of polyelectrolyte multilayers (PEMs) from natural or synthetic polyelectrolytes constitutes a very versatile and simple strategy to modify surfaces and modulate cell behavior. PEMs assembled from natural polyelectrolytes are very appealing for biological and medical applications due to their high biocompatibility. However, PEMs from natural polyelectrolytes display poor cell adhesion as they are soft materials with elasticity modulus of a few kPa. In this work we present results on the modulation of cell adhesion of different immortalized cell lines by PEMs. Substrates are assembled by the layer by layer technique and characterized by quartz crystal microbalance, atomic force microscopy, Z-potential and contact angle measurements and protein adhesion. Cell adhesion was quantified by its average spreading areas and inmunoflurescence. Two strategies are employed to vary cell adhesion. (I) A heterogeneous polyelectrolyte multilayer is assembled employing a rigid bottom block including a synthetic polyelectrolyte with a soft upper block of natural polyelectrolytes. (II) Polyelectrolyte multilayers from natural polyelectrolytes are thermal annealed after assembly. Physicochemical characteristics of the PEMs change upon thermal treatment. Depending on the composition of the polyelectrolyte multilayer, cell adhesion may be enhanced or reduced. Based on the impact on PEM properties and cell adhesion caused by the thermal annealing, a temperature gradient is applied to a PEM of (PLL/Alg) to induce a spatial variation of PEM properties resulting in a gradient on cell adhesion. The strategies shown here can be employed as simple alternatives to tailor PEMs properties by means of fully biocompatible procedures.

Keywords: polyelectrolytes, cell adhesion, cell proliferation, thermal annealing

(1178) **PURIFIED ALGINATE: BIOCOMPATIBILITY AND CYTOTOXIC STUDIES**

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Alginate is a natural polysaccharide obtained from brown seaweed. Due to many attractive features such as good biocompatibility, low toxicity as well as ease of hydrogel formation with divalent cations, it has been widely used in a variety of biomedical application, such as medical delivery and tissue engineering. These applications required highly purified materials. However the alginate extracted from brown seaweed and commercial alginate contain a large number of impurities, such as proteins, polyphenols and endotoxins, which might lead to an intense host immune reaction and reduce the biocompatibility of alginate. The brown algae, *Undaria pinnatifida,* native China is an invasive species which was introduced worldwide in different coasts, including the one of Puerto Madryn (Argentina). In this study, we compare *U. pinnatifida*'s sodium alginate extracted from two different tissue sources (Stem Alginate, SA, and Leaf Alginate, LA) with the commercial salt (CA, Sigma) and its purified (PSA, PLA, PCA) using a method based on chloroform/butanol. Bone Marrow Progenitor Cells (BMPC) were grown in an osteogenic medium in presence of alginate during 14 days. Biocompatibility was evaluated by measuring the activity of the enzyme alkaline phosphatase (ALP) and production of collagen type 1 (COLt1) as parameter of osteogenic differentiation. Cell citotoxicity was evaluated measuring nitric oxide (NO) liberation and viability was assessed using MTT method during 48hs in murine macrophage-like cell RAW 264.7. Results showed, in all cases, that unpurified samples induce a decrease in cellular viability ($p<0,001$) and ALP and COLt1 ($p<0,001$) levels. Meanwhile, purified ones maintain normal cell viability and ALP levels and increase COLt1 levels ($p<0,01$) and do not present citotoxicity. In conclusion, purified sodium alginate materials present a reduction of contamination by using a simple method that generates a better biocompatibility and cell viability than the unpurified ones.

Keywords: Alginate, purification, biocompatibility, cell viability.

(631) **URETERAL REGENERATION: DEVELOPMENT AND HOST INTEGRATION OF A URETERAL GRAFT FROM PORCINE TISSUE**

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Ureteral injuries account for about 3% of urogenital traumas. Decellularized tissues have emerged as an alternative to ureteral repair but the available protocols have failed in functional host integration. The aim of this study was to develop and validate in vivo a ureteral graft from porcine ureteric scaffold, seeded with adipose mesenchymal stem cells (aMSCs). 4 ureteral samples from healthy pigs were used. Tissues were decellularized using Triton X-100 1% and SDS 0.1% under intraluminal continuous perfusion in a bioreactor designed by our group. Decellularization and structural integrity were characterized by histological analysis, β-actin western blot, residual DNA content and scanning electron microscopy. Extracellular matrix (EMC) proteins and VEGF were studied by immunohistochemistry. Furthermore, 41 growth factors were analyzed by protein array. Recellularization was performed with aMSCs extracted from sheep adipose tissue and it was evaluated by histological analysis. The ureteral graft was implanted into a host sheep and functionality was evaluated by ureterography. At ten weeks, the implant was extracted and the tissue integration was evaluated by histological analysis. Decellularized grafts showed high structural integrity and low DNA and β-actin levels. EMC proteins and VEGF were observed. After cellularization with aMSC, the grafts analysis showed the presence of groups of aMSCs and 32 growth factors were detected. Sheep implants were functional, showing peristaltic movement and regeneration of all ureteral tissue components. These results indicate that the protocol used is successful to achieve a decellularized ureter with an intact native architecture and recellularization with aMSCs. In addition, this porcine ureteral scaffold seeded with aMSCs showed a high functional integration with the host tissue. Therefore, this type of graft may be a suitable alternative to ureteral regeneration.

Keywords: ureter, regenerative medicine, scaffold, adipose stem cells

(439) **VALIDATION OF UMBILICAL CORD BLOOD AS**

A NEW CELL THERAPY PRODUCT FOR NEONATAL HIPOXYC-ISCHEMIC ENCEPHALOPATHY

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Cord blood (CB) has provided a source of hematopoietic stem and progenitor cell (HSPC) for treating hematologic disease. However, there is a growing number of studies supporting the use of CB therapies for neurologic conditions including neonatal hypoxic-isquemic encephalopathy (HIE). In these cases, CB is collected, processed, fractioned and storage until the product is infused within the first postnatal days (up to 72hs). According to INCUCAI´s Resolution 119/2012, the facility that prepares cell products shall identify, design and validate the processes that affect the quality of cellular products including that intent for research. Namely, the conditions for collection, transportation, processing and fractioning of the product, storage and transportation back to the hospital. Our goal is to determine the viability and contaminations of a CB product after being collected, processed, fractioned, settled for transportation and storage until used. Collected CB (10 units) were processes following established procedures using automated system. CB cells were aliquotted into individual dose syringes, cooled to 4°C, placed in a validated container, in a 36°C chamber to simulate transport in summer time. Viability was assessed by CD45+ cells at 3 time points (post-processing, 48 and 72hs) by FACS analysis and by Colony Forming Units (CFU) assay. Sterility at 2 time points and transport conditions were also tested. Over 70% viability and absence of contamination were set as acceptance criteria. We observed over 70% viability in all CB units tested (pp: 94,3 ± 3,1; 48 hs: 89,4 ± 4,1; 72 hs: 87,4 ± 4,9). CFU assay were positive for all samples. Regarding sterility, we found 1 positive culture for anaerobic microorganism (72hs). We defined conditions for processing, fractioning, storage and transportation for a cell therapy product intended for research. Although the source of contamination cannot be defined, the agent found is commonly observed in oral and vaginal flora.

Keywords: Cord Blood – Cell Therapy - Transplant

REPRODUCTION AND FERTILITY 8

(428) **ANDROGEN EXCESS *IN UTERO* PRODUCE LONG TERM ALTERATIONS ON OVARIAN FUNCTIONS**

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An adverse environment during prenatal life programs the developing embryo and may conduct to diseases during the adult life. In previous works we have shown that prenatal hyperandrogenism is able to induce endocrine abnormalities that mimics those observed in human polycystic ovary syndrome at pubertal age. The aim of this work is to study the long term impact of prenatal hyperandrogenism on reproductive functions, at adult life.

Pregnant rats were hyperandrogenized with testosterone and a Control group was obtained by vehicle injection. To study the long term effect of fetal programming caused by androgen excess on reproductive functions, prenatally hyperandrogenized (PH) female offspring(N=100) and Controls(N=80) were characterized according to the estrous cycle as ovulatory (PHov) and anovulatory (PHanov) phenotypes at adult age and gonadotropins serum levels (LH and FSH) were measured. To study the effect of androgen excess on ovarian functions we quantified by qPCR the mRNA levels of steroidogenic enzymes (SF1, StAR and P450 aromatase) and the oxidant/antioxidant balance as levels of MDA and GSH, respectively.

At adult life Control rats showed (100%) regular estrous cycle, 51% of the PH group showed irregular estrous cycles (PHov), whereas 49% presented anovulatory cycles (PHanov). LH and FSH were not affected in any of the PH groups. Regarding steroidogenesis, SF1 and P450 aromatase levels were lower in the PHanov group than in controls ($p<0.05$), StAR levels were increased in the PH phenotypes as compared to Controls ($p<0.05$). MDA levels were increased in the PHanov group as compared to Controls ($p<0.05$) and the antioxidant GSH levels were decreased in both of the PH groups as compared to Controls ($p<0.05$).

In conclusion, prenatal androgen excess exposure impacts on reproductive functions at adult life affecting ovarian functions and causes alterations on the steroidogenesis pathway and the ovarian oxidant/antioxidant balance.

Keywords: PCOS, ovary, hyperandrogenism, steroidogenesis.

(1119) **EVIDENCE FOR OXYGEN-MEDIATED CONTROL OF CFTR AND CAVEOLIN-1 IN HUMAN PLACENTAS**

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It has been proposed that intermittent placental perfusion, secondary to deficient trophoblast invasion of the endometrial arteries, leads to an ischemia-reperfusion type insult in preeclamptic placentas (PE). Such variations in oxygenation can further alter the syncytiotrophoblast functions. We have previously reported that the cystic fibrosis transmembrane conductance regulator (CFTR) is significantly reduced in PE. In addition, we have recently reported alterations in the membrane lipid composition of the apical membrane of syncytiotrophoblast (hST) of PE. Therefore, we also detected that the expression Caveolin-1, the major structural component of caveolas, was reduced in these membranes.

Our aim was to identify the mechanisms implicated in the regulation of CFTR and Caveolin-1(Cav-1) expression. We hypothesized that intermittent hypoxia may be responsible for the altered expression of both proteins.

Localization of CFTR and Cav-1 expressions were studied by double immunofluorescence in normal placentas and PE *(n=13)*. To study the role of oxygen, explants from normal placenta were cultured in normoxia, hypoxia, and hypoxia/reoxygenation (H/R) and the mRNA and protein expressions of CFTR and Caveolin-1 were determined.

In normal placentas, both proteins co-localized in the apical membrane of the hST, but the colocalization was lost in PE. CFTR expression decreased 2-fold *(n=6; p<0.01)* in explants cultured under hypoxic conditions and H/R treatment did not restore CFTR expression. Caveolin-1 expression decreased 2.5-fold *(n=6; p<0.05)* in hypoxia and it was almost undetectable in H/R.

Our findings suggest that the intermittent hypoxia may be the responsible for CFTR and Cav-1 reduced expression in PE. We speculate that the reduced expression of Cav-1 may decrease the number of caveolae inducing a reshaping of the type of lipid microdomains and creating an unfavorable environment for the insertion of CFTR into the plasma membrane.

Keywords: CFTR, Caveolin-1, trophoblast, preeclamptic placentas

(1316) **CSD-C2, A COLD SHOCK DOMAIN RNA BINDING PROTEIN AND ITS ROLE IN DECIDUALIZATION**

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Abstract: RNA binding proteins (RBPs) have been described in cancer cell progression and differentiation, although there is still much to learn about their mechanisms. Here we describe the role of the RBP CSD-C2 in differentiation using *in vivo* decidualization as a model. Kinetic analysis of *Csd-c2* mRNA expression during rat pregnancy in uterine horns (0, 2, 4 days post-coito (dpc)) and implantation sites (ISs) (6, 7, 8, 9, 10 dpc) by qPCR showed that it increased at 7 dpc, reaching its maximum mRNA expression at 8 dpc (P<0.001). The *Csd-c2* mRNA expression correlated with CSD-C2 protein increment at 8 dpc (8 vs 0 dpc, P<.001; 8 vs 6 dpc, 8 vs 4 dpc P<0.05). To localize the specific decidual area for *Csd-c2* expression we performed a laser capture extraction for slices from 8 dpc ISs. The content of *Csd-c2* mRNA determined by qPCR was significantly higher in antimesometrim (AM), followed by its expression in the Junctional Zone and Underneath myometrium (JZ+UM) and barely noticeable in the mesometrium (M) (AM vs JZ+UM, P<0.05; AM vs M, P<0.01). These results show that *Csd-c2* mRNA expression was differentially regulated depending on time and areas of decidua development, with the most variation in antimesometrium and, to a lesser degree, in the junctional zone. Inmunohistochemistry of CSD-C2 showed a preferentially cytoplasmic localization at antimesometrium and junctional zone, and nuclear localization in underneath myometrium and mesometrium. Cytoplasmic localization coincides with differentiated areas, marked by Desmin, while nuclear localization coincides with proliferative zones marked by Ki67. Uterine suppression of CSD-C2 using intrauterine injected specific siRNA led to abnormal decidualization in early pregnancy, with more extended antimesometrial area and with poor mesometrium development if compared to control siRNA-injected animals (Notched box-plot). These results suggest that CSD-C2 could be a master regulator of differentiation during decidua development.

Keywords: CSD-C2, endometrium, differentiation, RNA Binding Proteins

(196) **INHIBITION OF mTOR, PPARgamma AND PPARdelta DURING EARLY ORGANOGENESIS INDUCE DECIDUA AND FETO-PLACENTAL ALTERATIONS**

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Our previous studies have shown that mTOR inhibition induces changes in PPARgamma and PPARdelta levels in the decidua during early organogenesis, a period in which it serves for embryo development and histotrophic nutrition. Fatty acid binding protein 4 (FABP4, involved in decidualization and lipid transport) and perilipin2 (PLIN2, involved in lipid accumulation) are PPAR targets. Objective: To evaluate FABP4 and PLIN2 levels in the decidua (day 9 of pregnancy) and measure morphological parameters (day 14 of pregnancy) in rats treated or not with rapamycin (mTOR inhibitor), T0070907 (PPARgamma inhibitor) and GSK0660 (PPARdelta inhibitor) from days 7 to 9 of pregnancy. Methods: Female rats were mated and received sc injections of rapamycin, T0070907, GSK0660 or vehicle during days 7, 8 and 9 of pregnancy. On day 9 of pregnancy, the decidua was explanted, PLIN2 levels were evaluated by Western blot and FABP4 levels by immunohistochemistry. On day 14 of pregnancy, resorption rate, placental weigh and fetal size were evaluated. Results: Administration of the mTOR inhibitor rapamycin reduced FABP4 levels (22%, p <0.05) and increased PLIN2 levels (24%, p<0,01). Administration of the PPARgamma inhibitor T0070907 reduced the levels of FABP4 (40%, p<0,001) and PLIN2 (51%, p<0,001). Administration of the PPARdelta inhibitor GSK0660 did not change FABP4 levels but reduced PLIN2 levels (37%, p<0,01). On day 14 of pregnancy increased reabsorptions rate (control 6%, rapamycin 92%, p<0,001) were observed in rapamycin-treated rats and reduced feto-placental growth was observed in the rats treated with the three inhibitors. Conclusion: The early postimplantation stage is crucial in the determination of fetal growth, as inhibition of mTOR, PPARgamma and PPARdelta in this short period affects feto-placental growth, an alteration possibly related to impairments in decidual function as suggested by the impaired levels of FABP4 and PLIN2 proteins.

Keywords: Decidua, mTOR, PPARs.

(1354) **PATERNAL MODERATE ALCOHOL CONSUMPTION MODIFIES MOUSE PERI-IMPLANTATION EMBRYO DEVELOPMENT IMPAIRING INNER CELL MASS AND TROPHOBLAST GROWTH AND MORPHOLOGY.**

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Previously, we showed that paternal alcohol intake partially inhibits sperm capacitation, increases nuclear decondensation rate and deregulates the dynamics of pronucleus formation and fertilization. Aim: To evaluate the effect of paternal alcohol consumption and its consequences on mouse early embryo development focusing on trophoblast (TB) differentiation and inner cell mass (ICM) as critical embryo stages invading maternal decidua. Methods: CF-1 fertile males were exposed (treated group, T) or not (control group, C) to 15% (v/v) ethanol in drinking water *ad libitum* for 15 days. They were mated with a fertile nontreated CF-1 female (1:1). Positive mating females were sacrificed at day 2 of gestation to obtain 2 cell embryos which, then, were cultured for 7 days. Embryo differentiation, growth and morphology were evaluated during pre- and peri-implantation phases *in vitro*. Then, embryos were classified as type A (ICM: protruding aggregates of compact cells; TB: symmetric monolayer of flat and elongated cells) or type B (ICM: disaggregated, few scattered or no cells, TB: asymmetric trophoblast outgrowth). Frequency differences between C and T were tested by Fisher´s exact test. Results: Male alcohol consumption for 15 days delayed the embryo differentiation and growth by detention/fragmentation and altered the embryo morphology. Differences between type A and B distribution in C and T were statistically significant for ICM and TB in both groups. For ICM: Type A 53% C vs 10% T (p<0.0001), for TB: 60% C vs 26% T (p<0.01). When we evaluated the trophoblast growth area at day 7 of culture there were no significant differences between both groups. Conclusion: Paternal alcohol consumption impairs early mouse embryo peri-implantation, affecting ICM and TB development. These effects might be crucial for further embryo survival, considering that ICM participates in the embryo formation and TB plays a relevant role in placental development.

Keyword: alcohol consumption, embryo development, trophoblast

(1750) **ANANDAMIDE STIMULATES THE MIGRATION OF HUMAN ENDOMETRIAL STROMAL CELLS AND TUBULOGENESIS OF HUMAN FIRST TRIMESTER TROPHOBLAST.**

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Vascular adaptations at the maternal-fetal interface are crucial for successful implantation and placentation. Decidual cells regulate the activity of the endovascular trophoblast and actively participate in spiral artery remodeling. Anandamide (AEA) is the main endocannabinoid described so far related to the process of implantation. AEA levels are primarily regulated by fatty acid amide hydrolase (FAAH), its degrading enzyme. Dysregulations in AEA levels and FAAH activity have been associated with implantation failure and decidual defects. However, the role of AEA in vascular processes at implantation sites has not been elucidated. Thus, the aims of the present study were: 1) to analyze the correlation between FAAH expression and a known mediator of vascular processes (IL-10); 2) to investigate the effect of AEA on endometrial stromal cell migration and trophoblast tubulogenesis. First, Wistar females were sacrificed

in days 5 (Dp5) and 10 (Dp10) of gestation. Uteri (Dp5) and decidua (Dp10) were collected and processed for qRT-PCR and western blot. Then, T-hESC (human endometrial stromal cells) and HTR-8/SVneo (human first trimester cytotrophoblast) cell lines were incubated with AEA 1 nM and tested for migration (8 and 10 h) or tubulogenesis (6 h), respectively. The mRNA expression of IL-10 augmented in deciduas from Dp10 compared to Dp5 uteri ($p<0.05$), and correlated with the expression of FAAH enzyme ($p<0.05$). The incubation with AEA 1 nM increased the migration of endometrial stromal cells at 10 h ($p<0.05$) and also stimulated trophoblast tubulogenesis ($p<0.05$). Our results show that the expression of FAAH is correlated with the expression of a vascular mediator as IL-10, and suggest that AEA modulates the behavior of endometrial stromal cells and the trophoblast present at the maternal-fetal interface.

(655) **MOLECULAR MARKERS ASSOCIATED TO THE MAINTENANCE OF THE GERMINAL RESERVE IN INFANT AND ADOLESCENT HUMAN OVARY**

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During fetal human ovary development, 7 millions of oogonia originate at mid-gestation; most of them are lost before birth and even before reaching puberty. Fertile period in woman requires the preservation and a moderate use of the remaining primordial and primary follicles ovarian reserve. Follicular development initiates when some follicles from the ovarian reserve are recruited into a growing pool. The continuous recruitment into the growing pool, controlled by intra-ovarian stimulatory and inhibitory factors both in oocytes (O) and granulosa cells (GC), leads to the decrease of the ovarian reserve. Mechanisms involved are not yet completely enveiled, especially in the infant-adolescent period. FOXO3 and PTEN are factors involved in the PI3K-Akt signaling pathway apparently associated to preserve follicles in a dormant state. Foxo3 protein is a transcriptional factor which is exported to the cytoplasm upon phosphorylation when follicles are recruited from the reserve in mice. PTEN protein acts inhibiting FOXO3 phosphorylation indirectly. In this study, the expression pattern of FOXO3, pFOXO3 and PTEN was analyzed by immunohistochemistry and western blot (WB) in human infant-adolescent ovaries (n=12) from 9 to 19 years old. FOXO3 was detected in O nuclei in primordial, primary, secondary, antral follicles and corpus luteum (CL). pFOXO3 was found in O cytoplasm of some primordial and primary follicles. PTEN expressed in CL and GC from some antral and atretic follicles and it was also slightly detected in O cytoplasm in a few primordial follicles. As far as we could track in the literature, this is the first description of FOXO3/pFOXO3/PTEN markers in the infant-adolescent ovary. The spatio-temporal expression pattern of these markers suggest a synchronized balance which favors cell cycle arrest, apoptosis and inhibition of follicular growth.

Keywords: ovarian reserve, follicle recruitment, infant-adolescent ovary.

(1133) **HYPOXIA INDUCIBLE FACTOR-1 ALPHA (HIF-1α) UPREGULATES AQP4 EXPRESSION IN HUMAN PLACENTAS**

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In preeclamptic placentas (PE), aberrations in the remodeling of the spiral arteries lead to fluctuations in O_2 tension, resulting in an ischemia-reperfusion (hypoxia/reoxygenation [H/R])-type injury. Previous reports show that HIF-1α is significantly overexpressed in PE due to a proteasome dysfunction, contributing to the dysregulation of numerous genes, including syncytiotrophoblast transporters such as aquaporins (AQPs). *In-silico* analysis showed 3 putative binding sites for HIF-1α in AQP4 promotor region. Recently, we described that both $CoCl_2$ (a HIF-1α inducer) and hypoxia (H) treatment enhanced HIF-1α and AQP4 expression in placental explants and both returned to basal levels after reoxygenation. However, despite HIF-1α overexpression and the proteasome dysfunction, AQP4 is decreased in PE.

Our aim is to study AQP4 expression and protein degradation pathways in placenta. Our hypothesis is that AQP4 mRNA expression is increased in H and in PE via HIF-1α and that protein degradation occurs via the lysosome pathway.

This study was approved by an ethical committee. We performed AQP4 semiquantitative RT-PCR in normal and PE placental explants cultured in different O_2 conditions or in the presence of $CoCl_2$ and evaluated AQP4 expression. Incubations with MG-132 or NH_4Cl, proteasome and lysosome inhibitors respectively, were also performed. We found that AQP4 mRNA increased in H, $CoCl_2$ and PE (2.3-fold, 1.5-fold, 5-fold; n=4, p<0,05). Proteasome inhibition was not able to abolish the H/R-induced AQP4 decreased. However, lysosome inhibition led to a 1.7-fold increase in AQP4 levels in H and 2.3-fold in H/R (n=4, p<0,01).

In summary, our data show that AQP4 expression is abnormal in PE possibly because of fluctuations in O2 tension. Although in PE AQP4 expression should be enhanced, our results suggest that the intermittent hypoxia may contribute to increasing its lysosomal degradation. However, the consequences of AQP4 dysregulation in preeclampsia remain unknown.

Keywords: HIF-1α, AQP4, trophoblast, preeclamptic placentas

(995) **NATURAL COMPOUNDS PRESENT IN *SCUTELLARIA BAICALENSIS* AND *ROSMARINUS OFFICINALIS* INDUCE CELL CYCLE ARREST IN HUMAN ENDOMETRIAL STROMAL AND MAST CELL LINES**

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Current medical therapies for endometriosis (EDT) are often unacceptable due to their long term side effects. Natural compounds are being investigated because of their potential beneficial impacts on symptoms of cancer and EDT. Wogonin (WG) is a flavonoid isolated from the *Scutellaria baicalensis* root. Carnosic Acid (CA) and Rosmarinic Acid (RA) are antioxidants found in *Rosmarinus officinallis* leaves. We previously demonstrated that these compounds inhibit endometrial stromal cell proliferation. Our aim was to evaluate the effect of WG, CA and RA on cell cycle progression in a human endometrial stromal cell line (T-HESC) and a human mast cell line (HMC1.1), with the latter chosen because of the association of mast cells with pain in inflammatory conditions such as EDT. T-HESC cells and HMC1.1 cells were incubated with different doses of WG, CA, RA or the appropriate vehicle control for 48hs or 24hs respectively. Cell cycle distribution was assessed by flow cytometry in T-HESC. WG 40μM and CA 2.5μg/ml decreased the percentage of cells in G0/G1 and increased the percentage of cells in G2/M phase ($p<0.01$ vs. basal). The progression of cells from G2 to mitosis is mainly controlled by the formation of the Cdk1-cyclinA2/B1, and p21 inhibits these complexes. Concentrations of CyclinA2, CyclinB1, Cdk1, p21 and cdc25A mRNAs were analyzed by qRT-PCR in HMC1.1. WG 40μM reduced cyclinA2, cyclinB1, Cdk1 and cdc25A and increased p21 mRNA concentrations in mast cells ($p<0.05$ vs. basal). Also, CA 7.5μg/ml decreased Cdk1, and CA 5μg/ml increased p21 expression ($p<0.001$ vs. basal). The inhibition of

T-HESC proliferation induced by WG and CA might be associated to cell cycle arrest at G2/M phase. Similarly, CA and WG inhibited human mast cell growth through G2/M phase cell cycle arrest causing reduced levels of cyclin A, cyclin B, Cdk1 and cdc25A, all proteins required for G2/M transition. These results enforce the view that natural compounds may offer benefit for patients with EDT.

Keywords: endometriosis, wogonin, rosmarinic acid, carnosic acid.

(1196) **ROLE OF THE CAVEOLAE IN THE REMODELING OF MATERNAL SPIRAL ARTERIES DURING THE FORMATION OF HUMAN PLACENTA**

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Extravillous trophoblast (EVTs) cells of the human placenta migrate and invade the uterine decidua and adopt an endothelial phenotype to modify utero-placental arteries. These events also require a successful cooperation between EVTs and endothelial cells (ECs).

Caveolae are membrane domains that compartmentalize intracellular signaling pathways to orchestrate different cellular events, as cell migration and invasion. However, the importance of caveolae during placentation remains unknown.

Our aim was to evaluate the role of caveolae in EVTs migration, endovascular differentiation and interaction with ECs.

Swan 71 cell line was treated with 5 mM methyl-beta-cyclodextrin (MCD) to disrupt caveolae. Conditioned medium (CM) was collected. Cav-1 expression was tested by Western blot and immunofluorescence. Migration was assessed by wound healing assay. Endovascular differentiation was evaluated by formation of tube-like structures in plates coated with Matrigel.

To evaluate the interaction between EVTs and ECs (EA.hy926), proliferation (evaluated by MTT assay), migration and tube formation assays were performed in the presence of CM obtained from Swan 71 cells.

Disruption of caveolae significantly decreased Cav-1 expression (*n=5; p<0.05*) and Swan 71 cell migration at 8h (32.78±1.14 vs 17.78±0.56), 18h (66.74±1.43 vs 51.79±1.16), 24h (84.45±1.16 vs 64.95±0.72) *(n=5, p<0.01)*. Swan 71 cells formed tube-like structures and MCD treatment increased significantly the amount of closed cells in the tubular network (16.7±1.5 vs 26.4±1.0) and the number of junctions (653.4±59.5 vs 844.0±21.5) *(n=7; p<0.05)*. No differences were found in proliferation, migration and tube formation assays of ECs treated with the CM from Swan 71.

These results show that the lack of caveolae in the EVTs significantly attenuates cell migration and enhanced its endovascular differentiation during the remodeling of the spiral arteries, suggesting a role of caveolae at the early stages of placental development.

Keywords: caveolae, extravillous trophoblast, migration, tubulogenesis.

GENETICS AND MOLECULAR BIOLOGY 17

(77) **A CANDIDATE GENE ASSOCIATION STUDY IDENTIFIES NOVEL RESISTANCE-ASSOCIATED LOCI FOR CAPRINE BRUCELLOCIS: *TIRAP*, *IRF3* and *KARLN*.**

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Brucella melitensis is the etiological agent of brucellosis in goats and is the main cause of human brucellosis. The selection of resistance-related genes is considered one of the best long-term means to improve control to bacterial infection in livestock animals. We performed a candidate gene association study to test if polymorphisms at bacterial-infection related genes influence the resistance to *Brucella* infection in goats.

Using the NCBI dbSNP database and a literature review, we identified polymorphic INDELs lying in five host genes linked to *Brucella* spp. and other bacterial infections: IRF3-rs540, FKBP5-rs294, TIRAP-rs561, PTPRT-rs588 and KARLN-rs989 (GENE-marker). DNA samples from 64 cases (seropositive) and 78 controls were used. INDELs were resolved by PCR-capillary electrophoresis and genetic associations were determined by the Fisher's exact test.

Allelic frequencies were significantly different between cases and controls at IRF3-rs540 and KARLN-rs989 ($p \leq 0.01$). Moreover, IRF3-rs540, KARLN-rs989 and TIRAP-rs561 genotypes were associated with presence/absence of *Brucella*-specific antibodies in goats ($p \leq 0.01$). The major homozygous genotype (AA) at IRF3-rs540 was associated with susceptibility to *Brucella* infection (OR=0.45). Using RegRNA, we observed that the allele A introduce a premature stop codon in an IRF3 uORF. The KARLN-rs989 minor allele (b) was almost exclusively present in the controls. Furthermore, Bb/ bb genotypes were associated with resistance to *Brucella* spp. infection (OR=9.54). The heterozygous genotype at TIRAP-rs561 (Cc) was also associated with resistance (OR=4.44). We hypothesize that KARLN-rs989 and TIRAP-rs561 might be in linkage disequilibrium with missense SNPs at KARLN exon 8 and TIRAP exon 4, respectively. This study contributes to the understanding of genetic variation in host control of *Brucella* infection.

Keywords: *Brucella*, goats, genetic resistance, polymorphisms.

(337) **PATHOGENICITY ANALYSIS OF SMALL IN-FRAME DELETIONS IN DMD/DMB PATIENTS: REACHING CERTAINTY OF UNCERTAINTY**

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Duchenne Muscular Dystrophy (DMD) and Becker Muscular Dystrophy (BMD) are X-linked genetic diseases caused by mutations in the *DMD* gene. DMD is a severe dystrophy that occurs due to absence of dystrophin and affects 1:3.500 born males, whereas BMD is less severe due to less expression or function of the protein and affects 1:18.000. Molecular alterations in *DMD* gene are gross deletions/duplications in 80% of cases and small mutations in 20%. Large rearrangements are identified by Multiplex Ligation-dependent Probe Amplification (MLPA), while point mutations are detected by gene sequencing. The "reading frame theory" establishes a correlation between phenotype and mutation type, which agrees with the observed phenotype in 92% of cases. According to this theory, patients carrying a mutation causing a disruption on the translational reading frame (out-of-frame mutation) show a clinical progression to DMD, while patients with a genetic alteration that do not affect the translational reading frame (in-frame mutation) develop a milder phenotype, BMD-like.

From a cohort of 175 patients with clinical diagnosis of DMD/BMD, analyzed by MLPA and 40 of them also studied by Next Generation Sequencing (Whole Exome Sequencing), we have detected two small in-frame variants c.10101_10103delAGA and c.120_131delCTTCAGTGACCT in two patients with a DMD phenotype. As the observed phenotype did not adjust to the "reading frame theory" and there were none or scarce cases carrying these mutations previously reported, our aim was to increase the predicted pathogenic effect of these two mutations found. Two different strategies were implemented, an intrafamilial segregation analysis and a functional and structural bioinformatic analysis of the dystrophin protein. This work offers an example of different methodologies to corroborate the pathogenic effect of Variants of Unknown Significance (VUS), one of the major challenges of the Next Generation Sequencing data interpretation.

Keywords: Dystrophinopathies, Variant of Unknown Significance, Structural bioinformatic analysis, Segregation analysis

(702) **CCR5 AND CCR2 POLYMORPHISMS AND THEIR ASSOCIATION WITH CHRONIC CHAGAS CARDIOMYOPATHY (CCC) IN ARGENTINIAN POPULATION**

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Several studies have proposed different genetic markers as chemokines and cytokines genes for susceptibility to develop CCC. Many genes may be involved, each one making a small contribution. Thus, an appropriate approach for this problematic is to study numerous SNPs in individuals sharing genetic background.

Our aim was to analyze *CCR2* and *CCR5* SNPs located in the promoter region by TaqMan allelic discrimination assay, and their association with CCC in Argentinean populations.

A case-control study was carried in 480 *T. cruzi* seropositive adults from Gran Chaco endemic region and patients attending Buenos Aires hospitals. They were classified in 2 groups according to the Consensus on Chagas-Mazza Disease: non-demonstrated (non-DC) or demonstrated (DC) pathology groups.

Due to our studied population did not fit Hardy-Weimberg equilibrium, we subclassified them according to geographical/ethnic origin. Thereby, SNPs frequencies between creole population from endemic regions and Buenos Aires patients were similar but differ from wichi population from Gran Chaco. The association analysis showed that the T allele in rs1800024 was more represented in non-DC than in DC group (p=0.041) in non-wichi population, becoming a protective factor. Among wichi individuals the G allele in rs1800023 was more frequent in DC group (p=0.016) and may be a risk factor to CCC. Moreover, we found that only in wichi population the HHE haplotype displayed a higher prevalence in non-DC group.

These results are consistent with a previous study showing that although wichi and creoles live in the same geographical area, they are genetically different and for this reason, the results differed according to the population studied. It is tempting to speculate an association between the above described genetic differences and the clinical manifestations of CCC. Indeed, right bundle-branch block, the most frequent abnormality in CCC, had a clear tendency for lower prevalence in the wichi population.

Keywords: CHAGAS DISEASE, Trypanosoma cruzi, POLYMORPHISMS, CHRONIC CHAGAS CARDIOMYOPATHY

(731) **THE IL-17F rs763780 SNP IS ASSOCIATED WITH HUMAN TUBERCULOSIS SUSCEPTIBILITY AND DISEASE SEVERITY IN ARGENTINA.**

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Mycobacterium tuberculosis (*Mtb*) causes nearly 10 millions of new tuberculosis disease cases annually. However, most individuals exposed to *Mtb* do not develop tuberculosis, suggesting the influence of a human genetic component. In this work, we investigated the association of the IL-17F rs763780 SNP (T→C), which generates a protein that is a natural IL-17F antagonist, with tuberculosis disease in Argentina. We found that individuals carrying the C allele were overrepresented in the TB population (TB N=201; HD N= 200; p<0.001). Strikingly, we found that *Mtb*-stimulated PBMCs of HD secreted higher amounts of IL-17F than *Mtb*-stimulated PBMCs of TB (p<0.01), but we did not found any differences in the IL17F secretion between individuals carrying the different genetic variants. We also observed that *Mtb*-stimulated PBMCs of HD carrying the C allele produced the lowest levels of IFN-γ in culture supernatants (p<0.05), percentage of $CD4^+$ T cells producing IFN-γ (p<0.05) and the lowest proliferation index (p<0.05), indicating that these individuals would mount a poorer immune response against the bacterium. Within the TB population, *Mtb*-stimulated PBMCs of patients with the poorest immune response against the bacteria displayed the lowest level of IL-17F in culture supernatants (p<0.01). Moreover, we detected the lowest levels of IFN-γ (p<0.001) and highest levels of IL-17A (p<0.05) in culture supernatants of *Mtb*-stimulated PBMCs of TB carrying the C allele; and we measured the highest percentage of IL-17A producing $CD4^+$ T lymphocytes (p<0.05) and the lowest proliferation index (p<0.05), immunological parameters associated with the disease severity. In fact, we evidenced that the majority of the patients carrying the C allele had a greater number of bacilli in sputum smears than patients carrying the TT genotype (p<0.01). Therefore, the C allele of the IL-17F rs763780 SNP is proposed as a marker of tuberculosis susceptibility and disease severity in Argentina.

Tuberculosis, IL-17F, SNP, immune response

(963) **PHYLOGEOGRAPHIC STUDY OF THE INSECT PEST NEZARA VIRIDULA (HEMIPTERA: PENTATOMIDAE) IN NATURAL POPULATIONS OF ARGENTINA**

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Phylogeographic studies in populations of *Nezara viridula* could provide useful information to optimize the control strategies. The comparative analysis of a fragment of 718 bp of the citocromo oxidase I (COI) gene in 98 individuals from Argentina revealed limited levels of nucleotide and haplotype diversity (π = 0.00039 and Hd = 0.138, respectively). In samples from soybean crops only the common and most frequent haplotype was observed; while in samples obtained from mixed crop (soybean and maize) and a peanut crop, exclusive haplotypes of low frequency were also detected. It is probable that during the reduction of the populations produced by the intense insecticide exposure that characterizes soybean crops, very low frequency haplotypes would be lost faster. Neutrality tests indicated population expansion in the samples from the mixed crop and the peanut crop. It is probable that these *N. viridula* populations, belonging to crops with lower insecticide requirements, were expanded recently and that the gene flow levels and time elapsed were not enough to disperse the exclusive haplotypes. A phylogenetic analysis using COI gene sequences available from the GenBank of populations from Europe, South America, Asia, and Africa revealed that the haplotype detected in Africa was separated by numerous mutational steps and presented a basal position in the phylogenetic tree. These results are consistent with previous studies supporting the hypothesis of an African origin of *N. viridula*. The haplotype network, the phylogenetic tree, and a Bayesian method of grouping individuals revealed that individuals from Japan were grouped with individuals from Brazil. In adition, the haplotypes from Europe and South America formed another haplogroup. The results obtained are consistent with previous studies, suggesting a colonization from Europe to the coasts of South America and that in more recent times could have existed gene exchange between South America and Japan.

Keywords: *Nezara viridula*, Phylogeography, COI gene

(1052) **CHAGAS' DISEASE IN ABORIGINAL AND CREOLE COMMUNITIES: MOLECULAR FINDINGS AND ITS RELATION WITH HEART DISEASE.**

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Abstract: The objective of this work was to associate the molecular aspects obtained from infected in chronic form by *T. cruzi* with electrocardiographic and epidemiological findings.

T. cruzi infection was searched in a total of 139 individuals inhabiting the Province of Chaco. A total of 100 out of 139 individuals belonged to Aboriginal communities at a Province of Chaco (Qom, Wichi). Conventional serological tests were assayed through Indirect Hemagglutination and ELISA. Subjects were considered infected if at least two tests were positive. The DNA was purified with CTAB method (Hexadecyltrimethyl Ammonium Bromide). PCR based detection of the 330-bp minicircle variable region of parasitic kinetoplastid DNA (kDNA-PCR) was carried out in blood samples of seropositive subjects using primers 121 and 122. Parasitic loads were determined by means of TaqMan Real Time PCR (qPCR) targeted to a 166- bp segment from *T. cruzi* satellite DNA (SatDNA). Field cardiac evaluation was determined by means of 12-lead ECG using a Cardioprint 100. Individuals were classified as Normal or Abnormal ECG (International Consensus of 2010 Scientific Committee on Chagas Disease-FAC). Categorical variables were compared using the $\chi 2$ test or the Fisher Exact test. P values <0.05 were considered as significant. The results obtained show a higher prevalence of cases with heart disease among the Wichi-Qom and Wichi populations, in male patients and in the age range of 40 years or more. There was no association of the parasitic aspects studied with the finding of chronic disease with demonstrable cardiac pathology. The values of parasitic load obtained are within expectations for patients with chronic infection, except for some specific cases of aboriginal in Monte Impenetrable, where high parasite burden could indicate the occurrence of reinfection events.

Keywords: Trypanosoma cruzi, DNA, PCR, Qom, Wichi

(1329) **CONSEQUENCES OF SHORT SEQUENCE POLYMORPHISMS IN THE FORMATION OF G-QUADRUPLEXES THAT CONTROL THE EXPRESSION OF GENES ASSOCIATED WITH HUMAN DISEASES.**

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Guanine quadruplexes (G-quadruplexes or G4) are non-canonical nucleic acid structures formed by the stacking of at least two guanine tetrads bonded by *Hoogsteen* hydrogen bonds. Intramolecular G4 are dynamic structures transiently formed in G-rich ssDNA during transcription and replication. Several genes related with human diseases (mainly proto-oncogenes) were reported to be transcriptionally regulated by G4 formed within their proximal promoter regions. G4 forming sequences (G4FS) are defined by the consensus $G_{2\text{-}5}N_{1\text{-}7}G_{2\text{-}5}N_{1\text{-}7}G_{2\text{-}5}N_{1\text{-}7}G_{2\text{-}5}$. Subtle variations in G4FS, not only in the G-tracts but also in the connecting loops or proximal flanking sequences, may alter the G4 stability and/or topology, with putative consequences on gene expression control. The aim of this work was to find naturally occurring short sequence variations (SSV, including single nucleotide polymorphisms and short deletions and insertions) in G4FS that may affect the transcriptional regulation of genes related with human diseases. First, an *in silico* search was performed using the database of sequence variants available in the Ensembl genome browser (http://www.ensembl.org) to find SSV overlapping with the G4FS or in the proximal flanking sequences (5 nucleotides to each side) of G4 with reported functions in the transcriptional regulation of genes related with human diseases. Then, oligonucleotide sequences containing the G4FS, the polymorphic variants, and mutant versions that disrupt the G4 consensus were used to test G4 formation, stability and topology by *in vitro* assays (dot-blots with a single-chain anti-G4 antibody, circular dichroism spectroscopy, Thioflavin T fluorescence and intrinsic fluorescence spectroscopy). Results indicate that some SSV affect the stability and/or topology of the studied G4, defining several candidates to further test the effect of these variations on G4-mediated transcriptional regulation by *in cellulo* experiments.

Keywords: G-quadruplexes, single nucleotide polymorphism, gene expression control, proto-oncogenes

(1403) **A RARE CASE OF DOUBLE ANEUPLOIDY MOSAICISM 47,XXX/47,XX,+14 IN A PATIENT WITH MULTIPLE CONGENITAL ANOMALIES.**

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The presence of two different numerical aneuploidies in the same individual is a very rare event. The mechanism underlying the origin of double aneuploidies is unclear. Mosaic trisomy 14 is a rare chromosomal anomaly and there are very few cases reported of live born children. It is characterized by growth retardation, dysmorphic features such as low set ears, broad nose, micrognathia, cleft or highly arched palate, short neck, congenital heart disease and genitourinary abnormalities. Trisomy X is the most common trisomy in females (1/1000). As some individuals are asymptomatic, it is estimated that only 10% of patients are diagnosed. The most common physical features are tall stature, epicanthal folds, hypotonia, clinodactyly, seizures, renal and genitourinary abnormalities and premature ovarian failure.

The aim of this study is to report a patient with a double aneuploidy mosaicism with two unrelated cell lines. The proband, a female, was the only live born child from a gemelar pregnancy, the other sibling was reabsorbed during the first trimester. Clinical assessment stands cerebral anomalies, bilateral iris coloboma, cleft palate, a congenital teeth, Fallot tetralogy, left hand with cubital deviation, finger pads, clinodactyly, pilonidal sinus and an anterior anus. Standard cytogenetic GTW banding and array-CGH studies were performed.

The first cytogenetic analysis showed a 47,XXX karyotype. Due to the discordant genotype/phenotype correlation, an array CGH analysis was performed. We identified a mosaicism involving chromosomes 14 and X, that was later confirmed by a second cytogenetic analysis where the karyotype was 47,XX,+14[8]/47,XXX[19].

It is difficult to ascertain the mechanism of formation of this mosaicism. As it was a gemelar pregnancy, we hypothesize that the patient could be a chimera. Moreover, we emphasize the value of the right combination of cytogenetic and molecular techniques to arrive to a correct diagnosis.

Keywords: Multiple congenital anomalies, double aneuploidy

(1685) **MODY TYPE DIABETES: RESULTS OF A LARGE STUDY IN DIABETES PATIENTS FROM ARGENTINA.**

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Introduction: Maturity-onset Diabetes of the young (MODY) are a group of monogenic forms of diabetes. Each type related to mutations in one of 14 known genes which participate in pancreatic development and function, so in case of mutations, synthesis and/or secretion of insulin is altered.

Those patients are characterized by a family history of diabetes with autosomal dominant inheritance, an early age at diagnosis, a non-insulin-dependent clinical presentation and absence of anti-β-cell antibodies.

The most frequent cause of MODY are mutations in the GCK/MODY2 gene and in the HNF1A/MODY3 gene, whereas MODY5 is caused by mutations in the HNF1B gene.

Objectives:

Analyze and diagnose patients with clinical characteristics of MODY 2, 3 or 5 studying the related genes, using molecular biology methodologies. Establish the prevalence of those types of Diabetes and investigate the rate of de novo mutations in our population.

Materials and Methods:

We studied 109 patients selected due to their clinical characteristics performing the screening for mutations in the related gene using Sanger sequencing or NGS. All variations found were analyzed by bioinformatic tools to strengthen the assignment of pathogenicity.

Results:

We found mutations in the genes studied so we could diagnose 41 patients with MODY2, 14 with MODY3 and 2 with MODY5. The prevalence of each type was 37.6%, 12.8% and 1.8% respectively related to the population having clinical characteristics. We also found that four patients without family history of Diabetes had mutations in the GCK gene and one patient in the HNF1B gene.

Conclusion:

We found mutations in 57 out of 109 patients studied, we performed a correct diagnosis on those patients which is crucial for their correct pharmacological treatment and prognosis. We also stablished the prevalence of those subtypes of MODY in our population and made a contribution to stablish the importance of study patients without family history despite the stablished recommendations.

(39) **GENE EXPRESSION PROFILE OF HUMAN PLACENTA FROM *T. cruzi* INFECTED MOTHERS**

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Background. More than one million women in fertile age are infected with *Trypanosoma cruzi* worldwide Anti-*T.cruzi* seropositivity in mothers has been associated with adverse pregnancy outcome but there is still a knowledge gap regarding this effect.

Our aim was to compare the gene expression profile of term placental environment from *T. cruzi* seropositive (SP) and seronegative (SN) mothers.

Methods. A RNA-Seq experiment was performed in 9 pools of 2 different placental RNA samples each: 3 pools belonging to placentas from SN and 6 pools from SP. Each pool consisted of a binomial of a female/male newborn and a vaginal/caesarean delivery.

Results. Only 42 genes showed a significant fold change between SP and SN groups. Among the down-regulated genes (fold change≤-1.5 and p-value=0.05) were *KISS1* and *CGB5.* In the up-regulated genes group (fold change≥1.5 and p-value=0.05) were: *KIF12, HLA-G*, *PRG2*, *TAC3, FN1* and *ATXN3L.* To identify pathways significantly associated with maternal *T. cruzi* infection, a gene-set association analysis was done implementing the GSAASeqGP software. The placental transcriptomic profile of infected mothers consisted of an enrichment of immunological genes sets (inflammatory response and lymphocytic activation were over-expressed) and numerous biosynthetic processes were down-regulated.

Conclusions. It is worth noting that several differentially expressed genes in SP placentas (*KISS1*, *CGB5, HLA-G*, *PRG2* and *TAC3*) code for proteins associated to preeclampsia and miscarriage. This is the first placental transcriptomics study in natural human infection that may contribute to the understanding the placental behaviour in maternal *T. cruzi* infection. Our results suggest that although most children are born uninfected, they are affected by maternal *T.cruzi* chronic infection during pregnancy.

Keywords: maternal *T. cruzi*-infection, pregnancy, placenta, gene expression.

METABOLISM AND NUTRITION 4

(243) **ANTI-B2 ADRENERGIC AUTOANTIBODIES MAY CONTRIBUTE TO INSULIN RESISTANCE IN PATIENTS WITH CHRONIC CHAGAS DISEASE**

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An increased prevalence of obesity and diabetes has been described in patients with chronic Chagas disease (CCD); inflammatory/metabolic changes induced by *T. cruzi* persistence in adipose tissue have proposed as a possible underlying mechanism. Since antibodies directed to β2 adrenergic receptors (anti-β2AR) may arise during the host reaction to the parasite, we hypothesized that adrenergic activation of metabolically active tissues by anti-β2AR might contribute to the metabolic derangements detected in CCD patients. After developing an in-house ELISA for the detection of anti-β2AR and testing their functional effects in a corticotroph cell line, we performed a cross-sectional study, including 80 CCD patients and 41 healthy matched controls (CON) in order to explore a possible association between anti-β2AR levels and features of the insulin resistance (IR) syndrome.

Following a routine physical evaluation, a 75g Oral Glucose Tolerance Test was performed; samples for plasma glucose and serum insulin levels were obtained at 0, 30 and 120 min. Fasting lipid levels and anti-β2AR antibodies were also determined.

As compared to CON subjects, a greater proportion of patients with impaired fasting glucose (IFG) and/or impaired glucose tolerance (IFG) was found in the CCD group (41% vs. 71%; p=0.001); besides, significantly higher HOMA-IR values were found in this group (2.3 ±1.4 vs. 1.6 ±0.9; p=0.003). Anti-β2AR (+) patients (n=54), showed increased HOMA-IR values as compared to anti-β2AR (-) ones (2.6 ±1.4 vs. 1.6±1.3; p=0.030]. Furthermore, anti-β2AR levels were positively correlated with HOMA-IR (r= 0.438; p=0.004). A logistic regression analysis showed that only waist circumference and anti-β2AR levels resulted relevant for IR prediction (AUC: 80%; 95% CI: 0.699-0.899; p<0.001).

According to these results, anti-β2AR, present in ~68% of our patients, may exert a concurrent role in the pathogenesis of IR-linked metabolic abnormalities in subjects with CCD.

Keywords: Adrenergic beta-2 Receptor Agonist, antibodies, Chagas disease, insulin resistance.

(1613) **EFFECT OF MUSCLE MASS ON BONE MINERAL COMPOSITION IN POSTMENOPAUSAL WOMEN**

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Osteoporosis and obesity are two public health problems that are growing in prevalence around the world. These disorders, which appear to be unrelated, share several characteristics at the molecular and clinical levels, and at the predisposing risk factors. Body mass (BM) is composed of three compartments: total lean mass, which includes muscle mass (MM), fat mass (FM) and bone mass.

Several studies suggest that BM is positively correlated with bone mineral density (BMD), indicating the beneficial effect of FM. Others instead argue that MM has a greater positive effect on BMD. On the basis of current knowledge, it is unclear whether FM or MM has the greatest effect on BMD. Objective: To study the effect of FM and MM on BMD in postmenopausal women. Materials and Methods: Observational, descriptive and transversal study. Intentional sampling by spontaneous demand. A bioelectrical impedance analysis was performed using the In-Body (720) body composition analyzer. Linear regression was performed between BMD, MM and FM. StatGraphics statistical software was used. Results: The sample was represented by 52 postmenopausal women aged 60 ± 5 years belonging to the university community (UNSa). There was a strong correlation between MM and BMD ($p < 0.0001$; $R = 0.91$) vs. FM with BMD (p 0.28; $R = 0.15$). Conclusion: MM could be considered a protective factor of BMD in postmenopausal women.

Keywords: postmenopausal disorders, osteoporosis, lean mass.

(1595) ***Trypanosoma cruzi* INFECTION AFFECTS ADIPOCYTE FUNCTION *IN VIVO* AND *IN VITRO***

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Adipose tissue (AT) influences energy homeostasis and inflammation and also acts as *Trypanosoma cruzi* (Tc) reservoir. We recently showed that C57BL/6 mice infected with Tc displayed an exacerbated pro-inflammatory milieu associated with metabolic abnormalities like manifest hypoglycemia, hypoleptinemia and a markedly loss of AT mass, which may play a role in lethality. Probably, pro-inflammatory factors released during infection may be involved in lipolysis and also influence adipocyte function. In order to characterize AT wasting, we evaluate in vivo how Tc infection affects AT histology, features of inflammatory infiltrate and the expression of factors involved in inflammation, lipolysis and adipogenesis. Other series of studies were carried out *in vitro* using 3T3-L1 adipocytes exposed to plasmas derived from healthy (Co) and Tc-infected mice. Results: After 17 days post-infection (17 dpi), adipocyte number and size diminished [size(pixel^{2}x10^5) media±SEM; Co=2.7±0.2; Tc=1.3±0.1, $p<0,05$], while plasma glycerol and triglycerides were increased. Noticeably, blockade of TNF-α action (using TNF-R1/R2 KO mice) does not prevent loss of body mass or lethality (Relative weight, 17dpi, Co=0,5±0,1; Tc=0,2±0,1 KO=0.6±0.2; KOTc=0.2±0.1). In the AT, Tc mice revealed an increased proportion of T, CD11b$^+$ and CD11c$^+$ cells and a decreased percentage of Treg cells compared to uninfected ones (all, $p<0,05$). mRNA levels of adiponectin and leptin decreased in the AT from Tc animals compared to the Co, whereas TNF-α was increased. On the other hand, in vitro studies using 3T3-L1 adipocytes showed that Tc-derived plasmas caused a diminution in the expression of C/EBPβ (transcription factor that promotes adipocyte differentiation), adipose triglyceride lipase, hormone-sensitive lipase and adiponectin compared to Co-derived plasmas. The results suggested that immune response increases lipolysis rate as a result of a high metabolic demand accompanied by adipocyte dysfunction.

Keywords: Trypanosoma cruzi, adipose tissue, inflammation

(1013) **THE INCREASE IN SARCOPLASMIC RETICULUM-MITOCHONDRIA INTERACTION FAVORS APOPTOSIS IN PRE-DIABETIC HEARTS**

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The interaction between sarcoplasmic reticulum (SR) and mitochondria plays a pivotal role in physiological and pathological situations. Several proteins, like mitofusin 2 (Mfn-2), glucose regulated protein (GRP75) and voltage-dependent anion channel (VDAC) are involved in this tight relationship and participate in Ca2+ trafficking between both organelle. We previously showed that calcium-calmodulin kinase II (CaMKII) phosphorylation of ryanodine receptor 2 (RyR2), SR Ca2+ leak and mitochondrial membrane depolarization are critically involved in the apoptotic pathway of the pre-diabetic heart. We hypothesized that the enhanced apoptosis in pre-diabetic vs. control mice, is at least in part due to an increased in the interaction between mitochondria and SR.

To test this idea we used wild type (WT) and AC3I mice, which express an inhibitor peptide of CaMKII at heart level, fed whit a control diet (CD) or a fructose-rich diet (FRD) to induce pre-diabetes. By using electron microscopy we found that the distance between SR and mitochondria was markedly decreased in FRD mice with respect to CD mice (18.0±0.6 vs 9.8±0.2nm, CD vs DRF respectively), and this result was prevented in AC3I mice (15.3±0.5nm). Furthermore, we found an increase in FRD mice of VDAC (100±11 vs 148±18), Mfn-2 (100±4.81 vs 120.13±6.01) and GRP75 (100±16.46 vs 154.63±16.19) with respect to CD mice.

These results allow us to speculate that the FRD-induced decrease in SR–mitochondrial distance in association with tethering protein overexpression. This fact would favor Ca2+ transit between both organelle, contributing to mitochondria damage and apoptosis in pre-diabetic heart, presumably by CaMKII effect.

Keywords: Heart, Mitochondria, Calcium, Apoptosis

(471) **SARCOPENIA AND BODY COMPOSITION IN A GROUP OF OLDER WOMEN FROM ARGENTINA**

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Sarcopenia is characterized by low muscle mass and physical performance. The aim of this study was to analyze the association between the body composition, muscle strength and physical performance in older subjects. 23 women (60 - 84 years old), who attended recreational centers for elderly in Buenos Aires province, Argentina, were assessed. Body weight (kg) and height (m) were determined and body mass index (BMI) was calculated (kg/m^2). Fat free mass (%FFM) and Fat mass (%FM) were evaluated by deuterium dilution technique. Handgrip strength (HS, kg) was measured using a Jamar ® Hydraulic Hand Dynamometer. Physical performance was evaluated by Gait speed (GS, m/s) and Timed get up and go test (TGUG, s). 30% overweight and 50% obesity were observed; %FM was 43.1 ± 4.7 (IC95% = 41,1 - 45,1). BMI and %FM were associated ($r= 0,87$; $p<0,0001$). HS, GS and TGUG (mean ± DS (IC95%)) were 20,07 ± 3,50 (18,56 - 21,59); 1,42 ± 0,33 (1,23 - 1,57) and 8,73 ± 2,16 (8,01 - 9,38), respectively. HS significantly decreased with age ($p< 0.0279$) but no significant differences were found neither in GS ($p< 0,8052$) nor TGUG ($p< 0,7332$); however, TGUG tended to increase in older women. %FFM was associated to GS ($r= 0,58$, $p< 0,0046$) and it was inversely associated to TUGT ($r= -0,51$, $p< 0,0163$).

In this preliminary report, it was found a high obesity degree and association between FFM and muscle strength and physical performance tests in older women, which could be important risk factors for adverse events like falls, fractures, daily living disabilities and independence loss observed in sarcopenia status.

Supported by PB04 and IAEA RLA 6073.

Keywords: sarcopenia, body composition, elderly, physical performance

(1250) **ROLE OF TUMOR NECROSIS FACTOR-ALPHA RECEPTOR 1 (TNFR1) SIGNALING PATHWAY IN THE PROGRESSION OF HEPATIC STEATOSIS AND LIVER DAMAGE**

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In obese individuals, the excess fat accumulates in the liver and it is associated with hepatic low grade inflammation. Pro-inflammatory cytokines as TNF-α, IL-6 or IL-1β promote sustained inflammation that increase the susceptibility of hepatocytes to apoptotic cell death and therefore exacerbate liver damage. We evaluated the effects of high caloric feeding on the phenotype of TNFR1 knockout mice to assess its role in the progression of steatohepatitis, apoptosis and liver damage. C57BL/6J wild type (WT) and knockout C57BL/6-Tnfrsf1atm1Imx/J (TNFR1 KO) mice (n=6) were fed with regular chow diet (CHOW) or a 40% high-fat diet (HFD) for 16 weeks. Liver pro-inflammatory cytokines mRNA was increase in HFD-KO compared with HFD-WT (RT-PCR; IL-1β: 6-fold increase; TNFα: 2-fold increase; IL-6: 4-fold increase), but only plasmatic IL-1β levels showed a statistically significant increase in HFD-KO mice (ELISA; HFD-WT: 189 pg/ml; HFD-KO: 1078 pg/ml). In line with this, liver immunoblot membrane expression of its receptor was increased in HFD-KO (+40%) vs HFD-WT ($p<0.05$). Histological sections showed moderate to severe lobular inflammation and lipid accumulation with clear fat vacuoles involving zone 3 in the liver of HFD-KO mice (H&E staining) compared with mild inflammation and lipid accumulation in HFD-WT mice. Additionally, hepatic triglyceride content was higher in HFD-KO livers (colorimetric assay;+46% vs HFD-WT; $p<0.05$). In this regard, HFD-KO mice showed an increase of hepatic damage assessed by: plasmatic alanine aminotransferase (ALT) activity (+25%; $p<0.05$). In addition, apoptotic process was higher in HFD-KO livers [Caspase-3 fluorescence activity (u.a.): HFD-WT: 2039±495; HFD-KO: 35000±17100 ($p<0.05$) and Apoptotic index (+143%; $p<0.05$)]. Based on these results, we suggest that the disruption of TNFR1 signaling pathway increases interleukin-1β levels, liver inflammation and promotes the progression to steatohepatitis exacerbating HFD-derived liver damage.

Keywords: Liver, inflammation, TNFR1, steatohepatitis, apoptosis.

(212) **INFLAMMATORY CYTOKINES-INDUCED BETA-CELL DEATH AND VACUOLE MEMBRANE PROTEIN 1 EXPRESSION IN β-CELLS**

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Lack of insulin production by means of destruction of β-cells drive mainly by antigen specific T cells characterized type 1 diabetes. Autophagy is a highly conserved process of degradation and recycling pathway of organelles and proteins; it is crucial for maintaining cellular homeostasis as well as remodeling cellular components during normal development. Dysfunctions in this process have been associated with a variety of pathologies including cancer, neurodegenerative and inflammatory diseases, such as diabetes. We have found that in response to experimentally-induced diabetes, the autophagy pathway was activated. VMP1 is an autophagy-related protein that stimulates autophagosome formation and was found elevated during the course of several pathologies, including diabetes.Aim: Determine whether VMP1 is involved in β-cell demise in the naturally occurring progress of disease employing the non-obese diabetic (NOD) mice, as a model of autoimmune diabetes.Methods: Female NOD mice (4-28wk); immuno-fluorescence (IF); cell culture.Results: Insulitis (immune cells infiltrating the islets) severity and VMP1 expression within β-cells, increased with age in NOD mice while the intensity of immune-reactive insulin, assessed by IF decreased. Local production of inflammatory cytokines during insulitis triggers β-cell death. Mimicking this process in vitro, we challenged the rat insulinoma INS-1E and isolated mouse islets with IL-1β+IFN-γ(CYT). We detected an increase in the expression of VMP1 in both INS-1E and β-cells within islets after CYT exposure resembling those findings observed in islets from non-manipulated, naturally aged NOD mice. Discussion: VMP1 expression is induced by pro-inflammatory cytokines during the process of β-cell dysfunction and death, occurring in autoimmune diabetes. Experiments are being carried out to unravel the mechanistic action of VMP1 in β-cells under autoimmunity in diabetes.Keywords: Autoimmune diabetes, β-cell death, cytokines, autophagy.

(889) **ACTIVATION OF M1 PROFILE IN MACROPHAGES OF EPIDIDYMAL ADIPOSE TISSUE IS ASSOCIATED TO AN IMPAIRMENT IN ADIPOCYTE FUNCTION.**

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Obesity has become one of the main public health problems and is usually related to unbalanced diet consumption and sedentary lifestyle. In Argentina, from 2005 to 2013 the prevalence of obesity has increased 42,5%, and is still growing. The aim of this study was to evaluate the innate immune function in adipose tissue (AT) of Swiss mice supplemented with a fructose rich diet (FRD) in drinking water (20%w/v) for 10weeks. Caloric intake and body weight were recorded every 48hs. Epididymal adipose tissue (EAT) was dissected and weighted at the end of the experiment and was processed for histology, qPCR and flow cytometry (FACS) analysis. FRD mice showed an increased in caloric intake ($P<0.001$) and higher EAT mass ($P<0.0001$). The mRNA of genes related to AT function (Ob and adiponectin) were altered in FRD mice. Additionally, expression of pro-inflammatory markers (IL6, TNFα and IL1b) and anti-inflammatory markers (CD206 and IL10) were increased and decreased in EAT from FRD mice, respectively. Furthermore, FRD adipocytes were hypertrophic ($P<0.05$) and released higher leptin *in vitro* ($P<0.01$). When FRD adipocytes were incubated with LPS produced higher amount of IL6 ($P<0.01$). FACS analysis of Stromal Vascular Fraction cells from EAT showed higher percentage of CD11b+, Ly6C+low and CD11c+ ($P<0.05$) cells,that can be assumed as M1 macrophages, indicating a higher pro-inflammatory state. Moreover, there was a reduction in the percentage of CD11b+ CD206+ and CD11c- cells, indicative of an anti-inflammatory profile ($P<0.05$; macrophages M2). Finally, FRD adipocytes were able to activate RAW264.7 *in vitro* (macrophage cell line) when were co-incubated with low concentration of LPS, showing higher activation of RAW264.7 M1 profile (IL6 expression, $P<0.05$). In conclusion, FRD induced an inflammatory state by increase in macrophages M1 in EAT and adipocytes capable of maintaining this phenotype. Overall, these alterations may contribute to the EAT dysfunction. PICT2015-2352.

Keywords: Fructose rich diet, Adipocytes hypertrophy, Macrophages M1

(1879) **EFFECT OF GRAPE POMACE EXTRACT RICH IN POLYPHENOLS ON ADIPOSE TISSUE ANGIOGENESIS AND INFLAMMATION IN RATS WITH HIGH-FAT DIET**

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Adipogenesis and angiogenesis may counteract epididymal adipose tissue (eWAT) dysfunction under exposure to high-fat diet (HFD). Polyphenols has been found to exert anti-inflammatory effecs on eWAT. The aim of this study was to evaluate the effect of grape pomace extract (GPE) on eWAT angiogenesis/adipogenesis and inflammation from Spontaneously Hypertensive (SHR) and Wistar-Kyoto (WKY) rats receiving a HFD. SHR/WKY rats were divided: Control group SHR/WKY; SHR/WKY-HFD group receiving fat (40% (w/w)); and SHR/WKY- HFD supplemented with GPE (300 mg/Kg/d) for 10 weeks. HFD consumption led to increased body weight in WKY rats compared to both Ctrl and SHR strain. HFD significantly increased eWAT weight and adipocyte size in WKY rats, suggestive of hypertrophy. WKY rats supplemented with GPE had smaller adipocytes without changes in adipose weight, suggestive of hyperplasia. HFD did not affect eWAT weight and the diameter of adipocytes in SHR rats. GPE supplementation led to higher eWAT

PPARγ protein levels in both SHR and WKY rats and higher mRNA levels in SHR compared to WKY/SHR HF and Ctrl groups. In HF rats the protein levels of vascular endothelial growth factor A (VEGF-A) increased in both strains, while VEGF-R2 were higher only in SHR rats. In addition, GPE supplementation enhanced VEGF-A and VEGF-R2 protein levels in WKY compared to WKY HF and Ctrl rats. In contrast, the VEGF-A mRNA expression was enhanced by GPE in SHR. HFD caused higher levels of TNFα and MCP-1 mRNA, and CD68 protein in SHR rats, which was prevented by GPE supplementation. Protein levels of resistin, were higher in WKY HF and in SHR groups, while those of Nox4 were higher in the WKY/SHR HF groups. GPE supplementation significantly reduced HF-induced increase of resistin and Nox4 protein in both strains. Considering these results, supplementation with food rich in bioactive compounds can attenuate eWAT inflammation perhaps by its effect on increase eWAT angiogenesis and adipogenesis.

Keywords: Adipogenesis, angiogensis, adipose tissue, grape pomace extract, high- fat diet.

PHARMACOLOGY 9 (PHYTOPHARMACOLOGY)

(392) ACETYLCHOLINE AND GABA RESPONSES AT THE NEUROMUSCULAR JUNCTION OF THE NEMATODE *Caenorhabditis elegans* ARE NAGATIVELY MODULATED BY TERPENOIDS

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Parasitic nematodes infect many species of animals throughout the phyla, including humans. These nematodes place a major burden on human health and livestock production. Many anthelmintic drugs are losing their effectiveness because drug-resistant nematode strains are emerging. There is a need for concerted efforts to incorporate novel drugs. Medicinal plants provide an alternative source of potential anthelmintic compounds, and the free-living nematode *Caenorhabditis elegans* is a good animal model for drug screening and for the identification of drug actions. The levamisole-sensitive acetylcholine receptor (L-AChR) and the GABA-A receptor are involved in nematode muscle contraction and are targets of widely used anthelmintic drugs. By using plant extracts, we identified three terpenoids -thymol, carvacrol and eugenol- that have the capability to paralyze *C. elegans,* with a potency order of carvacrol>thymol>eugenol. Behavioral assays in *C. elegans* strains lacking Cys-loop receptor subunits showed that L-AChR and GABA-A receptor are involved in the terpenoid-induced paralysis. Also, thymol potentiates the paralyzing actions of established anthelmintic agents, indicating that it can be used in combination with these drugs as a strategy to reduce acquisition of resistance. Electrophysiological assays in *C. elegans* L1 muscle cells revealed that terpenoids are not capable of eliciting macroscopic currents but they significantly reduce ACh- and GABA-elicited responses. Single-channel activity of L-AChRs in presence of terpenoids is significantly reduced but the channel properties remain constant. These results are compatible with the action of the compounds as antagonists or allosteric inhibitors of muscle Cys-loop receptors. Thus, by acting at the neuromuscular junction, terpenoids are novel anthelmintic candidates. We propose that medicinal plants that contain terpenoids can be an alternative source of anthelmintic compounds.

Keywords: Cys-loop receptors, *Caenorhabditis elegans*, Medicinal plants, anthelmintics.

(1684) THE ALKALOIDS SAUROINE AND SAUROXINE FACILITATE THE EJACULATION IN THE FICTIVE EJACULATION MODEL

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Phlegmarirurs saururus (Lam.) B. Øllg., an autochthonous species in our country, is used in the ethnomedicineas anaphrodisiac. Previously we demonstrated that the decoction and the alkaloid extracts of this species produce a proejaculatory effect in spinalized male rats.As a result, the objective of the present investigation was to assesswhetherthat effect was produced by its main alkaloids, sauroine and sauroxine. It is known that the ejaculatory response is regulated by the CNS in both the brain and the spinal cord, so if we make a cut at the level of T6 (spinning), we remove the brain control and let the activation of the Spinal Generator of Ejaculation. Thus, sexually experienced male Wistar rats were anesthetized and subjected to surgery to be spinalized. The animals were divided into 6 groups of 3 males each.Control group received saline, reference group was administered with 10 mg/kg of sildenafil, and treatment group received 0.1 and 1 μg/kg of sauroine or sauroxine, all given intravenously. Results showed that both alkaloids facilitate the ejaculation, in view that both produce a diminution in the Latency of Discharge ($P<0.05$). This means that the motor pattern of ejaculationthat produce the expulsion of the seminal content was activated faster. At the same time, these alkaloids increased the Number of Motor Patterns ($P<0.05$), doing that the animals increase their ejaculatory capacity ejaculating a mayor number of times in relation to the controls. Likewise, sauroxine (as it happens with the decoction) produced an increase in the Number of Discharges, promoting an increase in the ejaculatory potency. The effects produced by sauroine and sauroxine were better than those for sildenafil, in all the cases. With these findings, we can affirm that both alkaloids cause a marked aphrodisiac effecton the male spinalized rats explained by the capacity of the alkaloids to promote the ejaculation.

Keywords: Sauroxine, Sauroine, Spinalization, Fictive Ejaculation.

(42) COMPLETE ESSENTIAL OILS OF *LAURUS nobilis* INDUCING ANTINOCICEPTION BY NMDA RECEPTOR INHIBITION IN C-REFLEX AND SPINAL WIND-UP MODEL IN THE RAT.

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The complete essential oil of *Laurus nobilis* was used to test their antinociceptive efficacy in normal rats. It was obtained by hydrodistillation and applied intraperitoneally (i.p.). Thirty rats (Rattus norvegicus) of the Sprague-Dawley strain, normal male weighing between 250 and 350 g were used. They were maintained under a light-dark cycle of 12/12 Hrs., with food and water *ad libitum*. All experiments were performed in accordance with the ethical guidelines of the International Association for the Study of Pain and the Committee on Bioethics of the University of Santiago of Chile. The rats were subjected to a nociception test (C reflex and spinal Wind-up). The results showed that the complete essential oil applied at higher doses (0.06 ml/Kg) causes a complete abolition of the spinal wind-up, while the C reflex was unchanged, indicating a clear antinociceptive effect. At lower concentrations (0.012 ml/Kg), there was a lowering in the wind-up by 85% within ten minutes of the essential i.p. oil application. Interestingly, there was an effect of naloxone (0.08 mg/Kg i.p.). When applied, a change occurred that almost entirely reversed the antinociception caused by the complete essential oil from *Laurus nobilis*. We can conclude that there is a significant antinociceptive effect of the essential oil of *Laurus nobilis* to rats subjected to electric nociception. In addition, it was observed that naloxone (0.08 mg/Kg) reversed the antinociceptive effect (wind-up) produced by Laurus, indicating a possible effect mediated by opioid mechanisms.

Keywords: *Laurus nobilis*, Pain, C reflex, Spinal Wind-up.

(1885) EVALUATION OF THE EJACULATORY ACTIVITY PRODUCED BY *SATUREJA PARVIFOLIA* (PHIL.) EPLING

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Continuing with the studies on aphrodisiac plants, we worked in this occasion with *Satureja parvifolia* (Phil.) Epling (Lamiaceae). Very well known, especially in the north of Argentina, the "muña-muña" is reputed in the ethnomedicine as an aphrodisiac. So far, it lacks scientific studies related to this effect. The objective of the present work was to evaluate the infusion of the species in the Fictive Ejaculation Model. It is known that the ejaculatory response is regulated by the CNS at both, the spinal and brain levels. This model allows the experiment to become independent of the brain to evaluate the effect of a particular substance on the Spinal Generator of Ejaculation. We worked with male Wistar rats trained in sexual behavior until being considered sexually expert. The animals were divided according to the Latency of Ejaculation (LE) in premature (those with LE ≤ 10 minutes) and intermediate (LE ≤ 20 minutes). Premature rats were selected. They were anesthetized and submitted to surgery to section the spinal cord at the level of the T6 vertebra, above the Spinal Generator. The animals were divided into 3 groups, of three animals each. Physiological solution (Control Group) or infusion of *S. parvifolia* (Treatment groups) were administered intravenously in doses of 10 and 30 µg/kg. The results showed that the infusion at 10 µg/kg facilitates ejaculation in premature rats producing an increase in the Number of Discharges (ND, number of contractions of the bulbospongiosus muscles) ($P<0.01$). This important parameter evaluates ejaculatory potency, so it can be said that the infusion increases it. It is very interesting to observe that at higher concentrations the effect caused is a decrease in ND. These results could be indicative of a dual effect according to the concentration. More experiments should be performed to determine whether it is possible to associate the concentration with the ability to slow down ejaculation in premature animals.

Keywords: *Satureja parvifolia*, Spinalization, Aphrodisiac, Premature ejaculators.

(261) **FLAVONOIDS AS ALLOSTERIC MODULATORS OF ALPHA7 NICOTINIC RECEPTOR**

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Ubiquitously present in plants, flavonoids are a class of polyphenolic compounds comprising a benzopyrone moiety. These compounds have been reported to decline incidence rate and development of several neurodegenerative disorders, such as Alzheimer's and Parkinson's diseases. Two flavonoids, genistein and quercetin, have been also described as type I positive allosteric modulators (PAMs) of α7 nicotinic acetylcholine receptor, which increase the amplitude of macroscopic responses evoked by ACh without changing the desensitization rate. The molecular mechanism underlying these macroscopic effects remains unknown. Our main objective is to unravel the molecular basis of flavonoid allosteric potentiation of α7 at the single-channel level. Receptors were expressed in mammalian cells and receptor function was evaluated by patch-clamp recordings. We analyzed the concentration-dependent effects of a prototype flavonoid for each class: quercetin as flavones, genistein as isoflavones and 5,7-dihydroxy-4-phenylcoumarin as neoflavonoids. We here reported for the first time that neoflavonoids act as PAMs of α7. All flavonoids increased the mean open channel lifetime and the mean burst duration of α7, although the magnitude of the change differed among the different compounds. In order to define the structural and functional determinants of potentiation, we evaluated flavonoid potentiation in an α7 receptor carrying a quintuple mutation at the transmembrane region that makes it insensitive to potentiation by type II PAMs and in a chimeric α7-5HT_3A receptor with the extracellular domain of α7 and the transmembrane region of 5-HT_3A. Statistically significance differences were established at p-values<0.05. We conclude that, in addition to the well-known effects as antioxidants, the unique properties of flavonoids as natural α7 PAMs make them candidate drugs for the treatment of different neurodegenerative disorders.

Keywords: flavonoids, alpha 7 nicotinic receptor, positive allosteric modulators, patch-clamp.

(690) **INTERACTION BETWEEN HYPOTHYROIDISM AND THE INFUSIONS OF *Melissa officinalis* AND *Bahuinia forficata* IN RAT HEARTS EXPOSED TO ISCHEMIA-REPERFUSION**

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Drinking infusions of *Melissa officinalis* ("melisa", *Mel*) or *Bahuinia forficata* ("pezuña de vaca*", Pzñ*) is contraindicated in hypothyroid patients because they potentiate the endocrinological alteration. The aim of this work was to evaluate whether these plants have any effect on the postischemic cardiac stunning in interaction with the hypothyroidism (HypoT). HypoT was induced by drinking methimazole (0.02%) for 15 days. Euthyroid (EuT) and HypoT rats received infusion of *Mel* or *Pzñ* (5% w/v) in drinking water during 7 days. Isolated hearts were perfused inside a calorimeter at 37°C to measure left ventricular pressure (LVP, in mmHg) and total heat rate (Ht, in $mW.g^{-1}$) during exposition to moderate I/R (20 minI/ 45min R). **In** EuT hearts: *Mel* improved the postischemic contractile recovery (PICR) up to 105.0 ± 16.4 % of initial P (Pi) vs 69.4 ± 6.0% of Pi in non-treated C-EuT ($p<0.05$, n=6-7) and muscle economy (Eco= P/Ht) at 15 min R (7.9 ± 1.9 % vs 4.1 ± 0.8 % in C-EuT, $p<0.05$) without changing diastolic tone. *Pzñ* slightly reduced PICR to 40.4 ± 6.7% of Pi (n=4) without changing Eco, but with a significative increase in the diastolic tone. In HypoT hearts: *Mel* and *Pzñ* drastically reduced the PICR (54.7 ± 3.3 % and 12.5 ± 5.3 % of Pi respectively vs 92.6 ± 5.2 % of Pi in non-treated HypoT (C-HypoT, $p<0.05$, n=5-4-5) and Eco (2.9 ± 0.4 % and 1.0 ± 0.7 % of initial, respectively, vs 5.1 ± 0.8 % in C-HypoT, $p<0.05$) with a significative diastolic contracture. The dysfunction induced by *Mel* in HypoT was reduced by perfusing cyclosporine-A (0.2 $\mu g.mL^{-1}$ Cys-A) before I/R, suggesting that the mPTP opening caused dysfunction.Results suggest that: a) *Mel* prevented the stunning in EuT ischemic hearts but increased dysfunction in HypoT hearts b) *Pzñ* worsened stunning in both, but more in HypoT than in EuT hearts. *Grant: UNLP X-795*.

Keywords: melisa, Bahuinia, ischemia-reperfusion, heart, stunning

(656) **NATIVE MEDICINAL VALERIAN PLANTS AND THEIR EFFECTS ON ACETYL AND BUTYRYL CHOLINESTERASES**

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It has been shown that acetylcholinesterase (AChE) activity is increased in patients with Alzheimer's disease (AD), whereas expression and concentration of butyrylcholinesterase (BChE) is compensatory and is increased. Three out of the four approved drugs for the treatment of AD are AChE inhibitors (donepezil/galantamine/rivastigmine); however, they present undesired side effects due to AChE inhibition in peripheral and autonomous nervous systems.

They could be overcome by selective inhibition of BChE.

Our country harbours several thousands of plant species, which lack scientific information although many of them are used in folk medicine. Our hypothesis is that native plants have unexplored compounds with multiple biological activities on central nervous system.

Herein we studied the capacity of hydroalcoholic (70%) extracts (underground parts, 10 %) from 5 Argentinian valerianas: *V. carnosa, V. macrorhiza, V. clarionifolia, V. effusa* and *V. ferax*, and *V. officinalis* (as the reference plant) (Caprifoliaceae) to inhibit AChE/BChE (from mice brain homogenate/plasma and mAChE/recombinant hBChE) by using the Ellman´s method. *V. clarionifolia* and *V. macrhoriza* ethanolic extracts inhibited AChE (IC_{50} (95% confidence interval): 1.29 (0.81-2.05) mg/ml and 1.08 (0.49-2.37) mg/ml, respectively). Although, main effects were observed on BChE, as *V. clarionifolia*, *V. macrorhiza* and *V. carnosa* showed IC_{50} of 1.86 (1.43-2.43) μg/ml; 82.04 (66.43-101.30) μg/ml and 1.46 (0.99-2.14) mg/ml; respectively. Moreover, even a better inhibition was observed with a partial purification step (ethyl ether extracts) from these three Valerianas´ ethanolic extracts (IC_{50}: 0.57 (0.41-0.81) μg/ml; 4.53 (3.79-5.43) μg/ml and 263.60 (164.60-422.00) μg/ml; respectively). Similar inhibitory profiles were observed with both recombinant and murine enzymes. Our results showed that Argentinian Valerians are promising tools for the discovery of novel native herbal products for the treatment of AD.

Keywords: native medicinal plant; *Valeriana clarionifolia*; Alzheimer disease, cholinesterase.

(1775) **ANTICANCER AND ANTIMETASTATIC ACTIVITY ON A549 CELL LINE OF A NEW COMPOUND**

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Objective: Many flavonoids are natural chelators and flavonoid-metal complexes have shown to possess significant cytotoxic activity. Chrysin is a flavone distributed in plants which was reported to have many biological activities. In this study, a complex of chrysin with magnesium(II) (Mgchrys) was synthesized and its anticancer and antimetastatic activity on A549 cell line were determined.

Methods: Mgchrys has been synthesized in ethanol at pH=9 and characterized by spectroscopy techniques, elemental and thermogravimetric analysis. The effect of the compounds on the human lung cancer cell line (A549) viability was measured (MTT assay). The study on the normal lung epithelial cell line (MRC-5) was included. The morphological changes and intracellular ROS (reactive oxygen species) content (using CM-H2DCFDA probe) were evaluated. In addition, the effect of the compounds at non-cytotoxic concentrations on adhesion, migration and invasion was investigated.

Results: The IC_{50} values of chrysin were determined to be >100, 66.4±4.2 and 37.3±3,3 μM at 24, 48 and 72 h, respectively. Mgchrys improved the action of the free ligand being its IC_{50} values 83.9±2.9, 28.2±3.5 and 21.1±3,2 μM at the same time. Ligand and complex did not behave like cytotoxic agents in the normal cell line. Oxidative stress is the probable mechanism of action because the compounds increased levels of ROS. Some morphological changes (increment of cytoplasm condensation, presence of pycnotic nuclei) were observed when the cells were incubated with Mgchrys. The adhesion to fibronectin ability of A549 cells treated with Mgchrys decreased 40 % in comparison with the control. Chrysin and $MgCl_2$ did not affect adhesion. Mgchrys showed significant inhibition of migration (45%) as compared to the ligand (16%). A 47% reduction on cell invasion was observed when cells were treated with Mgchrys.

Conclusion: This study shows that Mgchrys could be considered as a promising chemotherapeutic agent.

(1763) **THE MAST CELL AS A THERAPEUTIC TARGET IN NEUROPATHIC PAIN**

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Mast cells are one of the most important cell types responsible for neurogenic inflammation, the main neurochemical substrate of neuropathic pain. These cells release biologically active mediators such as serotonin in response to the activation by immunological and non-immunological stimuli. Mast cell activation by proinflammatory neuropeptides, such as substance P (SP) and neurotensin (NT), is one of the main mechanisms involved in neurogenic inflammation.

The present study was designed to examine the effects of a lactone isolated from *Artemisia douglasiana* Besser (dehydroleucodine) and a xanthanolide isolated from *Xanthium cavanillesii* Schouw (xanthatin) on mast cell activation induced by SP and NT.

Peritoneal mast cells from male adult Wistar rats were purified in Percoll, preincubated in the presence of test lactones (dehydroleucodine or xanthatin) and then challenged with the mast cell secretagogues SP and NT. Concentration-response and kinetic studies of mast cell serotonin release evoked by SP and NT (measured by HPLC with electrochemical detection), and evaluation of mast cell viability and morphology by light microscopy, were carried out. For statistics, Type 1 variance (ANOVA-1) was performed, followed by Tukey-Kramer test. $P<0.05$ was considered statistically significant.

SP and NT increased serotonin release when compared to basal groups ($P< 0.001$ and $P< 0.001$, respectively). These effects were significantly reduced by dehydroleucodine and xanthatin in a dose-dependent manner. Serotonin release studies, together with morphological studies, showed the effectiveness of the lactones to stabilize mast cells.

The present research provides strong evidence in favour of the hypothesis that dehydroleucodine and xanthatin inhibit SP- and NT-induced serotonin release from peritoneal mast cells, acting thus as mast cell stabilizers. Dehydroleucodine and xanthatin may provide new pharmacological strategies and a new line of research in neuropathic pain therapy.

Keywords: dehydroleucodine, xanthatin, mast cell, serotonin, neuropathic pain

INFECTOLOGY 8

(406) **CHRONIC CHAGAS'DISEASE: CAUSES OF DEATH IN INFECTED TREATED WITH TRYPANOCIDAL DRUGS AND UNTREATED**

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Mortality associated with Chagas' disease remains inadequately estimated, particularly for those with the indeterminate form of the disease.

The objective of this work was to evaluate the total mortality rate and the causes of death in a cohort of chronic infected by *T. cruzi*, who received trypanocidal treatment or not.

Inclusion criteria: age at baseline> 15 and <50 and follow-up time ≥15 years.

One hundred eighty four patients were analyzed: 65 received treatment (benznidazole: 36; nifurtimox: 29) and 119 did not.

The treated and untreated groups were comparable in age (31.6 ± 9.9 vs 30.0 ± 9.2 years old) and follow-up time (26.3 ± 7.0 vs. 28.4 ± 7.7 years).

At the beginning of the follow-up, 3.1% (2/65) in the treated group presented chronic Chagas cardiomyopathy (CCC) and 10.1% (12/119) in the untreated group (p=0.143). At the end of follow-up, the proportion of CCC was significantly higher among the untreated (25/119) than the treated (6/65) (21% vs 9.2%), p <0.05.

Out of the total number of patients followed, 34 deaths were documented: 13/65 (20%) in the treated and 21/119 (17.6%) in the non-treated, non-significant difference (p=0.694).

In the treated group, causes of death were: neoplasm 5/13, ischemic heart disease 2/13, postoperative complications 2/13, and one patient in each of the following: CCC, stroke, accidents and unk-

nown cause.

In the untreated group, causes of death were: CCC 8/21, neoplasm 4/21, ischemic heart disease 2/21 and unknown 7/21.

The development of neoplasm was similar in treated and untreated, 6/65 vs 6/119 respectively (p=0.27). There was also no difference between the drug administered: benznidazole 5/36; nifurtimox 1/29 (p=0.213).

The proportion of CCC death was higher in the untreated group, while the development of neoplasm was the same in both groups.

Keywords: Chronic Chagas disease, Trypanocidal treatment, Follow-up, Causes of death.

(415) **TRYPANOCIDE TREATMENT OF CHILDREN WITH CHRONIC CHAGAS DISEASE. SEROLOGICAL AND CLINICAL EVOLUTION DURING 25 YEARS ON AVERAGE**

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The only anti-*Trypanosoma cruzi* drugs for clinical use are: nifurtimox (Nx) and benznidazole (Bz).

In chronic infection, although the parasite plays an important role in the pathogenesis of chronic Chagas cardiomyopathy (CCC), trypanocidal treatment has not been proven to prevent it.

The objective of this work was to evaluate the clinical and serological evolution in treated children over a mean follow-up of 25 years.

A total of 84 chronically infected patients were studied: 42 received treatment (7 Nx; 35 Bz) before 15 years of age (mean age 6.8±3.9 years old) and 42 did not (control group).

Treated and untreated were comparable in final age (30±8.9 vs 28.6±6.7, p=0.37) and years of follow-up (23.7±7.7 vs 26±5.7; p=0.11).

Serological evolution using 3 conventional serology tests (CS) in the treated patients were: 88% (37/42) became negative; 5% (2/42) final CS inconclusive and 7% (3/42) final SC (+). The untreated group remained SC (+).

The time of negativization differed according to the age of treatment of the children. It was 13.7±6.4 years after treatment for those treated up to 7 years old (23/37) and 20.3±5.5 years in those treated up to 8 - 14 years old (14/37) (p=0.001).

Three of the treated patients showed electrocardiographic alterations: 1) IRBBB at age 19; 2) AVB1° at age 19. At the time of treatment (6 years old) presented Q in V1 and AVL; 3) axis -30°, 46 years old with obesity and diabetes. Treatment age, 14 years old, ECG axis -10°.

Among the untreated patients, 3 had alterations in ECG compatible with CCC: 1) LAFB at 28 years old; 2) RBBB + LAFB at 22 years of age; 3) VE at 25 years old. Another 6 infected had altered ECG: 3 had IBBB at age 29, 34 and 42; 2 had axis -10° and -30° at the age of 29 and 27 respectively, and 1 patient with atrial overload and hypertension at the age of 44.

Electrocardiographic alterations compatible with CCC were only present in the infected patients who did not receive treatment.

KEYWORDS: Chronic Chagas' disease - Treatment of children- Follow-up – Cardiomyopathy.

IRBBB: Incomplete Right Bundle Branch Block; AVB: Atrioventricular Block; LAFB: Left Anterior Fascicular Block; RBBB: Complete Right Bundle Branch Block; VE: Frequent Ventricular Extrasystole

(1760) **BENZNIDAZOLE-LOADED MULTIPARTICULATE DRUG DELIVERY SYSTEMS IMPROVE EXPERIMENTAL CHAGAS DISEASE PHARMACOTHERAPY**

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Benznidazole (BZ) is the selected drug for Chagas disease treatment, showing a parasitological cure rate of 60-80% during the acute phase. High frequency of administration, long-term treatment, and several side effects are issues that negatively affect therapeutic success. We have developed BZ-loaded multiparticulate drug delivery systems (MDDS) that showed modified release of BZ. These pharmaceutical strategies would reduce side effects of BZ and/or allow reducing its frequency of administration. The present work aimed to evaluate the efficacy and safety of BZ-loaded MDDS compared to the reference treatment (BZ 100 mg/kg daily) in a murine model of Chagas disease. BALB/c mice were ip infected with 1000 Tulahuen trypomastigotes, and after 15 days post-infection (dpi) were orally treated with BZ-loaded MDDS or pure BZ at 50 and 100 mg/kg daily or intermittent (2 or 5 day intervals). In order to accurately assess efficacy, at 105 dpi mice were immunosuppressed with 4 doses of cyclophosphamide at 3 day intervals. Then, mice were sacrificed and parasitemia, parasite heart-load, relative weight of spleens, livers and hearts, and tissue injury biomarkers were analyzed. Treated animals presented a survival higher than 80% compared to mice infected and non-treated (INT) which showed a survival of 9% (p<0.001). At different schemes of therapies, both pure BZ or loaded in the MDDS at 100 mg/kg, were able to override the parasitemia in comparison to INT mice (p<0.001), and at 50 mg/kg the treatments allow reducing the parasitemia levels (p<0.05). Before immunosuppression, the percentage of ROS-producing circulating cells was higher at 50 mg/kg (p<0.001) compared with BZ at 100 mg/kg, which could be explained due to parasite persistence. BZ-loaded MDDS treatments showed a significant reduction in heart damage (p<0.05) compared to the reference treatment. Thus, BZ-loaded MDDS seems to be safer than pure BZ at 100 mg/kg preserving the efficacy for the treatment of Chagas disease.

Keywords: Chagas disease therapy, *Trypanosoma cruzi*, Benznidazole, Drug delivery systems, Standardized protocols

(892) **IDENTIFICATION OF BENZNIDAZOLE METABOLITES IN URINE FROM PATIENTS WITH CHAGAS DISEASE**

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Chagas disease is a major public health problem in Latin America. Benznidazole is an effective treatment but has several side effects.

Information about benznidazole metabolism it is scarce although there is limited evidence that conjugation with glucuronic acid by glucuronosyltransferase (i.e. phase II reaction) is involved, but it is not clear if benznidazole is directly conjugated or an intermediate phase I metabolic step takes place.

The objective of this study was to characterize benznidazole metabolites in human 24 hours urine samples from patients with Chagas disease receiving benznidazole treatment.

Samples from 3 adult female patients in the chronic stage of Chagas disease, treated with benznidazole 7 mg/kg/day for 30 days were obtained near the end of treatment. Urine from 3 healthy female adult were used as controls.

Samples were analyzed using ultra-high performance liquid chromatography coupled to a triple quadrupole mass spectrometer (UHPLC-ESI-QqQ) in Q_1-Full Mass Scan, Neutral Loss (NL) and Multiple Reaction Monitoring (MRM) mode.

Initially samples were studied using Q_1 -Full Mass Scan mode and NL mode to identify mass loss of 176 units corresponding to glucuronic acid loss after fragmentation. Once relevant peaks were identified by comparison to control urine MRM mode was developed using their transitions to confirm results.

Benznidazole glucuronides were not observed, but two different benznidazole metabolites possibly from phase I reactions were observed: aminobenznidazole (m/z 231) and hydroxyaminobenznidazole (m/z 247). Both metabolites were found in free form and conjugated with glucuronic acid (m/z 407 and m/z 423).

Identification of these metabolites opens the door for future studies to clarify the metabolism of benznidazole in patients and its potential consequences including drug interactions.

Keywords: benznidazole, metabolites, mass spectrometry.

(218) **IMMUNOHISTOCHEMICAL ANALYSIS OF SKIN BIOPSIES OF CHAGAS DISEASE PATIENTS WITH DERMATITIS CAUSED BY BENZNIDAZOLE**

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Abstract: Benznidazole is one of the two drugs available for treatment of Chagas disease with a suspension rate of approximately 17-30% in chronically *T. cruzi*-infected adult patients primarily due to the appearance of dermatitis. We have previously shown that some of these patients have IFN-γ producing T cells reactive to benznidazole. The aim of this study was to analyze the phenotype and function of cells present in skin lesions of patients treated with benznidazole who presented dermatitis. Four skin biopsies were preserved in formaldehyde 10 %. Tissue samples were then dehydrated, cleared, embedded in paraffin, cut into 4–5 µm thick sections and stained with Hematoxylin and Eosin. Immunohistochemistry was performed using specific antibodies for human CD4, CD8, perforin, IL5 and IFN-γ molecules. All of the biopsies analyzed presented inflammatory infiltrate with eosinophilia. CD8 T lymphocytes but not CD4 were found with this technique. Secretion of perforin was observed in all cases whereas IL-5$^+$ and IFN-γ$^+$ cells were not found. These findings strengthen the evidence supporting that adverse reactions to benznidazole are cell mediated with the involvement of CD8$^+$ T cells.

Keywords: benznidazole, dermatitis, Chagas disease, hypersensibility.

(477) **TRYPANOCIDAL ACTIVITY OF POLYAMINES TRANSPORT INHIBITORS**

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Polyamines play a critical role as regulators of cell growth and differentiation. In contrast with other protozoa, *Trypanosoma cruzi* is auxotrophic for polyamines; therefore, their intracellular availability depends exclusively on transport processes. Thus, the inhibition of these processes can alter the viability of the parasite. The polyamine analogues used in this work were successfully tested in cancer cells, bacteria, fungi and also showed a potent antiplasmodial effect, inhibiting polyamine transport. We evaluated the activity of these compounds on polyamine transport in *T. cruzi* and we assessed its effects on parasite viability. Three polyamine derivatives, AMXT 1501, Ant4 and Ant44, inhibited the putrescine transport in epimastigotes (the insect stage of *T. cruzi*) with calculated IC_{50} values of 3.29, 5.01 and 3.98 μM, respectively. The Ant4 analogue showed a trypanocidal effect on trypomastigotes (the bloodstream stage of *T. cruzi*) with an IC_{50} of 462 nM, while in epimastigotes the IC_{50} was significantly higher (16.97 μM). In addition, we studied the effect of the combination of benznidazole, a drug used in treating Chagas disease, with Ant4 on the viability of epimastigotes. The combined treatment produced a significant increase on the inhibition of parasites growth compared with individual treatments. In summary, these results suggest that Ant4, a putrescine conjugate, is a promising drug for the treatment of Chagas disease because it showed a potent trypanocidal effect by inhibiting polyamines transport.

Keywords: *Trypanosoma cruzi*, polyamine transporter.

(215) ***IN VITRO* STUDY OF AMPHOTERICIN-B AND CLOMIPRAMINE COMBINATION UPON *Leishmania braziliensis***

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Current drugs for the treatment of Leishmaniasis show disadvantages due to toxicity and high cost. Among these, Amphotericin-B (AMPHO), which causes the death of the parasites due to alterations in their cell membranes, also has adverse effects upon the host. Consequently, the search for new drugs, focused on molecular targets that are present in the parasite but absent in the host, continues. Clomipramine (CLO) is a potent inhibitor of trypanothione reductase, an enzyme exclusive to the Kinetoplastida order.

The aim of the present work was to analyze the *in vitro* combined effect of AMPHO and CLO upon *L. braziliensis* promastigotes. We performed a cytotoxicity assay on Vero cells by incubating 2.5x10^5 cells/mL with AMPHO = 3.25–50 µg/mL and CLO = 7.8– 250 µg/mL for 4 days, and a combined effect assay in which 3x10^6 promastigotes/mL were incubated with AMPHO = 0.002–0.032 µg/mL and CLO = 0.3–2 µg/mL. We determined the IC_{50} values, calculated the Combination Index (CI: CI>1 antagonist, CI=1 additive, CI<1 synergist) and isobolograms were graphed. The effect of the drugs on the ultrastructure of *L. braziliensis* was evaluated by transmission electron microscopy.

IC_{50} values were 0.018 µg/mL for AMPHO and 1.098 µg/mL for CLO upon promastigotes, which were 1033.3 and 104.8 times lower than the cytotoxic concentration, respectively. IC_{50} values upon Vero cells were 18.6 µg/mL for AMPHO and 126.34 µg/mL for CLO. CI upon Vero cells was 1.04 indicating additive effect and upon *L. braziliensis* was 0.18 indicating a markedly synergistic effect. IC_{50} values of the combinations upon *L. braziliensis* were located below the additivity line in the isobolograms, indicating a synergistic effect between AMPHO and CLO. The ultrastructural analysis of treated parasites revealed signs of autophagy, which was evidenced by increased autophagosomes.

The association of AMPHO and CLO can therefore be considered as a more effective and safer potential strategy for Leishmaniasis pharmacotherapy.

Keywords: *Leishmania braziliensis*, amphotericin-B, clomipramine, synergism

(533) **FOODBORNE DISEASES: EPIDEMIOLOGICAL STUDY OF PREVALENT PATHOGENS IN THE NORWEST OF ARGENTINA REGION (NOA)**

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Foodborne Diseases (FBD) affect more than 77 million American people every year, these diseases are caused by food ingestion or water polluted. The etiological agents could have different origins but the most important is bacterial. In fact, 95% of the annual deceases are due to enteropathogens like *Salmonella* and *Shigella*. On these bases, we decide to perform an epidemiological study of the pathogens responsible for infantile diarrhea, and its prevalence in the NOA population. For this propose, we isolated and characterized clinical strains from stool samples collected over 2013-2017 period in different NOA Header Hospitals. Of 17,100 cases analyzed, we isolated 1774 samples for further study. Unlike other countries, we

observed that from this total (1774), the most abundant genus was identified as *Shigella* (88.75%), and *Salmonella* in second place. It is important to note that the highest percentage of isolated *Salmonella* sp strains was obtained in the Catamarca Hospital, specifically during the 2015 year. However, the greatest number of pathogen isolation was carried out in the Tucumán province with a wide difference range. In concordance with the global tendency, we found that some isolated strains presented a multidrug-resistance phenotype. In addition, we observed that these isolates displayed a wide range of plasmidic profiles, bacteriocin production and motility behavior. A deep analysis allowed us to generate a clinical isolates collection composed of more than 500 samples. Taken together, all data analyzed here suggest that at least in the NOA region, *Shigella* is the prevalent pathogen, being *Shigella flexneri* the most frequently isolated, mainly in the 3-5 years population old.

Keywords: Foodborne diseases – Salmonella – Shigella – pediatric patients

(1909) **STUDY OF *Clostridium septicum* INTRACELLULAR SURVIVAL IN PHAGOCYTIC CELLS**

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Clostridium septicum is a strict anaerobic pathogen that causes gas gangrene, affecting humans and most warm-blooded animals. The pathogen spreads rapidly to different organs causing severe toxemia. It has been proposed that some clostridia pathogens could be transported by phagocytic cells to reach distant organs. The present study aims to compare *C. septicum* survival inside peritoneal murine macrophages and RAW 264.7 cells. Phagocytic cells were infected with *C. septicum* ATCC 12464 at different multiplicities of infection (MOI): 5:1 10:1 y 20:1 in macrophage murine and MOI 10:1 and 50:1 in RAW 264.7 cells; at 2, 4, y 20 h of incubations. The cells were cultured in DMEM medium with 10% fetal bovine serum. RAW 264.7 cells were treated after 2h of incubation with penicillin G to kill the non-phagocytized bacteria. Cells were lysed and cultured in Perfringens Selective Agar (SPS) medium at 37°C in anaerobic conditions. The bacteria and macrophage association was observed by optical and electronic microscopy. The bacteria internalized were significantly higher ($p \leq 0.05$) at 20 h of incubation and at MOI 10:1 and 20:1. At MOI 50:1 macrophages were affected by the *C. septicum* cells. The bacteria cells in contact with air rapidly lost viability. Only 0.02% and 0.013% (MOI 50:1 and 10:1 respectively) of *C. septicum* cells were viable after a 2 h period of incubation and no *C. septicum* viable cells were found in 10 h of incubation outside the macrophage without penicillin G treatment. In all the experiences, very low number (0.0001- 0.008 %) of internalized bacteria remain viable inside the macrophage. No significant difference was obtained between phagocytic activity of the both macrophage types assayed. Electronic microscopy indicates that *C. septicum* cells can escape from the phagosome and persist in the cytoplasm of macrophages, where the O_2 level is lower than the extracellular environment.

(1930) ***TRYPANOSOMA CRUZI* SERINECARBOXYPEPTIDASE IS A SULFATED GLYCOPROTEIN, AND A MINOR ANTIGEN IN HUMAN CHAGAS DISEASE INFECTION**

Luciana Lía Soprano

In this work, the presence of sulfated N-glycans was studied in a high mannose type glycoprotein of *Trypanosoma cruzi* with serinecarboxypeptidase (SCP) activity. Taking advantage that the latter co-purifies with cruzipain (Cz) from Concanavalin-A affinity columns and after the obtainment of rabbit sera specific for SCP and Cz, respectively, by immunizations with each of the *T. cruzi* glycoproteins, we evaluated the immune-crossreactivity between both molecules. In addition, the Cz-SCP mixture was desulfated finding that the cross-reactivity seemed to be due to the presence of sulfate groups in both molecules. Therefore, knowing that Cz was the first sulfated glycoprotein described and considering the antigenicity of these groups, SCP was excised from SDS-PAGE and the N-glycosidic chains were analyzed by MALDI-TOF mass spectrometry confirming the presence of short chain sulfated high mannose type oligosaccharides. Besides, we analyzed the presence of sulfated epitopes in lysates of the different stages of the parasite demonstrating that in trypomastigotes a band with apparent molecular weight similarly to SCP was highly recognized. On the other hand, SCP was confronted with sera of infected people with different degree of cardiac dysfunction. Although most sera recognized the protein in the different groups, no statistical association could be found between the presence of sera antibodies specific for SCP and the severity of the disease. In summary, our findings demonstrate i) the presence of sulfate groups in the N-glycosidic chains of *Tc*SCP, ii) the involvement of these sulfated epitopes in the immune cross-reactivity between SCP and Cz, in epimastigotes, and iii) an enhanced presence of sulfated epitopes in trypomastigotes, probably involved in parasite-host relationship and/or infection. Interestingly, our results showed for the first time that SCP is a minor antigen recognized by most of chronic Chagas disease patients.

INFECTOLOGY 9

(249) **COMBINED TREATMENT WITH FENOFIBRATE PLUS A LOW DOSE OF BENZNIDAZOLE MODULATES CARDIAC DYSFUNCTION AND INFLAMMATORY RESPONSE IN AN EXPERIMENTAL MODEL OF CHAGAS DISEASE**

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Chagas disease is the main cause of dilated cardiomyopathy in the Americas. During the acute infection, inflammatory response is critical for the control of parasite proliferation and evolution of Chagas disease. Benznidazole (Bz), the current antiparasitic drug, induces adverse drug effects (ADE) of varied severity in numerous patients, leading to treatment discontinuation or withdrawal.

Peroxisome proliferator-activated receptors (PPAR), are known to modulate inflammation. In a previous work, we showed that treatment with Fenofibrate (Fen), a PPAR-α ligand, or Bz for 30 days (d), improves several aspects of cardiac pathology. Since the dose of Bz may influence ADE onset, we sought to reduce it while keeping its effectiveness.

BALB/c mice were sequentially infected with a non-lethal strain of *Tc* (CA-I, K-98 clone, DTU TcI) for 42d, followed by reinfection with a lethal strain (RA, DTU TcVI), for 30d. Parasitemia was detectable by microhematocrit method from 21d to 42d post-infection. While Bz at 25 mg/Kg/day cleared parasites after 15d of treatment, it did not preclude cardiac pathology resembling what is found in human chronic chagasic cardiomyopathy. Therefore, we assessed the therapeutic efficacy of a combined treatment with Bz (15d) plus Fen (30d). This treatment turned the parasitological parameters negative ($p<0.0001$), restored to normal the ejection and shortening fractions, left ventricular end-diastolic and end-systolic diameter, and isovolumic relaxation time ($p<0.0001$). Moreover, it reduced cardiac inflammation and fibrosis, decreased the expression of proinflammatory (IL6, TNFα and NOS2, $p<0.001$) and heart remodeling mediators (MMP9 and CTGF, $p<0.0001$), and reduced serum creatine kinase activity ($p<0.0001$). These results suggest that combined treatment (Fen plus low dose of Bz) contributes to the resolution of cardiac pathology in a model of mixed infection with *Tc.*

Keywords: Trypanosoma cruzi; heart dysfunction; PPAR **α**; Fenofibrate; Benznidazole

(350) **EXPRESSION OF TGFB, IL-12 AND PPARA BY PERIPHERAL BLOOD MONONUCLEAR CELLS FROM PATIENTS WITH CHAGAS DISEASE. EFFECT OF FENOFIBRATE TREATMENT *IN VITRO***

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Chagas disease is one of the main causes of cardiac failure in Latin America. About 30-40% of the infected people will develop chronic chagasic cardiomyopathy leading to significant disability, with high economic and social impact.

Control of host infection requires the development of an appropriate immune response that involves a delicate balance between proinflammatory and anti-inflammatory mechanisms.

In this work we evaluated the expression of IL-12 and TGF-β in peripheral blood mononuclear cells (PBMC) of healthy (n=6) and Chagas disease patients with different stages of cardiac involvement (n=20). We observed a trend to increased expression of TGF-β and IL-12 according to the severity of cardiac involvement. A positive Spearman correlation coefficient was found between the levels of expression of TGF-β and IL-12 in PBMC, irrespectively of the clinical status of Chagas disease patients ($r=0.7921$, $p<0.0001$).

Also, we evaluated the expression of the peroxisome proliferator-activated receptor (PPAR) –α as a modulator of inflammation. We did not find significant differences in its expression in PBMC in patients of the different clinical stages (0, I, II and III) *vs* the PBMC of the uninfected control group ($p>0.05$).

Then, we evaluated the effect of 100 μM Fenofibrate (a synthetic PPARα agonist) on the expression of TGF-β and IL-12 by cultured PBMC. PBMC with increased TGF-β (patients of stages II and III) displayed reduced expression of this cytokine ($p<0.05$) and a trend to diminished expression of IL-12 upon Fenofibrate treatment.

These results show that Fenofibrate might be able to modulate the expression of TGF-β and IL-12 in PBMC from chronic highly symptomatic Chagas disease patients (stages II and III).

Keywords: Chronic Chagas disease, PBMC, Fenofibrate

(988) **BIOLOGICAL CHARACTERIZATION OF A *Trypanosoma cruzi* STRAIN ISOLATED FROM A PATIENT WITH CHAGAS REACTIVATION FROM MENDOZA, ARGENTINA.**

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Chagas disease is an endemic Latin American zoonosis caused by the parasite *T. cruzi* that affects almost 8 million people worldwide. Benznidazole (BNZ) and Nifurtimox are the only drugs available today. These treatments are highly-toxic and cause serious side effects. Most *in vitro* studies of *T. cruzi* strains have revealed different susceptibilities to BNZ, displaying a wide range of IC50 values. In this work we have studied the BNZ susceptibility and cellular tropism of a natural *T. cruzi* isolated from a patient from Mendoza (TcM) and compared with a laboratory strain originally isolated in Brazil (TcY). Isolation was carried out from a blood sample of an adult patient with Chagas reactivation due to immunosuppressive therapy. Samples with blood trypomastigotes were placed on cell monolayers to produce the infection and subsequent isolation in cell culture. To analyze BNZ susceptibility, the proliferation of both strains in the epimastigote form (in axenic culture) and amastigote form (in cell culture) were studied *in vitro* in the presence of increasing concentrations of BNZ (1, 2, 5, 12, 25, 50, 75, 100 μM) for 72 and 48 h respectively. Our results showed that epimastigotes and amastigotes of TcM strain displayed similar values of IC50 of BNZ (11 ± 0.6 μM and 11 ± 0.55 µM respectively) whereas IC50 for TcY were 33 ± 1.7 μM for epimastigotes and 19 ± 1 µM for amastigotes. We also performed infection assays in epithelial (Vero), fibroblast (MEF) and muscle (H9C2, HSkM) cell lines to characterize the tropism of this isolate. In contrast to TcY, TcM displayed high affinity for muscle cells. We concluded that TcM is more susceptible to BNZ than TcY and that exhibits a myotropic behavior. From these results and considering that drugs studies are usually performed with laboratory strains of *T. cruzi*, we recommend revising the doses established for BNZ therapy of Chagas disease.

Keywords: Chagas, *T. cruzi*, Strains, Benznidazole, Susceptibility

(1414) **LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP) AS A DIAGNOSTIC TOOL FOR CONGENITAL CHAGAS DISEASE: OBSERVATIONAL PROSPECTIVE STUDY**

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Early diagnosis of congenital Chagas disease (CChD) in newborns is essential because babies show high indices of cure. Currently, diagnosis is based on microscopic examination and serology. Molecular diagnosis is a promising alternative, although it is not well suited in laboratories with limited resources. LAMP assay has the advantage of not requiring expensive equipment. The aim of this work was to apply a colorimetric LAMP assay to detect CChD by the naked eye.

A prospective study was carried out from a cohort of newborn patients who attended the hospital "R. Gutierrez" for CChD. Laboratory studies: microhematocrit test, serology (ELISA and HAI), PCRq targeted to Satellite DNA (Duffy et al., 2013) and LAMP targeted to 18S ribosomal RNA genes (Rivero et al., 2017). Diagnosis criteria: positive microhematocrite test before 8 months of age or two positive serologic tests after 8 months of age.

A total of 118 babies born to *T. cruzi*-infected mothers were included, 82 of the 118 (69,5%) completed follow-up until final diagnosis. A total of 11 of the 82 (13,4%) patients presented CChD. LAMP showed 98% of concordance with conventional diagnosis (sensitivity of 82%, specificity of 100%, PPV of 100% and VPN of 97%). This study showed that this LAMP assay would be useful in the detection of CChD.

The advantages of this novel tool include the speed with which the assays can be completed and the fact that it can be performed without expensive equipment. A LAMP assay targeted to repetitive motifs with a higher number of copies could improve sensitivity by detecting DNA from samples with low parasitic load.

The study was approved by the Ethics and Research Committee of the Hospital de Niños "Ricardo Gutiérrez". This work was supported by FOCANLIS and FONARSEC

Keywords: Congenital Chagas disease; diagnosis, Loop-Mediated Isothermal Amplification, *Trypanosoma cruzi*.

(1755) **PROOXIDANT AND ANTIOXIDANT EFFECT OF VITAMINA C ON *Trypanosoma cruzi***

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Trypanosoma cruzi is the causal agent of Chagas disease. Many reported drugs take anti- *T. cruzi* effect by generating ROS and even the host cell produces these species in order to protect itself from the infection. Therefore, this fact reveals the parasite vulnerability to oxidative damage. This work aims to clarify the oxidative damage produced by vitamin C (vit C) in the presence of heavy metal ions traces, present inside the parasite. Employing epimastigotes of T.cruzi treated with vit C (5-30 μM) during 2-8 hs, we evaluated the level of free thiols (DTNB reaction), intracellular oxidative stress (using the oxidant-sensitive fluorescent probe H2DCFDA) and superoxide dismutase (SOD) activity (by inhibition of a superoxide-driven NADH oxidation). The results showed a decrease in the percentage of thiols groups after 5 hs approximately. This decrease is the same for the three concentrations assayed, acknowledging that the power of vitamin C pro-oxidant effect is independent of vit C concentration. After 8 hs, the percentage of thiols group increased for the three concentrations but, in this case, the major increase was noticed for 30 μM of vit C and the minor one for 5 μM of vit C. Thus, vit C anti-oxidant effect is dependent of vit C concentration. When the

parasites were treated with Bnz during 2- 9 hours, we noticed that after 5 hours a significant decrease in the content of thiols group appeared only for the higher concentration assayed. After 8 hs, the content of thiols decreased for both concentrations (15 μM/30 μM). After 9 hs, the content of thiols was recovered reaching more than 80% for both concentrations. Nevertheless, the combined treatment, vit C + Bnz, did not show an additive behavior in accordance with the results observed on an acute murine model of Chagas disease. The intracellular oxidative stress and SOD activity in epimastigotes treated under the same conditions showed results that support the change observed in the level of free thiols.

PALABRAS CLAVES: Trypanosoma cruzi, Vitamin C, Benznidazole, Oxidative Stress

(1717) ***Echinococcus multilocularis*** **SHOWS STAGE-DEPENDENT SECRETION OF EXTRACELLULAR VESICLES WITH SMALL RNA CARGO**

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Cestode parasites are flatworms with complex life cycles that affect almost all vertebrate species and can cause severe neglected tropical diseases such as echinococcosis and cysticercosis. Cross-species communication is crucial for parasites to generate a proper environment for their establishment and reproduction within the host and may be achieved by extracellular vesicle (EV) transfer. EV are membranous structures that carry various molecules, such as microRNAs (miRNAs) which are short non-coding RNAs that regulate gene expression. The secretion of EV carrying miRNAs was reported in helminth parasites and recently we described this RNA secretion mechanism in the cestodes *Mesocestoides corti* and *Taenia crassiceps*.

Here, we aimed to determine if larval stages of the zoonotic cestode *Echinococcus multilocularis* secrete RNA-containing EV and to predict candidate host and parasite target mRNAs of cestode intravesicular miRNAs.

For this, EV-enriched samples from parasite conditioned media and hydatid fluid (HF) were obtained by differential centrifugation. By transmission electron microscopy, we show that metacestode EV are retained by the laminated layer and cannot reach the extra-parasite milieu. Capillary electrophoresis analyses showed that HF and protoscoleces EV contain small RNA (sRNA) (<200 nt). The putative role of cestode EV miRNAs was predicted with the miRanda algorithm on cestode and host genes, showing that let-7-5p and miR-71-5p have the largest number of targets in both organisms. In cestodes, the most probable targets are homeobox containing genes which are related to development and differentiation while mouse targets are related to signaling pathways and inflammation.

Here, we show the importance of *Echinococcus* larval morphology on EV fate which may condition EV cargo delivery into either parasite or host cells. Also, the identification of secreted sRNAs may provide useful information for the assessment of new candidate biomarkers of cestodiasis.

Keywords: cestode; extracellular vesicle; microRNA

(873) **THE GENOMIC POSITION OF THE RIBOSOMAL PROTEIN GENES IMPACTS THE PHYSIOLOGY AND EVOLUTION OF *VIBRIO CHOLERAE.***

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Recent works suggest that bacterial gene order links chromosome structure to cell physiology. Comparative genomics shows that genes expressed early in the growth cycle are located close to replication origin (*oriC*). This is the case of genes involved in the flow of the genetic information such as those encoding ribosomal proteins (RP). Such positional bias may be an evolutionarily conserved growth-optimization strategy since these genes would benefit from a higher dosage during exponential phase due to multi-fork replication. We addressed this issue using *V. cholerae*, a fast-growing pathogen, as model organism. Using genome-editing techniques, we altered the genomic position of the *S10-spc-α* locus (S10) an array of genes conserved among all three Domains of life, that encodes half of the RP. Interestingly, a longer distance between S10 and *oriC* tightly correlated with a reduction in growth rate, fitness and infectivity of the mutant strains. Since the addition of further copies of the locus did not improve *V. cholerae* growth, we conclude that the locus has a physiologically optimized genomic location. Deep sequencing of the DNA and RNA extracted from the most affected derivatives in fast growing conditions shows a reduction in the dosage and the expression of the whole *oriC* region. The experimental evolution of these strains for 1000 generations shows that the RP location influences how these strains evolve since growth rate and fitness suffer a long-term impact. Our results indicate that S10 positioning connects genome structure to cell physiology in *V. cholerae*. The genomic positioning RP and probably other genes involved in the flux of genetic information conditions bacterial physiology and evolution. We believe that in a near future, many more genes whose genomic position impacts bacterial phenotype will be identified.

(1546) **IN-HOOD HEAT GUN STERILIZATION OF CELL-CULTURE AND SMALL-ANIMAL SURGICAL INSTRUMENTS.**

Ana Laura Sirvan, Oscar Filevich

BIOMED - UCA - CONICET

One of the most common causes of failure in experiments involving culture of cell lines and primary cells involves contamination by either bacteria or fungi. These fungi and/or bacteria often invade the sterile culture environment through the very same instruments used in the manipulation of the cultured cells or tissues (glass pipettes, metal tweezers) and during animal surgery (small scissors, tweezers, and pipettes).

We devised a method, using a readily available, household-grade 2 KW heat gun for heat-sterilizing the required instruments inside the flow hood, immediately before use. Instruments may be re-sterilized in just a few minutes (<5') "just in case" if in doubt of accidental contamination during surgery. No flame is involved, which makes the procedure fairly safe and clean and leaves no combustion products over the tools. Cold-to-hot transition takes only seconds, unlike other similar techniques, and cooling to RT is also very quick.

We routinely use this method for sterilizing the tools and substrates used in culturing cell lines, and for culturing glial cells and neurons extracted from (non-sterile, whole animal) newborn mice brains.

Parameters for air speed, temperature, object exposure time and surface temperature are presented, as well as inexpensive ways of determining these parameters for any hot-air flow.

We hope this method will be widely adopted, since it is quicker than traditional autoclaving and can be used in-hood immediately before surgery, thus improving the throughput of manual cell and tissue manipulation for cell culture.

Keywords: Sterilization, Contamination, Cell culture, Autoclave.

(1699) **OPTIMIZATION OF THE ENZYME-LINKED IMMUNOSPOT FOR THE MEASUREMENT OF TRYPANOSOMA CRUZI-SPECIFIC ANTIBODY PRODUCING CELLS**

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Abstract: Plasmablasts, short-lived antibody-producing cells, can be detected in the circulation during an active infection. Thus, the

measurement of T. cruzi specific plasmablasts and memory B lymphocytes in the circulation could represent sensitive biomarkers to evaluate the efficacy of treatment against T. cruzi. Herein, we have set up the measurement of plasmablasts and memory B cells specific for T. cruzi in the circulation of chronic Chagas disease patients by ELISPOT assay. Peripheral blood mononuclear cells (PBMC) were stimulated with the R848 polyclonal activator and recombinant IL-2 to induce memory B cells into plasmablasts. ELISPOT plates were seeded with different concentrations of a) human polyclonal anti-IgG antibodies to detect total IgG secreting cells; b) a T. cruzi lysate derived from epimastigotes of the Tulahuén strain or T. cruzi-derived recombinant proteins to detect parasite specific antibody-producing cells. 100,000-500,000 PBMC were seeded per well to detect T. cruzi-specific IgG production and 25,000-50000 PBMC for total IgG detection. As negative control, 1% BSA was used while tetanus toxin antigens was used as positive control. After 24 h of incubation, the plate was developed with streptavidin and BCIP/NBT. The spots were analyzed using Immunospot v 5.0. 50.000 and 5000-50.000 PBMC were set up as the optimal conditions for detection of total memory B cells and plasmablasts, respectively, using in both cases 15 µg/ml of polyclonal anti-human IgG. The final conditions for detection of T. cruzi-specific memory B cells were 35 μg/ml of epimastigote lysate or 10 μg/ml of T. cruzi-derived recombinant proteins and 250000 PBMC following polyclonal activation; while 5 μg/ml of epimastigote lysate or 10 μg/ml or recombinant proteins and 500.000 unstimulated PBMC were set up to detect T. cruzi-specific plasmablasts. These findings show the feasibility to detect T. cruzi-specific memory B cells and plasmablasts in the chronic phase of Chagas disease.

Keywords: B cell, ELISPOT assay, *T. cruzi*, Plasmablast.

(1663) **ANTIBIOTIC RESISTANCE OF *Escherichia coli* ISOLATED FROM NORWAY RATS (*Rattus norvegicus*) FROM FARMS OF NORTHEAST BUENOS AIRES**

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Norway rats (*Rattus norvegic*us) represent a public health problem, since they are reservoirs of several zoonotic pathogens. The presence of Norway rat populations in the study area is associated with animal breeding farms in which rats reproduce all year long because of the continuous availability of food and water. Given the increasing occurrence of *Escherichia coli* strains resistant to antimicrobials in the farms and the discussion about environmental reservoirs, the purpose of this study was to perform a preliminary screening to determine the dispersion of antibiotic resistance of *E. coli* isolates recovered from Norway rats on farms from two localities, Marcos Paz and San Andrés de Giles in the province of Buenos Aires.

A selected group of 28 isolates of *E. coli* recovered from Norway rats were examined. Isolates were obtained from cultures of large intestine samples on McConkey agar plates. To determinate the antimicrobial resistance, the Kirby–Bauer disk diffusion susceptibility test was performed according to the CLSI.

28.5% *E. coli* isolates were resistant to multiple drugs (resistance to at least three 3 or more antimicrobial classes). Resistance to ampicillin and cephalothin was higher (46.4% and 42.9%, respectively), while resistance to 3GCs (ceftriaxone) was low (14.3%). Resistance to different antimicrobial families was observed such as tetracycline, nalidixic acid (32%) and streptomycin (28%). Only one isolate showed resistance to colistin. None of the strains showed resistance to imipenem and fosfomycin.

The ability of rats to be a potential source of environmental antibiotic resistant pathogens has received little consideration, and their importance of this host as dispersed of these organisms-requires further investigation. Thus, the preliminary results of this study shows that rats may act as a source of the dissemination of multi-resistant bacteria and resistance genes from the productive system to the human.

Keywords: antibiotic, Escherichia coli, resistance, norway rats

INFECTOLOGY 10

(347) **FUNCTIONAL RELATIONSHIP BETWEEN THE CAPSID DOMAINS OF SIMIAN AND FELINE IMMUNODEFICIENCY VIRUSES GAG POLYPROTEINS**

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The assembly of lentiviral particles is dependent on the multimerization of the Gag polyprotein at the plasma membrane of the infected cells. We have previously demonstrated that the capsid (CA) domain of Gag establishes the protein-protein interactions that drive the formation of lentiviral particles. To gain a better understanding of the functional equivalence between the CA proteins of simian and feline immunodeficiency viruses (SIV and FIV, respectively), we first generated chimeric SIVs containing FIV CA-derived regions. The phenotypic characterization of these chimeras showed that the SIV bearing the FIV CA amino-terminal domain (CA-NTD) is assembly-defective whereas the SIVs carrying the entire FIV CA or its carboxyl-terminal domain (CA-CTD) assemble into virions as efficiently as wild-type SIV. Further analysis of the latter chimeras demonstrated that they incorporate the envelope glycoprotein and contain wild-type levels of viral genomic RNA and reverse transcriptase; however, these viruses are non-infectious due to a post-entry impairment. Surprisingly, when we studied the FIV Gag chimeras carrying SIV CA regions we found that they were all assembly-defective. Interestingly, all of them are able to interact with wild-type SIV Gag and be rescued into extracellular particles, regardless of the SIV CA sequences present in the chimeric FIV Gag. Overall, our data support the notion that although the SIV and FIV CA proteins share 51% amino acid sequence similarity and exhibit a similar organization, i.e., an NTD joined by a flexible linker to a CTD, their functional exchange between SIV and FIV is strictly dependent on the context of the recipient Gag precursor. Collectively, our results illustrate the functional relationship between the CA proteins of primate and nonprimate lentiviruses.

Keywords: lentiviruses, Gag polyprotein, capsid domain, virion assembly

(1882) **ESTABLISHMENT OF A PROSPECTIVE COHORT OF ADULT HIV POSITIVE PATIENTS IN LATIN AMERICA (LATINA PROSPECTIVE COHORT): BASELINE DATA ANALYSIS**

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LATINA (Prospective Latin American Cohort of adult HIV positive patients in Latin America) is a multinational initiative currently including 13 sites distributed in: Argentina, Mexico, Perú and Colombia. Data presented here correspond to basal information from the first 41 months of recruitment (up to April 2017). Inclusion criteria: 1- recent HIV diagnosis (within 1 year) and 2- at least 2 prior visits at cohort site. Diagnosis for AIDS defining diseases was based on CDC criteria and standardized criteria were developed by the Executive Committee for serious non aids events. Database was periodically checked for incompleteness and consistency.

All data was consolidated in an Access database and analyzed

using Statistics V17

So far, 1807 patients have been included. All of them had complete demographic data, completeness of data addressing the remaining data included show a range of incompleteness ranging from 5 to 24%.

Median age is 31 (IQR 26-41), 86.2 % are male, 46% white and 53.5 latino. Median of 11 years of formal study (IQ range: 8-14). Health coverage comes mainly from public sector 77.8%

Transmission occurred in in 88% of cases through sexual intercourse either heterosexual in 30% of cases or homosexual/bisexual 58%

A total of 20.1% of patients had a progression of disease (POD) condition.

2.6% of patients had hypertension, 1.7% presented with dyslipidemia. Cancers not related directly to HIV were present in 0.6% of patients and cardiovascular disease in 0.7%. 2% had HBV coinfection while 0.9% HCV. 1.1% of patients had major depression.

CD4 and CV count median values were 401 (IQR 207-590) and 451000 (IQR 6875-178242) respectively.

Though HIV is not decreasing in most Latin American countries, there is little information about clinical status at diagnosis and therapeutic course of patients initiating follow up.

Data presented here reflects the regional situation even in the era of broad access to antiretroviral therapy, with significant proportion of late presenters, high prevalence of hypertension, dyslipidemia and serious HIV non marker diseases.

The current challenges for the cohort are: to increase the cohort, improve the quality of data through automatic on line query generation process and refining monitoring process.

(788) **ANTIVIRAL EFFECT OF TROGER'S BASE DERIVATIVES AGAINST HERPESVIRUS INFECTION**

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Abstract: In one hand, the Herpes simplex virus (HSV), the prototype of double DNA strand viruses, have played a prominent role in the development of antivirals; in the other, Troger's base (TB) structural features have been utilized in the design of DNA binders. This encouraged us to the study of the antiviral properties of this type of compounds.

In this work we reported the synthesis and the antiviral effect of six novel TB derivatives against HSV infection.

The key step in the synthesis was the skeleton construction, through the acid-catalyzed condensation between an aniline and paraformaldehyde. All final compounds were chromatographically purified and characterized by EM and 1D and 2D NMR techniques.

Regarding the biological activity, it was found that the compounds were not cytotoxic in Vero cells, presenting CC_{50} values >300μM. To evaluate the antiviral activity different concentration of the six TB derivatives were added after virus adsorption and 24 h later the supernatants were harvested and the viral titers were obtained by plaque assay. Most of the analyzed compounds inhibited virus yield of three strains of HSV-1 (HSV-1 KOS, and HSV-1 TK^- strains B2006 and Field) and two strains of HSV-2 (HSV-2 G and HSV-2 MS). The compounds also modulated cytokine production in J774A1 macrophages cultures induced by bacterial LPS.

The results obtained shown that these TB derivatives inhibit HSV in Vero cells and modulate cytokine production in macrophages.

Keywords: Troger's base derivatives, antiviral, herpesvirus

(117) **MICROBIOLOGICAL AND PHYSICOCHEMICAL STUDIES OF KETOKONAZOLE:β-CYCLODEXTRIN:CITRIC ACID MULTICOMPONENT INCLUSION COMPOUNDS**

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Abstract: *Candida* is an opportunistic yeast that causes severe diseases in immunocompromised individuals. Ketoconazole (KET) is an antifungal agent suitable for the treatment of candidiasis and other systemic fungal infections, classified as a class II drug in the Biopharmaceutics Classification System because of its low solubility and high permeability. Cyclodextrins (CD) multicomponent complexation is used to improve the poor aqueous solubility of different drugs. As citric acid (CA) is a pH-modifier used to increase solubility of weakly basic compounds, the aim of this work was to assess the effect of KET:βCD:CA multicomponent complexation in the solubility and antifungal activity of KET.

The effects of complexation on drug solubility, was determined in water, by means of phase-solubility studies. In solid state, the multicomponent systems, prepared by physical mixture (PM) or kneading (K), were studied by Fourier-transform infrared spectroscopy, thermal analysis and scanning electron microscopy. The microbiological activity was evaluated by the agar diffusion test.

The presence of βCD and CA caused a significantly increase in KET solubility with S_0 of 0.542 μg/ml in water, 2.6 mg/ml in presence of CA (10 mM) and S_{max} of 3.8 mg/ml with CA and βCD (13.2 mM). Taking into account the results obtained by the different techniques in solid state, it is possible to confirm the formation of inclusion complex between KET and βCD in presence of CA, in systems prepared by K. The PM shows a behaviour comparable with the pure compounds. Furthermore, the antifungal activity against *Candida albicans* y *C.parapsilosis* was significantly improved (p<0.05).

In summary, the multicomponent system prepared in our study, with simultaneous enhancement of KET solubility and microbiological activity, might have an important pharmaceutical potential in the development of a better antifungal formulation of KET.

Keywords: ketoconazole, cyclodextrin, citric acid, solubility, antifungal activity.

(1033) **CLINICAL AND GENOMIC CHARACTERIZATION OF INFECTIONS WITH THE MAIN HEPATITIS B VIRUS SUBGENOTYPE CIRCULATING IN MAR DEL PLATA**

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The most prevalent Hepatitis B virus (HBV) subgenotype (subgt) circulating in Mar del Plata is F1b. Nucleotide analogs are the current antivirals used for the treatment of chronic infections. The aim of this work was to clinically characterize infections with HBV F1b subgt and to determine the presence of mutations related to antiviral resistance. Thirty seven serum samples, previously genotipified as F1b subgt, from treatment-naïve patients with no co-infections, attended at public health institutions, were included. Patient's clinical information was collected and analyzed. DNA was extracted, HBV pol gene was amplified by nested PCR and sequenced. The sequences were phylogenetically analyzed. The presence of clinical relevant mutations was determined. In this cohort, the mean age of the patients was 44 years (ys), being 75.7% males (45.08 ys) and 24.3% females (32.25 ys). A 93% of the infections have been sexually transmitted, in agreement with the most usual actual infection route. According to serological markers, 75.8% were acute infections and 24.2% were chronic. Within the acute group, 96% were symptomatic and 88% had elevated transaminases levels. All chronic patients were HBeAg positive, and 2 were anti-HBcIgM positive, probably due to reactivations. The analysis of the nucleotide sequences of the POL gene showed mutations in 10 samples (7 acute, 3 chronic). The analysis of POL proteins showed that 5 samples (4 acute, 1 chronic, 13.5%) presented mutations at the reverse transcriptase (RT) domain. Any of these mutations were previously associated to antiviral resistance. One mutation, (Frt148Y) was

present in 3/5 samples, suggesting that it may be characteristic of the F1b subgt from Mar del Plata. In conclusion, new acute HBV infections in Mar del Plata are being produced by the F1b subgt. Most of the studied virus from acute patients do not show mutations at the RT domain related to antiviral resistance, as expected in a treatment-naïve cohort.

Keywords: HEPATITIS B VIRUS, SUBGENOTYPES, MAR DEL PLATA

(139) **IDENTIFICATION OF ANTIMICROBIAL COMPOUNDS AGAINST BRUCELLOSIS TARGETING RIBOFLAVIN SYNTHASE**

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Brucella spp. are facultative intracellular bacteria, that are adapted to life within cells of a large variety of mammals. These bacteria are the causative agent of brucellosis, a zoonotic infection with the highest incidence in the world affecting livestock and humans and for which a safe and completely reliable vaccine is still unavailable. Riboflavin (vitamin B2) is the universal precursor for the biosynthesis of FMN and FAD, two versatile redox cofactors in nature. This vitamin is biosynthesized in microorganisms and plants but must be obtained from dietary sources by animals. Owing to the absence of this pathway in animals and humans and the fact that endogenous riboflavin synthesis is necessary for the growth of many pathogens, this pathway is regarded as a rich resource of targets for the development of new antimicrobial agents. In this work, we describe a High-Throughput Screening (HTS) approach for the identification of inhibitors of the enzymatic activity of Riboflavin Synthase, the last enzyme of the riboflavin biosynthesis pathway, along with its subsequent validation as potential drugs candidates in an *in vitro* model for brucellosis infection. From a set of 44,000 highly diverse low-molecular weight compounds, a total of 163 exhibited a 30% threshold inhibition at 30 μM concentration. Further secondary screening with dose-response curves allowed for the identification of ten molecules with 50% inhibitory concentrations (IC50s) in the low micromolar range. Interestingly, a subset of five compounds sharing a 2-Phenylamidazo[2,1-b][1,3]benzothiazole chemical scaffold also displayed bactericide activity against *B. abortus*. Furthermore, we tested the effect of the two most promising compounds on the survival of intracellular bacteria and we found that they are able to inhibit *Brucella* growth in a murine macrophage cell line. These findings represent a promissory advancement in the pursuit of antimicrobial compounds against brucellosis.

Keywords: Brucellosis, Riboflavin Synthase, High-Throughput Screening, intracellular replication, Drug development.

(1017) **INTERACTION OF *BRUCELLA* WITH POLARIZED EPITHELIAL CELLS**

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Brucella, the bacterial agent of the worldwide distributed zoonosis brucellosis, primarily infects host phagocytes, coursing an intracellular life cycle within a membrane-bound compartment known as the *Brucella*-containing vacuole. Even though we currently have a great body of knowledge regarding the molecular mechanisms these bacteria use to modulate the phagocyte biology in order to establish an intracellular replication niche we still do not understand how *Brucella* adheres and trespasses epithelial barriers. Our laboratory has recently demonstrated that *Brucella* adheres and invades a polarized epithelial cell culture such as the **M**adin-**D**arby **C**anine **K**idney (MDCK) cells through the basolateral face. In order to fully understand how *Brucella* interacts with this cell type we performed a complete intracellular replication curve and determined the fate of the bacteria at different times post-infection by immunofluorescence confocal microscopy. Our results indicate that *Brucella* traffics in MDCK cells, modulating actin polymerization in the process and triggering its own egress in order to infect new cells. Moreover, infection of polarized cells grown in transwells suggested that the egress occurs through the basolateral face of the cell. Confocal analysis of these infected cells stained with antibodies against the apical and basolateral markers gp135 and p58 confirmed this hypothesis. Our studies provide a first approach in order to understand how this pathogen interacts and trespasses epithelial barriers, the first line of defense it encounters in the infectious process.

Keywords: Brucella, adhesion, intracellular traffic, polarized epithelial cell

(1457) **"NUTRITIONAL VIRULENCE": THE ROLE OF SERINE DURING THE INFECTION OF *BRUCELLA***

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Upon invasion of host cell, the ability to survive in the harsh intracellular milieu is crucial for many pathogenic bacteria as is the case of *Brucella*. How pathogens adapt their metabolism to the new nutritional conditions encounter in the intracellular environment has been one of the most underestimated aspects of bacterial pathogenesis. In this work we study SerB (BAB1_1410), a phosposerine phosphatase catalizing the last step of serine synthesis. We demonstrated that recombinant SerB has optimal phosphatase activity at pH=7 with a strong preference for Mg^{2+} over Mn^{2+}.To analyze the importance of serine in the biology of *Brucella*, we constructed clean deletion mutants of *serB* in *Brucella suis* and *Brucella abortus* and tested their ability to grow in both complex (TSB) and defined media (GW). Although no significant differences were observed in TSB, deletion of *serB* affected the ability of *Brucella* to grow in GW and this defect was reverted by supplementing the medium with serine, indicating that synthesis of serine is required under this growth conditions. To assess whether SerB is necessary during the intracellular stages of *Brucella*, in vitro infection of J774 macrophage-like cells and epithelial HeLa cells were assayed. In both models, *B.abortus serB* and *B.suis serB* were unable to sustain intracellular replication. As it was observed in the axenic culture, supplementation of the cell culture medium with serine was capable to revert the defect, indicating that synthesis of serine is necessary to sustain intracellular infection during the first phase of infection. Mice infection demonstrated that SerB mutants are attenuated thus confirming the cell culture infection assays. Taken together, these results indicated that synthesis of serine is a central requirement for the virulence of *Brucella*.

Keywords: phosphatase, serine, *Brucell.*

(1472) ***BORDETELLA BRONCHISEPTICA* LAPD INTERACTS WITH TWO DIFFERENT DIGUANYLATE CYCLASES**

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Cyclic-di-GMP (cdG) is a widely distributed second messenger in the bacterial world. It is produced by diguanylate cyclases (DGCs) and can direct many cellular processes like motility and biofilm formation. Over the last years our group has been working on phenotypes regulated by this molecule in the genus *Bordetella*. We have previously described that cdG regulates biofilm formation in *B. bronchiseptica*. This process relies, at least in part, on an effector system homologue to the *Pseudomonas fluorescens* Lap system: LapD, LapG and BrtA.

We speculate that there might be at least one DGC that physically interact with LapD in order to provide cdG in a specific manner to the mentioned Lap system. Using a bioinformatic methodology we have looked into every *B. bronchiseptica* membrane DGC and predicted that BdcG, a putative DGC, physically interacts with LapD. We have also considered that BdcA could interact with LapD because our previous results showed a strong correlation between BdcA activity and biofilm formation.

In order to advance on the knowledge of the recently found BdcG,

we analyze DGC activity using two different methodologies (heterologous expression on a *P. fluorescens* reporter strain and cdG direct measurement). Neither of the approaches could confirm if BdcG is an active DGC.

Bacterial Two Hybrid experiments were used to figure out if mentioned DGCs interact with LapD. Statistically significant differences between LapD-BdcA, LapD-BdcG and controls were found in β-galactosidase assays. Those results indicate that LapD physically interacts with BdcA and BdcG.

Furthermore we have also generated simple and double knock-out mutants of *bdcA* and *bdcG*. We hypothesized that deletion of DGCs may impaired biofilm formation. Neither simple nor double mutants showed a biofilm formation defect.

Taking in mind that *B. bronchiseptica* genome has 10 sequences that encode for DGCs our results suggest that other DGCs are part of the regulation network through Lap proteins.

Keywords: *Bordetella bronchiseptica*, biofilm, c-di-GMP, Bacterial Two Hybrid, protein-protein interaction

(1904) **OMP19 PLAYS A KEY ROLE IN *Brucella abortus´*s ABILITY TO ESTABLISH CHRONIC INFECTION AFTER ORAL INGESTION**

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Oral infection is one of the principal ways in which brucellosis is acquired. Animals usually sniff and lick fetal and placental tissues from abortions caused by brucella. Transmission of brucellosis to humans may occur by consumption of infected, unpasteurized animal milk products. We have previously demonstrated that U-Omp19 from *Brucella* spp. is a broad-spectrum protease inhibitor: inhibits main gastrointestinal and lysosomal proteases; moreover Omp19 has a role in *B. abortus* virulence after intragastric –gavage- infection. In this work, the role of Omp19 in *B. abortus* oral infection was studied in depth. In this case BALB/c mice were directly inoculated at the oral cavity with *wt* or a mutant *B. abortus* strain without Omp19 (Δ*omp19)*. At 20 days post infection there were very few colonies of Δ*omp19* strain at the spleens indicating this strain was highly attenuated ($P<0.01$ vs *wt*). Omp19 protects *B. abortus* from main gut proteases since *Δomp19* strain was more susceptible to mouse intestine lysates and to pancreatin (commercial pig pancreatic extract) than *wt* strain ($P<0.001$). When incubated with intestinal extracts the *wt* strain could grow but a bacteriostatic effect was evident in Δ*omp19* strain. This bacteriostatic effect was also found *in vivo* in gut isolated Δ*omp19* strain 2h after oral infection of mice. Confocal microscopy images revealed that when exposed to pancreatin wt strain formed normal colonies, but Δ*omp19* strain formed no or small colonies, with a significant reduction in the number of cell divisions ($P<0.05$). Cell cycle progression analysis by flow cytometry indicated a pancreatin-induced cell cycle arrest at G1 in Δ*omp19* strain that was not evident in *wt* strain. Thus, Omp19 deficiency leads to *B. abortus* cell division defects and cell cycle arrest when exposed to pancreatic proteases. Altogether these results demonstrate that Omp19 has a crucial role in the establishment of chronic *Brucella* infection after oral route.

Keywords: *Brucella*, protease inhibitor, Omp19, oral infection.

BIOPHYSICS 8

(1922) **THE PORE REGION OF HUMAN AQUAPORIN-1 IS INFLUENCED BY DISTANT RESTRICTIONS IN α-HELICES. A MOLECULAR DYNAMIC SIMULATION (MDS) APPROACH OVER THE CRYSTALLOGRAPHIC STRUCTURES 1FQY AND 4CSK**

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Human Aquaporin-1 (hAQP1) is an integral membrane protein that transports water as canonical function. We have reported that hAQP1 permeability is modulated by changes in membrane tension. To investigate whether this modulation is related to the dynamic conformation of the pore, 1FQY and 4CSK were studied by MDS. For both structures three systems were defined and analyzed: a) tetramer restricted in α-carbons (Cα) in contact with the lipid bilayer (RT); b) tetramer without any further restrictions that those provided by the tetramerization itself (FT); and c) monomer (M). Several descriptors were used for analysis, such as the classical RMSD and Solvent Accessible Surface Area in residues of the NPA and ar/R sites, and original ones as geometric description of 9 sections in the pore and the number of water molecules inside it. When statistical procedures like PCA and PARAFAC were performed, systems were discriminated. Results: 1) the contribution of restricting Cα in tetramers differs from the contribution of tetramerization itself; 2) monomers behave differently within FT and RT; 3) at level of the pore, no descriptor *per se* explains the differences observed between FT and RT systems; 4) localized restrictions in RT cause modifications in far and not restricted sites inside the pore. Conclusions: The distinct behavior shown by FT, RT and M systems open the possibility to deep in a possible influence of the membrane properties that could modify the permeability of the pore. Our work shows quantitative evidence that restrictions affect, subtle but significantly, the dynamics of the protein at level of the aqueous pore. We also report the need to exhaustively explore the assumptions in the use of MDS, since we have detected differences in the behavior of two structures that describe the same protein. Our findings also confirm the need for further investigations in the relationship of the aquaporins with their lipid environment to understand their fine regulation.

Keyword: AQP1, Molecular Dynamic Simulation, Membrane Tension, Pore Structure

(172) **A QUANTUM CLASSICAL STUDY OF THE REACTIVITY OF LOW MOLECULAR WEIGHT THIOLS AND PERSULFIDES TOWARDS HYDROGEN PEROXIDE**

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Hydropersulfides are thiols (RSH) derivatives species which belong to the group of sulfane sulfur compounds. They have been recently reported to be prevalent in mammalian systems, presenting relatively elevated concentrations of up to 0.1 mM. These thiols derivatives have been considered to have important biological function because of their unique chemical properties exhibiting nucleophilic (and so presenting many points in common with thiols chemical properties) but also electrophilic character. Moreover, it is usually assumed than persulfides (RSS^-) present enhanced nucleophilicity compared to the corresponding thiolates (RS^-). In addition, hydropersulfides presents higher acidity than thiols, with an estimated pKa difference of around 2 units and thus a bigger availability of persulfide anion at physiological pH could be reached.

Persulfides may play an important role in cellular redox regulation and possible other physiological functions related with signaling and

catalysis. Despite the growing attention of the bio-medical community in this novel species, the information reported in bibliography is still sparse and many questions about their reactivity and its relevance in biological systems have not been answered yet. In this work, we present an exhaustive study of selected thiolates and persulfides reaction toward hydrogen peroxide employing hybrid quantum mechanics-molecular mechanics (QM/MM) molecular dynamics (MD) simulations, using an umbrella sampling approach aiming to understand the whole processes in aqueous solution, in a realistic solvent representation at room temperature.

In each case, this simulation scheme allowed us to obtain thermodynamical information such as the free energy profile in addition to microscopic insight about electronic structure changes throughout the reaction. Our results suggest that contrary to what is commonly assumed by the community, persulfides are nucleophiles, at most, lightly stronger than corresponding thiolates.

Keywords: Hydropersulfides, Reactive Oxigen Species, Oxidative Stress, Mechanism, QM/MM.

(1756) **ANOTHER TURN OF THE SCREW IN THIOREDOXIN CASE: THE QM/MM CLUE.**

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Thioredoxin is one of the most studied proteins from experimental and computational point of view and it is an important model system because the active site primary structure (-CXXC-) is in other thioredoxin-like oxidoreductases. The reactivity of this active site is based on two consecutive nucleophilic substitution of thiolate to yield a disulfide bond, to shift the target system from an oxidized to a reduced state or vice versa. The principal features that make this proteins more or less reductive are related to the thiol/thiolate and disulfide relative stabilities *i.e.* p*K*a, environment polarity, hydrogen bonds, strain restrictions etc. However it is difficult to quantify how much these factors influences the relative stabilities of species.

We estimated the reaction free energy of thioredoxin successive nucleophilic substitutions, using computational Molecular Dynamics simulations based on Quantum Mechanics/Molecular Mechanics and Free Energy sampling technique (Umbrella Sampling). The target ligand was dithiothreitol. The free energy of activation can be related with pH-independent kinetic constants and it gives account of the intrinsic nucleophilicity of thioredoxin thiolates. The simulations allow the study of mixed disulfide species (product of the first nucleophilic substitution), which is not experimentally available.

Results show that the protocol developed for these simulations works to account the fine structural and dynamics features that influence the thioredoxin reactivity. The first step exhibits a reaction free energy close to zero and it has a free energy of activation ~10 kcal/mol. The second step is barrierless and the product (oxide thioredoxin and reduced dithiothreitol) is stable by 30 kcal/mol respect to mixed disulfide (the reactive). In our knowledge this is the first time that the complete free energy profile, including substrate, all intermediate, transition states and product, is quantitatively described for thioredoxin.

Keywords**:** Free Energy, Umbrella Sampling, Thioredoxin, QM/MM.

(1707) **CHARACTERIZATION OF RAT CALTRIN STRUCTURE AND ITS INTERACTIONS WITH MODEL MEMBRANES AT THE AIR-WATER INTERFACE USING MOLECULAR DYNAMICS SIMULATIONS AND OTHER BIOINFORMATICS TOOLS.**

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Rat caltrin (calcium transport inhibitor), the small and basic protein of the seminal plasma, binds to the spermatozoa during ejaculation and inhibits sperm extracellular Ca^{2+} uptake. Thus, it prevents the sperm spontaneous acrosomal exocytosis along the female reproductive tract. Although the sequence and some biological features of rat caltrin were studied, its physicochemical properties and 3D structure are still unknown. In this work we predicted the rat caltrin 3D structure, by molecular homology modeling and threading, which maintained its secondary and tertiary structures along molecular dynamics simulations. The molecular structure was further characterized by circular dichroism. Surface electrostatic potentials and electric fields were calculated using the Poisson-Boltzmann equation and the overall protein dipole was also evaluated. Bioinformatics tools and available web servers were used to deeply analyze physicochemical characteristics such as Kyte and Doolittle Hydropathy score, solvent accessibility, Wimley-White whole-residue hydrophobicity and helical wheel projections. The equilibrium spreading pressure was estimated by Gibbs adsorption isotherms. Interactions between rat caltrin and phospholipids model membranes were defined by penetration (cut off) studies. Rat caltrin was able to penetrate into the membranes, mainly in negatively charged surfaces and expanded lateral phase states, and the amino acid residues involved in the protein-membrane interaction were also predicted. To further characterize the protein binding to membrane surfaces we carried out simulations in the presence of negatively charged bilayers. Results presented have significant relevance to understanding the molecular mechanisms of caltrin to modulate physiological processes associated with fertilization.

(1921) **CONSTANT-pH MOLECULAR DYNAMICS IN A COARSE-GRAINED MODEL**

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IQUIFIB

In this work we show results of a preliminary development of an algorithm to study the effect of pH in proteins using molecular dynamics techniques, in particular, to study pKa shifts of protonable residues. Our implementation is based in the transferable AWSEM forcefield (Associative memory, Water-mediated, Structure and Energy Model). We use a Metropolis Monte Carlo algorithm to choose the discrete protonation state of protonable residues. This couples the effects of pH to protein dynamics. The advantage of this coarse-grained implementation is that we will be able to explore conformational changes such as unfolding/folding and large domain movements which often occur in solution, in a transferable potential. Our preliminary results in protein models which are often used to study charges effects show that the pKa obtained are in good correlation with experimental reported results and other coarse-grained constant-pH simulation packages. Our results suggest that the first and simplest implementation works better than previously reported coarse-grained methods for constant-pH dynamics. Moreover, for surface residues results are much better than for buried ones suggesting that other considerations should be taken into account, which are being applied for next versions of the algorithm.

Keywords: constant-pH, AWSEM, pKa, folding, coarse-grained.

(1780) **HIDDEN MARKOV AND BAYESIAN INFERENCE MODELING OF ACETYLCHOLINE RECEPTOR DIFFUSION IN THE MEMBRANE**

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We are currently using various fluorescence microscopy techniques to study the supramolecular structure and dynamics of the nicotinic acetylcholine receptor (nAChR) expressed at the surface of CHO-K1/A5, a clonal cell line developed in our laboratory. Images are obtained using stochastic optical reconstruction microscopy (STORM), a form of single-molecule localization nanoscopy. The dynamics of individual receptor molecules are followed in living cells using single-particle tracking (SPT) methods. In addition

to mean-square displacement analyses of ensemble trajectories, we are also employing hidden Markov modeling (HMM) methods (Chung et al., 2010; Monnier et al., 2015) in view of their ability to identify stochastic switching within a single molecular trajectory, thus opening the possibility to reveal heterogeneous motional regimes along individual molecular trajectories. The addition of Bayesian model inference to the HMM analysis ("HMM Bayes") further facilitates the selection of the simplest stochastic motional model describing the molecular trajectory. We have also applied another Bayesian inference approach (Teskouras et al., 2016) to determine the stoichiometry of the fluorophore-carrying moiety of the nAChR. This Bayesian procedure counts photobleaching steps, takes into account stochastic noise variation and is able to discriminate overlapping photobleaching events. When applied to the nAChR tracks along the surface of the cell, we could discriminate subpopulations of receptors which differ in their proportion of Brownian and other motional regimes. Additionally, nAChR molecules occasionally display super-diffusive motion, exhibiting alternation between this active form of diffusion and conventional thermally-driven Brownian motion.

(815) **MODELING CELLULAR INFORMATION PROCESSING OF PULSATILE INPUTS AND THE EMERGENCE OF RESONANCES**

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In many biological contexts it is important to understand how the cell signaling system responds to time-dependent inputs. For example, gene expression in neuronal cells is affected by the time-dependent signals that the cells receive from their afferent neurons, and this is essential for memory formation. Signaling pathways stimulated by inputs that change rapidly over time need to process a significant amount of information. How much information they are able to process per unit of time is proportional to its bandwidth, which is determined by measuring the system's response to fluctuating signals at different frequencies. The larger the bandwidth of a pathway, the shorter its response time and the more accurately its response to a rapidly varying signal.

It has been shown that certain small signaling networks behave as low-pass filters where the response is maximized at the zero input frequency. The focus of the work presented here is to identify conditions for which simple signaling topologies can optimize a given response at certain intermediate input frequencies, where by optimizing we mean, for example, maximizing the production or the level of activation of a protein at these frequencies. We refer to this optimization as resonance.

Resonance is typically measured in quasi steady-state (long lasting pulsatile signals). By using a combined computational and theoretical approach, we show that resonance can be obtained for pulsatile input signals that are active for a short number of periods as compared with the time scale of the signaling component processing that input,. We call this effect transient resonance since the systems we consider do not exhibit resonance when using long lasting pulsatile signal. Transient resonance also emerges for the same signaling system, with the long lasting pulsatile signal, provided that the downstream singling components are fast enough to read pre-steady-state information.

(1200) **MODELING, REFINEMENT AND INCLUSION IN A LIPID BILAYER OF AN ACETYLCHOLINE RECEPTOR AND ITS INTERACTION WITH FREE FATTY ACIDS**

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The free fatty acids (FFAs) act as noncompetitive antagonists of the nicotinic acetylcholine receptor (nAChR) and its site of action would be in the lipid-AChR interface being the key factor for receptor blockade the position and isomery of the double bond of the FFAs. The nAChR is a ligand-triggered ion channel, is composed of 4 different polypeptide chains in a pentameric arrangement ($\alpha 2\beta\gamma\delta$). Each subunit has an extracellular N-terminal domain, four transmembrane domains and a small C-terminal domain. Because of the AChR is a membrane protein little high-resolution information is available on its atomic structure. Our objective was to obtain a computational model of AChR inserted in a lipid bilayer and to study its interaction with its lipid environment and its interactions with the different free fatty acids in study (*cis*-18:1 ω-6, *cis* 18:1 ω-9, *cis*-18:1 ω-11 o *cis*-18:1 ω-13). We made use of MODELLER to make multiple alignments of the subunits with sequences from other family members. The development of the model and its refinement, that include both extracellular and transmembrane domains, was also carried out with MODELLER. Using CHARMM-GUI Membrane Builder we built and simulated lipid bilayers (composed with a ratio 1:1:3 of cholesterol, POPA (palmitoyl oleoyl phosphatidylamine) and POPC (palmitoyl oleoyl phosphatidylcholine) respectively), with AChR incorporated to the membrane. We are particularly interested in studying surrounding lipids to TM4 since previous studies suggest that M4 helix acts as a lipid-sensor that relates the properties of the bilayer to the AChR. We replace the lipid membrane closest to TM4 with one of the FFAs of interest using PYMOL.

Finally, we performed electrostatic analysis, normal modes calculations and stereochemistry tests to evaluate the quality of the model. We performed molecular dynamics with GROMACS for each system built.

Keywords: free fatty acids (FFAs), nicotinic acetylcholine receptor (nAChR)

(1146) **MOLECULAR DYNAMIC SIMULATIONS OF THE COMPLETE GUMB PROTEIN, INVOLVED IN XANTHAN GUM BIOSYNTHESIS**

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Many bacteria produce extracellular polysaccharides. Several bacterial polysaccharides are associated with pathogenesis development, and industrial applications because of their physical properties. It has been previously shown that the outer membrane protein GumB is involved in the synthesis/secretion of the exopolysaccharide xanthan in *Xanthomonas campestris*. Crystals of native protein and its Se-Met derivative were obtained, allowing the determination of GumB tetramer structure. The electrostatic surface representation of GumB tetramer shows it has two identical patches of positive residues delimiting a cavity. As xanthan is an acidic polymer, the positive cavities may be involved in xanthan binding, serving as a "tutor" for its secretion. Also, we observed that no water molecules are found in the positive cavities. On the other hand, the structure of the 25 Nterminal residues were not unraveled in the crystal, although they were present in the protein used for crystallization. We hypothesized that the N-terminal residues could be localized dynamically in the cavity. To test this hypothesis, we modeled the Nterminal residues by two methods: i) we modeled a polyproline II (PPII) helix using the sequence of GumB, because secondary structure prediction suggest that the N-terminal is unordered or adopt PPII structure, and ii) we constructed an automated model using Wza (a well-studied GumB homolog) as template. Both models were submitted to minimization and molecular dynamics simulation using GROMOS96 53A6 force field parameters and run with GROMACS package. Up to now, the protein conformation observed in the crystal structure is stable during the simulation. The N-terminal regions are observed to move freely and explore positions closer the core tetramer. Longer simulation are being carried out to obtain statically reliable results. We expect that the results obtained here allow us to construct a model about the role of GumB in xanthan biosynthesis.

Keywords: xanthan gum, GumB, exopolysaccharide, MD simula-

tion

(297) **QM/MM CALCULATIONS OF ACTIVATION PARAMETERS IN ENZYMATIC REACTIONS**

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Abstract: Since the early beginnings of chemical simulations, obtaining the activation enthalpy, entropy and free energy for molecular reactions has always been one of the major goals. In the last few decades, the ever-increasing computer power allowed the development of several methodologies such as metadynamics, Jarzynski, and umbrella sampling for the calculation of free energy profiles in complex systems, such as enzymatic reactions using multi scale QM-MM schemes. However, determining accurate activation enthalpies and entropies remained a more difficult issue. In this work we present a systematic study comparing three methodologies for the calculation of the activation parameters in enzymatic environments using a Gaussian basis set QM-MM implementation for graphical processing units developed in our group. Said methodologies are described as follows:

1. Calculation of activation enthalpies by means of the determination of free energy profiles at several temperatures and using a Van´t Hoff analysis;

2. Estimation of the activation enthalpy and free energy by means of potential and free energy profiles computed by restrained optimizations and umbrella sampling calculations, respectively.

3. Estimation of the activation parameters by means of geometry optimization of the reactants, transition state and products, followed by a normal mode analysis in order to compute the activation enthalpy, entropy and free energy.

Chorismate mutase catalyzed conversion of chorismate to prephenate has been employed as a benchmark case, since there is a significant amount of data, both experimental and theoretical, reported in literature.

Keywords: activation entropy, QM-MM, umbrella sampling

ONCOLOGY-ONCOIMMUNOLOGY 17

(598) (**ERBB-2 NUCLEAR VARIANTS DRIVE TUMOR GROWTH AND PREDICT CLINICAL OUTCOME IN TNBC**

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Triple negative breast cancer (TNBC) refers to the group of tumors with poor prognosis without clinically significant levels of estrogen and progesterone receptors, and which lack ErbB-2 overexpression or gene amplification. Our hypothesis is that BCs defined as TN indeed express ErbB-2 which instead of being localized at the membrane is present in the nucleus where it modulates tumor growth. We detected ErbB-2 variants of different molecular weight (MW) in the nucleus of TNBC cell lines by western blot: MDA-MB-453 expressed full-length ErbB-2 (185 kDa, p185ErbB-2); MDA-MB-468 expressed a variant of lower MW (165 kDa, p165ErbB-2); HCC-70 and MDA-MB-231 expressed p185 and p165ErbB-2. Different ErbB-2 transcript variants were reported, among them an alternative spliced isoform lacking exon 16 (ErbB-2D16) is associated with high oncogenic and metastatic potential in BC. Here, we studied ErbB-2 transcript variants expression by conventional PCR using splice-specific primers. All TNBC cells expressed high levels of the ErbB-2 variant C (NM_001289936) which is predicted to be localized in the nucleus since it lacks the N-terminal signal peptide. We also showed that ErbB-2D16 was expressed in TNBC cells. We further discover a strong NErbB-2 presence in TNBC cells by immunofluorescence and confocal microscopy. These results were confirmed in a cohort of 57 TNBCs in which 26 patients showed NErbB-2 positivity. We revealed NErbB-2 as a significant predictor of worse overall survival in TNBC. Finally, we explored the biological relevance of NErbB-2 by transfection with the hErbB-2DNLS mutant which is unable to translocate to the nucleus and also acts as dominant negative inhibitor of endogenous NErbB-2 translocation. Blockade of NErbB-2 presence inhibited TNBC cell proliferation and tumor growth in TNBC xenografts. In conclusion, our results identified NErbB-2 variants as key players in TNBC and highlighted NErbB-2 as potential biomarker and therapeutic target in these tumors.

Keywords: Triple Negative Breast Cancer, Nuclear ErbB-2, Transcript Variants, Tumor growth, Clinical Biomarker

(1870) **NON SQUAMOUS CELL CARCINOMAS UNDER 40 YEARS OLD. MOLECULAR CHARACTERIZATION AND RESPONSE TO TARGET THERAPY**

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Background: Lung cancer is the leading cause of cancer deaths in Argentina. The median age at diagnosis is 70 years. The incidence of non squamous lung carcinomas (NSLC) in patients under 40 years seems to be increasing. About 25% of NSLC harbor an actionable oncogenic driver. The objective of this study is to describe the clinicopathologic, molecular characteristics and response to target therapies in patients under 40 years diagnosed with NSLC at our institution.

Methods: We conducted a retrospective study of adults between 18 and 40 years included in two prospective studies of molecular profiling of NSCL at our institution: PUL003 from 3/2012 to 12/2014 (108 patients) and PUL006 ongoing since 6/2015 (101). Variables analyzed were: gender, age, performance status (PS), smoking status, histologic subtype, stage at diagnosis and treatment response. Molecular profiling included ALK fusions by immunohistochemistry and FISH, EGFR mutations by Sanger sequencing and, in some cases, Next Generation Sequencing (NGS) with Ion AmpliSeq Colon and Lung Panel or Oncomine Comprehensive Assay with ION Torrens S5.

Results: Seven patients were included: 1/108 from PUL003, 5/101 from PUL006 and 1 in the interval. Median age was 33 years (31-37), 4/7 male, PS 0-1 7/7, and 5/7 non-smokers. Six had adenocarcinoma, 1 undifferentiated carcinoma, 6/7 were stage IV and 1/7 stage I. Six (85%) harbored a known targetable oncogenic driver. EGFR sensitizing mutations in 3/7, ALK translocations in 2/7 and 1 HER2 exon 20 insertion. Only one sample, without NGS, was negative for the studied oncogenes. Four patients received Thyrosine Kinase Inhibitors and 3/4 exhibited partial response. NGS provided additional information in 3 cases either to detect a driver or to explain mechanisms of resistance to TKIs.

Conclusions: NSLC in young adults is an emerging disease with distinguishable clinicopathologic and molecular characteristics. NGS technologies enabled the diagnosis and personalized treatment.

Key Words: lung cancer, young, NGS, NSLC

(694) **NUCLEAR FUNCTION OF ERBB-2/ERBB-3 HETERODIMERS IN BREAST CANCER CELLS RESISTANT TO TRASTUZUMAB AND LAPATINIB**

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ErbB-2 overexpression or amplification is associated with poor prognosis in breast cancer (BC) and is therapeutically targeted by monoclonal antibodies (trastuzumab, TZ) or by kinase-inhibitors (lapatinib, L). Despite clinical efficiency, resistance to said drugs is a major issue. ErbB-2 translocates to the nucleus (NErbB-2), where it acts as transcription factor (TF, e.g. to modulate ERK5 expression) or as coactivator of TF Stat3 (e.g., to regulate cyclin D1 -CCND1- or $p21^{CIP1}$ expression). We showed that NErbB-2 function is vital for proliferation of ErbB2+ BC cells sensitive (BT474) and resistant (JIMT1) to TZ and L. Here we explored the molecular mechanisms within NErbB-2 function. We demonstrated that heregulin (HRG), ligand of ErbBs, stimulates ErbB-2 and ErbB-3 nuclear (NErbB-3) translocation and colocalization with Stat3 in BT474 and JIMT1 cells. Basal levels of NErbB-2/NErbB-3 were higher in JIMT1 than in BT474 and were not modulated by TZ or L. To block NErbB-2 presence we used ErbB-2ΔNLS mutant unable to translocate to the nucleus. It also acts as a dominant negative inhibitor of endogenous NErbB-2. We found that ErbB-2ΔNLS colocalized with ErbB-3 at the cytoplasm and abrogated NErbB-2 and NErbB-3 migration in JIMT1 cells. We also found that HRG induces CCND1, $p21^{CIP1}$ and ERK5 expression and that ErbbB-2ΔNLS inhibited their levels in JIMT1 cells. *In silico* analysis of microarray datasets of BT474 cells sensitive *vs* resistant to L (GSE16179) showed that levels of NErbB-2 target genes (CCND1, $p21^{CIP1}$) were increased in resistant cells. We developed a preclinical model in JIMT1 cells and demonstrated that transfection with ErbB-2ΔNLS significantly inhibits *in vivo* tumor growth. *Ex vivo* culture of JIMT1 xenografts showed that blockade of NErbB-2 function induced release of LDH (lactate dehydrogenase) necrosis marker to the supernatant. These findings highlight the relevance of targeting NErbB-2 function as a novel therapeutic strategy in TZ and L-resistant BC.

Keywords: breast cancer, resistance to anti-ErbB-2 therapy, nuclear ErbB-2, trastuzumab, lapatinib

(810) **OVEREXPRESSION OF FGF2 MODULATES HORMONE RECEPTOR EXPRESSION AND INDUCES ENDOCRINE RESISTANCE AND BREAST CANCER PROGRESSION.**

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Endocrine therapy is the standard treatment for patients with luminal breast cancer. However, resistance may develop as a consequence of enhanced growth factor signaling. Fibroblast growth factor 2 (FGF2) consists of a secreted low molecular weight form (LMW-FGF2) and several nuclear high molecular weight forms (HMW-FGF2). We previously demonstrated that endocrine resistant mammary carcinomas display levels of progesterone receptor (PR) isoform A (PRA) lower than those of isoform B (PRB) and high levels of HMW-FGF2 compared to responsive tumors. Also, LMW- or HMW-FGF2 overexpression in human hormone responsive T47D-YA cells, engineered to only express PRA, induced endocrine resistance. To further understand the mechanism of FGF2-induced endocrine resistance, we performed RNA-seq studies comparing both, LMW- and HMW-FGF2-transfected cells compared to control cells. We identified deregulated pathways related to hormone resistance, tumor invasiveness and cellular adhesion in FGF2-overexpressing cells. *PR* and *estrogen receptor* α (ER) were downregulated while *androgen receptor* was upregulated in cells overexpressing FGF2. As observed in the T47D-YA model, T47D cells, which express endogenous levels of both PR isoforms and were transfected with LMW- or HMW-FGF2, also developed endocrine resistance both *in vitro* and *in vivo*. Moreover, these tumor xenografts showed high mitotic activity, vascular emboli, and lung metastasis that were absent in control xenografts. In concordance with RNA-seq assays, Western blot studies revealed a decrease in ER and PR expression in FGF2-overexpressing cells compared to control cell lines, together with a more pronounced decrease of PRA than that of PRB, resulting in a low PRA/PRB ratio. In conclusion, our results suggest that an increase in intrinsic FGF2 associated with low PRA/PRB ratios provides a novel mechanism to explain endocrine resistance and metastatic dissemination.

Keywords: Breast cancer; FGF2; hormone resistance, progesterone receptor, estrogen receptor.

(697) **THE IMPORTANCE OF HYALURONAN IN BREAST CANCER PROGRESSION AND RESISTANCE TO ERBB-2 THERAPY**

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Hyaluronan (HA), one of the main components of the tumor microenvironment, promotes oncogenic signals, activates migration and metastasis and induces resistance to antineoplastic agents through its interaction with its receptor CD44. Accumulation of HA is associated with poor prognosis and resistance to anti-ErbB-2 agent trastuzumab (TZ) in breast cancer (BC). ErbB-2 receptor is overexpressed in 15-20% of BC patients (ErbB-2+) and constitutes an important therapeutic target. In addition to its membrane function, ErbB-2 migrates to the nucleus (NErbB-2) where it acts as a transcription factor (FT) or coactivator of TF, modulating proliferation, metastasis and resistance to anti-ErbB-2 therapies in BC. Although crosstalk between ErbB-2 and HA/CD44 pathways has been report, how the molecular interactions between said pathways mediate resistance to TZ remains poorly known. Here, we found that HA stimuli induced nuclear translocation of ErbB-2 in T47D BC cells. JIMT1 BC cell line constitutes a classical model of resistance to TZ. In this cell line, which expresses high levels of HA and NErbB-2, constitutive levels of nuclear CD44 were increased when stimulated with exogenous HA. Treatment with the chemical inhibitor of HA synthesis 4-methylumbelliferone (4MU) decreased not only HA levels but also NErbB-2 in JIMT1 cells.

We also explored the role of HA synthesis inhibition in cell proliferation and migration: Treatment with 4MU impaired proliferation of JIMT1 cells similarly to the one observed when ErbB-2 was excluded from the nucleus via transfection with hErbB-2ΔNLS mutant. Even more, wound-healing assays showed that 4MU inhibited JIMT1 cell migration.

In summary, we reveal that HA induces ErbB-2 and CD44 nuclear translocation, thus leading to the assembly of transcriptional complexes that would induce proliferation and migration of BC cells resistant to TZ. Our findings also highlight blockade of HA presence with 4MU as a novel therapeutic strategy in TZ-resistant BC.

Keywords: breast cancer, hyaluronan, tumor microenvironment, extracellular matrix, nuclear ErbB-2

(565) **MINIMAL DISSEMINATION PATTERN AND OUTCOME IN TRILATERAL RETINOBLASTOMA**

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Trilateral retinoblastoma (TRb) is a rare manifestation in hereditary retinoblastomas that presents intracranial tumors, in the pineal or in supra/paraselar region. Intensive systemic chemotherapy may improve its cure rate but leptomeningeal dissemination is usually the fatal outcome and little is known about its dissemination patterns. Few publications exist of the clinical findings and the mutations in TRb. Our aims were to detect the minimal dissemination (MD) outside the CNS and in the cerebrospinal fluid (CSF) at diagnosis and during follow-up in TRb patients. Also we studied mutations, tumor locations, treatment and final outcome. We evaluated MD in 5 patients with TRb detecting the mRNA of CRX and/or the ganglioside GD2, in samples from bone marrow (BM) and CSF. Sequencing and MLPA assay of the RB1 gene were used for screening mutations.

Four patients presented intracranial tumor in the pineal and 1 in the selar/supraselar area. All patients were offered an intensive chemotherapeutic regimen with consolidation with stem cell rescue (SCR) if complete response was achieved. 1 patient did not receive this treatment and died for progression of the disease. Two patients achieved a complete response and underwent SCR. Three patients had a leptomeningeal relapse and died. One patient remains disease-free for 60 months. Only one case presented MD at diagnosis in the CSF, and none in the BM. Upon leptomeningeal relapse, no case had concomitant MD in the BM. In all these cases, cells were positive for GD2 and/or CRX. All the mutations were deletions of 1 or 2 bp spread in different exons of Rb1 gene in 4 patients, and 1 patient had deletion of the whole gene.

The mutations of Rb1 gene did not show any pattern associated with TRb. CSF dissemination always concluded in the death of the patient, without concomitant systemic dissemination denoting the importance of increasing treatment intensity directed to the CSF compartment instead of intensifying systemic treatment.

Palabras claves: INTRACRANIAL TUMOR, RB1 GENE, CEREBROSPINAL FLUID, BONE MARROW, CHEMOTHERAPY

(1356) PATCHED RECEPTOR CAN INDUCE TUMOR INDUCTION OR PROGRESSION IN A DROSOPHILA MELANOGASTER MODEL OF CANCER

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The Hedgehog signaling pathway has essential roles during embryonic development that are echoed in adult mammals by its involvement in cancer. The best characterized ligand, Sonic Hedgehog (Shh), binds its receptor Patched (Ptc), effecting a de-repression of Smoothened (Smo). Upon activation, Smo promotes activation of the Gli transcription factors which is called "the canonical pathway". However, newly evidences have shown that there are two more pathways into this signaling. The non canonical type I results in the activation of Patched receptor, upstream and independent of Smo and Gli transcriptional action, while the type II occurs downstream of Smo and requires coupling of Smo to heterotrimeric G inhibitory (Gi) proteins but independent of Gli transcriptional factors. Objective: To study the role of the non canonical type I in cancer induction or progression.

Results: Overexpression of Drosophila Ptc (dPtc) induces a rough phenotype in the adult eye showing that Ptc can act as a tumor inducer. In addition, co expression of dPtc together with the RetMEN2B mutation leads to a greater rough phenotype showing that dPtc can be also a tumor progression gene. To study the molecular pathways which are involved in those responses, we isolated imaginal discs from the third instar larvae stage. We found that Ptc overexpression activates drosophila Erk and MAPKs. To extrapolate those findings in mammals, we used cell cultures as MCF10A and mouse embryonic fibroblast isolated from Smo knock out mice ($Smo^{-/-}$). We found that stimulation of Ptc, independent of Smo, can activate phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204), phospho-p38 MAP Kinase (Thr180/Tyr182), as well as, phospho-Akt (Ser473) in both cell lines. Conclusion: Here, we reported for the first time that Ptc can be a tumor promoter or maintainer where it activates oncogenic signaling pathways as MAPKs and AKT. These actions are independent of its traditional canonical signaling pathway.

Keywords: Signaling pathways, molecular pharmacology, Oncology, thyroid Cancer.

(1378) HISTAMINE H2 RECEPTOR MODULATES MRP4/ABCC4 EXPRESSION IN ACUTE MYELOID LEUKEMIA

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Acute Myeloid Leukemia (AML) is a heterogeneous hematopoietic disorder where blood precursors lose their ability of normal differentiation and proliferation. Chemotherapy is the first-line treatment, complemented with differentiation therapy and immunotherapy (including histamine and IL-2). In previous studies, we demonstrated the importance of the cAMP pathway in AML differentiation and the role of H2 histamine receptor (H2R), phosphodiesterases and MRP4 (cAMP efflux) in this regulation. cAMP's ability to regulate MRP4 levels was described, as well. The aim of this study was to evaluate the modulation of MRP4 expression in leukemic cells treated with histamine and to test the therapeutical potential of a combined treatment using histamine and a MRP4 inhibitor. Histamine and H2 agonist stimulation increased the expression of MRP4 mRNA and protein levels ($p<0.01$) in different AML models (U937, HL60, KG1a). Similarly, a U937 clone that overexpresses H2R, showed higher levels of intracellular cAMP ($p<0.001$), as well as higher MRP4 mRNA and proteins levels ($p<0.01$) compared to the parental line. In this clone, H2R agonists and inverse agonists modulated MRP4 levels in a positive and negative way, respectively. Furthermore, HEK293 cells transfected with H2R showed an increase on the MRP4 promoter activity dependent of the stimulus with histamine and H2 agonists, in a reporter gene assay. Given that an upregulation in MRP4 expression would not be beneficial for the differentiation therapy, we tested the combined effect of H2R agonist with two MRP4 inhibitors (Ceefourin-1 and MK-571) in U937 cell proliferation and differentiation. Our results show that MRP4 inhibition has an anti-proliferative and differentiating effect (CD88 expression) enhanced by H2R stimulation. Taken together, these results contribute to the rational basis for a polypharmacological approach for AML with H2R ligands and MRP4 inhibitors.

Keywords: Acute Myeloid Leukemia, Histamine H2 Receptor, MRP4, cAMP, Differentiation Therapy.

(1933) SURVIVIN AS A MODULATOR OF AUTOPHAGY AND SENESCENSE IN PANCREATIC ADENOCARCINOMA CELL LINES

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Pancreatic tumors are highly resistant to chemotherapy. The use of Gemcitabine (Gem) raises the levels of survivin expression and this is accompanied by increases in basal autophagy levels.

Recently, it has been shown that exist a link between the processes of autophagy and senescence. Therefore, we proposed to evaluate whether survivin would be mediating these biological processes in our cell lines. For this purpose, we treated the lines MIAPaCa-2 wt and Sur -/- with Gem (10, 100 and 1000 µg/ml) alone or in combination with 3MA for 48 hs and determined the percentage of SA-β-Gal positive cells, to evaluate senescence. On the other hand, we treated the cell lines with Gem (10, 100 and 1000 μg/ml) alone or in combination with 3MA for 48 hs to determine the percentage of apoptosis by TUNEL assay. We could observe in the wt line that

the treatment with Gem increases the levels of senescence in a dose-dependent manner reaching values of 80% ($p < 0.001$) and they decrease when autophagy is inhibited (3MA); while in the Sur -/- line the treatment with Gem increases the levels of senescence similarly, but the treatment with Gem and 3MA induces a lower decrease in the percentage of senescence to which is observed in the wt line ($p < 0.001$), of similar value which obtained by treatment with 3MA alone. It is also observed that in the Sur -/- line no differences were found in the percentage of TUNEL + cells with or without 3-MA, neither increase in the number of apoptotic cells was found ($p > 0.05$), while the wt line, inhibition of autophagy produces sensitizing cells to the pro-apoptotic action of Gem ($p < 0.001$).

From these results, we postulate that pancreatic tumor cells use the autophagic process to induce senescence in the treatments with Gem. We can also propose to survivin as one of the possible links between these processes.

Keywords: Survivin, autophagy, senescense, apoptosis

ONCOLOGY-ONCOIMMUNOLOGY 18

(635) **TRISTETRAPROLIN (TTP) ABLATION AND K-RAS ACTIVATION PROMOTE ABNORMAL ORAL EPITHELIUM PROLIFERATION AND DIFFERENTIATION**

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Oral squamous cell carcinoma is among the most prevalent cancers in the world characterized by high morbidity and few therapeutic options. RNA-binding proteins (RNA-BPs) that impact the stability of transcripts have a significant role in tumor progression. Tristetraprolin (TTP) is a RNA-BP that regulates multiple proinflammatory mediators which promote tumorigenesis. We have previously developed TTP conditional *knock out* mice specific for oral cavity (TTP KO). TTP KO mice developed moderate dysplastic lesions in the tongue with deregulated expression of cytokeratins and proliferation markers. Here, to asses TTP role in oral carcinogenesis we breed the TTP KO mice with a K-Ras knock in line (compound mice: K14-CreER^{tam} /TTP$^{-/-}$/ K-ras$^{G12D+/-}$). Tissues from these mice were used to study proliferation and differentiation by immunohistochemistry. The compound mice exhibited an oral phenotype after a few weeks of tamoxifen induction leading to a significant reduction in survival time (compound mice: 40.25± 7.05 days; K-ras $^{G12D+/-}$: 56.63± 1.99; TTP KO: 120±0.0. Kaplan–Meier survival curve, $p<0.001$). All compound mice developed tongue dysplasias and oral papillomas. Some of the tongues presented infiltrated mast cells. Increased cell proliferation (PCNA) and cytokeratin 14 (K14) expression not restricted to the basal layers was observed in tongues and papillomas from the compound mice. However, oral papillomas from K-ras $^{G12D+/-}$ mice showed PCNA expression restricted to the basal layers and low K14 expression. PCNA and K14 expression in the hyperplastic tongues from K-ras $^{G12D+/-}$ mice was similar to the ones from the compound mice. Thus, we provide evidence that TTP loss concomitantly with Ras activation trigger epithelial changes although these alterations are not enough for full oral tumorigenesis. However, the phenotype of these compound mice warrant further analyses in order to understand the role of the inflammatory infiltrate observed in the tongues of these mice.

Keywords: epithelium, oral cancer, TTP, Ras.

(470) **ALTERED CELL SECRETOME REVEALS HEME OXYGENASE 1 AS A MODULATOR OF BONE PROGENITOR AND PROSTATE TUMOR CELL INTERACTIONS**

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Abstract: Prostate Cancer (PCa) is the second leading type of cancer in men. PCa cells display abnormalities in their adhesive properties, which result in an augmented capacity to resist chemotherapy and colonize other organs such as bone. Heme Oxygenase-1 (HO-1) acts as a cellular rheostat counteracting oxidative and inflammatory damage. We previously showed that HO-1 over-expression impairs tumor growth and angiogenesis *in vivo*. Given that inflammation is critical for the development and progression of PCa and that bone is the most common homing organ for PCa metastases, we used a co-culture transwell system for tumoral and bone progenitor cells and quantified filopodia structures at the leading edge of PCa cells. Briefly, PC3 cells pre-treated or not with hemin (potent inducer of HO-1; 50uM, 24h) were co-cultured with the murine MC3T3 or RAW 264.7 cells for 24h. Conditioned media (CMs) were collected and added to new PC3 cultures for 24h.

Results show that the CM from PC3/MC3T3 or PC3/RAW264.7 co-cultures, have a negative impact both in the number of filopodia per cell and contacts among them. However, hemin pre-treatment of PC3 cells prior to co-culture, prevented the reduction in the number of filopodia and cell contacts.

High-throughput secretomic analysis of CMs (nanoLC-MS/MS(Orbitrap) identified more than 30 proteins differentially released, in the CMs, of note: cadherins, collagen and critical proteins associated to cell detachment. Interestingly, the bioinformatics screening for these cytoskeletal-related proteins showed a significant misregulation in prostate adenocarcinoma compared to normal tissue.

Altogether, our findings demonstrate that HO1 modulation in PCa cells in co-culture with bone progenitor cells induces the release of key cytoskeleton-regulating proteins that impact on cell morphology, favoring a less invasive phenotype towards homing organs.

Keywords: filopodia, cell adhesion, bone metastases

(161) **ANGIOTENSIN-(1-7) COUNTERACTS THE TRANSFORMING EFFECTS TRIGGERED BY ANGIOTENSIN II IN BREAST CANCER CELLS**

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Angiotensin (Ang) II, the main effector peptide of the renin-angiotensin system, has been implicated in multiple aspects of cancer progression such as proliferation, migration, invasion, angiogenesis and metastasis. Ang-(1-7), is a biologically active heptapeptide, generated predominantly from AngII by the enzymatic activity of angiotensin converting enzyme 2 (ACE 2). Previous studies have shown that Ang-(1-7) counterbalances AngII actions in different pathophysiological settings. In this study, our aim was to analysed the impact of Ang-(1-7) on AngII-induced pro-tumorigenic features on breast cancer cells (MDA-MB-231). We found that Ang-(1-7) abrogated AngII induced migration and invasion of the MDA-MB-231 cells as well as pro-angiogenic events such as the stimulation of MMP-9 activity and VEGF expression. In addition, similar to Ang-(1-7), the treatment of cells with an anti-angiogenic VEGF antibody (bevacizumab), abolished AngII –induced cell migration of MDA-MB-231 cells. Together, these results demonstrate for the first time that Ang-(1-7) counteracts tumor aggressive signals stimulated by AngII in breast cancer cells.

(86) **CHARACTERIZATION OF CELLULAR PLASTICITY OF HUMAN GBM LINES WITH STEM CELL PROPERTIES USING THREE-DIMENSIONAL (3D) CULTURES**

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The study of molecular and cellular mechanisms involved in the formation of tumors, which originate from cells that undergo "reprogramming", is critical to understand their characteristic functional heterogeneity and therapeutic resistance. In this category, Glioblastoma, a highly aggressive brain tumor, attacks the brain by infiltrating and making difficult for its surgical removal. We studied the plasticity of different established patient-derived human GBM cells using three-dimensional (3D) cultures as an experimental model system. Although different patient-derived lines presented heterogeneous expression levels of the both stemness markers CD133 and KANSL2, a consistent positive correlation between their expressions was found as well as their cell self-renewal capacity in the 3D cultures. The overexpressed KANSL2 cells showed induced transcriptional activation of developmental master genes and enhanced the initiator ability of tumor spheres validating its role as a positive regulator of tumor stem cell identity. Interestingly, using the different population of CD133 expression cells, we found that the migratory capacity of these cells with stem properties is inversely proportional to the levels of mesenchymal markers.

A better understanding of the in vitro behavior of these cells will improve our knowledge for the development of new therapeutic pathways and their potential use for therapeutic screening.

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(1031) **IN VITRO ANTICANCER ACTIVITY OF TWO NOVEL RU HIDROXIQUINOLINES COMPOUNDS AGAINST 2D AND 3D CELL MODELS.**

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The development of ruthenium complex has shown to be very potential as anti-tumor agents. These compounds have high specificity for cancer cells.

The aim of this project is to evaluate the antitumoral activity of two novel Ruthenium hidroxiquinoline complexes, HQRuBr (4) and $HQRuBr_2$ (5), in cancer monolayer and spheroids models. The cytotoxic assays were carried out on a panel of human cancer cell lines including MG-63 (osteosarcoma), A549 (lung), MCF7(breast), MDA-MB-231(breast) and one normal cell line L929 (mouse fribroblast), using MTT and alamar blue assay. The Ru compounds decrease cell viability in all cells lines tested, MG-63 (IC_{50} complex 4 13,6 μM and complex 5 10,4 μM), A549 (IC_{50} Complex 4: 52,9 μM and Complex 5: 26,7 μM), MCF7 (IC_{50} complex 4: 46.1 μM and complex 5: 20.7 μM), MDA-MB-231 (IC_{50} complex 4: 38.2 μM and complex 5: 15.8 μM) and L929 (IC_{50} complex 4: 43,5 μM complex 5: 26.7 μM) ($p<0.001$). Furthermore, the wound healing assay showed Ru complexes decline MG-63 cells migrations, nearly a 50% and single cell lamellipodium formation diminished too. The genototoxic activity on MG-63 cell line was determinate using micronucleus and comet assay; these processes expose increased tail moment and the formation of micronucleus in a concentration range of 2.5 to 10 μM ($p<0.001$). The cell cycle was analyzed by flow cytometry, showing that compound 4 and 5 arrest the cell cycle in G1 phase.

Finally, both compounds diminished the cell viability on spheroids (MG-63, A549 and MCF-7) affecting the volume and spherical shape ($p<0.005$).

In summary, these two Ru complexes show antitumoral activity, both caused cytotoxicity and genotoxicity in all tumoral cell lines in a concentration dependent manner but the compound 5 has a stronger activity than compound 4. Therefore, our results show that compound 5 is the most interesting candidate for potential antitumor uses, and it would be interesting to test this complex in further in vivo studies for cancer treatments.

Palabras clave: ruthenium, cancer, spheroids.

(150) ***IN VIVO* ANTITUMOR EFECT OF A TRIAZOLYL PEPTIDYL PENICILLIN IN MURINE MELANOMA CELLS**

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The triazolylpeptidyl penicillins (TAP) are novel hybrids compounds having in their structure a penicillanic core linked to a peptide portion via a triazole group. In a previous study, we showed that the derivative containing the dipeptide Leu-Phe (TAP6) inhibited the proliferation of mouse melanoma cells *in vitro* by arresting cell cycle and inducing an apoptotic response. In this work, we decided to investigate the *in vivo* effectiveness of this derivative in a syngeneic C57BL/6J mouse melanoma model. To this end, B16-F0 cells ($1x10^5$) diluted in 200 μl of RPMI were injected subcutaneously in the right flank of each mouse. 10-12 days after cell inoculation, mice were injected with 0.2 ml of vehicle (70 % (v/v) polyethylene glycol 400 in PBS) or different doses of the penicillin derivative, via i.p. three times per week for two weeks. A dose-dependent effect was evident, being the reduction of tumor volume ~70% and 50% at doses of 20 and 10 mg/kg, respectively. A significant reduction of PCNA expression, a cell proliferation marker, was detected in 20 mg/kg TAP6 treated- tumors. Apoptosis *in vivo* was also assessed by evaluating the expression of different apoptosis-mediator proteins. Thus, results obtained by Western blot showed a significant increment in the expression levels of Bax (~2 fold), TRAIL (~1.5-fold) and Fas (~3.5-fold) proteins, and a significant decrease in the amount of full-length Bid protein, Bcl-2, Bcl- X_L and PARP in tumor lysates from 20 mg/kg treated-mice. Immunohistochemistry assays of 20mg/kg treated-tumor slices revealed higher levels of active caspase-3. The administration of 80 mg/kg of TAP6 to non-tumor-bearing mice showed no histopathological effects on different organ-tissues. Taken together, our results showed that TAP6 markedly suppressed melanoma cell proliferation *in vivo* by inducing an apoptotic cell death. This novel penicillin derivative should be considered as a promising therapeutic agent for cancer treatment.

Keywords: triazolylpeptidyl penicillin, antitumor action, apoptosis, murine melanoma cells

(1113) **INFRARED IMAGING AND FLUORESCENCE MEASUREMENTS IN PHOTODYNAMIC THERAPY OF A FIBROSARCOME ANIMAL MODEL**

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Abstract: Infrared imaging and In vivo spectrofluorometric analysis are non-invasive procedure that can be used to follow photodynamic therapy (PDT) of tumors. Infrared imaging detects radiation emitted by the tissue as a result of its temperature and produces a temperature field. The second technique analyzes the light emitted by the tissue containing a fluorescent photoactive drug.

Using a thermal imaging camera (Agema 550) and portable spectrofluorometer with an excitation source emitting at 400 nm wavelength and a spectral analyzer ranging from 500 nm to 800 nm, the evolution of the m-tetrahydroxyphenyl chlorin (m-THPC) photosensitizer at the tumoral tissue of BALB/c murines with fibrosarcoma located at their flank was followed up. Thermal images were registered

during the illumination while fluorescence was measured before, immediately after illumination and at interval of 24 h for 48 – 256 h. We investigated the usefulness of both techniques for monitoring the extent of the photodynamic reaction in the tumor tissue illuminated with a light dose of 20 J/cm2 from a 637 nm LED lamp with an output of 1,06 W. The average fluorescence intensity in the tumor reached a maximum after 24 - 72 h. Subsequently, illuminations 24, 48, 72 and 96 h post-injection were performed, and the fluorescence was measured immediately before and after each illumination, observing in average a decrease in the intensity. Eventually, 24 h post-illumination, the fluorescence at certain parts of the tumor increased in comparison with that measured immediately after illumination. Temperature monitoring revealed non-uniform temperature distribution in the illuminated areas. The temperature gradually increased in the presence of photosensitizer in comparison with control mice. During the PDT temperature increase from 28,7°C to 35,2 °C. The use of both techniques allows a more confident evaluation of the PDT treatment and are expected to contribute to improve protocols for PDT application.

Keywords: photodynamic therapy, infrared imaging, fluorescence

(966) **HEME-OXYGENASE 1 (HO-1) PROMOTES MESENCHYMAL TO EPITHELIAL TRANSITION THROUGH N-CADHERIN SIGNALING IN PROSTATE CANCER**

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Castration resistant prostate cancer (CRPC) is one of the most important clinical challenges in prostate cancer (PCa) therapy. Epithelial-mesenchymal transition **(**EMT) plays a critical role in CRPC thus, therapeutic targeting of EMT has the potential to open new avenues in the treatment paradigm of CRPC reversing the invasive mesenchymal phenotype to a more differentiated tumor epithelial phenotype. We have previously shown the strong anti-tumoral action exerted by heme oxygenase 1 (HO-1) in PCa. However its association with EMT is still poorly elucidated. For this purpose we carried out a comprehensive RNA-Seq analysis to compare EMT associated gene expression profiles between PCa cells overexpressing HO-1 pharmacologically (hemin treatment) or genetically (transfected with pcDNA3HO-1 vector) and their respective controls. The obtained EMT signature genes modulated by HO-1 consisted of both epithelial and mesenchymal state-associated mRNAs.

The Gene Ontology classifications reflected as the top cellular localization annotations: cell surface, extracellular, or membrane-bound. The top biological processes were cell locomotion and adhesion, extracellular structure organization, embryonic organ morphogenesis and ion homeostasis. Among the identified mesenchymal cell state-associated factors, HO-1 down-regulation of N-Cad, B-catenin, Lef1, ITGA, RHOA, DVL1 and LEF1 mRNAs displayed the most significant altered genes. In particular, HO-1 was able to regulate both up- and downstream N-Cad signaling pathways involving Notch-4, B1 integrin, SMAD7, CDC2, Twist and FN1. N-cad is highly implicated in bone formation and metastasis and HO-1 regulation also displayed significant alterations of Frizzled, Dsh and CDH2 mRNA expression levels. Results were further validated by RTqPCR.

This study may cast a new light on CRPC treatment highlighting a multifaceted role for HO-1 in altering EMT signaling, supporting it as a potential therapeutic target for the metastatic disease.

Keywords: EMT, prostate cancer.

(1288) **ELECTRIC CELL–SUBSTRATE IMPEDANCE SENSING (ECIS) FOR THE STUDY OF ADRENOMEDULLIN ON HUMAN RENAL CLEAR CAKI-2 CELLS IN HYPOXIC CONDITIONS**

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Angiogenesis is a crucial process in tumor development and metastasis. The hypoxic feature of solid tumors contributes locally and systemically to tumor progression. Clear cell renal carcinoma (CRCC) is the most common histological subtype of renal cancer (80-90% of cases). It is characterized by an intense vascularization implying a close relationship among angiogenesis, cellular response to hypoxic stress and tumor progression. There is limited evidence about the use of electric cell–substrate impedance sensing (ECIS) assay to evaluate angiogenesis and / or metastatic potential in CRCC. In addition, the function of adrenomedullin (AM) and the effects of its inhibition on solid tumors (such as CRCC) are not fully known. The aim of this study was to assess the effects of AM, a pro-angiogenic molecule, and its inhibition in CRCC using the ECIS assay. Briefly, Caki-2 cells were cultured on ECIS 8W10E electrode plates (Applied Biophysics, Troy, NY). Cellular impedance variations were measured against different concentrations of cobalt chloride (1-100 μM) for simulating hypoxic conditions and with/ without the inhibitory agents: 16311(10 to 1000 nM) and 22-52 (0,01 to 1 μM). Monolayers were summited to 1400µA current at 60Hz for 20 sec for migration assay.

Results showed that 100 μM CoCl2 was the most effective dose for mimicking hypoxia in Caki-2 cultures. Expression of AM was evaluated by Western Blotting (WB), RT-PCR and qPCR at 0,12, 24 and 36 h post cobalt chloride. AM expression increased significatively between 12 and 24 h in hypoxic conditions by WB and RT-PCR ($p \leq 0.001$). qPCR revealed a significant increment of AM RNAm at 24 h of hypoxia ($p \leq 0.01$). Finally, AM inhibitors caused the decrease in cell growth and migration by ECIS assays. This preliminary study contributes to a better understanding of CCRC tumor biology and encourages us to propose AM as a potential anti-tumor target in relation to angiogenesis.

Key words: Hypoxia, renal cancer, angiogenesis, adrenomedullin, ECIS.

(1123) **MRP4/ABCC4 AS A NEW THERAPEUTIC TARGET: META-ANALYSIS TO DETERMINE SUBSTRATE BINDING SITES AS A TOOL FOR DRUG DESIGN**

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Abstract: Over the last years, numerous MRP transporters have been suggested as possible pharmacological targets for the treatment of cancer for several reasons: their increased expression in tumor cells, their ability to generate multidrug resistance and transport endogenous substrates that can eventually increase tumor malignancy. Due to the structural complexity of their substrate binding pocket, designing specific pharmacological agents with the ability to selectively modify their affinity to certain substrates represents a challenge in current medicinal chemistry. We have recently established that inhibiting cAMP extrusion by MRP4 could represent a possible effective therapeutic strategy for pancreatic ductal adenocarcinoma treatment. Using available information regarding substrate specificity, homology models and mutagenesis assays, we recapitulate the up-to-date knowledge about MRP structure. We also aligned amino acid sequences to identify the candidate MRP4 residues where cyclic nucleotides may bind. We identified two possible binding sites that are present both in the outward and inward conformation of the transporter. In the first binding site, residues L838 to D842 from transmembrane helix 9 (TM9) are postulated to bind cAMP in the inward conformation, while residues T364, F368, E374 and R375 from TM6 are postulated to bind cAMP in the outward conformation. Residues G779 to R782 from TM8 could bind cAMP in both conformations. Regarding the second binding site, in the inward conformation we postulate that A739 to S743 of extracellular loop 4, together with A982 from TM12 may participate in

cAMP binding. In the ourward conformation, the second binding site is composed by C956 and A957 from TM11 and F993 from TM12. Binding site identification may serve as a basis for the future development of inhibitors of MRP4 cAMP specific transport. Also, this meta-analysis could serve to establish structural requirements for any substrate specificity of other MRP transporters.

Keywords: MRP4, cAMP, drug design

GENETICS AND MOLECULAR BIOLOGY 18

(648) **SEQUENTIAL AND COOPERATIVE ACTION OF TonEBP AND NFkB TRANSCRIPTION FACTORS IS NEEDED FOR OSMOPROTECTIVE GENE EXPRESSION.**

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Tonicity-responsive enhancer binding protein (TonEBP) belongs to the Rel superfamily of transcription factors (TF) and is considered the master regulator of osmoprotective gene expression. TonEBP exists as a dimer that completely encircles its DNA target (TonE). Nuclear factor kappa B (NFkB) also belongs to the Rel family and also forms dimers when it binds to its response elements (kB) on its target genes. In renal cells, TonEBP and NFkB, are activated by hyperosmotic environment and regulate COX2 expression, which is considered an osmoprotective gene. The present work explores whether TonEBP and NFkB act together or as individual TFs in their regulatory action on osmoprotective gene expression under hyperosmotic conditions. For this purpose, we blocked the activity of TonEBP and NFkB, individually or together and then, evaluated the expression of their reporter genes (MCP1, IkB for NFkB and BGT1, AR, SMIT for TonEBP) as well as COX2. To do this, cultures of the renal epithelial cell line MDCK were grown in isoosmotic or hyperosmotic (298 or 500 mOsm/Kg H_2O) media in the absence or presence of TonEBP siRNA, a NFkB inhibitor (parthenolide, Parthe) or both. After 24 h, osmoprotective gene expression was evaluated by RT-PCR. The possible interaction between both TFs was studied by co-immunoprecipitation and immunofluorescence microscopy. Parthe downregulated COX2 and unexpectedly, TonEBP reporter genes. TonEBP-silencing knocked down COX2 and NFkB reporter genes. Parthe treatment blocked TonEBP expression but, TonEBP silencing did not affect NFkB. These results show that both TFs are needed to the expression of their reporter genes, and suggest a sequential action where NFkB modulates TonEBP expression and then, both together activate gene transcription. Immunofluorescence shows the possible colocalization of both TFs in the nucleus. Such interaction was confirmed by co-immunoprecipitation. This finding indicates that both TFs act together to regulates gene expression.

Keywords: Hyperosmolarity, osmoprotection, gene expression

(1499) **CHANGES IN CREB3L2 TRANSCRIPTION FACTOR IN CELL DIFFERENTIATION MODELS**

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CREB3L2 is a member of the CREB3 family of bZip transcription factors that show tissue-specific expression patterns. Although they have been described to participate in cell differentiation in various tissues by regulating the secretory machinery, very little is known about their role in the nervous system. Our goal is to analyze the regulation of the secretory pathway and the participation of CREB3L2 in cell development and differentiation models, specifically in neuronal cells. We are working on two models: one of them are PC12 cells, which can differentiate to neuron-like cells upon treatment with NGF, and the other one are hippocampal cells in culture obtained from rat embryos. We found that NGF increases significantly mRNA and protein levels of CREB3L2, while the other members of the CREB3 family do not respond the same way. Moreover, we show that NGF induces a time-dependent increase in protein levels of different markers of the early secretory pathway. The NGF-induced effect on CREB3L2 is abolished when cells are incubated with UO126 and H89, pharmacological inhibitors of MEK1 and MEK2 MAPK kinases and PKA, respectively. We also have evidence that, in hippocampal neurons in culture, there is a time-dependent increase in protein levels of CREB3L2 and secretory pathway factors as well. Our data suggest that: a) differentiation process goes along with an increase in proteins of the early secretory pathway; b) only CREB3L2 but not the other CREB3 transcription factors accompanies this process; and c) increase in CREB3L2 is both ERK and PKA dependent, proposing a dual regulation of this transcription factor. Further analyses are needed to investigate the molecular mechanisms underlying these changes and to elucidate the specific function of CREB3L2 in both models.

Keywords: CREB3L2, PC12, neurons, secretory

(1107) **STUDY OF TELOMERIC TRANSCRIPT EXPRESSION DURING EPITHELIAL-MESENCHYMAL TRANSITION**

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Telomeres are transcribed into telomeric repeat-containing RNA (TERRA). TERRA expression is elevated in human cancer tissues, however little is known about their regulation in cancer progression. We have shown that TERRAs are induced by oxidative stress that alters microtubule integrity. TERRA induction was mimicked by treatment of cells with either, the microtubule-disrupting agent colcemid or taxol, a microtubule-stabilizing drug, suggesting that TERRA induction is delicately regulated by mechanotransduction. During epithelial-mesenchymal transition (EMT), epithelial cells reorganize their cytoskeleton as they transition into mesenchymal cells. Thus, the aim of our study was to characterize TERRA expression during this process. The mouse mammary epithelial cells NMuMG undergo EMT following TGFβ1 stimulation. Transdifferentiation of NMuMG after 96h treatment with 5ng/ml TGFβ1 induced a change in their morphology from an epithelial to an elongated, fibroblastic phenotype; reported to result from microfilament reorganization. This change correlated with decreased expression of the epithelial marker E-cadherin and increased expression of mesenchymal markers ZEB1, ZEB2, SNAIL, and SLUG as assessed by Western blot, demonstrating the occurrence of EMT. Importantly, qPCR analysis showed an induction of TERRAs during the EMT of NMuMG, result in line with our hypothesis that TERRA expression can be regulated by cytoskeleton remodeling. To further explore its relevance in EMT, we measured TERRA levels after treating NMuMG and transdifferentiated NMuMG cells with 0,5mM H_2O_2. NMuMG undergo TERRA induction shortly after H_2O_2 treatment. In contrast, TERRA induction was abrogated in transdifferentiated NMuMG, suggesting that changes in the cytoskeleton undergone during EMT altered TERRA regulation in response to H_2O_2. In summary, we show for the first time that TERRAs may be induced during EMT and they could be potential

lly novel early markers of cancer progression.

Key words: TERRA, EMT, cancer progression.

(262) **GENE EXPRESSION MISREGULATION IN DNA REPAIR DEFICIENT CELLS**

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DNA damage caused naturally by UV light exposure in skin cells triggers not only lesion repair mechanisms but also a global gene expression response that ultimately modulates cell functions. We

recently demonstrated that repair of damaged DNA by the Nucleotide Excision Repair (NER) system generates single stranded DNA intermediates that in turn activate the ataxia telangiectasia mutated and Rad3 related (ATR) kinase. Active ATR triggers global hyperphosphorylation of the Carboxyl Terminal Domain (CTD) of RNAPII major subunit affecting gene expression at the quantitative and qualitative (alternative splicing isoforms) levels (Muñoz et al., 2017). Moreover, using CRISPR-Cas9 technology, we found that ablation of XPE, a lesion recognition factor, partially decreased the UV effect on AS, further demonstrating the crosstalk between repair and gene expression (Muñoz, 2017). To pursue the idea of the repair process acting as a gene expression controller in a genotoxic scenario, we knocked-down different repair factors to evaluate repair and gene expression upon UV irradiation of skin cells. The NER factors can be divided in two groups, those in charge of lesion recognition (XPE, XPC) and those in charge of the actual repair (XPA, XPB, XPD, XPF, XPG). We found that impairment in lesion recognition (XPE, XPC) or in the actual repair (XPA, XPB) have different consequences at the gene expression level.

While it is clear that all XP patients have an increased risk of developing skin cancer, some other puzzling clinical features are characteristic of the specific factor being mutated: while XPA and XPB (repair factors) patients develop blistering burns on minimal sun exposure, patients with defects in recognition (XPC and XPE) do not (DiGiovanna, 2012). Therefore we propose that some of the clinical features of XP patients are due to defects in gene expression modulation triggered by the DNA repair pathway.

Key words: DNA damage, UV light, DNA repair, NER, gene expression

(1215) **CNBP, A NUCLEIC ACID CHAPERONE PROTEIN, MODULATES THE TRANSCRIPTION OF A GENE INVOLVED IN CRANEOFACIAL DEVELOPMENT THROUGH G-QUADRUPLEX UNFOLDING.**

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In a previous work we found a set of developmental genes containing conserved G-quadruplexes (G4) in their promoters. The folding and transcriptional enhancer role of some of the identified G4 were addressed by *in vitro* and *in cellulo* studies, and further confirmed *in vivo* by G4-specific disruption through antisense oligonucleotides (ASO) microinjection in zebrafish embryos. ASO disruption of *noggin3* (*nog3,* one of the studied genes) caused craniofacial malformation phenotypes consistent with *nog3* function in pharyngeal development. In this work, an *in silico* analysis predicted that the G4 controlling *noggin* transcription (*nog3*-G4 for the zebrafish gene and *NOG*-G4 for the human gene) overlapped with the binding motif of cellular nucleic acid binding protein (CNBP). CNBP is a nucleic acid chaperone protein with preference for G-rich single stranded nucleic acids, reported as promoting cell proliferation and involved in craniofacial embryonic development. Electrophoretic mobility shift assays showed that CNBP bound to folded *nog3*-G4 and *NOG*-G4 with lower affinity than to the unfolded nucleic acids. Circular dichroism and polymerase stop assays revealed that CNBP promotes the unfolding of *nog3-G4* and *NOG*-G4, in agreement with similar results obtained for DNA and RNA-G4 reported to control the expression of a set of proto-oncogenes. ChIP performed in HEK293 cells expressing CNBP-EGFP demonstrated the interaction of CNBP with the region containing *NOG*-G4. Furthermore, CNBP siRNA knockdown in HeLa cells led to an increased *NOG* transcription when compared with controls. In agreement, CNBP overexpression in zebrafish embryos caused a down-regulation of *nog3* transcription *in vivo.* Results suggest that CNBP role in craniofacial embryonic development and cell proliferation is performed, at least in part, by resolving G4-DNA secondary structures. Besides, results demonstrate the existence of G4-DNA *in vivo* and their role in the transcriptional regulation of vertebrate genes.

Keywords: noggin, CNBP, G-quadruplex, embryo development, zebrafish

(560) **EFFECTS OF OSMOTIC AND SALINITY STRESS ON EPIGENETIC MARKS ON *GL2*, A GENE INVOLVED IN EPIDERMAL ROOT DIFFERENTIATION AND ROOT HAIR DEVELOPMENT IN *ARABIDOPSIS THALIANA***

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In plants, root epidermal cells are differentiated in two cell types: trichoblast cells and atrichoblast cells. The first one produces root hairs, a long and thin structure involved in absorption of water and nutrients from soil. *Gl2* is a crucial gene determining the fate of root epidermal cells. *Gl2* is expressed in non-hair cells, where it inhibits the expression of genes that promote root hair development and elongation (i.e. *RHD6*, *RSL4*). On the other hand, it is known that epigenetic marks arisen by cytosine methylation and histone methylation or acetylation are stable but can be modified by environmental factors. The aim of this work is to determine if changes in soil conditions i.e. saline or osmotic stress are able to modify epigenetic marks on *Gl2*. These marks might in turn bring about changes in the expression of this gene thereby regulating the pathway responsible for length and/or density in root hairs. For that purpose, plants were grown on plates containing half-strength MS medium for three weeks. One group was supplemented with 20 mM NaCl and the other with 50 mM mannitol. The control group was grown on half-strength MS medium only. Roots were then cut off and immediately frozen in liquid nitrogen. DNA was isolated following a standard protocol and then subjected to bisulfite treatment, which converts unmethylated cytosines into uracil, whereas methylated cytosines remain unchanged. We found that under 20 mM NaCl, cytosine methylation increases 10% over control methylation levels (51% vs. 41%, p=0.0874) in the gene body of *Gl2*. In addition, the mannitol treatment caused no significative changes in methylation levels. Overall, these findings indicate that high salinity in soil causes an increment in DNA methylation on the *Gl2* gene body, probably increasing its expression as usually occurs in plant genes, resulting in inhibition of root hair development.

Keywords: Root hairs, epigenetics, abiotic stress, *Arabidopsis thaliana.*

(664) **CHARACTERIZATION OF E3 UBIQUITIN LIGASE HERC1 AS AN IMPORTANT REGULATOR OF TUMOR CELL INVASION AND MIGRATION**

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Despite formidable advance in the prevention, early detection and treatment of a great number of cancers, the development of metastasis represents the principal cause of death in patients with solid tumors. The invasive potential of malignant cells is characterized by a series of molecular and genetic modifications that increase their invasive potential, allowing them to penetrate the extracellular matrix and spread in the surrounding tissues. These changes are associated with alterations in a wide variety of posttranslational modification patterns, including the Ubiquitin-Proteasome System (UPS). The UPS plays a fundamental role in almost every single cellular process, and therefore it is not surprising that genetic alterations, abnormal expression or dysfunction of different components of this cascade are often associated with malignant transformation of tumor cells and the development of metastatic processes.

Using shRNAs that target different UPS genes we identified Herc1 as a key player in the control of tumor cell migration and invasion. Herc1 is a huge protein involved in intracellular membranes trafficking and its role in the control of the metastatic capability of tumor cells has not been reported yet. Using different phenotypic experi-

ments, such as Boyden chamber, proliferation, wound healing and colony formation assays we were able to demonstrate that the depletion of Herc1 decreases the invasiveness of triple negative cancer cells, highlighting its potential as a novel putative therapeutic candidate for cancer treatment.

Keywords: migration; invasion; UPS; screen; HECT.

(1340) **TREACHER-COLLINS-FRANCESCHETTI SYNDROME GENE 1 (*TCOF1*): REGULATION BY G-QUADRUPLEXES AND CNBP (CELLULAR NUCLEIC ACID BINDING PROTEIN).**

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Treacher Collins Syndrome (TCS) is a congenital disease characterized by craniofacial defects. Over 90% of the cases are due to mutations in the *TCOF1* gene, which codifies a nucleolar protein. We have reported a TCS model in zebrafish that fully recapitulates the craniofacial abnormalities observed in patients. Cnbp overexpression rescued the TCS-like phenotype in zebrafish, in a dose-dependent manner. A positive correlation between *CNBP* and *TCOF1* expression in mesenchymal cells from both control and TCS subjects was found. Based on these data, we speculated a possible regulation of *TCOF1* expression by Cnbp.

Cnbp is a nucleic acid chaperone that interacts with and unfolds G-quadruplexes (G4) non-canonical nucleic acid structures. Consensus binding sites for Cnbp were detected both in *Danio rerio* (z2393) and *Homo sapiens* (h791 & h2160) *Tcof1* promoters. These sites overlap with putative G-quadruplexes sequences (PQS). Synthetic single-stranded oligodeoxyribonucleotide sequences representing the human and zebrafish PQSs were used to assess *in vitro* whether they fold as G4 by Circular Dichroism (CD) and DNA intrinsic fluorescence. CDs and fluorescence performed on PQSs folded in the presence of 100 mM K^+ showed spectra indicative of parallel topologies of G4. Addition of purified Cnbp decreased CD signals of z2393 and h2160 suggesting an interaction and unfolding. Quenching of Cnbp tryptophan fluorescence by both oligos also suggested such interactions (Kd in Nm range). ChIP analysis in zebrafish embryos confirmed binding of Cnbp to the *tcof1* promoter *in vivo*. RT-qPCR experiments in zebrafish embryo depleted or overexpressing Cnbp showed altered expression of *Tcof1*. Moreover, Hela cells treated with siRNA to knock-down *CNBP* showed decreased *TCOF1* expression measured by RT-qPCR.

In conclusion, results suggest that Cnbp contributes to the *TCOF1* expression by binding to G4 located in the promoter region and likely by unfolding these DNA secondary structures.

Keywords: gene expression, Treacher-Collins Syndrome, G-quadruplex, CNBP.

(1445) **INTRACELLULAR MICROCIN J25 REPRESSES THE ACTIVITY OF THE STRUCTURAL GENE PROMOTER**

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INSIBIO

Microcin J25 (MccJ25) is a 21-amino-acid, plasmid-encoded antibacterial peptide produced by *Escherichia coli*. Its target of action is bacterial RNA polymerase. MccJ25 synthesis requires four genes and is induced in stationary phase. The structural gen mcjA encodes a 58-residue precursor, which is posttranslationally modified by the *mcjB* and *mcjC* gene products. The *mcjD* gene specifies an exporter and also provides immunity against the peptide. It was observed that microcin production is similar when the mcjA gene is cloned in a low- or a high-copy number plasmid. A possible explanation could be that the peptide represses its own production when it accumulates above certain threshold. To test this hypothesis, we cloned a DNA fragment containing the mcjA promoter in the promoter probe plasmid pRS415, generating a transcriptional fusion with the *lacZ* reporter gene. The recombinant plasmid was introduced in a strain previously transformed with a MccJ25-producing plasmid. β-galactosidase activity was measured during exponential and stationary phases. Levels of β-galactosidase were tenfold lower in the strain producing microcin than in the non-producing control carrying a mcjA gene inactivated by Tn5 insertion. These results support the notion of a negative autoregulation of MccJ25 synthesis. We are investigating whether this effect of MccJ25 is direct, or mediated through its action on RNA polymerase.

Keywords: microcin J25 - gen mcjA - autoregulation

(1615) **CHARACTERIZATION OF THE REGULATORY REGIONS OF THE *CYP21A2* GENE**

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21-hydroxylase deficiency accounts for 90–95% of congenital adrenal hyperplasia cases. This autosomal recessive disorder has a broad spectrum of clinical forms, ranging from severe or classical to the mild late onset or nonclassical. The gene encoding 21-hydroxylase, *CYP21A2* (6p21.3) is adjacent to the pseudogene *CYP21A1P* with which it shares 98% sequence identity. Three distal regions located 2.5, 5.6 and 6.4 kb (referred here as cAMP, PZ and VitD, respectively) upstream from *CYP21A2* have been suggested as transcriptional enhancers. Previously, we studied the cAMP and PZ regions in 66 samples with at least one non-determined pathogenic allele, after sequencing *CYP21A2* and its proximal promoter, and excluding deletions/duplications and large gene conversion by MLPA. We reported a novel variation in the PZ region in 3 patients, involved in misregulating the transcriptional activity of the gene, demonstrating the importance of the study of these regions. The aim of this study was to identify genetic variants in the 3 regions in the remaining patients with at least one non-determined mutation. We included 47 patients for the analysis of the VitD region, and 25 patients for the PZ and cAMP regions. Each region was amplified by PCR and analyzed by direct sequencing. To date, we analyzed the sequences of 30 and 20 patients for the VitD and PZ/cAMP regions, respectively, and we did not identify sequence variants. Only a few works analyzed distal regulatory regions for the screening of putative disease-associated mutations that could lead to alter *CYP21A2* gene transcription. The screening of larger series of patients is necessary to further evaluate the implications of the regulatory regions of the *CYP21A2* in the clinical manifestation of 21-hydroxylase deficiency.

Keywords: Regulatory regions of the *CYP21A2* gene, 21-hydroxylase deficiency, *CYP21A2* gene

GENETICS AND MOLECULAR BIOLOGY 19

(388) **HETEROGENEITY IN THE PATTERN OF FAMILIAL HYPERCHOLESTEROLEMIA MUTATIONS IN A POPULATION OF THE SOUTH-EAST OF BUENOS AIRES**

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Familial Hypercholesterolemia (FH) is a common genetic cause of premature coronary heart disease (CHD), widely underdiagnosed. Genetic testing allows the early identification and then the decision

of treatment contributing to prevent the CHD. So far, we did not have genetic data from the same area of the country. Here we describe the mutation pattern across the main FH genes, LDLR, APOB, PCSK9, plus LDLRAP1 gene of the recessive form, in patients assisted at a Medical Center depending on the Health Direction from General Pueyrredon-BA. From the *"First Prevalence Study of Familial Hypercholesterolemia in Argentina"* 247 blood samples of patients ≥18 years with c-LDL ≥190mg/dl were selected. According to the DUTCH Clinic Lipid Network criteria, 72 selected patients have clinic diagnosis of probable FH (score 6-8) or definitive (score>8). DNA was obtained and the four genes were sequence in a MiSeq, Illumina. The variants were annotated using reference sequences Hg19 and classified according to ACMG-2015, *in silico* tests were applied for novels and VUS variants. Mutations, related to FH, were find in 20 subjects (30%): 17 in the LDLR (85%) and 3 in APOB (15%). All variants were in heterozygosis; two, on the LDLR, were seen in more than one patient: c.1027G>A (p.G343S) in 4 and c.1567G>A (p.V523M) in 2 cases; 2 were novels; the most frequent APOB variant in Caucasian, c.10580G>A (p.R3527Q), was observed once and the Lebanese mutation in LDLR, that had been previously found more frequent in a general study, was not observed. Conclusion: FH mutations show an important heterogeneity in our population. Knowing the spectrum of mutations will allow us to develop strategies that are adequate to approach their study in our population.

Familial Hypercholesterolemia, prevalence, genetic variant, mutations

(461) **ANALYSIS OF AN ARGENTINEAN FAMILY WITH CONOTRUNCAL CONGENITAL HEART DISEASE**

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Congenital heart disease (CHD) is a group of structural abnormalities of the heart and great vessels. In Argentina, the infant mortality (IM) is around 10.8 /1000. Congenital anomalies (CA) are the second leading cause, accounting for 25,9% of total IM. CHD is the most common CA and one of the major cause of perinatal mortality, with a prevalence of 1/125 births.

Genetic CHD pathogenesis is largely unknown but it is widely reported that genetic factors play an important role. 22q11.2 deletion is found in a significant proportion of patients with contruncal CHD.

We present a family with two children affected with CHD. One presents pulmonary atresia, ventricular septal defect, facial dysmorphisms intellectual disability and afebrile seizures. His brother has Tetralogy of Fallot. Chromosome analysis was performed on GTG-banded metaphases. Additionally, SALSA P250 and P424 MLPA kits were used to detect copy number variations (CNVs) and a total of 160 genes related to CHD were analyzed by Next Generation Sequencing. No chromosomal anomalies or genomic imbalances were found, while 3 nucleotide changes in three different genes - VEGF, MYH6 and MYH7- with high impact on protein function were found. We emphasize the value of targeted NGS in familiar CHD to know the genetic causes of this disease.

Key words: Congenital heart disease, targeted gen sequencing

(491) **GENETIC CAUSES OF MULTIPLE CONGENITAL ANOMALIES (MCA) AND ISOLATED CONGENITAL HEART DISEASE (CHD)**

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Congenital anomalies (CA) are morphological and/or functional disorders that originate before birth. Affecting 3 to 5 % of newborns, they represent the second leading cause of infant mortality in Argentina, after perinatal conditions. In approximately 50% of the patients, the underlying causes are unknown. Cases with MCA are those with 2 or more unrelated birth defects. MCA are present in 2,26/1000 births. CHD are the most frequent CA, with a prevalence of 4,06/1000 births.

The goal of this work was to identify the genetic causes of MCA and isolated CHD cases from our population.

We studied 220 patients (127 MCA and 93 isolated CHD) born between June 2015 and August 2017 in 13 public hospitals participating in the National Network of Congenital Anomalies of Argentina (RENAC). Peripheral blood and DNA was obtained from all patients and a karyotype was performed in MCA patients. Patients with conotruncal CHD (cCHD. n=51) were studied by MLPA. Array-CGH was performed in 30 MCA selected patients.

Sixty six MCA patients displayed a normal karyotype, 9/127 presented cytogenetic abnormalities: a trisomy 13, 4 trisomy 18, a 47,XXX/47,XX,+14, a (t(1;2)(q25;q21)), and 2 supernumerary marker chromosome. The karyotype could not be performed in 27 patients due to culture failure. These results are in accordance with the expected frequency of chromosomal abnormalities.

Among 51 cCHD patients, 12 presented a typical 22q11 deletion, one 22q11 short deletion, one 15q14 duplication and one *TBX1* gene deletion. Our 22q deletion frequency (0,24) is slightly higher than reported for cCHD.

By array-CGH, we identified 44 CNVs (copy number variants):9 pathogenic in 7 patients; 12 VUS (variants of uncertain significance) in 8 patients, 14 likely benign in 8 patients and 9 benign CNVs in 7 patients. The diagnostic yield of array- CGH was 23.3%. With the combine algorithm performed, we determined a causative genetic cause in 30 patients.

Key words: Multiple congenital anomalies, congenital heart disease, chromosomal abnormalities, array-CGH

(725) **RESEQUENCING OF DNA SAMPLES FROM PATIENTS WITH FAMILIAL HYPERCHOLESTEROLEMIA SUGGESTS ADVANTAGES FOR THE APPLICATION OF GENOMIC SEQUENCING PARTICULARLY IN YOUNG CASES**

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Familial Hypercholesterolemia (FH) is the most common genetic dyslipidemia characterized by high levels of LDL-c and associated with elevated coronary heart disease risk. FH unambiguous identification in young people is not easy, particularly in children, and an early identification is important because of the cumulative effect of LDL on the arteries. The absence of founding effects, the large number of mutations and several genes involved in its origin, make difficult their complete genetic assessment. Here we present the results of the resequencing of DNA samples of cases with FH in which genetic variants had previously been identified. 13 samples: 6 ≤18, 2 between 18-30 and 5 ≥30 years of age; presenting 12 different genetic variants that have been previously identified by Sanguer sequencing of selected regions, were resequenced by NGS. We included 5 genes: LDLR, APOB, PCSK9, APOE and LDLRAP1. Fifteen variants candidate to be related to the phenotype, were identified: the 12 expected variants, 11 in the LDLR and 1 in APOB, and other 3 variants placed in regions not sequenced before. An LDLR VUS variant, c.1783C>T (p.R595W), detected in a sample of a child carrier of another variant in the LDLR gene with a well known pathogenic effect, the Lebanese mutation. A new variant on APOB gene, c.814_816delTAC (p.Y272del), VUS, and an APOE recently reported pathogenic variant, c.487C>T (p.R163C), both of them in the same sample that belongs to a child carrier of a previously identified VUS LDLR variant c.-135C>A placed in a regulatory C-repeat. Conclusion: new generation sequencing is a powerful tool

and it would be especially suitable for the cases of children and adolescents with clinical suspicion of FH.

Familial Hypercholesterolemia, NGS, genetic variant, mutations

(795) **STUDY OF MUTATIONS IN JAK2 , MPL AND CALR GENES IN PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA**

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Essential thrombocythemia (ET) is a nonreactive, chronic myeloproliferative neoplasm in which sustained megakaryocyte proliferation leads to an increase in the number of circulating platelets. ET is characterized by an unusual thrombocytosis and risk of thrombotic and hemorrhagic disorders and a small number of patients show disease transformation towards myelofibrosis, myelodysplasia or acute myeloid leukemia. The presence of JAK2V617F mutation has been described in 50% of cases of ET and recently, mutations in exon 10 of *MPL* and exon 9 of *CALR* genes were described in patients with ET without mutations in *JAK2*. Objective: to evaluate the presence of mutations in *JAK2*, *CALR* and *MPL* genes in patients clinically diagnosed with ET and to study the correlation of each type of mutation with hematological characteristics. The presence of the described mutations was studied in 26 patients, performing real time PCR amplifications, with subsequent screening by High Resolution Melting Analysis and confirmation by sequencing. *JAK2V617F* mutation was found in 62% (16) of patients, *CALR* mutations in 23% (6), *MPL* mutation in 4% (1) and in 11% of patients (3) no mutations were detected (triple negative, TN). Correlation with a high level of hemoglobin, leukocyte and hematocrit ($p<0,05$) was found in patients carrying *JAK2V617F* mutation in comparison with *CALR* positive patients. An additional finding was the detection of an intronic 5bp deletion SNP, rs56241661 [-/TCTTA], located in *JAK2* gene in the 3 TN patients where no mutations were found. This SNP is cataloged as part of the 46/1 haplotype that apparently predisposes to the development of myeloproliferative neoplasms. Accurate molecular diagnosis of ET is of great importance due to the difference in the clinical course between ET with mutations in *CALR* gene and ET associated with mutations in *JAK2* or *MPL* genes.

Key words: Essential thrombocythemia; JAK2, CALR, MPL, clinical course

(969) ***CYP21A2* MUTATION UPDATE: COMPREHENSIVE ANALYSIS OF DATABASES AND PUBLISHED GENETIC VARIANTS**

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Abstract: Congenital adrenal hyperplasia (CAH) is a group of autosomal recessive disorders of adrenal steroidogenesis. Disorders in steroid 21-hydroxylation account for over 95% of patients with CAH. Clinically, the 21-hydroxylase deficiency has been classified in a broad spectrum of clinical forms, ranging from severe or classical, to mild late onset or non-classical.

Known allelic variants in the disease causing *CYP21A2* gene are spread among different sources. Until recently, most variants reported have been identified in the clinical setting, which presumably bias described variants to pathogenic ones, as those found in CYPAlleles database. Nevertheless, a large amount of variants are being described in massive genome projects, as many found in dbSNP, but lack functional implications and/or its phenotypic effect.

In this work we gathered a total of 1340 genetics variants in the *CYP21A2* gene, from which 899 variants were unique and 230 have an effect on human health, and compiled all this information in an integrated database. We also connected *CYP21A2* sequence information to phenotypic effects for all available mutations. Data compiled in the present work could help physicians in the genetic counseling of families affected with 21-hydroxylase deficiency.

Keywords: CYP21A2, 21-hydroxylase deficiency, Congenital Adrenal Hyperplasia, Data Base Analysis, Genetic Variants

(1101) **ASSOCIATION OF *FCER1A* AND *FCER2* COMMON VARIANTS WITH IGE SERUM LEVELS AND ASTHMA EXACERBATIONS IN CHILDREN**

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Introduction: Several studies identified single nucleotide polymorphisms (SNPs) in *FCER1A* to be major genetic determinants of IgE levels. Pharmacogenetic studies found that *FCER2* SNPs increase the risk of asthma exacerbations and influence serum IgE levels in asthmatic patients.

Objectives: To determinate the genotype frequency of rs2251746 and rs2427837 genetic variants (*FCER1A*) and rs28634072 (*FCER2*) and to evaluate the association of these SNPs with IgE levels and exacerbations in children with asthma from Argentina.

Methods: Children of both sex, mean age 8.6 years (SD 3.6), with mild, moderate and severe asthma (n:260) were genotyped for rs2251746, rs2427837 and rs28634072. Total serum IgE level was measured by nephelometry and aged-adjusted values were determined (n:227). A comparison of the genotype distribution among different populations was performed (X^2 test). Total serum IgE levels were analyzed for each genotype (Kruskall-Wallis test). The number of exacerbations was assessed in the severe group (N:117) for each variant (X^2 test).

Results: All SNPs genotyped were in Hardy–Weinberg Equilibrium. The minor allele frequencies for all SNPs was significantly lower than the observed in other asthmatic populations ($p<0.0001$). A significant difference of IgE serum levels was observed among the genotypes for *FCER1A* polymorphisms ($p=0.02$ for rs2251746; $p=0.03$ for rs2427837). Patients homozygous for the minor allele for *FCER1A* variants showed the highest IgE level. No significant association of rs28634072 with IgE levels was detected. No statistical association was observed among the assessed variants and the presence of exacerbations.

Conclusion: In this series of patients, the frequency of the minor allele for all variants was significantly lower than the described in other asthmatic population. *FCER1A* variants were significantly associated with IgE level. The variants studied do not seem to condition the presence of exacerbations in children with severe asthma.

Keywords: *FCER1A, FCER2*, genetic variants, IgE, Asthma.

(1554) **STUDIES OF THE ROLE OF CYP2C9 SNPS ON ACUTE INTERMITTENT PORPHYRIA MANIFESTATION**

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Abstract: Porphyrias are a group of metabolic hereditary pathologies in which only the presence of the mutation in the gene codifying the deficient enzyme is not sufficient for porphyria manifestation. Many porphyrinogenic drugs as barbiturates, anaesthetics, anti-inflammatories, antibiotics, anticonvulsants and others are known as triggering factors. So, genetic variants in xenobiotics metabolization enzymes have an essential role in symptomatology manifestation. It was suggested that SNPs in CYP2C9, that affect its activity and/or expression, would play a role in the manifestation of the most frequent acute porphyria in our population, Acute Intermittent Porphyria (AIP). With this aim, we started the first analysis in world literature studying the presence of allele CYP2C9*2 (rs: 1799853),

which diminished its enzyme activity, in both, a control population and AIP patients diagnosed at CIPYP. We selected samples of 50 individuals, 13 healthy volunteers and 37 AIP patients, 18 symptomatic and 19 asymptomatic. Molecular typing was performed by PCR and automatic sequencing. The Fisher exact test was used to detect differences in alleles and genotype frequencies, odds ratio and 95% confidential interval. Considering the total population (50) the frequencies found for C/C and C/T genotypes were 0.72 and 0.20 respectively while genotype T/T was not present in Argentinean population. There is no significant difference between genotype and allelic frequencies comparing control and AIP patients. When we compared these parameters between the group of symptomatic and asymptomatic AIP patients no significant difference was found; p= 0.195, OR=2.6 and IC (95%) = 0.55-12 for genotype variants and p= 0.2130, OR=2.33 and IC (95%)=0.53-10 respectively. According these results, CYP2C9*2 allele do not have any role in AIP manifestation. We are following this study increasing the number of individuals and also searching other variants in this CYP.

Keywords: porphyrias, acute intermittent porphyria, CYP2C9

(1566) **STRUCTURE-BASED ANALYSIS OF GENETIC VARIANTS IN *NKX2.5*, *GATA4* AND *ZIC3* AND THEIR IMPLICATION IN CONGENITAL HEART DISEASE**

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Congenital heart diseases (CHDs) are the most prevalent type of human structural birth defect worldwide, affecting between 4 to 9 per 1000 births. The etiology of CHDs includes both genetic and environmental factors. In 5-10% of the patients, CHD is caused by a single gene variation. Although several genes have been described, *NKX2.5, ZIC3* and *GATA4* are some of the most frequently involved.

The aim of this work was to perform a structure-based analysis of genetic variants (GVs) found in the three genes, using the crystal structure of human NKX2.5 and building molecular models of *ZIC3* and *GATA4* human genes.

With the aid of MODELLER v9.18, we modeled the human ZIC3 and GATA4 proteins using homologous proteins domains as templates retrieved from the Protein Data Bank (PDB). NMR and X-ray crystal structures were used for NKX2.5 as templates. Since too many templates were available, a clustering analysis was performed in order to remove redundant information. DISOPRED3 was also used to annotate naturally disordered regions. For NKX2.5, we have used FoldX to generate point mutations and to analyze changes in protein stability or protein interaction with DNA.

We generated a molecular model for each of the core regions of GATA4 and ZIC3. In addition, we performed a structural evaluation of the pathogenic implication of 32 *GVs* in the NKX2.5 involved DNA interaction region and protein destabilization.

These models can be used to frame known Single Nucleotide Variants (SNV) and predict the effects of unknown variants that may appear in the future. This would be especially useful in these genes as they have been associated with sudden death and other cardiac defects, and an early diagnosis can mean palliative measures as the inclusion of a peacemaker in time.

Keywords: NKX2.5, congenital heart disease, genetic variants, MODELLER, structure-based analysis

(1878) **DETECTION OF HYPOPITUITARISM GENES IN ARGENTINEAN CHILDREN USING A NEXT GENERATION SEQUENCING PANEL**

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Hypopituitarism is caused by genetic and environmental factors. Over 30 genes have been implicated in isolated and combined pituitary hormone deficiency (CPHD), but the majority of cases are of unknown etiology. Mutations in the *PROP1* gene are the most common known cause, but the frequency of mutations in this gene varies greatly by ethnicity. The rate of mutations in *PROP1* or other known hypopituitarism genes has not been analyzed in Argentinean children. We designed a custom array to assess the frequency of mutations in known genes involve in pituitary organogenesis and also included new candidates, based on research studies, by next generation sequencing technology.

Methods: Molecular inversion probes were designed to capture 693 coding exons of 30 known genes and 37 candidate genes. We captured genomic DNA from 51 pediatric patients from Argentina with CPHD (N=43) or IGHD (isolated growth hormone deficiency, N=8) and their relatives and conducted next generation sequencing on an Illumina platform.

Results: We obtained deep coverage over targeted regions and demonstrated accurate variant detection by comparison to whole-genome sequencing in a control individual. We found a dominant mutation *GH1*, p.R183H, in a three-generation pedigree with isolated growth hormone deficiency.

Conclusions: Molecular inversion probes capture and deep sequencing is an efficient and inexpensive method to detect mutations in patients with hypopituitarism.

Keywords: NGS, gene panel, pituitary, genes

IMMUNOLOGY (ADAPTATIVE IMMUNITY) 5

(26) **STUDY OF A POLYMERIC ADJUVANT (MONTANIDE GEL 01) IN A VACCINE AGAINST BOVINE RESPIRATORY DISEASE**

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Bovine Respiratory Disease (BRD) is a multi-factorial disease that causes high morbidity and mortality in cattle. The disease involves a sequential cascade of events initiated by stress; thus, predisposing animals to viral infections which facilitate rapid bacterial invasion from the upper respiratory tract to lungs. Among bacteria causing BRD *Mannheimia haemolytica* (MH), *Pasteurella multocida* (PM) and *Histophilus somni* are the most important. Although BRD vaccines are worldwide produced and applied, there is a need of new therapeutic-adjuvant strategies in order to minimize the economic impact of respiratory syndrome.

Montanide Gel 01 is a polymeric adjuvant, based on a dispersion of stable gel particles of sodium polyacrylate in water, which enhance the immune response by an antigen sustained release and activation of innate immune cells.

The aim of this work was study the action Montanide Gel 01 *(Seppic),* when it is employed in the BALB/c mice immunization with MH and PM, both bacteria causing BRD. Mice were subcutaneously immunized on days 0, 15 and 27 with inactivated MH or PM formulated with Montanide Gel 01 at 5% or 10%, or with aluminum hydroxide (AH). Commercial vaccine was employed as positive control. Control mice received the adjuvants with PBS. Blood samples were obtained on days 0, 10, 27 and 38 and serum anti-PM and anti-MH IgG, IgG1, and IgG2a levels were measured by *in house* indirect

ELISA.

Montanide Gel 01 induced an increase in the anti-MH IgG, IgG1 and IgG2a levels compared with AH vaccine ($p< 0.0001$; $p< 0.001$; $p< 0.01$, respectively). In relation with anti-PM antibodies, IgG2a levels were higher than the commercial vaccine ($p< 0.001$). Specific IgG and IgG1 levels showed the same tendency although no significant differences were found.

To conclude, Montanide Gel 01 induced a strong humoral immune response. Further studies in cattle will allow us analyze Montanide Gel 01 as a possible adjuvant candidate to use in BRD vaccines.

Keywords: Vaccination, adjuvant, Bovine Respiratory Disease, antibodies

(38) ROLE OF SERUM ANTIBODIES FROM PROTEIN DEFICIENT RATS IN ANTIBODY DEPENDENT CELLULAR CYTOTOXICITY MECHANISM AGAINST NEWBORN LARVAE OF *TRICHINELLA SPIRALIS*

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Abstract: This study was aimed to analyze how protein deficiency affects serum antibodies production and their ability to mediate antibody dependent cellular cytotoxicity (ADCC) against *T. spiralis* migrant stage (newborn larvae, NBL).

Weaning Wistar rats (n=10/group) received protein deficient (PD, 6.5% casein) or control diets (C, 20% casein). After ten days, both groups were orally infected with *T. spiralis* muscle larvae (PD_I, C_I); non-infected rats served as controls (PD_{NI}, C_{NI}).

Sera were obtained on days 6, 13 and 33 post-infection (p.i.), and effector antibodies against NBL (IgE, IgG1) were detected by indirect immunofluorescence. In ADCC assays, NBL suspensions, sera from animals of each group and peritoneal cells from C_{NI} rats were co-incubated for 20 h. Results were expressed as NBL mortality percentages (M%) and were analyzed using the two-way ANOVA test.

Specific antibodies were present since days 6 and 13 p.i. in C_I and PD_I, respectively. PD_I had lower titers of anti-NBL isotypes than C_I on days 13 (IgE: 16 *vs.* 32-64; IgG1: 32-64 *vs.* 64-128) and 33 p.i. (IgE: 32-64 *vs.* 256; IgG1: 1024 *vs.* 2048). As in the C_I group, ADCC was observed in the PD_I group when sera from days 13 (M%: PD_I 21.00±1.41 *vs.* PD_{NI} 6.61±2.04 $p<0.01$) and 33 p.i. (M%: PD_I 26.64±3.39 *vs.* PD_{NI} 6.61±2.04 $p<0.0001$) were used. However, no significant differences in NBL mortality percentages were found between PD_I and C_I sera at both time points (M% in C_I group: day 13 p.i. 22.00±1.98; day 33 p.i. 33.77±3.22).

Although the kinetics and titers of the serum antibodies against *T. spiralis* are affected by protein deficiency, these alterations do not seem to affect their ability to mediate NBL death by ADCC mechanism.

Keywords: Trichinella spiralis; protein deficiency; specific antibodies; ADCC.

(157) REDUCED FREQUENCY OF ACTIVATED FOXP3$^+$ REGULATORY T CELLS ALLOWS THE EMERGENCE OF SPECIFIC CD8$^+$ T CELL IMMUNITY DURING *T. CRUZI* INFECTION

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CD4$^+$ Foxp3$^+$ T cells (Tregs) present a dual role in infections as they limit immunopathology but also restrain immunity to the pathogen. During *T. cruzi* (Tc) infection Tregs response has been poorly characterized. We previously determined that during this infection, Tregs become activated, upregulate a wide range of suppressive markers and acquire a transcriptional program specialized in controlling Th1 responses. However, Tregs frequency is significantly reduced in the periphery of infected mice as a result of a low proliferation rate and impaired induction of peripheral Tregs.

Here, our aim was to assess the biological relevance of reduced Tregs frequency in Tc infection. First, we adoptively transferred Tregs generated *in vitro* from naïve CD4 T cells cultured with IL-2, TFG-β and *all-trans* retinoic acid. Injection was performed at day (d) 11 post-infection (pi) in WT mice. At d17pi, Tregs recipient mice showed increased parasite burden in blood, spleen and liver concomitantly with decreased frequency and numbers of total and Tc-specific CD8 T cells compared to non-transferred animals. No differences were observed between the two groups in the levels of biochemical damage markers. As a second approach, Tregs were specifically depleted by the injection of diphtheria toxin (DT) in DEREG mice at d5pi. Significantly reduced Tc levels and augmented parasite specific CD8 response were observed in spleen and liver of DT-treated DEREG mice in comparison to PBS-injected counterparts at d19pi. CD8 functionality was also improved by Tregs depletion, as shown by the significant increase in the frequency of splenic specific CD8 cells able to degranulate (CD107a$^+$) and produce IFN-γ and TNF upon parasite-specific stimulation in DT-treated DEREG mice. These results outstand Tregs role during acute Tc infection, suggesting that a reduction in activated Tregs frequency may be necessary to allow the development of the CD8 T cell immunity responsible of parasite control.

Keywords: Treg cells, *Trypanosoma cruzi*, CD8 cells

(338) PLASMACYTOID DENDRITIC CELL AND T CELL INTERACTION IN EYE DRAINING LYMPH NODES MEDIATE IMMUNE RESPONSE IN HERPES SIMPLEX VIRUS-1 KERATITIS

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Plasmacytoid dendritic cells (pDCs) represent a highly functional subset of bone marrow (BM)-derived cells that play a key role in anti-viral responses and link innate and adaptive immune responses. Here we investigate the presence of activated pDCs in draining lymph nodes (dLNs) and their role in modulating T cell responses in the acute herpes simplex virus (HSV)-1 keratitis. **We induced** corneal infection by inoculation of HSV-1 after scarification. Then, submandibular dLNs were excised and quantified for pDCs by flow cytometry or immunofluorescence staining of cryosections for pDC markers (CD11c, PDCA-1), T cells (CD3) and activation markers (CD86 and MHC-II). Intravital multiphoton microscopy (IV-MPM) was performed in living mice to image dLNs in bone marrow chimera mice reconstituted from Tred mice (T cells are red) and pDC-eGFP mice (pDCs are green) in steady state and after corneal inoculation with HSV-1 to determine the kinetics of pDCs and their interactions with T cells. We observed a significant increase in the number of pDCs in dLNs ($P<0.05$) after corneal HSV-1 infection, with an increased percentage of activated pDCs (MHCII$^+$CD86$^+$) ($P<0.05$) and an increase in total T cells (CD45$^+$ CD3$^+$) ($P<0.05$), compared to naïve mice. IV-MPM imaging in bone marrow chimera mice showed more high motile pDCs after corneal inoculation of HSV-1 with high velocity and track length ($P<0.05$) compared to steady state pDCs. We also observed low motile elongated pDCs that established contact with T cells with durations of >10 minutes demonstrating direct interaction. Our data shows that activated pDCs are increased during HSV-1 keratitis in dLNs where they directly interact with T cells and mediate anti-viral adaptive immune response. We showed for the first time, in vivo imaging of submandibular LNs during herpes keratitis allowing the study of pDCs and their interaction with T cells.

Keywords: Plasmacytoid dendritic cells, draining lymph nodes, HSV-1 keratitis

(358) **ORAL DNA VACCINE OF AN ENGINEERED CHIMERIC IMMUNOGEN BETWEEN A *Trypanosoma cruzi* ANTIGEN AND A NON-TOXIC SUPERANTIGEN DELIVERED BY ATTENUATED *Salmonella* INDUCES PROTECTION AGAINST *T.cruzi* CHALLENGE**

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Abstract*:* There is still an urgent need for an effective vaccine to prevent and treat Chagas disease.

Our group has developed superantigen mutants (mSAg) that lack T cell activation capability but bind MHC-II on antigen presenting cells.

In this work, we engineered a chimeric antigen (chimera) between mSAg and a protective *Trypanosoma cruzi* antigen. Our goal was to evaluate the ability of chimera-DNA immunization through a *Salmonella*-based delivery system to induce protection in a murine model of *T.cruzi*.

Chimera was genetically engineered, cloned in two expression vectors: pET32 and pcDNA3.1; and was produced as a recombinant protein in *E.coli*. Attenuated *Salmonella* was transformed with pcDNA3.1-chimera (Schimera).

C3H/HeN mice were immunized with four total doses as follows: I- 10^9 Schimera (orally); II- chimera (10 μg/dose) + ODN-CpG (intramuscularly); III- two Schimera + two boosts of chimera; IV- two Schimera + two boosts of chimera + ODN-CpG; V- 10^9 *Salmonella* with empty pcDNA (control). Two weeks after the last immunization, *T.cruzi* antigen-specific IgG titers and IgG1/IgG2a isotypes were determined. Animals were challenged with 1000 bloodstream trypomastigotes (RA strain). Parasitemia levels were registered every two days and survival was monitored daily.

Specific antibody titers against *T.cruzi* antigen were detected in groups II, III and IV, showing significant differences against the control group ($p<0.01$). In these groups, IgG2a titers were significantly higher than IgG1. In groups I, II and IV parasitemia levels were low until 23 dpi, showing significant differences against the control group at the peak of parasite loads (14 dpi; $p<0.05$). Survival of groups I, II, III and IV was of 100% until 100 dpi.

Our results indicate that immunization with this DNA-delivery system induces an efficient immune response that provides protection against *T.cruzi* challenge. Engineered chimeric immunogens represent useful and promising strategies against parasitic chronic infections.

Keywords*:* *Trypanosoma cruzi*; vaccine; superantigens; engineered chimeric antigen; attenuated *Salmonella*.

(616) ***trans*-SIALIDASE FROM *Trypanosoma cruzi* INDUCES IL-6 SECRETION IN BONE MARROW DERIVED DENDRITIC CELLS AND PROMOTES TH2/TH17 RESPONSES IN CD4+ NAÏVE T CELLS**

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trans-Sialidase (TS) is a secreted antigen from *T.cruzi*, which is shed in large amounts during infection in mammalian hosts. This protein has a repetitive *C*-terminal domain known as SAPA that elicits a strong humoral immune response. It has been observed in both infected animals and humans, an important rise in serum IL-6, thatin turn is known to influence and modulating the immune system. Given that antigen-presenting cells (APCs) are responsible for mounting adaptive immune response, we decided to evaluate IL-6 secretion in bone marrow-derived dendritic cells (BMDCs) from C57BL/6 mouse. We found that co-culture of BMDCs with trypomastigotes (CL Brener strain), induces IL-6 secretion an effect that was mimicked by the addition of TSs (both active and inactive isoforms) ($p\leq0.05$). Surprisingly, we found that recombinant SAPA protein was also able to induce IL6 secretion, that is, in a TS-independent form. Induced secretion of IL-6 by BMDCs was associated with the profiling of *Th17/Th2* response in $CD4^+$ naive cells after either antigen-unspecific CD3/CD28 stimulation or specific (using OT-II mouse model) activation ($p\leq0.001$). BMDC-$CD4^+$ physical contact seemed to be unnecessary. In sights of understanding the underlying mechanism of this process, we tested IL-6 secretion in C3H/HeJ mice-derived BMDCs (deficient in toll-like receptor 4 [TLR4] signaling). IL-6 secretion was abrogated under these conditions ($p\leq0.01$). Given that experiments were performed in the presence of Polymyxin B and that the effect was reversed when anti-SAPA antibodies were added to the cultures ($p\leq0.01$), we suggest the involvement of the TLR4 pathway in the SAPA-induced IL-6 secretion by BDMCs. In addition, it is known that this secretion is responsible of profiling a Th17/Th2 response in $CD4^+$ naive T cells, supporting the primary induced phenotypes observed after parasite/TS treatment.

Keywords: trans-Sialidase, SAPA, BDMCs, IL-6, $CD4^+$ T cells

(1046) **ROLE OF THE GTPASE RAB22A IN THE RECRUITMENT OF ER COMPONENTS TO DENDRITIC CELL PHAGOSOMES AND ENDOSOMES**

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Cross-presentation is the process by which antigen presenting cells (APCs) expose exogenous antigens-derived peptides in association with MHC class I molecules to $CD8^+$ T lymphocytes in order to trigger cytotoxic immune responses. In this context, dendritic cells (DCs) are the most potent APC able to achieve cross-presentation efficiently. Several studies have focused on deciphering the molecular mechanisms underlying this process, but the connection between the endocytic network and endoplasmic reticulum (ER)-derived compartments is still poorly understood. In this study, we have investigated the role of the small GTPase Rab22a during the delivery of ER resident proteins to DC endosomes and phagosomes. In a previous work, we have shown that Rab22a is crucial to regulate antigen cross-presentation, the phagosomal acquisition of MHC-I and the recycling of these molecules to the cell surface in DCs. Now, we show that the knock-down (KD) of Rab22a expression in DCs impairs the normal delivery of ER components exclusively to endosomes but not to phagosomes, suggesting a differential role of Rab22a in these compartments. We have validated these observations by performing a biochemistry assay (phagosomal purification), immunofluorescence and confocal microscopy, and a flow cytometry-based approach. Furthermore, we evidenced that endosomal maturation is drastically altered in Rab22a KD DCs, as compared to control cells. Interestingly, this phenomenon was not observed during the process of phagosomal maturation. Altogether, our results indicate that Rab22a displays major regulatory functions in endosome maturation and is important to guarantee the proper recruitment of ER components to DC endosomes.

Keywords: Dendritic cells, Rab22a, ER recruitment, endosomes, phagosomes.

(1298) **STUDY OF THE ROLE OF TISSUE RESIDENT REGULATORY T CELLS DURING *TRYPANOSOMA CRUZI* INFECTION**

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Tissue resident $CD4^+Foxp3^+$ regulatory T cells (tTregs) have emerged as a specialized Treg subset that exhibit phenotypic, func-

tional and transcriptional profiles particular to each tissue. tTregs not only regulate immune effector function as lymphoid Tregs but also modulate several non-immune biological processes and maintain tissue homeostasis. *T. cruzi* (Tc) triggers a strong effector response that controls parasite spreading and may promote tissue damage and immune pathology. Our previous results showed that Tc infection is linked to a limited Tregs response that is required for the emergence of protective CD8+ T cell immunity.

Our current aim is to evaluate whether tTregs plays any role in the regulation of tissue damage and pathogenesis in Chagas disease. To this end, Foxp3-GFP C57BL/6 mice were infected with 5000 Tc parasites (Tulahuen) and frequency and phenotype of Tregs were determined by flow cytometry in blood and spleen as well as in target tissues such as liver (L), skeletal muscle (SM) and heart (H) at different days post-infection (dpi). As reported in blood and spleen, the frequency of Tregs identified as CD4+Foxp3-GFP+ T cells decreased in L, SM and H along the infection. We next evaluated the expression of IL-18R and ST2, required for survival and considered markers of tTregs. Tc infection did not change the % of either IL-18R+ Tregs in any organ or ST2+ Tregs in spleen but it reduced % of ST2+ Tregs in L and SM. Finally, we studied whether Tc infection triggered the production of tTregs growth factors such as IL-33. We determined by ELISA that while IL-33 was detectable at low concentration in the plasma of non-infected mice, it became undetectable after 14 and 21 dpi ($p<0.001$). Our preliminary results suggest that tTregs are not induced during Tc infection and this may be associated to a greater damage in target organs. Further studies may establish whether boosting tTregs generation may be useful to limit immunopathology during Tc infection.

Keywords: Tregs, *Trypanosoma cruzi*, immunopathology.

(1706) **PLASMABLASTS CONSTITUTE AN EARLY CONTROL OF *TRYPANOSOMA CRUZI* REPLICATION AND MAY REGULATE INFLAMMATION AND IMMUNOPATHOLOGY DURING THE INFECTION**

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B cells are the only cells that differentiate into antibody-secreting cells (plasmablasts, PB; and plasma cells) and they can also shape and regulate T cell responses through cytokine production. We have found that PB generated in *Trypanosoma cruzi* infection have a high surface expression of the inhibitory molecule PD-L1 and that these cells were also present in other infections such as LCMV (Clone 13) infection and Malaria. Since the PD1/PDL1 pathway is involved with disease in these chronic infections, we studied the biology of PD-L1+PB generation and function. We have found that PD-L1+PB were not driven by cytokines such as type I IFN, IFNg, IL-6 and TNF or by TLR2 and TLR4, because PD-L1+PB were present in *T. cruzi* infected mice deficient in all the cytokines and TLR mentioned. In fact, PD-L1+PB were driven by an antigen specific mechanism, since MD4 mice, whose B cells are specific for Hel, did not generate PB after *T. cruzi* infection. Additionally, PB generation required Tfh collaboration since infected Bcl6f/fCD4Cre-pos mice presented a decrease in PB compared to Bcl6f/fCD4Cre-neg mice.

Blimp1f/fCD23cre-pos mice infected with *T. cruzi* presented a significant increase in parasitemia at day 9 post-infection(pi) ($p<0.0001$) but despite PB-absence these mice controlled parasitemia, showing a reduction of trypomastigotes in blood at day 12pi. Infected PB-deficient mice also presented a significant increase in PD1+CD4+T cells, particularly those IFNg+TNF+ at day 18pi ($p<0.001$), indicating that CD4+T cells were disregulated in absence of PB. Furthermore, livers of infected PB-deficient mice presented more lesions related to inflammation. In vitro, co-culture of cell-sorted PD-L1+PB with CD4+ and CD8+T cells showed that PB suppressed cytokine production by T cells in a PD-1/PD-L1 mechanism.

In conclusion, PB are key players during *T. cruzi* infection because they constitute an early control of parasite replication and may regulate inflammation and immunopathology.

Keywords: Trypanosoma cruzi, Plasmablasts, PD-1/PD-L1, Inflammation, B cells.

(1528) **A STABLE LIGAND OF ARYL HYDROCARBON RECEPTOR MODULATES THE BALANCE BETWEEN REGULATORY AND INFLAMMATORY RESPONSE DURING *TRYPANOSOMA CRUZI* INFECTION**

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A strong inflammatory and an efficient regulatory response are essential to restrict parasite replication and prevent immunopathology during *T. cruzi* infection. Despite its ability to control parasite replication, infected B6 mice are unable to expand regulatory T cells (Treg), resulting in the premature death by inflammatory liver failure. In T cells, the ligation of AhR, (a ligand-activated transcription factor that plays important roles in several biological processes) with the xenobiotic TCDD up-regulates the expression of Foxp3. We have reported that the treatment of B6 mice with TCDD 24 h prior *T. cruzi* infection is able to control the inflammatory response increasing the % of Treg but also increasing the parasite burden and impairing the survival. To determine the effect of TCDD treatment on the modulation of T cell response during the acute infection we treated B6 mice with TCDD (40 ug/kg) 24 h before being infected with *T. cruzi* and analyzed the spleen Treg and Th cell populations at day 10 pi. TCDD group showed increased parasite burden and levels of serum AST and ALT associated with impaired survival compared with control group. TCDD group presented markedly reduced spleens (*p<0.01*) containing a higher population of Treg cells (*p<0.05*) with significantly increased number of these cells producing TGF-β (*p<0.05*) compared with control group. The inflammatory populations Th1 and Th17, and also CD8+ *T. cruzi*-specific cells (TSKB20 tetramer+) were reduced in number and % in treated mice (*p<0.01*) with this last population showing increased expression of Annexin V (*p<0.05*), in agreement with studies showing a direct effect of TCDD in the apoptosis of activated T cells. Our results show that even if effective to induce a regulatory response able to balance the Th1/Treg and Th17/Treg ratios in *T. cruzi*-infected B6 mice, TCDD treatment is detrimental to the host by leading to the inability of mount an effective immune response to deal with the parasite replication.

Palabras clave: AhR, TCDD, infection, *T. cruzi*, regulation

IMMUNOLOGY (INNATE IMMUNITY) 6

(124) **TRYPTOPHAN-DERIVED METABOLITE, INDOLE-3-PROPIONIC ACID, PROMOTES HOMEOSTASIS AND PREVENTS INFLAMMATION OF THE INTESTINAL MUCOSA**

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Microbiota-derived metabolites serve as signals that can promote the homeostasis of the epithelial cell layer of the intestinal mucosa. These signals can shape immune responses to bacterial pathogens as well as to prevent dysregulated responses to microbiota that result in inflammatory bowel disease (IBD). We tested the hypothesis that the metabolite indole-3-propionic acid (IPA), generated by commensal bacteria following the tryptophan metabolism, provides the signals that promote intestinal mucosa homeostasis and in turn control inflammation to prevent IBD. The effect of IPA in the intestinal mucosa was investigated using a mouse model of colitis caused by oral infection with *Citrobacter rodentium.* Remarkably, we found that IPA treatment prevents colitis in mice ($p<0.0341$ by student t-test). Although most active indoles derived from the tryptophan

metabolism act as ligands of the aryl hydrocarbon receptor (AHR), previous reports have suggested that IPA acts through the nuclear receptor pregnane X receptor (PXR), independently of AHR. Here, we investigated if the effect of IPA is mediated by the activation of PXR promoting the integrity of the epithelial barrier present in the gut mucosa. Indeed, we found that IPA did not protect against colitis in PXR deficient mice. Moreover, the absolute numbers of lymphocytes producing IL-17A and IFNγ are increased in the intraepithelial lymphocyte compartment of the colon after IPA treatment (Absolute number (#) IL-17A+ γδ T cells WT Untreated (WTU) vs IPA treated (WTI) p=0.039 by One-way ANOVA and # IFNγ+ αβ CD8 T cells WTU vs WTI p=0.0088 by One-way ANOVA) and that this effect is also dependent on the presence of PXR. Furthermore, we found that the effect of IPA is mediated by the increased gene expression of E-cadherin, Reg3γ and SAA-1. In conclusion, we believe the discovery of metabolites like IPA that promotes the maintenance of the intestinal mucosa will provide exciting new strategies to prevent and treat IBD.

Keywords: Microbiota, metabolites, intestinal mucosa, Citrobacter rodentium, inflammatory bowel disease

(1492) **ACTIVATED MACROPHAGES AND DENDRITIC CELLS EXPRESS CLUSTERIN**

Maria Sol Carregal

Clusterin is a ubiquitous glycoprotein present in almost all tissues and body fluids. Although clusterin appears to be involved in a variety of physiological and pathological processes such as cell death regulation, complement inhibition and Alzheimer's disease, its function is still controversial. Whereas cytosolic clusterin inhibits apoptosis promoting cell survival, the role of secretory clusterin (sCLU) remains obscure. In this study, we analyzed the expression of clusterin in resting and activated human macrophages (M) and dendritic cells (DCs). To this aim, monocytes were differentiated into M or DCs by culturing monocytes during 7 days with M-CSF or GM-CSF plus IL-4, respectively. M or DCs ($1x10^5$/ml) were cultured in the absence (controls) or presence of LPS (10 ng/ml) for 48 h, and the concentration of clusterin was determined in cell supernatants by ELISA. Resting M and DCs did not produce clusterin, while LPS-activated M and DCs produced significant amounts of clusterin: 17 ± 3 and 15 ± 3 ng/ml (media ± SE, n=5, $p<0.01$ vs controls). The expression of intracellular clusterin was also evaluated in cell lysates by ELISA. We found that lysates ($1x10^6$ cells) from resting M or DCs did not contain clusterin, while those obtained from LPS-activated cells contained significant amounts of clusterin: 13 ± 3 and 12 ± 2 ng, for M and DCs, respectively (media ± SE, n=5, $p<0.01$ vs controls). We further studied the expression of intracellular clusterin in M and DCs by confocal microscopy. The images showed that intracellular clusterin was present in the cytosol of LPS activated cells but not in resting cells (n=3). These results demonstrate that activation of M and DCs by LPS induces the cytosolic expression and secretion of clusterin. Ongoing experiments using clusterin shRNA carrying lentiviruses will allow us to determine the role of clusterin on the survival, function, and antigen-presenting capacity of M and DCs.

Palabras clave: Dendritic Macrophage Clusterin

(1527) ***T. CRUZI* INFECTION INDUCES FIRST WNT/β-CATENIN AND THEN WNT/CA^{+2} PATHWAYS ACTIVATION IN MACROPHAGES WHICH FAVOUR THE PARASITE REPLICATION.**

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Abstract: Many studies have demonstrated that *T. cruzi* utilize the host Ca^{+2} signalling to establish the infection and several mechanisms have been proposed to explain the intracellular Ca^{+2} influx that occurs during this infection. In addition, it has been reported that NFATc1 is activated in response to *T. cruzi* infection in a TLR-independent manner but the critical molecules and signalling pathways that lead to NFATc1 activation have not yet been identified. Wnt signalling, essential for embryonic development, has recently been involved in the regulation of inflammatory processes. This signalling pathway is induced in macrophages (Mo) by inflammatory stimulus and depending on the composition of Wnt/Fdz complex, Wnt/β catenin or Wnt/Ca^{+2} pathways are initiated leading to amplify or control the inflammation. We have reported that after the recognition of *T. cruzi* by TLR in Mo, the expression of Wnt proteins and Fdz receptors are induced and Wnt/β-catenin pathway is activated, with the specific inhibition of this pathway or the inhibition of Wnt proteins secretion controlling the *T. cruzi* intracellular replication. In this study, we tested the hypothesis that Wnt proteins secreted after the infection might also activate Wnt/Ca^{+2} pathway with this pathway being responsible for the infection-induced NFATc1 activation. Western blot and immunofluorescence assays revealed that *in vitro* infected Mo up-regulates the expression of p-CAMKII (Thr286) and active NFATc1 ($p<0.01$) at 18 h pi, with both proteins being down-regulated after the treatment with IWP-L6, an inhibitor of Wnt proteins secretion ($p<0.05$). In addition, peritoneal Mo from 18 days-infected mice showed NFATc1 accumulation ($p<0.01$) that was prevented by *in vivo* IWP-L6 treatment ($p<0.0001$). These findings suggest that *T. cruzi* infection induces first Wnt/β-catenin and then Wnt/Ca^{+2} pathways activation with both signalling pathways being used by the parasite to subvert the Mo control of the parasite replication.

Keywords: Wnt; *Trypanosoma cruzi*; calcium; macrophages.

(1651) **EFFECT OF U-OMP19 FROM BRUCELLA SPP. ON INTESTINAL EPITHELIAL CELLS**

Mirta Lorena Coria

Our group have been working in the usefulness of a protease inhibitor (U-Omp19) from Brucella spp. as vaccine adjuvant. We previously demonstrated that U-Omp19 inhibits main gastrointestinal proteases protecting co-delivered antigens (Ags) from digestion thus increasing immune responses. Our current hypothesis is that after bypassing stomach and pancreatic proteases, vaccine formulations comprising U-Omp19 could face proteolytic digestion by brush border and intracellular proteases from enterocytes. Thus, in this work, we evaluated the effect of U-Omp19 on intestinal epithelial cells. Results demonstrated that U-Omp19 can inhibit protease activities from murine intestinal brush-border membranes ($p<0.01$) and cysteine proteases from human Caco-2 and HT29 epithelial cells ($p<0.01$). We also demonstrated by confocal microscopy that U-Omp19 co-administration with Ag on Caco-2 cells promoted Ag (OVA) accumulation within Lamp-2 + endosomal compartments ($p<0.001$). In addition, using transwell plates we have shown that U-Omp19 facilitated the transcellular passage of Ag (OVA) through Caco-2 epithelial cell monolayers ($p<0.01$). Transepithelial electric resistance (TEER) values remained unaltered during transcytosis of Ag and/or U-Omp19 confirming that the epithelial barrier integrity was unaltered by the formulations. Finally, oral co-delivery of U-Omp19 with Ag in mice induced the production of Ag-specific IgA at feces and the recruitment of CD103+ CD11b- CD8α + dendritic cell (DC) subset to Peyer Patches. This particular DC subset has a key role in the induction of IgA producing plasma cells at the lamina propria and it has been associated with Ag cross-presentation to CD8+ T cells. Our results demonstrate that U-Omp19 co-delivery protects Ag digestion by brush border and intracellular enterocyte proteases, facilitating Ag transport across the intestinal epithelial barrier and increasing the amount of Ag that could reach DCs for the induction of appropriate immune responses.

(559) **THE TUBERCULOUS PLEURAL EFFUSION ALTERS THE METABOLIC REPROGRAMMING OF M1 ACTIVATED MACROPHAGES**

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In response to infection, activation of the host immune cells is accompanied by a switch in the bioenergetic pathway from oxidative phosphorylation to aerobic glycolysis, which is requires for the production of antimicrobial and pro-inflammatory effector molecules. Particularly the pro-inflammatory macrophages or M1 undergo this metabolic reprogramming governed by HIF-1α. While M1 are key players to combat intracellular pathogens such as *Mycobacterium tuberculosis* (Mtb), the bacilli display several strategies to counterattack. Since this metabolic reprogramming is associated with M1 functions, we hypothesize that Mtb may perturb it to facilitate its persistence. Then we wondered whether Mtb infection can alter the metabolic reprogramming of M1. To test it, we polarized human macrophages towards M1 with IFN-γ/LPS and treated them with the acellular fraction of tuberculous pleural effusions (PE) mimicking those soluble factors released locally during the infection. Glucose and lactate were determined by colorimetric methods; HIF-1α, Glut-1, and glucose uptake by FACS; IL-1β and TNF-α by ELISA; and bacterial loads by colony forming units. The treatment with PE increased the expression of the glucose transporter Glut-1 ($p<0.05$) as well as the uptake and consumption of glucose ($p<0.05$) in M1. Surprisingly, the release of lactate was reduced in PE-treated M1 ($p<0.05$) together with the expression of HIF-1α ($p<0.05$) and the production of IL-1β and TNF-α ($p<0.05$). Finally, PE-treated M1 contained higher bacillary loads ($p<0.05$). In conclusion, while PE treatment promotes glucose uptake, this additional glucose is not used to fuel the aerobic glycolytic pathway associated to the M1 profile. Otherwise, aerobic glycolysis seems to be reduced by PE in association with a reduced ability to control the bacterial growth. So we propose that Mtb infection may alter the metabolic reprogramming of M1 cells impacting on their ability to control the infection.

Keywords: immunometabolism, macrophages, tuberculosis

(623) **TYPE I INTERFERONS PATHWAY HAS A RELEVANT ROLE DURING THE COURSE OF VULVOVAGINAL CANDIDIASIS**

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Type I Interferons (IFNs-I) constitute a cytokine family with diverse effects on immune cells. Recent studies have revealed that, besides viruses and bacteria, fungal pathogens can also induce IFNs-I production by professional APC. *Candida albicans* (*Ca)* is the principal agent of Vulvovaginal Candidiasis(VVC), a disease affecting 75% of women worldwide. We aimed to explore whether IFNs-I pathway modulates the immune response against *Ca* during VVC course. Female C57BL/6(WT) and *IFNAR-/- (*IFN-alpha receptor deficient) mice on estrus phase (estradiol-treated) were intravaginally inoculated with $5x10^6$ *Ca*-SC5314 (Infected group) or PBS (Uninfected group) at day(D) 0. Untreated mice were used as controls. Vaginal lavage (VL) and vagina were obtained at D2, 4 and 8 pi. CFU studies revealed that both mice strains remained infected throughout the study period but no significant differences were observed in fungal burden. However, IFNAR-/- mice showed a significant increase on PMNs recruitment at D8 as compared to WT mice($p<0.05$). IL-1b levels were lower on IFNAR-/- than WT mice at D2 and 4 pi($p<0.001$), IL-6 showed similar values between both groups and TGFb was decreased in IFNAR-/- mice at D4 and 8 pi ($p<0.05$)(ELISA). We also performed histological studies and determined both invasiveness (Inv.S) and inflammatory (Inf.S) score during VVC course. While in WT mice blastoconidia and pseudohyphae were attached to the cornified epithelium, in IFNAR-/- mice hyphae were observed to penetrate deep epithelial layers. Inv.S was significantly higher in IFNAR-/- mice compared to WT at D2($p<0.05$). Inf.S was higher on IFNAR-/- than WT mice at D2, however, at the end of the study, an inverse profile was observed($p<0.001$).Our results indicate that in absence of IFNs-I signaling, *C. albicans* mucosal invasiveness, tissue reaction, PMNs recruitment and local immune mediators in the vaginal tract were altered.This provides new evidence about the relevant role of IFNs-I during the course of VVC.

Keywords: Type I Interferons, *Candida albicans*, Vulvovaginal Candidiasis.

(1450) **TRANSFORMING GROWTH FACTOR-BETA (TGF-β) MODULATION OF HUMAN NEUTROPHIL EXTRACELLULAR TRAPS (NET) FORMATION INDUCED BY RESPIRATORY SYNCYTIAL VIRUS**

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Respiratory syncytial virus (RSV) infection is the leading cause of bronchiolitis and hospitalization in young infants and is responsible for almost 200000 deaths annually. Previous studies indicated that RSV induces NET formation and these NET capture RSV virions and prevent viral binding and infection of A549 epithelial cells. Additional studies showed that RSV triggers TGF-β production from lung epithelial cells and macrophages, and TGF-β facilitates RSV infection of epithelial cells. Here we aim to: 1) confirm the ability of supernatants of RSV-infected-epithelial cells, the usual source of virus, to induce NET, discriminating the contribution of mediators released by epithelial cells (ECS); 2) determine the ability of TGF-β to modulate RSV-induced NETosis; and 3) evaluate the ability of RSV-induced NET (NET-RSV) to promote the release of proinflammatory cytokines by 16HBE14o- bronchial epithelial cells. NETosis was evaluated with human neutrophils by determining the DNA concentrations in culture supernatants and by confocal microscopy. RSV (10^1 PFU/ml) induced NETosis in contrast to ECS employed at the same dilution ($p<0.05$, n=7). By contrast, RSV (10^4 PFU/ml) did not induce NETosis while ECS at the same dilution did (n=6), indicating that at these dilution, mediators present in ECS modulated NETosis induced by RSV. Besides, TGF-β (10 ng/ml) abrogated NETosis induced by RSV (10^1 PFU/ml; $p<0.05$, n=9) while did not inhibit the NETosis induced by PMA (n=4). Of mention, TGF-β did not affect basal levels of NETosis. Preliminary results indicated that NET-RSV added to 16HBE14o- cell cultures, but not NET-RSV obtained in the presence of TGF-β, promoted IL-6 release by these epithelial cells (n=2). Our findings suggest that TGF-β might modulate the inflammatory response to RSV by inhibiting NETosis. We can also speculate that TGF-β also contributes to facilitate epithelial cells infection by RSV by inhibiting NETosis, an aspect that remains to be determined.

Key words: Respiratory syncytial virus, neutrophil, neutrophil extracellular traps, Transforming Growth Factor-beta

(244) **FUNCTIONAL EFFECTS OF ANTI-B2 ADRENERGIC AUTOANTIBODIES FROM PATIENTS WITH CHRONIC CHAGAS DISEASE IN A CORTICOTROPH CELL LINE**

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Inflammation associated with parasite persistence in adipose tissue, resulting in altered adiponectin/resistin levels, has been held responsible for the higher prevalence of obesity and diabetes described in patients with chronic Chagas disease (CCD). Since host reaction to *T. cruzi* includes β2 adrenergic receptor autoantibodies (anti-β2AR AB), and β2AR are expressed in metabolically active tissues (liver, fat), we hypothesized that anti-β2AR AB might

play a pathogenic role in these alterations. After demonstrating the presence of anti-β2AR AB in patients with CCD, we aimed to perform a bioassay to assess their functional effects on a cell system expressing β2 receptors and a cAMP-responsive machinery.

Anti-β2AR AB levels were measured in adult CCD patients by ELISA. A subset of positive specimens was selected by their AB titers, and specific anti-β2AR Ig G fraction were affinity purified. Murine AtT20 cells were transfected with CRE-luc and POMC-luc reporter plasmids (cAMP pathway) and incubated with purified AB. Luciferase activity was compared with results obtained after treatment with clenbuterol (Cb, β2 agonist) or butoxamine (Bu, β2 antagonist). Inactivated anti-β2AR AB and sera from *T. cruzi*(-) patients served as controls.

Anti-β2AR AB induced significant increases in luciferase activity in relation to negative controls both in CRE-luc ($p=0.006$) and POMC-luc ($p<0.001$) transfected cells, similar to those elicited by Cb. Furthermore, this effect was antagonized by Bu in each transfection condition ($p=0.026$ and <0.001, respectively). The observed stimulation showed dose-dependent characteristics.

Our results indicate that anti-β2AR AB exert an agonist effect on β2AR pathways expressed in AtT20 cells, lending biologic plausibility to the hypothesis that these antibodies may contribute to the pathogenesis of metabolic disturbances in CCD patients. Studies on the effects of anti-β2AR AB performed in hepatic cell systems are in progress to answer this question.

Keywords: Adrenergic beta-2 Receptor Agonist, antibodies, Chagas disease, Pituitary Adrenocorticotropin-Secreting Cells, cyclic AMP.

(1711) **VIP AND CONDITIONED MEDIA FROM TROPHOBLAST CELLS INHIBIT AUTOPHAGIC PROCESSES IN NEUTROPHILS AND PROMOTE THEIR APOPTOSIS THROUGH ACTIVATION OF CAMP-PKA PATHWAY**

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Trophoblast cells (Tb) interact with different maternal immune cell populations at early pregnancy promoting an anti-inflammatory and tolerogenic response. Neutrophils (neu) are short-lived cells and apoptosis is considered to be the major death mechanism. Autophagy and apoptosis cooperate to modulate neu survival. Failure to properly regulate neu abundance and turnover can contribute to human disease. In addition, efferocytosis of dying neu dampens proinflammatory cytokine production and reprograms macrophages to a pro-resolution phenotype. Vasoactive Intestinal Peptide (VIP) is a pleiotropic peptide with immunomodulatory effects through its action on VPAC1 and VPAC2 receptors, both coupled to the activation of adenylate cyclase and protein kinase A (PKA). We have already shown that VIP and conditioned media (CM) from human first trimester Tb (Swan-71 cell line) inhibit PMA-induced NET formation, promote neutrophil apoptosis and revert the anti-apoptotic effect of LPS. Our aim was to evaluate the effect of VIP and Tb derived factors on neu autophagy and the mechanisms involved in neu apoptosis and efferocytosis.

Whole blood was obtained from healthy donors and neu purified on a Ficoll-Paque PLUS gradient and Dextran sedimentation. We found that CM and VIP inhibit PMA-induced autophagy, quantified by fluorescence intensity of LC3 puntae by confocal microscopy ($p\leq0.05$). On the other hand, H89 a PKA inhibitor, blocks the pro-apoptotic effect of VIP on neu apoptosis ($p\leq0.05$), determined by fluorescence microscopy with ethidium bromide and acridine orange. Moreover, the release of elastase, known to prevent efferocytosis, was reduced when neu were stimulated with PMA + VIP or CM, compared to PMA alone as evaluated by flow citometry. We conclude that VIP and Tb factors regulate autophagic and apoptotic processes in neu and suggests that they contribute to the maintenance of an anti-inflammatory microenvironment during early pregnancy.

(1937) **FACTORS INVOLVED IN THE ANGIOGENIC PROCESS IN THE TESTIS UNDER CHRONIC INFLAMMATION**

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Experimental autoimmune orchitis (EAO) is an established model of chronic testicular inflammation. The murine model mimics the pathological changes reported in immunological infertility in men. At the end of EAO induction (35 days, d), few signs of inflammation are present in the testis and no damage of seminiferous tubules occurs. At 55d, lymphomononuclear cell infiltrate increases concomitantly with germ cell apoptosis, leading to aspermatogenesis and infertility.

Hypoxia and oxidative stress triggers an adaptive response in which hypoxia-inducible factor 1α (HIF1α) and vascular endothelial growth factor (VEGF) are induced. Progression of EAO is associated with an increase of testicular endothelial cells and number of blood vessels. The aim of this study was to explore the role of HIF1α and VEGFA in the angiogenic process that occurs under testicular inflammation. EAO was induced in adult male Wistar rats by active immunization with testis homogenate and adjuvants. Rats were killed on days 35 and 55. Qualitative evaluation of hipoxia showed similar results in EAO vs normal (N) testis (immunohistochemistry, IHQ). HIF1α localized in endothelial and Sertoli cells (IHQ) similarly to VEGFA, as we previously reported. No significant changes in the expression of nuclear HIF1α was observed at 35 and 55d compared to N testis (n= 4-9) (Western blot, Wb). However, VEGFA expression was significantly higher at 35d in testicular fluid vs N rats decreasing at 55d (n= 5-10; $p<0.05$) (Wb). Our results showed that HIF1α did not increase concomitantly with VEGFA, although we cannot rule out the involvement of HIF1α in VEGFA up-regulation at an earlier EAO stage. Up-regulation of VEGFA levels preceding the increase in the number of blood vessels highlights the role of this factor in angiogenesis during experimental orchitis.

Key Word: orchitis, hypoxia-inducible factor1α, vascular endothelial growth factor

PLANT BIOLOGY 5

(707) **IRON ACQUISITION IN LOTUS CROPS SUBJECTED TO ALKALINE STRESS: RESPONSE TO INOCULATION WITH *Pantoea eucalypti* M91.**

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Alkaline soils are characterized by low availability of essential nutrients, including iron (Fe), causing Fe chlorosis and therefore affecting plant growth. In addition, soil microorganisms, through production of siderophores, may play an important role capturing this element under limited conditions. Here, we aimed to study the response of *L. tenuis* (Lt) and *L. corniculatus* (Lc) and an interspecific hybrid LtxLc obtained in our laboratory to alkaline treatment (10mM $NaHCO_3$) and inoculation with *P. eucalypti* M91 (PM91), a plant growth-promoting bacterial strain capable of producing siderophores at high pH. At first, we observed that alkalinity affected shoot growth of Lc ($p=0.0049$) and developed chlorosis in their leaves. Interestingly, when inoculated with PM91, Lc exhibited an increase in shoot growth ($p=0.039$). On the other hand, no detrimental effect was seen in growth of shoots and roots of Lt and LtxLc when exposed to alkalinity, but no effect of inoculation was observed either. Moreover, the Fe^{2+} content in shoots of Lc and LtxLc when cultivated under alkaline conditions was lower than controls ($p\leq0.01$), but when inoculated, the Fe^{2+} content in shoots increased in all cases obtaining even greater values than the ones reached at controls. In addition, all species increased their Fe^{2+} root contents under alkalinity, compared to controls. Furthermore, the Fe^{2+} accumulation was higher in the roots of alkaline treated Lc (2-fold) than in the other species. These results, together with the chlorosis observed in Lc grown under alkalinity, reveal a flaw in Fe^{2+} translocation from roots to shoots. However, inoculation improved the Fe^{2+} balance for this

species, resulting in high endogenous Fe^{2+} content in the shoots, with accompanying re-greening in young leaves. These findings suggest that particularly, Lc is the species more affected to alkaline stress; however inoculation with PM91 generates an increase in the Fe^{2+} content of the three studied accessions. Keywords: Alkalinity, *Lotus* spp., Fe, endophytic bacteria.

(1597) **NON-THERMAL PLASMA ENHANCE THE QUALITY OF SOYBEAN PLANT**

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Soybean is one of the most important crops worldwide. According to FAOSTAT, this crop production and harvested area has dramatically increased over the last 2 decades. Different strategies have been developed with the aim of improving seed quality. Non-thermal plasma (NTP) treatment is a fast, economic and pollution-free method. The objective of this study was to determine whether different non-thermal plasmas could be used to enhance seed performance.

Soybean seeds were exposed to a multi filamentous DBD discharge. The dielectric barrier of the discharge consisted in a Pertinax 2.5 mm plaque and 2 0.1 mm layers of Mylar and 3 Ternophase 0.4 mm plaques on top of which the seeds were deposited. Different gases (O_2 and N_2) were used to transport the active agents of the plasma. The seeds were irradiated for 1 minutes using either O_2 or N_2. To study the qualities of the plant, different oxidative stress markers and biometrical parameters were assayed.

The evaluated treatments demonstrated an increase in germinative power and vigor. In this work, we found that leaf area, plant weight and chlorophyll content. were increased by the NTP treatments. In addition, we observed that the NTP treatments had an increase in the activities for most of the oxidative enzymes; on the other hand, TBARS content was not modified respect to the control.

We conclude that this environmentally friendly technology is a good method for the improvement of the biochemical qualities of the soybean seeds and increases the crop production.

(860) **MOLECULAR CARACTERIZATION AND EXPRESION PATTERNS OF C2H2-TYPE ZINC FINGER TRANSCRIPTION FACTORS INVOLVED IN THE DEVELOPMENT OF GRASS INFLORESCENCE**

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The grass family includes species among which are outstanding cereals that constitute a very important part of human intake. The final form of a grass inflorescence is the result of a balance between the ability of the axillary meristems to form branches or terminate in spikelets. The *Cys2-His2-type (C2H2) zinc finger* transcription factors seem to play a central role at this stage of development. The aim of this work is to explore the molecular and functional divergence of these proteins in grasses. By comparison of *C2H2 zinc finger* peptide sequences available on databases and others generated in our laboratory, we observed a little conservation of the sequences towards the C-terminal. From this sequence alignment we reconstructed the molecular evolution of the genes coding for *C2H2 zinc finger* proteins. As a result, we identified two successive duplications around the origin of the grasses that generated at least three exclusive lineages of grass *C2H2 zinc finger* proteins, for which little information on their biological role is available. To begin with their characterization, we investigated the expression preference of the coding genes throughout the whole body of the plant (root, stem, leaf and inflorescence) of the model species *Setaria viridis*. In particular, such genes are preferentially expressed in inflorescence with developing branches. The expression decays at late stages of inflorescence development (during initiation and differentiation of floral organs). So far, our results show that the genes coding for the *C2H2 zinc finger* proteins have been diversified in grasses and they may play an important role in the evolution of the branching system of grass inflorescences.

Keywords: cereals, evolution, branching systems.

(1762) **OVEREXPRESSION OF ARABIDOPSIS *BBX21* ENHANCED PHOTOSYNTHESIS RATES AND ALLEVIATES PHOTOINHIBITION IN POTATO PLANTS**

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B-box (BBX) proteins are a class of zinc-finger transcription factors containing a B-box which are highly conserved along green plant linages. Some members of BBX proteins are key factors in regulatory networks of growth and developmental processes. BBX21 is a member of this family that promotes photomorphogenesis and dehydration tolerance, and inhibits shade avoidance in *Arabidopsis thaliana* seedlings. Regarding the relevance of BBX21 in light and ABA signaling and the null information of its role in adult plant development, we generated and studied the performance of *Arabidopsis thaliana BBX21* heterologous overexpression in *Solanum tuberosum* (potato) plants under natural light conditions. The experiments were conducted in a greenhouse with controlled temperature cultivating three independent *AtBBX21* overexpressing (*BBX21*-OE) lines and the non-transformant control. *BBX21*-OE potato adult plants have a more robust phenotype with higher tuber yield than non-transformant plants. In addition, *BBX21*-OE plants produce more chlorophyll and show higher photosynthesis rates and tolerance to photoinhibition in than non-transformant. Biochemical studies demonstrated that *BBX21*-OE plants produced a larger amount of anthocyanins and total phenolic compounds that correlated with a higher expression of chalcone synthase gene involved in the early step of anthocyanin synthesis. Taking together, BBX21 appears as a potential candidate to improve crops plants through biotechnological approaches.

Keywords: BBX transcription factors, development, photosynthesis, potato.

(890) **STUDYING THE ROLES OF MITOGEN-ACTIVATED PROTEIN KINASES (MAPK) IN PLANT DEVELOPMENT**

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A main question in developmental biology is how cells make decisions. These decisions are based on determinants that cells inherit from its precursor, molecular dialogues with neighboring cells and signals that cells perceive from its environment. The signal transduction cascade of mitogen-activated protein kinases (MAPKs) is a key component in the integration of signals that determine cell identity and terminal fate. For this reason, ensuring specific responses between cues and responses is of vital importance for the normal development of the organism. An ideal model to study the integration of MAPK signals and development in plants is the cellular lineage that generates stomata in Arabidopsis. Stomas are the valves that control gas exchange in the epidermis and their precursors have the ability to tune the development program to information from surrounding cells and the environment. Using stomatal development as context, we found candidate proteins and genes that would participate in ensuring the generation of specific responses to MAPK activation. Preliminary results suggest that these candidates may be involved in developmental processes other than stoma formation. Here we present different strategies to 1) identify processes in which the MAPK cascade transduces information necessary for the normal formation of organs and patterns; 2) determine the mechanisms that plant cells use to ensure specificity between signals and their responses; 3) establish MAPK signaling study models that have minimal genetic redundancy and have agronomic potential such as

tomato.

Keywords: Plant development, MAPK signaling, Arabidopsis, tomato, stomata

(1072) **STUDY OF MITOCHONDRIAL GAMMA CARBONIC ANHYDRASES IN THE DIATOM HALAMPHORA COFFEAEFORMIS STR. BAHÍA BLANCA ESTUARY**

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Diatoms are responsible for 20–25% of total terrestrial primary production and approximately 40% of annual marine biomass production, which make the most dominant group of organisms sequestering carbon from the atmosphere. It has been proposed that the high photosynthetic efficiency of diatoms is mainly based on the close relationship between their mitochondria and chloroplasts. In previous studies in our laboratory, we have shown that the mitochondrial gamma carbonic anhydrases (CA's) forming the CA domain of the mitochondrial complex I are important components involved in complex I assembly, in development and in the basal carbon concentrating mechanism in plants. Now we are studying the role of the gammaCA's in diatoms. In all sequenced genomes of diatoms, we found at least three gammaCAs predicted to be in mitochondria as in plants. We cloned partial sequences of gammaCA's and other genes from a highly oil productive strain (Bahía Blanca Estuary, 38° 45' S, 62l 22' W) of *H. coffeaeformis*. This strain has around 80% nucleotide identity with a north-american *H.coffeaeformis* strain (SRX691230) for which a RNA-seq experiment has been performed. We then proposed that both are both different strains of *H coffeaeformis.* We found that the enriched CO_2 atmosphere has a direct positive impact in the growth of *H.coffeaeformis*.. In this context, we are studying, by silencing strategies, the role of the mitochondrial gammaCA's in the growth and development of south American *H.coffeaeformis* strain.

(528) **PHOTOMORPHOGENESIS REGULATION BY PROTEIN PHOSPHORYLATION**

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INGEBI

Light environment provides signals for plants to develop and accomplish their life cycle successfully. Those signals are perceived and transduced by photoreceptors. Phosphorylation is one of the biochemical mechanisms initiating light signalling cascade and is a challenging question in the photobiology field today. Here, we study early light-induced phosphoproteome in *Arabidopsis thaliana* through a *Label free LC-MS/MS* proteomic approach to identify proteins which significantly change their phosphorylation status in a light-responsive way. Total proteins were extracted from three treatments: 5-d-old etiolated WT seedlings (WTD) and 5-d-old etiolated WT and *phyA phyB cry1 cry2* quadruple mutant seedlings exposed for 20 min to white light pulse (100 μmol.m^{-2}.s^{-1}) (WTL,TETL) just before harvest. We identified 2097 phosphopeptides corresponding to 1319 proteins. 32 phosphopeptides changed significantly their phosphorylation status in response to the light treatment. 26 of them were more phosphorylated in WTL while the other 6 were less phosphorylated in WTL compared to WTD. 29 phosphopeptides changed their phosphorylation status in a photoreceptor independent manner. 3 phosphopeptides were more phosphorylated in TETL compared with WTL, suggesting that their dephosphorylation in light is mediated by the photoreceptors. Currently, we are studying the function of some of these phosphoproteins and the importance of the phosphorylation events during light signalling.

Keywords: Photoreceptors, Light Signalling, Phosphoproteome

(1411) **UNRAVELLING THE BIOCHEMICAL BASIS OF FRUIT CHILLING INJURY: PROTEOMIC AND METABOLOMIC DIFFERENCES BETWEEN MEALY AND NON-MEALY PEACH FRUIT**

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Peaches are highly perishable and deteriorate quickly at ambient temperature. Cold storage is commonly used to prevent fruit decay; however, it affects fruit quality causing physiological disorders collectively termed 'chilling injury' (CI). One of the principal phenotypic expressions of CI in peach is flesh mealiness or lack of juiciness. In the present work, the differences between mealy and non-mealy fruits of Springlady cultivar, were assessed using proteomic and metabolomic analysis. Harvested fruits were stored at 0 ºC and 90% relative humidity for 21 days followed by 4 days (time required for ripeness) at 20 ºC. The apparent juice content was evaluated using a visual scale of 0 to 5 (0 = Healthy and 5 Maximum mealiness). Individual fruits with contrasting flesh mealiness were selected for proteomic and metabolomic studies. Quantitative proteomic profiling, performed using the Orbitrap technology, revealed drastic differences in the amount of 213 proteins between mealy and non-mealy fruits. The differentially expressed proteins were identified and classified considering their biological role. Significant differences were found in proteins involved in lipid, cell wall and protein metabolism; as well as in enzymes involved in secondary metabolism, such as chalcone-flavone isomerase and carotene desaturase. A marked decrease in the levels of enzymes involved in the control of the redox state, such as superoxide dismutase and a glutathione peroxidase, was detected in mealy with respect to non-mealy fruits. Metabolomic analyzes, performed by GC-MS, showed a decrease in the level of sugars, such as sucrose, galactose and raffinose, in mealy with respect with non-mealy fruits, as well as in most of the amino acids detected, with no significant changes in organic acids content. Overall, the present work identifies the biochemical basis of mealiness in peach fruits, and reveals that an altered redox state may be key in mediating many of such biochemical alterations.

Keywords: peach fruit, chilling injury, mealiness, proteomics, metabolomics

(1778) **ALTERNATIVE MRNA SPLICING FOR THE CONTROL OF FUMARASE ACTIVITY IN ARABIDOPSIS**

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Arabidopsis thaliana possesses two fumarase genes (*FUM*), *AtFUM1* (*At2g47510*) encoding for the mitochondrial Krebs cycle-associated enzyme and *AtFUM2* (*At5g50950*) for a cytosolic isoform required for the massive accumulation of fumarate. While *AtFUM1* codifies a single transcript, *AtFUM2* could codify two different isoenzymes (AtFUM2 and AtFUM2b) through an alternative maturation process of the primary transcript. The characterization of recombinant forms of these proteins indicated that AtFUM2b lacks of enzymatic activity due to absence of the amino acid residues that are encoded by the last exon, as the *AtFUM2b* transcript retains an intron containing a stop codon. Here, we further analyzed the physiological role of this *AtFUM2*-derived aberrant mRNA. In order to determinate whether it is translated *in vivo*, the *AtFUM2b* cDNA was cloned in frame with fluorescent green protein (GFP) coding sequence into a binary vector for the transient Agrobacterium-mediated transformation of *Nicotiana benthamiana* leaves. The results indicated that, despite *AtFUM2b* transcript was present, no fluorescence was detected in infiltrated Nicotiana leaves, noting that the protein is not translated *in vivo* and pointing out a regulatory role for the not fully processed *AtFUM2b* mRNA into the cell. On the other hand, the abundance of *AtFUM1*, *AtFUM2* and *AtFUM2b* transcripts in Arabidopsis plants grown under different conditions was quantified through RT-qPCR. In general, the three mRNA were differentially accumulated under the assayed conditions of biotic and abiotic stress, and with the *AtFUM2* gene expressed to a greater extent than the *AtFUM1*. Particularly, the *AtFUM2b* abundance was

significantly lower than *AtFUM2* and, in several conditions, both *AtFUM2*-derived transcripts accumulated inversely. Overall, our results indicate that in Arabidopsis the FUM activity is regulated at the level of RNA-processing for control of the C4-organic acids flux through metabolism.

Key words: fumarase, alternative splicing, Arabidopsis, C4 metabolism

NEUROSCIENCE 12

(353) DIFFERENTIAL FUNCTIONAL ROLE OF ALPHA-4BETA2 AND ALPHA7 NICOTINIC RECEPTORS ON HUMAN NATURAL KILLER CELLS

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Nicotinic acetylcholine receptors (nAChRs) are pentameric ligand-gated ion channels of the Cys-loop receptor family that serve as targets for acetylcholine and nicotine. nAChRs can be homomeric, which are assembled from five identical subunits, such as α7, or heteromeric, which are assembled from different subunits, such as α4β2. Although nAChRs have been studied mainly at the neuromuscular junction and in neurons, immune cells express several nAChR subunits but the functional relevance of the extra-neuronal cholinergic system remains still undefined. Previously, we demonstrated that human natural killer (NK) cells express α7 nAChR whose expression levels increase during NK stimulation with IL-12, IL-18, and IL-15. Activation of α7 down-regulates NKG2D receptors, and decreases NKG2D-dependent cell-mediated cytotoxicity and IFN-γ production. In the present study, we investigated the expression of α4β2 nAChR in human NK cells as well as its regulation in their functions. By RT-PCR, we detected in freshly isolated human NK cells mRNA corresponding to α4 and β2 nAChR subunits. Upon stimulation of NK cells with IL-12, IL-18, and IL-15 for 48h, the mRNA determined by qPCR and cell surface expression determined by flow cytometry of α4 nAChR did not change significantly, whereas those of β2 slightly increased with respect to fresh cells. Activation of α4β2 nAChR with the specific agonist 5-Iodo-A-85380 did not affect the expression of some major NK cell activating receptors, such as NKG2D, NKp46, and DNAM-1, as well as the levels of perforins and IFN-γ production. Activation of α4β2 nAChR by its specific agonist 5-Iodo-A-85380 did not affect the expression of CCR7 or CD62L, which are indicators of the migratory potential of NK cells to lymph nodes. Taken together, our results show that α4β2 is neither regulated by NK stimulation nor involved in NK cell effector functions, thus indicating that, among nAChRs, α7 is a central player in NK cell physiology.

Keywords: nicotinic acetylcholine receptors, natural killer cells, 5-Iodo-A-85380

(1050) EFFECTS OF THE CYTOKINES ON PROLIFERATION OF HIPPOCAMPAL ADULT NEURAL PROGENITOR CELLS.

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Abstract: The hippocampus, an area involved in learning and memory, is sensitive to the effects of chronic stress. Cytokines have been shown to affect some behaviour, including memory. Moreover, IL-1β, IFN-γ and IL-6 had been implicated in psychiatric disorders. Previously, we found that chronic stress induced a cognitive deficit that was correlated with a decrease in adult neurogenesis and Th1/Th2 imbalance. Recently, we observed a decrease in IFN-γ mRNA levels in hippocampus and lymph nodes and increase IL-4 expression in lymph nodes in stressed mice. On the other hand, adult hippocampal neurogenesis is initiated with the proliferation of neural progenitor cells (NPCs) within the dentate gyrus leading to the formation of new neurons. Regulation of this process occurs via numerous secreted factors such as cytokine. The aim of this work was to study the effect of IFN-γ, IL-1β, IL-4 and IL-6 on the NPCs proliferation. NPCs were obtained from hippocampus of adult BALB/c mice. NPCs were culture in vitro in optimal conditions with growth factors. In the last 72 h, each cytokine (0.1, 1 and 50 ng/ml) were added and BrdU 2 h before to fix the culture. The proliferation was measure by immunocytochemistry using DAPI and anti-BrdU-Cy3. We found that cytokine concentration of 50 ng/ml inhibit the proliferation of the NPCs respect to control without cytokine: IFN-γ (95%, $p<0.01$), IL-1β (100%, $p<0.001$), IL-4 (41%, ns) and IL-6 (43%, ns). Concentrations of 1 ng/ml of IFN-γ (85%, $p<0.01$) and IL-1β (77%, $p<0.01$) also reduced the proliferation of the NPCs. No significant differences were found in culture treated with IFN-γ (0.1ng/ml) and IL-4 and IL-6 (1 and 0,1 ng/ml) respect to control. These results indicated that high concentration of cytokines inhibit the proliferation of hippocampal NPCs. Low concentrations of cytokine no affect the NPCs proliferation. These findings are the first in this field. More studies are necessary to determinate the direct role of cytokine on adult NPCs.

Keywords: adult neural progenitor cells, hippocampus, cytokines.

(127) EXPRESSION OF SOX-11 AND SOX-4 AND ACTIVITY OF LINE-1 RETROTRANSPOSONS DURING NEURONAL DIFFERENTIATION.

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Sox-4 and Sox-11 are single-exon genes members of the Sox-C transcription factors family. Evidence has shown critical roles for both genes in multiple developmental processes, particularly during the central nervous system development, probably with redundant functions. On the other hand, Long Interspersed Nuclear Elements-1 (LINE-1) retrotransposons are repetitive elements that encode an RNA binding protein and an endonuclease with reverse transcriptase activity, under the control of a promoter in the 5´UTR; both proteins modulate LINE-1 mobilization. Human LINE-1 insertions occur mainly during early embryonic development, although somatic retrotransposition occurs also in adult neuronal progenitor cells. Despite a Sox-11 binding site has been described in the LINE-1 5´UTR, it has not been addressed before whether Sox-C members control LINE-1 activity in the neuronal lineage. With that aim, we decided to evaluate the expression of Sox-4 and -11 in LINE-1 retrotransposition conditions of neuronal differentiation of neuroblastoma cells cultured with retinoic acid (RA). First, we observed that LINE-1 promoter activity increases in human SH-SY5Y and mouse Neuro-2A cells treated with RA, as shown by wild type 5´UTR-driven luciferase activity, compared to control basal conditions. Next, we studied Sox-4 and -11 RNA levels by RT-qPCR, and found that expression of both genes increases in RA-treated SH-SY5Y cells. Finally, immunohistochemistry assays showed a higher abundance of Sox-11 protein in nuclei of both cell types challenged with RA, compared to control non treated cells. Thus, our results suggest that both Sox-4 and -11 expression and LINE-1 activity increase in neuronal differentiation conditions; also, that RA probably induces Sox-11 translocation to nucleus. Because the knock-down of these transcription factors has not been done yet, it remains to be determined whether one or both proteins indeed control LINE-1 activity during neuronal differentiation.

Keywords: neuronal differentiation, Sox-4, Sox-11, LINE-1

(294) FOLLOWING HYPOXIC GRADIENT: DIFFERENT REGULATION OF AUTOPHAGY FLUX IN INNER AND OUTER NUCLEAR LAYERS

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In order to maintain homeostasis post mitotic cells, as neurons, require efficient degradation systems that eliminate cellular waste. In this sense, defective autophagy flux has been associated with the development of many neurodegenerative diseases. Cytoplasmic accumulation and aggregation of p62, an adaptor protein involved in the incorporation of ubiquitinated proteins into the autophagosome, seems to be a key event in neuronal dysfunction. In this work we analysed in a mouse model of oxygen induced retinopathy (OIR): a) changes in autophagy flux in the retinal layers; and b) if pharmacological modulation of autophagy could prevent neuronal alterations. For this purpose, C57/BL6 mice were exposed to 75% O_2 from postnatal day (P)7 to 12, and then they were brought to room air (RA). Age-matched mice maintained in RA were used as control. Animals were sacrificed at P17 and P26. We observed at the inner retinal layers altered autophagy flux with increased levels of p62 at the neovascularization peak (P17). As a valid strategy to modulate p62, P12 mice were intraocularly injected with Spautin-1 (a specific autophagy inhibitor). Western blot of neural retinas and immunofluorescence staining showed that Spautin-1 decreased p62 levels and slightly increase LC3II, but did not modified detoxifying and stress proteins expression at P17. At the same time point electroretinogram (ERG) activity evidenced a decreased photoreceptor function. However, at P26 autophagy flux was restored, Glutamine synthase increased, ERG response improved and a minor number of TUNEL positive cells were observed. Structural and vascular modifications were also analysed. The results suggest that in the outer nuclear layer autophagy is preserved during hypoxia and constitutes a survival mechanism. Whereas in the inner layers, where cells are severely affected by hypoxia, the inhibition of autophagy decreased p62 levels and decreased neuronal death.

Keywords: hypoxia, autophagy flux, retinopathy, neurodegeneration

(1773) **BRAIN MITOCHONDRIAL DYSFUNCTION AND CHANGES IN NITRIC OXIDE METABOLISM INDUCED BY LEVOCABASTINE ADMINISTRATION**

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Mitochondrial function is modified by nitric oxide (NO). Levocabastine, an antagonist for low affinity neurotensin receptor (NTS2), alters NO metabolism in synaptosomal membrane fractions devoided of mitochondria. The purpose of the present study was to evaluate the potential involvement of NTS2 receptor activity in mitochondrial function and NO metabolism. Wistar rats were injected with levocabastine (50 µg/kg, i.p.) or vehicle (saline solution) and decapitated 30 min or 18 hours later. Oxygen consumption and total NO levels were evaluated in crude mitochondrial fractions. Enzymatic activities of mitochondrial respiratory complexes, monoamine oxidase (MAO) and nitric oxide synthase (NOS), as well as neuronal NOS (nNOS) protein expression were evaluated in purified mitochondria. Crude mitochondrial fractions were capable of generating NO, as detected by flow cytometry. Assays of oxygen consumption showed no response to oligomycin or FCCP additions in levocabastine-treated rats. Mitochondrial respiration assays in the presence of malate-glutamate or succinate showed that levocabastine decreased mitochondrial respiratory controls assayed ex vivo and in vitro. The activity of mitochondrial complexes I-III, II-III and IV decreased 60%, 63% and 42%, at 30 min after levocabastine administration and 83%, 82% and 74%, at 18 hours after the treatment respectively ($p<0.001$). NOS activity decreased 42% whereas nNOS expression enhanced 86% at 18 hours after levocabastine administration ($p<0.05$). MAO activity was 25% and 60% lower at 30 min and 18 hours after levocabastine treatment respectively ($p<0.001$). In vitro incubation of control fractions with 1 μM levocabastine also decreased the activity of NOS and mitochondrial complexes. It is concluded that mitochondrial dysfunction and alterations in NO metabolism induced by levocabastine treatment seem to occur as parallel actions and not mainly due to NTS2 receptor blockade.

Keywords: cerebral cortex, nitric oxide synthase, mitochondria, neurotensin receptor, levocabastine

(897) **RELAXED MITOCHONDRIA-ENDOPLASMIC RETICULUM CONNECTIONS AND AMYLOID β ACCUMULATION: A COMMON NEUROTOXIC PATHWAY?**

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Alzheimer's disease (AD) is a neurodegenerative and progressive disorder with no effective treatment to prevent or delay the onset of the disease. Intraneuronal amyloid β (iAβ) accumulation and bioenergetic failure have been suggested as early events in the progression of AD. Bioenergetic function is mainly regulated by communications that mitochondria make with a specialized region of the endoplasmic reticulum (ER), called mitochondria-ER connections (MERC), which involve variable distances of 5-30 nm. To evaluate MERC in an early AD model, we employed hippocampal primary neurons from transgenic (Tg) McGill-R-Thy1-APP and wild-type (control) rats. Neurons from Tg rats with 7 days in vitro (DIV7) display a diffuse pattern of iAβ accumulation associated with the outer mitochondrial membrane (OMM), secrete low levels of Aβ to the conditioned medium and, despite not showing mitochondrial ultrastructural alterations, exhibit lower capacity to provide ATP in situations where the energy demand increases. Cultures were transfected with plasmids coding drug-inducible synthetic interorganellar linkers targeting OMM or ER fused to fluorescent proteins that form a FRET pair upon addition of rapamycin. Live imaging data recorded by multi-colour epifluorescence microscopy revealed that neurons DIV7 from Tg rats display relaxed ER-mitochondria connections when involving distances of less than 10 nm as compared to control neurons. Given that these short distances might favour lipid transfer between ER and mitochondria, we assessed the levels of cardiolipin, a mitochondrial phospholipid which possess an important role in the assembly of the inner mitochondrial membrane. Preliminary results show that levels of cardiolipin are decreased in Tg neurons with no differences in mitochondrial number. Together these results suggest that the mechanisms by which iAβ accumulation impairs bioenergetics may be mediated by alterations in MERC that cause diminished lipid transport.

Keywords: mitochondria-ER connection; intraneuronal amyloid beta; bioenergetics; lipid transfer

(1825) **RIBOSOME PROFILING OF DIFFERENTIATED PC12 REVEALS NOVEL FUNCTIONS OF TRANSLATION REPRESSOR PDCD4**

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Programmed Cell Death 4 (PDCD4) is a tumor suppressor gene that regulates translation initiation by interactions with pre-initiation complex through eIF4A. Translation is inhibited when PDCD4 binds eIF4A and suppress its RNA helicase activity. Despite there are only few reports on PDCD4 in neuronal models, our group observed high expression levels of this protein in rat brain neurons. Since translation regulation is a key process in several functions in neurons, in this work we aimed to find out which biological functions are regulated by PDCD4 at translation level. For this we apply Ribosome Profiling methodology while silencing PDCD4 transiently in a neuronal cell line model (PC12). We use lentiviral vector to induce a transient knockdown with an shRNA against PDCD4. After mapping transcrip-

tomes and translatomes of the two conditions (shScrambled and shPDCD4), we normalize data counting (DESeq2) and calculate translation efficiency for each mRNA, obtaining 480 mRNAs with a translation efficiency fold change higher than 2. This list of PDCD4 translational target candidates was analyzed by gene ontology approaches (DAVID software) to know what biological processes were affected by this factor. We find that PDCD4 regulates translation of several proteins involved in energetic and metabolic process at the mitochondria, pathways of neuronal diseases and also some ribosomal proteins. In addition we find some others interesting molecular functions like: synaptic, immune response and neurite growth. Consistent with the later, we verified that in absence of PDCD4, neurite length is increased. Now we are moving forward to verify some PDCD4 translational targets and check the relationship between PDCD4 and some cellular functions mentioned above. Taken together, these results indicate that PDCD4 have relevant functions regulating mRNA translation in neuronal PC12 cells, involving metabolic process at mitochondria, synaptic function and neurite growth associated genes.

Keywords: PDCD4, Ribosome Profiling, Translation, Neurons, Mitochondria.

(993) **TRANSFERRIN INDUCES NEURITE OUTGROWTH IN A NEUROBLASTOMA CELL LINE**

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Transferrin (Tf) is a glycoprotein best known for its role as an iron transporter. However, it has been shown that Tf influences many cellular processes not directly linked with iron metabolism. Our group has studied Tf effects on oligodendroglial cells, where it has been shown to accelerate maturation and commit neural stem cells toward an oligodendroglial lineage. The effect of Tf on neurons, however, has never been studied. For this purpose, we decided to use an *in vitro* approach to evaluate the effects of Tf on neuronal differentiation. For our studies we chose the N2a neuroblastoma cell line as well as cortical neurons.

Our results indicate that Tf is able to increase cell survival ($p \leq 0.01$) through a decrease in apoptosis ($p \leq 0.01$). Tf also has a significant and positive effect on cell differentiation, since it increased the proportion of neurite-bearing cells ($p \leq 0.01$). It was also well established that Tf incorporation through its canonical pathway can activate the MAPK signaling pathway. We tested ERK activation through WB in response to Tf treatment at short times, ranging from 0 to 30 minutes, and observed that this protein is phosphorylated in a time-dependent manner, exhibiting a phosphorylation peak at 15 minutes and declining afterwards ($p \leq 0.01$). Co-cultures of N2a cells and oligodendrocytes were treated with Tf, which accelerated oligodendrocyte maturation but had no effect on N2a cell differentiation.

Keywords: N2a cell lines, neurons, transferrin

(369) **CRYOPRESERVATION OF EMBRYONIC NEURAL STEM CELLS (ENSCs) BY THE SLOW COOLING TECHNIQUE**

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Neural stem cells have become a great promise for tissue repair and regenerative therapies due to their potential to proliferate and differentiate into major neuronal cell lines and their ability to incorporate into preexisting neuronal circuits. The advances achieved in the application and use of these cells have made their cryopreservation a great challenge.

The goal of this work was to optimize a preservation protocol of Murin ENSCs at subzero temperatures by the Slow Cooling technique.

ENSCs were isolated from cerebral cortex of C57/BL mice embryos (13-15 days) and cultured as neurospheres (NE) in proliferation medium. On day 7 of culture NE were disgregated to obtain single cells, resuspended in DMEM/Ham's F12/10% Fetal Bovine Serum plus 10% DMSO and incubated for 20 min at 30°C to allow DMSO diffusion. After that, cryopreservation of ENSCs was performed using a device to control cooling rate (3.4°C/min and 6.2°C/min) until -80ºC. ENSCs were finally store in liquid nitrogen for <50 days and >60 days. After the set time, cells were rapidly thawed at 37°C and cultured in proliferation medium for 6-7 days; NE were disgregated and single ENSCs were cultured in differentiation medium. Cell viability and viable cell yield were evaluated in all the procedures by Trypan blue exclusion test. NE proliferation was determined by measure of NE diameter by light microscopy. The ENSCs ability to differentiate into neurons or astrocytes was estimated by immunocytochemical stains.

Cryopreservation of ENSCs by slow cooling produced a significant decrease in cell viability at both tested cooling rates; moreover the observed viable cell yield decreased significantly with the cryopreservation time. Additionally, NE proliferation declined with the raise of the cooling rate and the cryopreservation time. No differences were observed in ENSCs differentiation. However the best results were obtained with a cooling rate of 3.4ºC and a cryopreservation time less than 50 days.

Keywords: Neural Stem Cells, Cryopreservation, Slow Cooling

BIOTECHNOLOGY AND BIOINFORMATICS 6

(1622) **DEVELOPMENT AND EVALUATION OF NON COVALENT COUPLING METHODS FOR THE PRODUCTION OF ARTIFICIAL CELLULOSOMES**

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Lignocellulose is the most abundant renewable resource on the planet and it is an excellent substrate for the production of biofuels. Its enzymatic degradation generates sugars that upon fermentation produce bioethanol. For an economically viable production of biofuels it is essential to develop new methods to increase the activity and stability of the enzymes involved in lignocellulose degradation. The cellulosomes of some anaerobic organisms represent the most efficient machinery for the degradation of lignocellulose. These multienzymatic complexes co-localize different cellulolytic enzymes and cellulose binding domains, increasing their degradation activity through enzymatic proximity and substrate targeting effects. Our goal is to develop artificial cellulosomes using an oligomeric protein scaffold that is highly stable and highly expressed in bacteria for the colocalization of cellulases, hemicellulases, beta-glucosidases and cellulose binding domains. For the assembly of these multienzymatic particles we use a non-covalent coupling strategy through high affinity heterodimeric modules complementary fused to the scaffold subunits and the target proteins. In this work we present a comparative analysis of two alternative approaches for non-covalent coupling of enzymes to our oligomeric scaffold using heterodimeric coiled coil peptides and cohesion/dockerin modules. Advantages and disadvantages of each method regarding the expression level, solubility and aggregation tendency of the target and scaffold modules are presented. The analysis of the expression behavior of the isolated protein modules used as building blocks is also presented for comparison. We also show preliminary results on the functional analysis of cellulose binding domains used in the construction of the artificial cellulosomes. It is expected that this technology would be valuable to improve lignocellulose degradation.

Keywords: artificial cellulosome, scaffold, dockerin/cohesin, coiled coil, bioethanol

(376) **IDENTIFICATION AND DEEP ANALYSIS OF THE MOLECULAR REQUIREMENTS FOR MONOCOT MALIC ENZYMES GENIC EXPRESSION**

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NADP-dependent malic enzyme (NADP-ME) is widespread in nature and involved in different metabolic pathways due to the relevant physiological functions of its substrates (malate) and products (pyruvate and NADPH). In plants, this gene family harbours several members although the biological roles of each of them remain still unknown. Phylogenetic analysis of NADP-ME proteins from monocot species revealed the existence of four taxonomic lineages, consisting of ortholog peptides with conserved amino acidic and structural features. Here, we explored the conservation of molecular requirements responsible for the specific spatio-temporal expression patterns of each NADP-ME lineage, in order to deepen the understanding of their biological functions. In particular, previous transcriptional studies allowed us to classify a maize NADP-ME isoform as embryo-specific. An own design software was applied to perform a deep analysis on the regulatory sequences of more than ten orthologous genes of this clade and a novel cis-regulatory module (CRM) was identified. This conserved arrangement of motifs included two specific ABREs (ABA-Responsive Element) and other uncharacterized cis-elements. Moreover, the study of their proximal genomic context revealed the putative bidirectionality of these promoters, sharing the found cis-elements and their potential functionality with OVA4 (OVule Abortion 4), a gene with a reported role in embryo development. *In silico* transcriptional evidences were explored for these adjacent genes and also for potential trans regulators of their promoters in monocot seed development. These findings, along with distinct conserved features found for the other lineages, provide new insights and trigger new hypothesis in order to unravel the specific roles of each NADP-ME isoform, to validate their possible redundancy, and to understand the extents of the functional diversity of this family of metabolic enzymes.

Keywords: Malic Enzyme, phylogenetic footprinting

(814) **GENERATION OF METABOLIC ENERGY FROM ARSENIC IN MICROBIALITES WITHIN ARGENTINEAN PUNA**

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The ability of microorganisms to metabolize arsenic (As) may have arisen more than 3.4 billion years ago. The proposed metabolism is the oxidation of As (III) and reduction of As (V) under anoxic conditions. In 2009, modern stromatolites were reported in high-altitude Andean lakes (HAAL) within Argentinean Puna which have similar characteristics to the primitive Earth, since they are exposed to low O_2 pressure, high UV radiation, hypersaline alkaline waters and elevated levels of volcanic origin As. In 2013, it was reported for the first time the presence of genes involved in arsenite oxidation (aioBA) and respiratory arsenate reduction (arrBA) in haloarchaea (Euryarchaeota phylum) biofilms forming inside a volcano crater in Diamante Lake, Argentina. Moreover, it was shown that As metabolism in haloarchaea is so ancient that it would belong to LUCA (Last Universal Common Ancestor) before the divergence of Archaea and Bacteria. Here, we focused on the comparative study of As metabolism genes in 5 metagenomes of microbialites and microbial mats of the Argentinean Puna. The DNA extracted from the samples was sequenced by shotgun strategy with Illumina or Roche 454 technology. Quality control and preprocessing of sequence reads prior to assembly was done with PRINSEQ and Trimmomatic. MetaSPAdes was used for assembly, and the assembly performance was rated by two statistics: N50 (in kilobases) and read mapping rate (in percent). These statistics where obtained with MetaQUAST and Bowtie 2 respectively. Gene prediction was achieved with Prodigal and As metabolism genes were identified by BLAST comparisons against different protein databases. The number and diversity of the identified As metabolism proteins suggest that many microorganisms in these environments are capable of using arsenic as an energy source. So this mechanism could be widespread in other HAAL systems, as shown in findings at Diamante Lake.

Keywords: metagenomics, arsenic, arsenite oxidase, arsenate reductase

(961) **EFFECT OF HEAVY METALS ON GROWTH AND PGPR ACTIVITY OF *BACILLUS TEQUILENSIS* AND *KOSAKONIA RADICINCITANS*: ITS POTENTIAL USE IN PHYTOREMEDIATION**

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The capacity of plant growth-promoting rhizobacteria (PGPR) for improving plant development, either by synthesizing compounds that modulate their physiology, facilitating the uptake of nutrients or protecting them from pathogens is well known. However, less is known about how heavy metals influence these mechanisms. In previous works, *Helianthus petiolaris* was reported as novel heavy metals tolerant plant species and excellent candidate for soil phytoremediation in semiarid environments. Also, *Bacillus tequilensis* and *Kosakonia radicincitans* strains were isolated from *H. petiolaris* as heavy metals tolerant and plant growth-promoting rhizobacteria. In this context the aim of this work was to assess the influence of heavy metals on the growth of these strains, as well as its plant growth-promoting capabilities. The phosphate solubilization, phytohormones production, nitrogen fixation, siderophore production, biofilm formation, production of acylated homoserine lactones (AHL) and growth inhibition capacity on *Alternaria sp.* (endophytic fungi of *H. petiolaris*) were assessed, in order to understand the complex plant-PGPR-heavy metals interaction during a phytoremediation process.

Keywords: plant growth-promoting rhizobacteria, PGPR, heavy metals, phytoremediation.

(1214) **TAILORING OF AN EXTRACELLULAR LIPASE ACTIVITY FROM *Aspergillus niger* MYA135 BY SUBMERGED FERMENTATION: MEDIUM OPTIMIZATION, PERFORMANCE AND MOLECULAR CHARACTERIZATION**

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Lipases (EC 3.1.1.3) are important industrial enzymes due to their versatile applications. In this work, the medium engineering strategy was used for tailoring an extracellular lipase activity from *Aspergillus niger* MYA ATCC 135. Previously, among eleven variables, the carbon/nitrogen ratio and the concentration of both olive oil and $FeCl_3$ were identified as significant parameters. Thus, the level of those important input factors was optimized to maximize the lipase production using a central composite design. All experiments were performed in duplicate and analyzed with the Minitab software for Windows. The hydrolytic activity was measured with *p*-nitrophenyl palmitate (C16) as substrate. The molar extinction coefficient of *p*-nitrophenol (*p*-NP) under the given assay conditions was 0.00979 μM^{-1} cm^{-1}. One unit of enzyme activity (U) was defined as the amount of biocatalyst that released 1 µmol of *p*-NP per min. The enzymatic activity was expressed as U per liter of supernatant. As a result, the optimal culture conditions were the following: 3 % olive oil (X1), 0.20 g/l $FeCl_3$ (X2) and a carbon/nitrogen ratio (X3) of 0.37. The effect of each variable was also analyzed; the linear coefficient of X1 and X3, the interaction terms coefficients of X1and X2, and the quadratic coefficients of both $X2^2$ and $X3^2$ were significant, as their P values were below 0.05. In addition, the *P*-value for the lack-of-fit test (0.578) showed the adequacy of the model. This is also verified by the R^2 (80.10 %) and the Adj R^2 (73.24%) coefficients indicating the percentage of variability that is explained by the model. Besides, the thin layer chromatography was used to analyze the performance of these biocatalysts in the biodiesel synthesis. Finally, the gene en-

coding for the lipase activity was identified from cDNA obtained by retrotranscription using the saline medium with olive oil as inductor. This work was supported by FONCyT (PICT 2015- 2596), CONICET (PIP 339) and UNT (PIUNT E548/3).

Keywords: *Aspergillus niger*, lipase, medium optimization, cloning.

(1229) **OPTIMIZING ENZYMATIC OIL DEGUMMING EFFICIENCY: NOVEL PHOSPHATIDYLINOSITOL-SPECIFIC PHOSPHOLIPASE C ENZYME AND DEGUMMING PROCESS DEVELOPMENT.**

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In the last few years, there has been a constant increase in the demand for oils to be used as a food and production of fuels. This demand has generated a need for cost-effective methods for removing contaminating phospholipids, known as gums, during the refining process. Traditionally, physical and chemical degumming methods have been used. More recently, developments were made to use enzymatic degumming, which possesses several advantages over chemical and physical methods such as minimal chemical waste and higher yields of refined oil. Type C phosholipases (PLCs) provide a higher extra yield of oil compared to traditional methods, both by generating 1,2-diacylglicerol (DAG) from phospholipids, which is miscible with triacylglycerols (TAGs), and by releasing the TAGs trapped as a consequence of the reduced volume of gums. Most of the PLCs reported in the literature for oil degumming have specificity for phosphatidylcholine (PC) and/or phosphatidylethanolamine (PE), which together represent ~67% of the phospholipids present in soybean oil. Recently, we designed a novel PCPLC-Y, an engineered enzyme that can completely hydrolyze PC and PE. In this work, phosphatidylinositol-specific phospholipase C (PIPLC) candidates obtained from an *in silico* analysis were evaluated for oil degumming expecting to formulate this new enzyme in combination with PCPLC-Y in order to increase the efficiency of oil degumming. A PIPLC from *Lysinibacillus sphaericus* was shown to efficiently remove phosphatidylinositol, which represents ~24% of the phospholipids in crude oil, and when combined with PCPLC-Y, the three major phospholipids present in crude oil were completely hydrolyzed, providing an extra yield of oil greater than 2.1%, compared to standard methods. A remarkably efficient fed-batch *Escherichia coli* fermentation process producing ~14 g/L of the recombinant PIPLC enzyme was developed, which may facilitate the adoption of this cost-effective oil-refining process.

Keywords: Oil enzymatic degumming; phospholipid removal; green chemistry; phosphatidylinositol phospholipase C;

(1495) **ENHANCED WATER USE EFFICIENCY AND PHOTOSYNTHETIC PARAMETERS: STUDIES OF TRANSGENIC *Nicotiana tabacum* EXPRESSING MAIZE NADP-MALIC ENZYME IN GUARD AND COMPANION CELLS.**

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The consumption of plant materials increases at a rapid pace to satisfy the demands of a growing population for food and feed. These growing demands can only be met with improvements in plant productivity at given irrigation rates. In order to asses this proposes, a maize NADP-malic enzyme (NADP-ME) was expressed in guard cells and vascular tissues of *Nicotiana tabacum*, driven by the *Arabidopsis thaliana* Potassium channel 1 promoter. NADP-ME decarboxylates malate in the presence of NADP to produce pyruvate and CO_2. The biochemical modifications in those cells and tissues, produced by maize NADP-ME, are traduced in important phenotypic modifications in the whole plant. Compared to wild type plants (WT), the transgenic tobacco lines export sugars to the phloem at higher rate than WT; this leads to higher sucrose, glucose, and fructose levels in phloem exudates and veins, they also produce significantly more biomass per water used and flower earlier than WT. Here, we aimed to study water use efficiency (WUE), differential protein expression and metabolite analysis in the transgenic tobacco lines. Net CO_2 fixation and transpiration rates analysis showed that, although the stomata of the transgenic lines exhibited reduced pore sizes compared to WT, leaves of transgenic tobacco lines possess significant higher net CO_2 fixation rates at CO_2 levels above 400 ppm and light intensity above 200 µmol m^{-2} s^{-1}, resulting in higher instantaneous WUE than WT. Leaves quantitative proteomic profiling revealed drastic differences in the amount of specific proteins in the transgenic lines in comparison to WT. The proteins with highly modified content are related to cell cycle, flowering, hormone signaling, and carbon metabolism, among others. These findings show that the NADP-ME site-specific expression causes important variations in metabolomic and proteomic profiles, and it is an efficient strategy to enhance WUE in the C_3 plant *Nicotiana tabacum.*

Keywords: Water use efficiency, *Nicotiana tabacum*, maize NADP-malic enzyme, guard cells, vascular tissue.

(1639) **COLD-ADAPTED OLEAGINOUS YEASTS: EFFECT OF GLUCOSE CONCENTRATION ON LIPIDS PRODUCTION OF *Rhodotorula glutinis***

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Certain oleaginous yeasts as *Rhodotorula glutinis* can accumulate neutral storage lipids from 20 to 70% of biomass, consisting mainly in triacylglycerols (TAG), under appropriate cultivation conditions. Microbial TAGs represent a valuable alternative feedstock for biodiesel production. Lipid accumulation in oleaginous microorganism occurs under nutrient limitations, mainly nitrogen, with simultaneous excess of carbon source. Effect of glucose concentration (30, 40 and 100 g/L) and C/N ratio on the growth and lipid production of two strains of *R. glutinis* (R4 and R48) isolated from Antarctica was investigated using a nitrogen-limited medium (MI). Samples were taken at 72, 96 y 120 h of culture time for analytical determinations (pH, biomass, lipid production, percentage of lipid accumulation and residual glucose). *R. glutinis* R4 and *R. glutinis* R48 were capable to accumulate high amounts of intracellular neutral lipids (51-60%, w/w) at different glucose concentration in the nitrogen-limited medium, MI. Effect of initial glucose concentration and C/N ratios on the growth and lipid accumulation was observed mainly in *R. glutinis* R4. Results showed an increase of growth and lipid parameters with the culture time in both strains. Significant differences on lipid accumulation along the culture time were not observed for *R. glutinis* R48 (~49.94-57.71%). Results of statistical analysis for biomass and lipids production indicated that the culture time can be reduced at 72 and 96 h for *R. glutinis* R4 and *R. glutinis* R48, respectively, using 30 g/L of glucose. Lipid production and accumulation of R4 and R48 are comparable with other oleaginous yeasts reported in the literature. Based on these results, *R. glutinis* R4 and *R. glutinis* R48 have an interesting capacity for lipid production and they can be considered as a source of TAG with potential application for biodiesel production.

Keywords: Oleaginous yeasts, microbial lipids, biodiesel, Antarctica, C/N ratio

(1716) **HYALURONIC ACID PRODUCTION IN *Sinorhizobium meliloti*: EXPLOTING THE HOST EXOPOLYSACCHARIDE REGULATORY NETWORK WITH BIOTECHNOLOGICAL PURPOSES**

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Abstract: Hyaluronic acid (HA) became one of the most popular polymers in the field of biomedicine. Initially, HA was obtained from animal tissues; however, this source of HA is being gradually replaced by the bacterial production. This work describes the engineered of the non-pathogenic bacterium *Sinorhizobium meliloti* for HA production. Two radically distinct hyaluronan synthases genic sequences, from Gram positive *Streptococcus equisimilis* and Gram negative *Pasteurella multocida*, were codon-optimized for expression in *S. meliloti*. Both synthetic sequences were independently cloned in plasmid pWBT and were expressed in *S. meliloti*. Unexpectedly, in a Gram negative bacterium such as *S. meliloti*, HA production by a Gram positive HA synthase showed a significant better performance than that observed from the Gram negative HA synthase of *P. multocida*. This is the first report evaluating the HA production from two, widely used bacterial HA synthases, in *S. meliloti*. Polymer identity was confirmed by a specific HA-binding assay and hyaluronidase digestion. Furthermore, the synthetic sequences were integrated into the *S. meliloti* genome at the native EPS II gene cluster, harnessing the regulatory network and the high rate of production of this endogenous exopolysaccharide. HA yield from the *S. meliloti* strain expressing the synthetic HA synthase from *S. equisimilis* was 3.5 mg/l while the yield from the synthetic HA synthase from *P. multocida* was 0.05 mg/l. Work is in progress to increase the HA production in the recombinant *S. meliloti* strain by disrupting the synthesis of endogenous exopolysaccharides and by coexpressing genes encoding proteins responsible for HA precursors synthesis.

Keywords: hyaluronic acid, *Sinorhizobium*, exopolysaccharide.

(1817) **OLEAGINOUS YEASTS FROM EXTREME ENVIRONMENTS IN PATAGONIA AND ANTARCTICA. SCREENING AND ANALYSIS OF LIPID PRODUCTION USING MICRO-BREWERY EFFLUENTS**

Andrea Trochine, Luciana Cavallini, Ariel Favier, Antonio Uttaro, Diego Libkind

Yeasts capable of accumulating more than 20% of their dry weight in lipids are considered oleaginous. These lipids are mainly stored in the form of triacylglycerids (TAGs) in intracellular lipid bodies and may serve in biodiesel production, animal and human nutrition, among others. Accumulation occurs under nutrient deprivation (e.g. N) in the presence of excess C. Up to date more than 100 species of oily yeasts are known, some accumulating up to 70% of their dry weight in lipids. The aim of this work was to obtain information about the ability of a collection of extremophylic or extremotolerant yeast strains, isolated from Patagonia and Antarctica, to convert into the "obese" phenotype. For this purpose, more than 100 yeast strains were cultured and analyzed with nile red staining, under both low and high C/N ratio. Nearly 50 strains showed lipid bodies in media containing excess C and low N. Subsequent analyses of lipid production (GMY medium, 5 day culture at 20°C) using gravimetric analysis showed many of these are oily yeasts, with lipids accumulations ranging from 20 to 65%; and lipid yields from 1 to 4 g lipids/L culture. The lipid profile of some of these yeasts was analyzed by GC-MS, and showed most are rich in oleic acid (30 to 44%), linoleic acid (15 to 29%), palmitic acid (14 to 24%) and stearic acid (4 to 16%). Some include linolenic acid ranging from 2 to 10%. In addition, six strains of the amylase secreting yeast *G. pullulans* were analyzed for growth and lipid production in beer effluents, including wort runoff and boil remainings. Beer effluents were tested at a density of 5 brix with no other nutrient supplementation. In both effluents all strains showed biomass and lipid accumulation (10-15 g/L biomass and 20-34% lipid on dry weight basis). As a conclusion a number of yeasts show potential for their use in lipid production, including yeasts with potential for consolidated bioprocessing using starch containing or starch derived substrates.

Keywords: yeasts, oleaginous, lipids, brewery effluents

REGENERATIVE MEDICINE AND CELL THERAPY 4

(1826) **COLONY FORMING UNITS AS FUNCTIONAL ASSAY FOR RELEASING UMBILICAL CORD BLOOD PRODUCTS FOR HEMATOPOIETIC STEM CELL TRANSPLANT**

Romina Sainz, Matias Ghiguly, Carolina Bentham, Eleonora Pagano, Ana Agustina Miguel, Maria Rosario Silvestri, Julieta Rosello, Cecilia Gamba, Silvina Kuperman, Valeria Roca

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Umbilical cord blood (CB) is an alternative source of hematopoietic progenitor cells (HPC) for transplant of a great variety of diseases, such as hematological malignancies, marrow failure, immunodeficiencies, hemoglobinopathies and inherited metabolic diseases. Transplantation with CB allows for faster availability, since CB is cryopreserved in banks around the world. However, according to local regulation and international quality standards, cryopreserved products shall be monitored through a stability program and quality control tests should be performed prior to distribution. The facility should define the critical product characteristics to be tested prior to release: viability of an attached segment, confirmatory HLA typing, among others. More than one test might be needed to evaluate the potency of the product prior to its release for transplantation. Colony forming units (CFU) is an in vitro assay developed to support optimal growth of different types of HPC.

Our goal was to study CFU assay as a functional test for releasing a CB unit for transplant.

We tested viability of CD45+ cells by flow cytometry (FC) with 7-AAD staining (Mean±SD) in 11 CB units before cryopreservation and a thawed segment of each of them. For segments we also performed CFU assay and viability testing of CD34+ cells (Mean±SD) by FC.

Viability of CD45+ cells for non cryopreserved samples was 93 ± 7.5% (n=11), and for thawed samples 65 ± 14.7% (n=10). In the segments the viability of CD34+ cells was 75 ± 15.5 % (n=10). However, for one unit it was not possible to analyze the FC results of the attached segment. Instead, all samples tested (n=11) showed HPC colonies growth in CFU assay.

Our results showed that CFU assay could be a valuable test to asses CB potency, and could be defined as a release criterion, especially when results cannot be obtained by FC.

UMBILICAL CORD BLOOD - CELL THERAPY - TRANSPLANT

(220) **CONSTRUCTION OF MOLECULAR TOOLS AND TRANSGENIC MICE FOR IMPLEMENTING *IN VIVO* REJUVENATION BY CELL REPROGRAMMING**

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It was recently reported that animal rejuvenation and life extension can be achieved by partial cell reprogramming using repeated cycles of expression and silencing of the four pluripotency genes Sox2, Oct4, Klf4 and c-Myc (the Yamanaka genes) in old mice, transgenic for the Yamanaka genes. We have constructed an expression vector harboring the 4 Yamanaka genes (the STEMCCA cassette) under the control of a bidirectional Tet-On or Tet-Off regulatable promoter. With the future aim to study the redox biology of cell reprogramming (collaboration with Institut Pasteur de Montevideo), a redox-sensitive GFP fused to a glutaredoxin (hGrx-roGFP2) was included into the STEMCCA plasmid. The redox biosensor is highly sensitive to detect small changes in the intracellular glutathione-dependent redox homeostasis. The regulatability of the plasmids by doxycycline (DOX) was characterized in HEK293 cells expressing the four Yamanaka genes as assessed by ICC. Subsequently, a plasmid harboring the final STEMCCA cassette flanked by two homology arms 1,200 and 3,200 bp long will be microinjected in one-cell embryos along with RNA encoding for the enzyme Cas9 and a guide RNA targeted to the Rosa26 locus. The embryos will

be cultivated in M16+50μM SCR7 until they reach a two-cell stage and further transferred to pseudopregnant surrogate females. Once the STEMCCA transgenic mice are generated, we will investigate in vivo rejuvenation by subjecting them to several cycles of partial reprogramming according to the procedure described by Ocampo et al (In Vivo Amelioration of Age-Associated Hallmarks by Partial Reprogramming Cell 167: 1719-1733 (2016)). In vivo rejuvenation by cell reprogramming is an emerging technology that is revolutionizing the field of the biology of aging.

Keywords: Yamanaka genes regulatable, cell reprogramming, rejuvenation

(147) **PROLIFERATION AND SURVIVAL DURING HEPATIC DIFFERENTIATION OF AMNIOTIC EPITHELIAL STEM CELLS**

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The placenta and fetal membranes have recently been proposed as an important stem cells source for regenerative medicine. Amniotic epithelial cells isolated from human amnion (hAECs) offer considerable advantages that make them stand out between other stem cells. In addition, they express embryonic stem cells markers and have the ability to differentiate toward all three germ layers. Moreover, they are not tumorigenic and have immunosuppressive properties. These characteristics would make hAECs ideal candidates for tissue engineering and application in regenerative medicine. Hepatic failure is one of the major causes of morbidity and mortality worldwide. Recently, stem cells have been spotlighted as alternative source of hepatocytes because their specific potential for differentiation. The aim of this work was to study the proliferation and survival of hAECs, during hepatic differentiation. Hepatic differentiation was assayed by specific factors (EGF + dexamethasone) or by HepG2 conditioned medium (CM). We have analyzed the expression of some key cell cycle proteins. After specific factors (HD) treatment, we observed a significant increase (2 ± 0.5 fold) in Cyclin D1 mRNA expression and a decrease (1.7 ± 0.3 fold) in p53 and (1.6 ± 0.4 fold) p21 expression, measured by *q*RT-PCR. This treatment also caused a down regulation (1.3 ± 0.5 fold) in p53 and p21 expression and an increment (1.9 ± 0.06 fold) in Cyclin D1, measured by Western-blot. The opposite effects were observed with CM treatment. Finally, we have evaluated the MAPK signaling pathway activation, which is linked to cell growth and proliferation. Immunofluorescence and western blot analysis revealed the HD treatment significant increased (1.3 ± 0.1 fold) ERK 1/2 phosphorylation while CM diminished it (1.3 ± 0.04 fold). Our results suggest hepatic differentiation with specific factors promotes the proliferation and survival of hAECs, improving their quality and quantity for an eventual future transplant.

Keywords: Stem cells, hAECs, proliferation, hepatic differentiation, regenerative medicine

(1024) **RELEVANCE OF AKT SUMOYLATION IN MOUSE EMBRYONIC STEM CELLS**

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Abstract: Embryonic stem cells (ESC) have the ability to self-renew indefinitely and to give rise to cells of all three germ layers. The PI3K/Akt pathway is involved in survival and proliferation and is key for maintaining the levels and activity of ESC's essential transcription factors (TF) Oct4, Sox2 and Nanog. Recently, it has been reported that SUMO conjugation to Akt1 regulates the activity of this kinase, with consequences in the splicing pattern, proliferation and cell cycle in different cultured cell lines. However, the relevance of this post-translational modification (PTM) of Akt1 hasn't been studied in ESC.

Our hypothesis is that SUMOylation of Akt1 is relevant for survival, proliferation and maintenance of ESC fundamental properties. This work was aimed to study the effect of this PMT of Akt1 on the regulation of Nanog expression, and to set up the conditions to induce apoptosis and detect this process in mouse ESC.

First, ESC were transfected with expression vectors for Akt1 variants with different capacity of being SUMOylated along with a Luciferase reporter vector driven by Nanog promoter. We found a differential activity of this promoter depending on Akt SUMOylation levels, suggesting that this Akt PTM could modulate Nanog expression in ESC (Analyzed using randomized block design ANOVA and Tukey test). To further determine the possible involvement of Akt1 SUMOylation in the regulation of ESC's survival/apoptosis, we first set up the conditions for inducing apoptosis in our cell culture system. Culturing ESC in the absence of the cytokine LIF and the 2i inhibitor for up to 4 days induced apoptosis, as detected by DNA ladder assay, as well as by PARP-1 and Caspase 3 cleavage by Western Blot.

Our preliminary results suggest that the SUMOylation of Akt1 could play a role on the expression of Nanog. We consider that studying the regulation of the fundamental properties of ESC is essential for their future application in regenerative medicine.

Keywords: pluripotent stem cells, Akt, SUMO, Nanog, apoptosis.

(912) **EFFECT OF LENTIVIRAL DELIVERY OF A CHIMERIC TRANSFORMING GROWTH FACTOR BETA TYPE II RECEPTOR AGAINST LIVER FIBROSIS IN RATS**

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Liver fibrosis is a hallmark feature of chronic liver diseases, which affects millions of patients worldwide, and leads to liver failure. Current therapeutic options for these patients are limited. It is well established that transforming growth factor beta (TGF-β) promotes liver fibrosis. Thus, the development of therapeutic agents with a significant potential to achieve a specific and long-lasting block of TGF-β action *in vivo,* is of clinical relevance. The aim of this work was to study, in a carbon tetrachloride (CCl4)-induced liver fibrosis rat model, the effect of lentiviral-mediated overexpression of a chimeric human soluble TGF-β type II receptor ectodomain fused to the Fc portion of human IgG (TGFBR2/Fc). We compared three experimental groups: vehicle, CCl4, and CCl4 previous intrahepatic administration of a lentiviral vector encoding TGFBR2/Fc (Lv-TGFBR2/Fc). In this way, we observed partial recovery of body weight in rats treated with CCl4 + Lv-TGFBR2/Fc compared to CCl4 group. In addition, gross appearance of livers of Lv-TGFBR2/Fc + CCl4 group reversed the irregular shape and shrinkage observed in CCl4 group. Moreover, injection of Lv-TGFBR2/Fc diminished CCl4-induced liver enzymes augmentation, indicative of liver injury recovery. Histological analysis of liver sections revealed that Lv-TGFBR2/Fc significantly reduced the deposition of collagen fibers induced by CCl4 as well as practically restored liver architecture. These results suggest that lentiviral delivery of TGFBR2/Fc exerts a protective effect against liver fibrosis induced by CCl4 in rats.

Keywords: liver fibrosis, lentiviral vectors, soluble type 2 transforming growth factor beta-Fc receptor

(900) **DEVELOPMENT OF A MONOCLONAL ANTIBODIES PANEL FOR MONITORING GLYCOSILATED RECOM-**

BINANT HUMAN STEM CELL FACTOR PRODUCTION PROCESS.

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Stem Cell Factor (SCF) is an early-acting cytokine capable of promoting proliferation, differentiation, migration and survival wich depends on the cell type. It plays a crucial role in hematopoiesis, gametogenesis, melanogenesis, intestinal motility, and in normal development and function of nervous and cardiovascular systems. Thus, it is of mayor interest the production as well as the proper monitoring of each step during the process.

The aim was to produce and characterize a set of murine monoclonal antibodies (mAbs) against recombinant human SCF (rhSCF) in order to study the different stages of the glycoprotein production process.

The cell line producing rhSCF was generated through lentiviral transgenesis of HEK293 cells. Antibiotic selection was performed in order to increase productivity. A HisX6 tag was used to purify rhSCF from culture supernatant by Immobilized Metal-ion Affinity Chromatography. BALB/c mice were inoculated with purified rhSCF following an immunization plan comprising four different strategies. After fusion protocol, hybridoma cell lines which expressed specific immunoglobulins were cloned. Three clones with the highest productivities were amplified *in vivo* to produce murine ascites. mAbs were purified from ascites by protein A chromatography and characterized by their isotype, specificity and title.

Two mAbs were able to detect the glycoprotein in Western blot and indirect ELISA assays, showing adequate limits of detection and titles; and two mAbs were good candidates for capturing step in Sandwich ELISA, which permitted to develop an assay for rhSCF quantification in different samples. A panel of anti-rhSCF mAbs was developed and used to identify and quantify the protein during different stages of the production process. Such panel might also be useful for affinity chromatography and different determinations in regenerative medicine, developmental biology and immunology.

Keywords: hSCF, monoclonal antibodies panel

(680) **DIFFERENTIATION OF PLURIPOTENT STEM CELLS INTO MESENCHYMAL STEM CELLS IN A SUSPENSION CULTURE USING COMMERCIAL MICROCARRIERS**

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LIAN-FLENI

Mesenchymal stem cells (MSC) multipotentiality and immunomodulatory capacity are characteristics particularly appealing for the field of regenerative medicine. Despite the numerous clinical trials involving these cells, their large-scale production has drawbacks given the large amounts required for therapy and the loss of their therapeutic properties because they rapidly reach senescence. This highlights the need to have standardized and economically viable MSC procurement processes. Our laboratory has recently established a new way to generate MSC from Pluripotent Stem Cells (PSC). The aim of this project is to adapt this protocol to a suspension culture on microcarriers in order to develop a valid platform for obtaining MSC compatible for clinical use and without quantity limits.

First, we tested several coatings as well as quantities of cells seeded in order to optimize the conditions for the PSC adhesion to the microcarriers with respect to those previously established. We determined that a coating with a commercial extracellular matrix and an amount of 20.000-40.000 cells/cm^2 are adequate to obtain both a largest number of adhered cells and a homogeneous distribution of them. We also noted that constant stirring at 10 rpm favors the attachment. In addition to this, we improved the conditions of maintenance and amplification during the differentiation process. Moreover, we analyzed by flow cytometry the expression of pluripotency and mesenchymal lineage markers and integrins at different time points during the differentiation, proving that the cells obtained retain characteristics of a mesenchymal cell such as their adhesion to plastic.

These results show that it is possible to reproduce the protocol of obtaining MSC from PSC by adapting it to a culture in 3 dimensions. In the future, it remains to analyze other characteristics of the mesenchymal cells obtained, such as their immunomodulatory capacity and the scaling-up of this same protocol to 1L bioreactors.

Key Words: Mesenchymal, Differentiation, Microcarriers, 3D-culture.

(703) **ELECTROPHORETIC PROFILE OF SALIVARY PROTEINS IN EDENTULOUS PATIENTS**

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The aim of this study was to determine the pattern of salivary proteins in completely and partially edentulous patients (EP). Forty-eight subjects were grouped based on their number of teeth: group 1 - partially EP with ten or less than ten missing teeth (G1, n = 12), group 2 - partially EP with more than ten missing teeth (G2, n = 12), group 3 - completely EP (G3, n = 12), group 4 - patient with all teeth (G4, n = 12). Unstimulated whole saliva sample was collected from the subjects between 9 a.m and 2 p.m., after they had carefully rinsed their mouths with water. After collection, the saliva was transported on ice, centrifuged, and the supernatant was transferred to micro tubes and stored at -80 ºC. Concentrations of total protein was determined using quantitative colorimetric method based on the use of pyrogallol red (Proti U/LCR, Wiener lab, Rosario, Argentina). The expression profile of salivary proteins were analysed by electrophoresis on cellulose acetate membrane (Cellogel, Electrophoresis Co. SRL, Milano, Italy) and polyacrylamide gels (SDS-PAGE). Protein bands on cellogel strips were visualized by staining with Amido Schwartz. The molecular weights (MW) of the proteins were estimated by comparison with a commercial standard (Amersham BioSciences UK Ltd.). The bands were classified according to size and intensity of staining on gels stained with Coomassie blue and silver nitrate. Total salivary protein concentration was reduced in G3 (22.07±8.03 mg/dl, vs G4, $p = 0.0001$) and G2 (24.35±12.02 mg/dl, vs G1, $p = 0.026$). Analysis of the expression of salivary proteins by SDS-PAGE revealed individual variations in the protein expression. High molecular weight proteins (66 kDa) showed a similar expression pattern in the four groups. Effect of number of teeth on all cellogel electrophoretic protein fractions was found ($p < 0.05$). Identification and characterisation of salivary proteins in EP may be important in diagnosis and treatment.

Keywords: salivary proteins, edentulous patients, electrophoresis.

(283) **EXPOSITION TO INTERMITTENT HYPOXIA LEADS TO SUBMANDIBULAR GLAND ADAPTATION FAILURE**

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Hypobaric hypoxia (HX) is a stressful condition that induces activation of cellular components to maintain the physiology of several organs. If acclimation does not happen, deleterious effects occur. The **aim** of this study was to establish the submandibular gland (SMG) function and bioenergetics in a model of rats exposed to chronic continuous hypoxia (CCH) or chronic intermittent hypoxia (CIH) in order to elucidate whether these environmental conditions induce adaptive mechanisms in the organ. **Methods**: 45 adult female Wistar rats were divided into control (C), CIH (600 mbar, 18 hours from Monday to Friday) and CCH (23,5 hours every day at the same pres-

sure). At day 75 pilocarpine-stimulated total salivary secretion rate was measured. At day 90, animals were euthanized and SMG were resected to perform mitochondrial characterization, assess oxidative stress and inflammatory markers and evaluate histopathological characteristics by optic and electronic microscopy. ANOVA was used for statistics. **Results:** Salivary secretion decreased in animals exposed to hypoxia, being lower in CIH (95% vs. 59% in CCH). Oxygen consumption was higher in both hypoxic groups, but complex I activity was increased only in CCH. Mitochondrial hydrogen peroxide and superoxide dismutase activity remained unchanged, whether thiobarbituric acid reactive species in the whole tissue increased under hypoxia ($p<0,05$). Prostaglandin E_2 (PGE_2) content increased only in CIH vs C ($p<0,001$). Electronic microscopy revealed apoptotic nuclei and irregular secretion granules in CIH acini. Only CCH showed higher hypoxia inducible factor (HIF)-1α immunorreactivity and vasodilated blood vessels. **Conclusions:** CIH animals showed decreased salivary secretion that can be correlated with submandibular microstructural changes, PGE_2 increase and lack of HIF-1**α**. Mitochondria increased O2 consumption reflects an attempt of the organ to adapt to the hypoxic conditions, which seems to be more efficient in CCH than in CIH.

Key words: hypoxia – submandibular gland – bioenergetics - hyposalivation

(919) **EPIDEMIOLOGY OF LOWER-LIMB ULCERS: STUDY OF OUTPATIENT DURING 2013-2014 IN ARGENTINA**

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Lower-limb ulcer (LLU) affects approximately 1% of the population around the world and is a serious problem for patients, professionals and health systems. In Latin America (especially in Argentina) this is an undervalued problem due the lack of epidemiological studies. The aim of this study was to contribute to the knowledge about this problem in the North-West Region of Argentina.

Materials and Methods: A retrospective study of 640 outpatient medical records with ICD-10 codes related to LLU that were treated at A. Padilla Hospital, Tucumán, Argentina, during the period 2013-2014 was conducted. This study was approved by the SIPROSA Bioethics Committee and the Hospital Teaching and Research Committee. There was collected information about patients (age, sex, city, educational status, employment status, etc.), data of the ulcer (type, quantity, location, evolution, microbiology, treatments, etc.) and other epidemiological data.

Results: Frequencies, incidence, prevalence, clinical and dermatological associations, and therapeutic effectiveness were determined. This allowed us to evaluate the situation of LLU in our region. Statistical analyses were performed using standard statistical program, and the results were compared with similar studies published around world. Diabetic foot (55%) was the most frequently ulcer type, followed by venous (17%) and mixed (15%) ulcers. 30% of the patients were amputated (17% two or more times), mainly due to unresolved infection.

Conclusion: This study allows us to evaluate the severity of the problem and to find new opportunities for therapeutic improvement, reduction of associated costs, optimization of hospital funds administration and the design of public policies based on real knowledge of the problem.

Keywords: lower-limb ulcer, epidemiology, outpatient

REPRODUCTION AND FERTILITY 9

(1895) **ANTIOXIDANT EFFECTS OF METFORMIN PLUS ITS DURING *IN VITRO* MATURATION OF PORCINE COCS**

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The insulin-sensitizing drug metformin has antioxidant properties in a variety of models. Lee et al. (2005) have shown that metformin plus insulin supplementation during *in vitro* culture of porcine embryos increases the blastocyst rate considerably. Insulin- transferrin-selenium (ITS) is commonly added to oocyte *in vitro* maturation (IVM) media because it increases the maturation rate and has antioxidant effects. We aimed to determine if metformin added to IVM media containing ITS has antioxidant effects. Porcine COCs were obtained by follicular aspiration of slaughterhouse ovaries and matured *in vitro* in the supplemented M199 medium during 46-47 h. The medium was added to ITS (I) or ITS + metformin (I+M). COCs were pooled (ten COCs per tube, seven tubes per group) and homogenized in PBS on the ice and centrifuged at 4500 x g. Supernatants were stored at -80°C until use. Lipid peroxidation was determined by TBA-RS and total glutathione was measured by a colorimetric assay, both techniques adapted to 96 Well microplates. Lipid peroxidation decrease in the group I+M compared to group I (*t* test, $p<0.05$). Total glutathione did not show differences between groups. We conclude that metformin added to IVM medium containing ITS has antioxidant effects. It remains to investigate long term effects; it is potentially beneficial to IVF and the subsequent development of embryo and fetus.

Key words: metformin; oocyte; embryo quality

(1662) **ATP CONTENT OF THE PORCINE OOCYTE DURING IN VITRO MATURATION**

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Our aim was to evaluate the impact of the glycolytic pathway and the Krebs cycle on the oocyte ATP content and its relation with the maturation process in the porcine species. Cumulus-oocyte complexes (COCs) were obtained by aspiration of ovaries from slaughter sows. *In vitro* maturation (IVM) was performed in medium 199 with 50 µg/ml gentamicin sulfate, 10% fetal bovine serum and 0.57 mM of cysteine in presence (Control) and absence (Control -H) of 0.5 *µ*g/ml FSH and 0.5 *µ*g/ml LH, under mineral oil at 39 °C, 5% CO_2 in air and 100% humidity for 48 hours. The medium was supplemented with 10 mM sodium oxamate (OXA) or 10 mM sodium malonate (MAL), inhibitors of the enzymes lactate dehydrogenase (LDH) and succinate dehydrogenase (SDH), respectively. The ATP content was determined in immature and *in vitro* matured oocytes using a commercial kit based on the luciferin-luciferase reaction (Bioluminescent Assay, Kit, Sigma). Nuclear maturation was evaluated by the presence of the metaphase II by Hoechst staining. IVM rates were evaluated by Chi-square test. ATP results are expressed as media ± SEM and were analyzed by ANOVA. A significant decrease ($p<0.05$) in ATP was observed at the end of IVM in the Control-H group, whereas no difference was observed in the Control group respect to immature oocytes (0.11 ± 0.02, 0.19 ± 0.02 and 0.20 ± 0.03 pmol of ATP/oocyte, respectively). Supplementation with OXA or MAL did not modify ($p>0.05$) the ATP content (0.14 ± 0.02 and 0.24 ± 0.04 pmol ATP/oocyte, respectively vs Control, 0.23 ± 0.03 pmol ATP/oocyte) but decrease ($p<0.05$) IVM rates (34% and 39%, respectively vs control, 63%). These results indicate that gonadotrophins induce an increase in the ATP content during maturation, probably for stimulating metabolic pathways of ATP production. LDH and SDH enzymes participate in the nuclear maturation but their participation is not related to the ATP content of the matured porcine oocyte.

Keywords: oocyte porcine, in vitro maturation, ATP.

(1821) **DECREASE OF GENE EXPRESSION QUALITY MARKERS IN BOVINE OOCYTE DURING WINTER SEASON**

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The aim of this study was examined seasonal effects on expression of genes playing an essential role in quality of bovine oocyte. Santiago del Estero became in one of the region most important livestock production in Argentina. This region is characterized by wide thermal amplitude with very high temperatures which often exceed 35°C in summer and lower than 13°C in winter. Mammalian oocytes acquire their meiotic competence and fertilization potential in a stepwise manner. As a result, they are potentially exposed to various environmental stressors during follicular development. Folliculogenesis is controlled by growth factors as GDF-9 and BMP-15. The calpain-calpastatin proteases system has been implicated in various cellular signaling pathways including cell cycle regulation and cytoskeleton reorganization. In this work, we analyzed the expression levels of the CAPN1, CAPN2 and inhibitor CAST; BMP15, GDF9 factors and their receptors BMPRI and TGFβR1; GLUT4 and IGF1 as metabolic markers. Bovine ovaries were obtained from a local abattoir from Braford cows during the hot and cold seasons. Cumulus–oocyte complexes (COCs) were aspirated from 3 to 8 mm follicles. The oocytes were pipetted vigorously to ensure that they were fully denuded. To determine cross-contamination we carried out a semi-quantitative PCR using marker genes for oocyte: ZAR, granulosa cells: CYP 19A1 and theca cells: CYP 17A1. Real-time PCRs were carried out with GAPDH and 18S rRNA as reference genes. We found that GDF9, BMP15 and BMPRI mRNA expression decrease in cold season versus hot season. The same pattern was detected for CAPN2 and CAST expression. Meanwhile, TGFβR1, CAPN1, GLUT4 and IGF1 expression had no significative differences. Taken together, these results suggest there is a significant decrease in oocyte developmental competence during cold season. It remains to be investigated the direct effect of environmental factors on oocyte quality and its impact on in vitro production efficiency.

Keywords: bovine, oocyte, seasonality, gene expression

(1647) **EFFECT OF THE ACTIVATION AND INHIBITION OF THE PLASMINOGEN/PLASMIN SYSTEM DURING *IN VITRO* MATURATION OF BOVINE OOCYTES: A BIOLOGICAL AND SPECTROSCOPIC APPROACH**

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The Plasminogen (Plg)/Plasmin (Plm) proteolytic system plays a crucial role during the initial steps of mammalian reproduction. Plg must interact with its activators to become Plm, involved in a broad spectrum of proteolytic processes. Due that Plg is in the zona pellucida (ZP) and plasma membrane of bovine oocytes, this study examines the effect of the exogenous activation or inhibition of Plg/Plm during the *in vitro* maturation (IVM) of cumulus oocytes complexes (COCs). Three groups were studied: i) control (COCs), ii) with 3.5 IU/mL streptokinase, an exogenous Plg activator (COCs/SK) iii) with 10 mM ε-aminocaproic acid, a specific inhibitor of Plm proteolytic activity (COCs/ε-ACA). After 22 h of IVM, oocytes were processed and evaluated. It was observed that in COCs/SK the percentages of metaphasic II (MII) oocytes were not modified, while in COCs/ε-ACA a decrease in the number of MII oocytes was evidenced. Cumulus expansion after IVM was employed to evaluate the effect of Plm activity. By microphotographs it was observed that ε-ACA inhibits the cumulus expansion respect to the control and SK. Modifications in the ZP were evaluated by measuring the time of digestion of ZP (dtZP) with pronase. No significant differences in the dtZP between COCs and COCs/SK were observed. However, in COCs/ε-ACA the resistance of ZP to enzymatic digestion was increased. Physicochemical properties of the ZP were also studied by Raman microspectroscopy. Spectral differences were related to specific structural changes in glycoproteins upon IVM with ε-ACA. The intensity decrease in the sialic acid band agrees with previous results regarding the *in vitro* fertilization: the biological effect of ε-ACA would be interpreted like a reduced interaction between the ZP oocyte and the sperm. Considering all these results, the inhibition of Plm activity by ε-ACA produces a negative effect in the *in vitro* matured oocytes. On the contrary, negligible effects were observed with SK.

Keywords: oocyte, *in vitro* maturation, plasmin, zona pellucida, Raman

(1076) **EFFECT OF THE COCULTURE OF PORCINE LUTEAL CELLS WITH PORCINE CUMULUS OOCYTE COMPLEXES ON THE IN VITRO MATURATION RATE**

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Nuclear and cytoplasmic maturation have not been well described in porcine until the moment. The establishment and maintenance of a suitable microenvironment play an essential role in maturation and subsequent fertilization. The choice of porcine luteal cells (PLC) for coculture with cumulus oocyte complexes (COC) is based on the production of progesterone, a hormone with an antiapoptotic and antioxidant effect. The aim of this study was to evaluate the effect of the coculture with PLC on the in vitro maturation rates of porcine oocytes. Slaughterhouse ovaries were used for the PLC culture and COC aspiration. COC were matured in vitro for 44 h in 100 μL drops of supplemented TCM199 and with human menopausal gonadotropin (hMG) (control) and over a monolayer of PLC without the addition of hormones. Nuclear maturation rates were assessed with Hoechst 33342 stain. After in vitro fertilization (IVF) with fresh sperm with proof fertility, the suspected zygotes were dyed with Hoechst 33342, and sperm penetration, monospermic penetration, male pronuclear formation, and IVF efficiency were evaluated. The Fischer test was used and it was considered as significant a $p < 0.05$. No significant differences were observed in nuclear maturation rates between coculture treatment (72%, n = 104) and control (78%, n = 139). The coculture treatment significantly increased the monospermic penetration (60.47%, n = 96) and IVF efficiency (27.08%) in compare with the control (35.85% n = 137; 13.87%). No significant differences were observed in sperm penetration and male pronuclear formation. We conclude that nuclear maturation in coculture is similar than nuclear maturation with hormones and that monospermic penetration and IVF efficiency are improved with this treatment. Therefore, we could replace the conventional maturation with hormones using this coculture system, having a lower rate of polyspermic penetration, a key issue in the in vitro embryo production in pigs.

Keywords: coculture, porcine, oocyte, luteal cells, in vitro maturation.

(296) **METFORMIN ACTS DIRECTLY ON RAT GRANULOSA CELLS ACTIVATING AMPK AND REGULATING VEGF EXPRESSION.**

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Polycystic ovary syndrome (PCOS) is a common disorder that affects women in reproductive age. Its symptoms are heterogeneous and range from anovulation, oligo/amenorrhea and hyperandrogenism to obesity and insulin resistance. Metformin (MET) is an oral antihyperglycemic drug introduced in the treatment of PCOS to manage hyperglycemia. MET has been shown to improve ovulation, pregnancy and live birth rates in patients with PCOS. The mechanism by which MET improves reproductive parameters are

not fully understood. MET is a hydrophilic molecule so the organic cation transporters (OCTs) are actively involved in the cellular uptake of MET in different tissues. MET primary mechanism of action is through the activation of the AMP-activated protein kinase (AMPK), which acts as an energy sensor by monitoring the AMP/ATP status of the cell. The aims of the present work were to analyze a possible direct effect of MET on rat granulosa cells (rGCs) and to evaluate the presence of OCTs in this cell type. Materials and Methods: Sprague Dawley rats (21d) were injected subcutaneously with diethylstilbestrol (1mg/rat) daily for three days to stimulate the development of early antral follicles. rGCs were isolated by percoll gradient. RNA was isolated from the rGCs and RT-PCR was performed for OCT 1-3. Another set of isolated rGCs was stimulated with MET 0.01 or 0.1ng/ml with or without the OCT inhibitor cimetidine (CIM). Cells were harvested 48h later and proteins extracted for OCTs, phospho-AMPK (P-AMPK), AMPK and VEGF measurement by western blot. Results: Expression of OCT 1-3 was detected in rGCs. P-AMPK was increased ($P<0.01$) in rGCs while VEGF was decreased ($P<0.001$) after stimulation with MET. Inhibition of OCTs by CIM reversed these effects ($P<0.05$). Conclusion: We demonstrated that MET acts directly on rGCs. Our findings suggest that MET enters and exerts it effects on rGCs through OCTs. These results provide new evidence to explain the effect of MET on infertility treatments.

Keys Words: PCOS, METFORMIN, AMPK, OCT, VEGF

(292) **EXPRESSION AND DIRECT ROLE OF CCR2 RECEPTOR AND ITS CHEMOKINE LIGANDS WITHIN THE FELINE CUMULUS OOCYTE COMPLEX AND THE ANTRAL FOLLICLE WALL**

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The aim of this study was to: 1) evaluate the mRNA expression of CCR2, its ligands (MCP1, MCP2, MCP3 and MCP4) and genes related with periovulatory events (AREG and HAS2) within the cumulus oocyte complex (COC) and follicle wall after LH stimulus using a feline antral follicle culture, and 2) evaluate the mRNA expression of CCR2, MCP1, AREG and HAS2 within the COC, using a feline COC culture in the presence of MCP1. First, ovaries were removed from adult domestic cats (n=52). Antral follicles larger than 0.5 mm were mechanically dissected from the ovary and individually cultured (n=130) for 6, 12, 24 or 36h with or without recombinant human LH (75 mIU/ml). At the end of the culture, COCs and follicle walls were dissected to further analyze the mRNA expression by qPCR. A subset of COCs (n=20) were fixed for CCR2 and MCP1 immunofluorescence. In a second experiment, COCs (n=28) were isolated from antral follicles (n=7 cats) and cultured for 3h (time when periovulatory gene expression peaks in our COC culture) with or without recombinant human MCP1 (10 and 100 ng/ml) and mRNA expression was assessed by qPCR. Two-way ANOVA analyses (Time-Treatment) showed significant effects of mRNA expression for many of the genes. LH treatment significantly increased ($p<0.05$) CCR2 mRNA in COCs after 6h in culture, where the highest expression of HAS2 mRNA was observed. AREG mRNA expression also increased ($p<0.05$) in the follicle wall 6h after LH treatment. CCR2 and MCP1 immunostaining was present in both cumulus cells and the oocyte. COC culture showed an increase ($p<0.05$) of CCR2, MCP1, HAS2 and AREG in the presence of MCP1. In summary, LH stimulates the mRNA expression of CCR2 and its ligands in antral follicles (COC and/or follicle wall). Moreover, isolated COCs are able to respond to recombinant MCP1 in vitro. Together, these data suggest a direct role of CCR2 receptor in the feline COC, which in turn may regulate events that are necessary for ovulation and/or luteinization.

Keywords: ovary, feline, chemokines, follicle, cumulus oocyte complex

(1581) **THE EFFECT OF L-CARNITINE ON THE LEVEL OF RECOVERY OF MORPHOLOGICALLY HEALTHY OOCYTE, EARLY APOPTOSIS, AND VIABILITY OF MATURE PORCINE VITRIFIED-WARMED OOCYTES**

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L-carnitine (LC) plays an essential role in the catabolism of lipids and protects cells from the damage caused by reactive oxygen species (ROS). The aim of this study was to evaluate the effect of the addition of LC during *in vitro* maturation (IVM) on the level of recovery of morphologically healthy oocyte, early apoptosis, and viability of mature porcine vitrified-warmed oocytes. The cumulus-oocyte complexes (COC) were obtained by follicular aspiration from slaughterhouse ovaries and matured *in vitro* without LC (control) or with 0.6 mg/mL of LC (Sigma-Aldrich) in the maturation medium (TCM-199 supplemented) for 44 h at 39°C and 5% CO_2. Then, the oocytes were denuded and vitrified using a support similar to a Criotop. One week later, they were warmed and the level of recovery of morphologically healthy oocyte, early apoptosis (Annexin V), and viability (propidium iodide) were assessed. Data were analyzed by Statistix software (Fisher´s test). It was considered as significant a $p \leq 0.05$. There were significant differences in the level of recovery of morphologically healthy oocyte between the control (70.92 %, n = 141) and LC treatment (82.19 %, n = 146) ($p = 0.03$). No significant differences were detected in the level of early apoptosis (1.19 % vs. 5.61 %) and the viability (26.19 % vs. 17.76 %) between the control (n = 84) and LC treatment (n = 107), respectively. In conclusion, the LC treatment improved the recovery of morphologically healthy oocyte in compare with to the control. However, the level of early apoptosis and viability of porcine oocyte matured with LC in the IVM medium and then vitrified-warmed did not differ with the control.

Keywords: L-carnitine, porcine, oocyte, vitrification-warming, viability.

(1262) **ALTERED EXPRESSION OF ESTROGEN RECEPTORS α AND β IN BOVINE OVARIAN FOLLICULAR PERSISTENCE.**

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Steroid hormone receptors are members of the superfamily of ligand-regulated transcription factors that modulate gene transcription by different mechanisms and as a consequence they are able to activate or repress gene expression. In mammals, estrogens modulate the growth, differentiation and the female physiology through estrogen receptor (ER) α and ERβ. Cystic ovarian disease contributes to reproductive deficiency in lactating dairy cows. The endocrine profile, growth dynamics and histological characteristics of cystic follicles are similar to those of induced-persistent follicles in a model of subluteal progesterone administration. The purpose of the present study was to analyze the expression of ERα and ERβ by immunohistochemistry (IHC) in ovarian follicular structures during the development of follicular persistence induced in cows by long time progesterone administration. A low dose of progesterone was administered (n=5) for 0 (ovulation time, P0) and 5 (P5), 10 (P10), and 15 (P15) days after the expected day of ovulation using an intravaginal progesterone-releasing device. Control cows (group C) received no additional hormonal treatment. After IHC technique, digital image analysis was performed on ovary slides to quantify the immunostaining in preovulatory follicles of the control group as reference structure and persistent follicles of P0, P5, P10 and P15 groups. ERα expression was higher in preovulatory follicles of the control group than in the persistent follicles of the P5, P10 and P15 groups, in both granulosa and theca interna cells ($p <0.05$). Expression of ERβ was similar in the categories analyzed, both in granulosa and theca interna cells ($p> 0.05$). These results suggest that changes in the expression of estrogen receptors, mainly ERα, can lead to an altered response of steroid hormones, and thus contribute to the pa-

thogenesis of ovarian alterations such as follicular persistence and cystic ovarian disease.

Keywords: estrogen receptors, bovine, ovarian follicular persistence

(1467) **VESICLE-ASSOCIATED MEMBRANE PROTEIN 1 AND 3 PARTICIPATE IN CORTICAL GRANULE EXOCYTOSIS IN METAPHASE II OOCYTES**

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Following fertilization, cortical granules (CG) undergo exocytosis (CGE) to release their content into the perivitelline space, avoiding polyspermy and ensuring normal embryonic development. CGE, also known as cortical reaction, is a calcium-regulated secretion that represents a membrane fusion process during meiotic cell division of oocytes. Several studies suggest that CGE is a SNARE protein-mediated pathway; however, it is still unknown if VAMP (acronym for vesicle-associated membrane protein) is present and mediates CGE in mouse oocyte. We hypothesized that oocyte uses the same conserved membrane fusion machinery as neurons and human sperm, and that VAMP, one of the SNARE proteins required for membrane fusion, is present in mouse oocyte. Our aim was to identify and characterize the main VAMP isoforms related to regulated exocytosis in other secretory models: VAMP1, VAMP2 and VAMP3. We first investigated the expression of VAMP isoforms by RT-PCR. The results revealed that VAMP1, 2 and 3 are expressed in mouse oocyte. Western blot analysis indicated that VAMP1 and VAMP3 (but not VAMP 2) were present in mature mouse oocytes. Indirect immunofluorescence experiments revealed that VAMP1 and VAMP3 are predominantly observed in the CG-enriched cortical region during egg maturation. To evaluate the function of these proteins in CGE, endogenous VAMPs were perturbed by microinjection of antibodies prior to CGE activation. The microinjection of specific antibodies against either VAMP1 or VAMP3 in metaphase II oocytes inhibited CGE stimulated by SrCl2. Nevertheless, the microinjection of anti-VAMP2 antibody had no effect on CGE. Furthermore, recombinant tetanus toxin light chain (which cleaves VAMP) microinjection experiments showed that the microinjection of tetanus toxin was able to abolish CGE in activated metaphase II oocytes and that SNARE complex is in cis configuration. Altogether, our findings indicate that VAMP1 and VAMP3 have an active role in CGE process.

Palabras clave: VAMP, cortical granule, exocytosis, mouse oocytes

GENETICS AND MOLECULAR BIOLOGY 20

(861) **INHIBITION OF SPHINGOMYELIN SYNTHASE 1 ACTIVITY PROMOTES AN EPITHELIAL-MESENCHYMAL TRANSITION (EMT) IN DIFFERENTIATED COLLECTING DUCT CELLS**

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Abstract: In Epithelial-Mesenchymal Transition (EMT), contrary to Mesenchymal-Epithelial Transition (MET), cells lose their epithelial phenotype and acquire the characteristics of mesenchymal cells. EMT normally occurs during embryonic development, and in adult tissues is activated during inflammation, tissue regeneration, and it has also been related with fibrosis and cancer. The sphingomyelin (SM) synthase 1 (SMS1) participates during the final step of SM synthesis. In previous works, we have demonstrated that the inhibition of SMS1 activity induces the loss of cell-cell adhesions of collecting ducts (CD), and it also affects their morphology. Taking into account that these characteristics are similar to those described for EMT, we investigate whether the SMS1 inhibition could induce this process. To this end, primary cultures of differentiated CD cells were incubated for 24 h with D609, a SMS1 inhibitor. By immunocytochemistry and immunoblot we analyzed the expression of the mesenchymal cells markers: vimentin and α-smooth muscle actin (α-SMA). In basal conditions, CD cells formed monolayers with low expression of vimentin, and almost null in α-SMA. Overlapping cells with fibroblastoid morphology, which strongly express both proteins, were also observed. After D609 treatment, the number of CD expressing vimentin and α-SMA increased, denoting a *de novo* synthesis of α-SMA. The amount of overlapping cells was also increased. The immunoblot analysis showed a correlative increased in the level of both proteins. Tacking into account the changes observed in cell morphology and *de novo* synthesis of α-SMA, we suggest that the inhibition of SMS1 activity could alter the equilibrium between EMT-MET, generating myofibroblasts from preexisting CD cells, because of the inability to form cell-cell adhesions. In this context, we propose that SM synthesis is important to keep the EMT-MET equilibrium, and we highlight the activity of SMS1 as a modulator of this process.

Keywords: collecting duct cells, sphingomyelin synthase 1, epithelial- mesenchymal transition, differentiated epithelial cells.

(850) **CELLULAR MECHANISMS THAT MEDIATE THE COLLECTING DUCT (CD) FORMATION DURING POSTNATAL RENAL DEVELOPMENT**

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Abstract: In mammals, nephrogenesis is completed postnatally. In previous works, we have shown that primary cultures of renal papillary CD cells from neonatal rats, display a phenotype of migratory sheet of epithelial cells. Now, we investigated the mechanisms involved in the postnatal CD formation. In order to evaluate the differentiation degree of primary cultured CD cells, the ability of DBA (a CD marker) and BSL-I (a renal interstice marker)-binding, together with the expression of epithelial (cytokeratin-7, CK7, and adherens junction proteins, AJ) and mesenchymal biomarkers (vimentin and α-smooth muscle actin, α-SMA) were analyzed by immunofluorescence. We observed the coexistence of CD cells with different degree of differentiation in the same colony. Those of higher differentiation were DBA (+), intermediate filaments CK7 (+) and exhibited assembled AJs. While those of lower differentiation were vimentin (+), cytosolic CK7 (+), DBA/BSL-I (+), only BSL-I (+), or DBA/BSL-I (-), and they lacked mature AJs. The analysis of overlapping cells showed a mesenchymal phenotype. 3D reconstructions and xz profiles of confocal microscopy images showed that they were located adjacent to or below the plane of the colonies. Cell behavior studies in real time by phase contrast microscopy revealed that they could be inserted between the CD cells from the basal plane through a mesenchymal-epithelial transition, acquiring the epithelial phenotype of the cells that surround them. Taking into account the above results, we suggest that overlapping cells could originate *in situ* through an epithelial-mesenchymal transition from CD cells. Therefore, our results suggest the existence of a "dynamic balance" between CD and mesenchymal cells, strongly displaced towards the former. Most likely, between CD cells and the mesenchymal cells generated *de novo*, the mutual induction described above could occur.

Keywords: postnatal renal development, collecting duct, mesenchymal-epithelial transition, epithelial-mesenchymal transition

(1682) **LOSARTAN INHIBITS MIGRATION THROUGH Hsp70 CHAPERONE IN RENAL PROXIMAL TUBULE CELLS (PTC) FROM SPONTANEOUSLY HYPERTENSIVE RATS (SHR).**

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Angiotensin II (AII)/AT_1 receptor effects are dependent on reactive oxygen species (ROS) production. AII, induces renal injury through NADPH oxidase-dependent ROS generation. ROS function as signaling molecules contributing to migration, differentiation and cytoskeletal remodeling. Previously, we identified Hsp70 and CHIP as Nox4-interacting proteins, mediating ubiquitination and proteasomal degradation of Nox4 included within Losartan antioxidant effect on SHR PTC. Here, we evaluate Losartan (L) effect on migration, actin cytoskeletal organization and junctional-related protein in SHR PTC. Primary culture of PTC from SHR rats were stimulated with AII, treated with L or untreated (C). Live Cell Time-lapse Microscopy showed that L induces decreased cell displacement and slowed down cellular rate movement compared to C and AII PTC. Also, the cells remained attached and did not change their morphology compared to C and AII PTC. Inmunofluorescence shows actin filaments highly organized in L treated cells compared to C and AII treated cells. Furthermore, L increased the cortical E-cadherin levels. When the Hsp72 expression was silenced, L treated cells showed velocity and displacement values like C PTC. In addition, L was unable to stabilize the cytoskeleton showing an increase in misaligned actin filaments and decreased cortical E-cadherin. Western Blot, showed L increased vinculin and E-cadherin and decreased Nox4, p-ERK and p-p38 levels related to AII and C. Also, L decreased Rac1 and RhoA levels in membrane fractions, this effect was reversed in Hsp70 silenced cells. In conclusion, Losartan AT_1R blockage induces actin cytoskeleton stabilization and cell migration reduction. Moreover, through Hsp72 protein knockdown, we demonstrate that the chaperone is required for the cytoskeletal integrity modulation within the effect of L in PTC from SHR. A protective role of L could be suggested avoiding tubular cell detachment and stabilizing cell junctions.

Key words:Hsp70, Cell Migration, Losartan

(1471) **EARLY REQUIREMENTS OF FURRY IN CELL FATE DETERMINATION AND MORPHOGENETIC MOVEMENTS**

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Vertebrate embryonic axes formation is conducted by a group of cells with morphogenetic and inductive properties, named “the organizer”. These cells coordinate the formation of the axial mesoderm, gastrulation movements and induction of the nervous system. The *furry* (*fry*) gene encodes an evolutionarily conserved protein involved in multiple cellular functions in vertebrates and invertebrates. In *Xenopus*, Fry acts as a co-repressor of microRNAs inducing the expression of axial mesoderm genes. Dorsal depletion of *fry* produces shortening of the dorsal axis and interferes with head formation, possibly due to gastrulation defects and/or incorrect progenitor cells specification.

Since little is known about the role of *fry* in early development, we investigated its expression pattern by *in situ* hybridization .In *Xenopus*, *fry* is maternally expressed in the animal pole of both dorsal and ventral blastomeres. During gastrulation, *fry* is found in the axial mesoderm and at tailbud stage, its expression remains in notochord, somites and pronephros. Interestingly, zebrafish *fryl1* is also maternally deposited and expressed in the notochord at 12-somite stage. To investigate the nature of the developmental defects associated with *fry* depletion, we injected *Xenopus* embryos with a specific morpholino oligonucleotide. Dorsal depletion results in reduced expression domain of the organizer genes *goosecoid* and *chordin*, indicating that Fry is required for organizer formation. *Fry*-depleted embryos exhibit blastopore closure and axial mesoderm convergent extension defects, resulting in abnormal gastrulation movements. When *fry* is ventrally depleted, expression of dorsal organizer markers is unaffected whereas expression of the ventrolateral mesoderm marker, *wnt8* is diminished.

Together, our results show that Fry has conserved expression in the axial mesoderm and suggest that Fry has a dual role in early development: it is required for mesoderm specification and cell movements during gastrulation.

Palabras clave: *fry,* gastrulation, gastrula organizer, Xenopus, morpholino.

(384) **NUTRIGENOMIC, CALPAIN SYSTEM AND MEAT TENDERNESS**

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Abstract: Post mortem meat tenderization is primarily the result of calpain mediated degradation of key proteins within muscles fibers. Managing feeding system has a regulatory effect on biological processes that occur in the muscle, defining the final quality of meat. The aim of this study was to determine the effect of corn finishing strategy on the expression level of the calpain system proteins and meat tenderization of beef. Thirty steers grazing in summer pasture were used: 15 animals with corn supplementation (G1) and 15 steers without the supplementation (G2), under a completely randomized experimental design with three replicates (3 paddocks with 5 animals per group). The least squares mean for carcass measurements, taken during the slaughter period in the left side, and meat quality traits of *longissimuss dorsi* muscle, were compared using t-test. Feeding strategies generates significantly differences between groups. G1 showed higher hot carcass weight ($P < 0.001$), fat content ($P = 0.02$), and Warner-Bratzler shear force (WBSF) ($P = 0.01$). Gene expression and activities of the proteases (calpain 1, calpain 2) and the inhibitor (calpastatin) were measured using real time PCR and casein zimography. G2 showed higher protease:inhibitor ratios at mRNA ($P < 0.01$) and protein ($P < 0.05$) levels. The correlation analysis between mRNA expression and activity, with WBSF revealed a relationship with calpains and calpastatin in both systems ($r = -0.390$), suggesting that variation in beef tenderness could be modulated through the differential expression of these system. Under these conditions, it can be conclude that feeding strategies could be a tool to improve the quality of the carcass and meat. The present study introduce the potential of a nutrigenomic approach as a first step in the development of pro-active quality control systems which fulfil future demands from industry and consumers.

Keywords: meat tenderness, bovine, calpain system, gene expression, nutrigenomic

(1765) **MODULATING THE INTERNALIZATION OF CELL PENETRATING PEPTIDES INTO HUMAN SPERMS AND DENDRITIC CELLS**

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The development of tools that facilitate the cellular uptake of therapeutic molecules constitutes an active field of research. Cell penetrating peptides (CPPs) have been introduced as novel biocarriers, since they are able to translocate cell membranes by a mechanism which is still poorly understood. Experimental evidence suggests that CPPs internalization may occur by physical diffusion across cell membranes, may involve endocytic processes, or a combination of both. In this work, we explore the conditions that optimize the translocation of CPPs into human sperms and dendritic cells (DCs). We combine fluorescence microscopy and flow cytometry to analyze the effect of membrane potential, pH of the extracellular medium, and cell membrane composition, on the internalization of the TAT peptide. Ours results show that DCs internalize TAT via endocytic and diffusive pathways, while only the diffusive mechanism operates in sperms. Moreover, in both cases the kinetics of TAT translocation depends on the membrane potential at short times (5 min). For example, when cell membranes are hyperpolarized, TAT enters at higher rates, and in more cells (90%), in comparison with a control sample (50%). In contrast, when the cell membrane is depolarized, we observe a significant decrease, both in translocation rate and in the number of cells that show TAT in the cytosol (20%). Regarding the effect of pH, DCs incorporate TAT more quickly and effectively

at alkaline pHs, whereas the translocation in sperms is unaffected by changes in pH. In conclusion, we have demonstrated that TAT translocates the cell membrane of sperms and DCs after co-incubation for a few minutes, and that the translocation efficiency can be modulated by varying some simple experimental parameters.

Keywords: cell penetranting peptides, sperms, dendritic cells, cell membranes.

(1867) **OVIDUCTAL EXTRACELLULAR VESICLES INTERACT WITH BOVINE SPERMATOZOA AND ENHANCE CAPACITATION**

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Secretions present in the uterus and oviductal fluids affect oocyte and sperm function, whereas the underlying cellular mechanisms are unknown. Extracellular vesicles (EV) are composed of a lipid bilayer containing transmembrane proteins and enclosing cytosolic proteins and nucleic acids. EV have been recently identified along the female reproductive tract, including the uterus and oviduct, where they were called uteromes and oviductosomes, respectively. In a previous publication we showed that EV secreted by endometrial cells stimulated the level of capacitated human spermatozoa up to 4-fold, which may enhance *in vitro* fertilization rate. Since sperm capacitation is ultimately accomplished in the oviduct, we hypothesized that oviductosomes also induce sperm capacitation thus favoring fertilization. *In vitro* fertilization procedures cannot be performed in humans for ethical reasons. Therefore, in order to test our hypothesis, we first established an animal model. EV obtained from bovine oviductal fluid were characterized, and their *in vitro* interaction with spermatozoa was analyzed. A heterogeneous, ~50-500 nm diameter EV population with the characteristic cup-shape morphology was observed. PKH26-labeled EV were incubated with bovine sperm, and after only 15 minutes the lipidic fluorescent membrane marker PKH26 was detected in spermatozoa, suggesting that a fusion had occurred between EV and sperm membranes. Moreover, sperm capacitation triggered by EV collected from the ampula and istmus regions of the oviduct was determined by evaluating acrosome reaction and protein tyrosine phosphorylation. Similar to our observations with human uterosome-like vesicles, a rapid incorporation of oviductal EV to bovine spermatozoa was observed, which results in enhanced sperm fertilizing capacity. Thus, our preliminary results suggest a novel EV-mediated mechanism of intercellular communication between oviductal cells and spermatozoa.

Keywords: bovine oviductal fluid - extracellular vesicles - spermatozoa - capacitation.

(1774) **UTERINE EXTRACELLULAR VESICLES FUSE TO HUMAN SPERMATOZOA AND REGULATE INTRACELLULAR CALCIUM LEVELS**

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Cells can communicate via extracellular vesicles (EV), lipid bilayers containing transmembrane proteins, cytosolic proteins and RNA. EV consist of microvesicles and exosomes, and have been recently identified along the female reproductive tract, including the uterus. In a previous study we found that EV secreted by endometrial cells interact with human spermatozoa, and stimulate sperm capacitation up to 4-fold. Both capacitation and acrosome reaction are dependent on an increase in intracellular calcium level ($[Ca^{2+}]_i$). Based on our results, we hypothesized that the regulation of $[Ca^{2+}]_i$ could be mediated by binding or fusion of EV with the spermatozoa. Fluorimentric measurements of ($[Ca^{2+}]_i$) were performed using Fura-4 AM. Two different $[Ca^{2+}]_i$ kinetic patterns were observed when EV were added to spermatozoa: either a slow but sustained increase of $[Ca^{2+}]_i$, or a quick and transient increment of the $[Ca^{2+}]_i$. These results suggest that sperm response is mediated by two mechanisms: one dependent on the direct interaction between spermatozoa and EV, and another that requires the fusion spermatozoa-EV. Moreover, when EV were labeled with CMFDA -a fluorescent probe that is transformed into a non-permeable product once incorporated into the cells-, an increase in sperm fluorescence intensity was observed, suggesting that sperm cells incorporated EV. These data were corroborated by fusion experiments using the self-quenchable probe actacedyl rhodamine (R18). Furthermore, when endometrial cells were simultaneously treated with estrogen and progesterone, we observed that the ratio of microvesicles to exosomes was higher. Since these subpopulations were not physically isolated in our studies, to determine which one mediates sperm response to $[Ca^{2+}]_i$ -or both- will require further investigation. In conclusion, our results show that endometrial EV fuse to human spermatozoa and regulate sperm intracellular calcium concentrations.

Keywords: extracellular vesicles, endometrial cells, human spermatozoa, membrane fusion, intracellular calcium

(1872) **ROLE OF SPHINGOSINE-1-PHOSPHATE RECEPTOR 2 IN EPITHELIAL CELL DIFFERENTIATION**

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Previous results from our laboratory shown that in Madin-Darby canine kidney cells (MDCK), the exposure to a hypertonic medium (300 mM NaCl) induces a differentiated phenotype and this process is related to the sphingolipid metabolism. Sphingosine-1-phosphate (S1P) has been classically associated with the induction of a proliferative phenotype and anti-apoptotic activity. However, the participation of S1P in the cell differentiation is not well understood. S1P can act both intracellularly as second messenger and extracellularly as ligand for cell surface receptors (S1PRs). It has been reported that S1P levels decreases during transition to the differentiated state so that prevails mechanisms via S1PRs. For these reasons, we evaluated whether changes in the expression and/or localization of S1PRs are involved in the differentiation process. The expression level of S1PR2 in different stages of differentiation of MDCK cells was assessed by western blotting and did not show significant differences. Instead, immunofluorescence studies showed that during cell differentiation, S1PR2 was progressively enriched at the plasma membrane. In addition, we found that S1PR2 overexpression with plasma membrane localization, altered parameters related to differentiation like proliferation rate, maturation of adherent junctions and migration when compared to non-transfected cells. We concluded that a specific relocation of receptors is a determinant mechanism for acquisition of the differentiated phenotype in MDCK cells. These results suggest a new function for the S1P/ S1PR2 pathway.

Keywords: Sphingosin-1-Phosphate, S1PR2, cell differentiation, MDCK

(1435) **SPHINGOSINE KINASE 2 PRESERVE EPITHELIAL BARRIER DURING MDCK CELL DIFFERENTIATION**

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Sphingosine 1-Phophate (S1P) is a sphingolipid mediator of cellular fate. We have demonstrated that hypertonicity induces epithelial tissue organization by the establishment of mature adherent junctions. Organization and preservation of epithelial tissue requires an efficient cell extrusion followed by a closing of cellular gap to preserve tissue permeability. The aim of the work was to investigate the participation of sphingosine kinase (SK)/S1P pathway in the cellular extrusion in differentiated MDCK cells. For this, confluent MDCK cells were subjected to hypertonic medium with the concomitant knock down of SK1, SK2 or not (control) by siRNA, or pharmacological inhibition of SK. After 48 h, cell phenotype was visualized by fluorescence microscopy, evaluating actin cytoskeleton and Adherens Junction (AJ) formation. By performing a confocal z-plane recon-

struction we visualized extruding cells and found that the inhibition of SK evoked an alteration in the cell extrusion. SK2 knock-down produced deficient cell extrusion in live cell microscopy while SK1 knock-down do not shown any effect in the procces. We also found that SK inhibition impars the E-cadherin fragmentation necessary for cell detachment before the extrusion. In conclusion, we observe that SK/S1P pathway is involve in the cell extrusion procces and depends on SK2 activity.

Keywords: Sphingosine-1-phosphate, Sphingosine Kinase 2, Cell Extrusion, E-cadherine

GASTRO 1 / GASTROENTEROLOGY 1

(263) EFFECTS OF BILE ACIDS ON MITOCHONDRIAL PHYSIOLOGY FROM RAT INTESTINE

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The intestinal Ca^{2+} absorption is inhibited by sodium deoxycholate (NaDOC) and is increased by ursodeoxycholic acid (UDCA). In this work, we studied the effect of both bile acids on the mitochondrial dynamics, redox state and energy metabolism of intestine. Adult male Wistar rats were used: 1) controls, 2) NaDOC treated, 3) UDCA treated and 4) NaDOC + UDCA treated. Mitochondria were isolated from the duodenum of each group of animals by differential centrifugation. Spectrophotometric methods were used to quantify the activities of Krebs cycle oxidoreductases, complexes from electron transport chain (ETC) and enzymes of the antioxidant system. Gene expressions of Mfn-2, Drp-1 (mitochondrial dynamics proteins) and Pgc-1alfa (protein of mitochondrial biogenesis) were determined by RT-PCR. Glutathione (GSH) content, superoxide anion levels ($^{\cdot}O2^{-}$) and protein carbonyl content were also assayed. Results were analyzed by one-way ANOVA and Bonferroni *post hoc* test. NaDOC decreased the total GSH content and inhibited the activities of the malate dehydrogenase (MDH), isocitrate dehydrogenase (ICDH), complex III (ETC) and the Pgc-1 alfa gene expression. The combined treatment blocked the inhibitory effects produced by NaDOC. The activity of SOD and the contents of superoxide anion ($^{\cdot}O_2^{-}$) and protein carbonyls were increased by NaDOC, effects that were avoided by UDCA. The activities of ICDH and complex II (ETC) were increased by UDCA alone. In conclusion, the combined treatment avoids the oxidative stress triggered by NaDOC and blocks the inhibitory effect of NaDOC on the enzymatic activities from the Krebs cycle, the complex III (ETC) and the mitochondrial biogenesis. The stimulatory effect of UDCA on the intestinal Ca^{2+} absorption would be through an increase in the mitochondrial energy metabolism produced by bile acid.

Keywords: mitochondrial dynamics, bile acids, Ca^{2+} absorption

(1468) ENDOPLASMIC RETICULUM STRESS IN RAT ACUTE PANCREATITIS: ROLE OF THE ATRIAL NATRIURETIC FACTOR (ANF)

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In previous studies we showed that ANF attenuates the severity of experimental acute pancreatitis (AP) by reducing trypsinogen activation and the inflamatory response. Recent studies support that endoplasmic reticulum (ER) stress precedes these events. ER stress, which occurs when misfolded or unfolded proteins accumulate within the organelle, triggers the unfolded protein response (UPR) which aims to restore ER homeostasis. In this sense we previously reported that ANF inhibits the expression of BiP (major chaperone controlling UPR) and eIF (protein translation regulator) whereas it increases CHOP (apoptosis inducer) and improves ER swelling. In the present work we advanced in the knowledge of ANF effect on the signaling pathways involved in the UPR. AP was induced in Sprague-Dawley strain rats (200-220g) by four repetitive cerulein injections (40µg/Kg). Thirty minutes before the first cerulein injection animals were infused with either saline (control) or ANF (1µg/Kg/h) for 60 min. Following euthanasia (60 min after the last cerulein injection) pancreatic samples were harvested for immnunoblotting and fluorometric assays. ANOVA followed by a Student's *t* test modified by Bonferroni was used for statistical analysis. Results are expressed as the means±S.E.M. and *p* values of 0.05 or less were considered statistically significant. ANF did not change PERK activation or sXBP-1, factors which favor cell survival. However it decreased Bcl-2 expression in both control ($P<0.05$) and cerulein-treated rats ($p<0.001$) in accordance with CHOP previous findings and enhanced caspase 2 activity as revealed by a fluorometric assay in AP ($p<0.01$). In addition, preliminary results show that the expression of Bax in AP was also increased by ANF. *Present findings show that ANF stimulates the UPR signaling mechanisms involved in ER stress-induced apoptosis in experimental AP and further support ANF protective role given that apoptosis is inversely correlated with necrosis in AP.*

Keywords: ANF, AP, ER stress, UPR.

(1233) EXTRACELLULAR ATP REGULATION OF CACO-2 CELLS

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In eukaryotic cells, intracellular ATP can be released by mechanical and oxidative stress, exposure to toxins and calcium influx. These treatments mimic several conditions to which epithelial cells lining the intestine are exposed.

In the intestinal lumen, extracellular ATP (ATPe) can be hydrolyzed by ectonucleotidases located on the apical domain of the epithelium.

We analyzed the effect of several stimuli on ATPe regulation of the human intestinal Caco-2 cell line, a model of epithelial enterocytes.

Real time luminometry was used to measure ATPe kinetics, ATP release and ATPase activity.

In the absence of stimuli, Caco-2 cells displayed a regulated [ATPe] at approx. 20 nM, suggesting negligible ATP release. Addition of exogenous ATP (640 nM) led to an acute [ATPe] increase, followed by a non linear decay caused by ectoATPase activity, which amounted to 320 nM/min.

Next, we determined the effect of calcium influx (i.e., exposure to ionophore A231287), mechanical perturbation and exposure to E. coli suspensions on ATP release of Caco-2 cells.

All these stimuli led to similar –but quantitatively different- ATPe kinetics, with variable degrees of [ATPe] increase to a maximum (2-6 fold over basal levels), followed by an acute exponential decay.

Results were compatible with fast activation of ATP exit, where the rate of [ATPe] increase was partially balanced by ATPe hydrolysis due to ectoATPase activity.

In conclusion, ATPe kinetics of Caco-2 cells depends on the dynamic balance between ATP release and ATPe hydrolysis. ATP exit is transient in nature, and sensitive to various physiologically relevant stimuli. EctoATPase activity is significant, but nevertheless much slower that ATP exit during the acute phase of ATPe kinetics. With grants from CONICET (PIPC0459), UBA (20020130100027BA), ANPCYT (PICT0327) AND ECOS-Sud-MINCYT (A15S01).

Keywords: extracellular ATP, ectoATPase, ATP efflux.

(1788) GAL1 AND ITS SPECIFIC GLYCANS REGULATE MUCOSAL HOMEOSTASIS IN EXPERIMENTAL MODELS OF COLITIS

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Lectin-glycan interactions are crucial on many chronic inflammatory diseases. Galectin-1 (Gal1), a lectin that recognizes terminal *N*-acetyllactosamine in *N*- and *O*-glycans, can exert protective immunomodulatory activities in different chronic inflammation models, including TNBS-induced colitis in mice. However, the role of endogenous Gal1 in mucosal homeostasis and intestinal immunoregulation are still poorly understood.

We evaluated the development of TNBS-induced colitis in C57BL/6 wild-type (WT) mice and in animals devoid of Gal1 (*Lgals1*$^{-/-}$) or enzymes involved in the generation or masking of Gal1-glycoepitopes: Gcnt1 (generation of core-2 *O*-glycans) and St6gal1 (terminal α2,6 sialylation of *N*-glycans). Finally, we compared the development of colitis mediated by adoptive transfer of *Gcnt1*$^{-/-}$ or *St6gal1*$^{-/-}$ CD4^{+}CD45BR^{high} T cells in *Rag2*$^{-/-}$ mice.

WT and *Lgals1*$^{-/-}$ mice developed acute TNBS-induced colitis, although with a more severe disease in *Lgals1*$^{-/-}$ animals ($p<0.05$). Evaluation of macroscopic intestinal inflammation showed that *Lgals1*$^{-/-}$ mice exhibited a higher degree of inflammation when compared to WT TNBS-treated mice ($p<0.01$), and presented higher proportions of activated CD4^{+} and CD8^{+} T cells in mesenteric lymph nodes and colonic lamina propria in comparison to WT animals ($p<0.05$). Quantification of T helper cytokines on inflamed colon showed a differential response.

Gcnt1$^{-/-}$ mice developed a severe TNBS-induced colitis, whereas *St6gal1*$^{-/-}$ mice developed a lesser form of colitis ($p<0.001$). Gal1 binding to intestinal CD4^{+} and CD8^{+} T cells was only observed in *St6gal1*$^{-/-}$ mice. Adoptive transfer of *Gcnt1*$^{-/-}$ CD4^{+}CD45RB^{high} T cells led to a more pronounced colitis in comparison with *St6gal1*$^{-/-}$ ($p<0.05$).

These results highlight the critical role of endogenous Gal1 and its specific glycans in regulating mucosal homeostasis, suggesting potential avenues for modulating intestinal inflammation through the control of lectin-carbohydrate interactions.

Keywords: Galectin-1; glycans; colitis; inflammation.

(1591) **MITOPHAGY AND ROS PRODUCTION DURING ACUTE PANCREATITIS**

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Mitophagy, an autophagy pathway that selectively degrades damaged mitochondria, is an early cellular event in acute pancreatitis (AP). Mitochondria, as a source of ATP and other biological molecules, are necessary for autophagy; but are also the main cellular source of active O_2 species (ROS). Both, ROS and nitric oxide (NO), could regulate autophagy during pancreatitis. The aim of this work was to analyze mitochondrial function and dynamics, and cellular redox state during AP in a cellular model. Experimental model: AR42J pancreatic acinar-cells were treated with 7.4uM caerulein (CAE) during 15, 30 and 60 min. Mitochondrial function was assessed by determining the mitochondrial inner membrane potential using the probe TMRM by flow cytometry. For mitochondrial dynamics, OPA1 (fusion protein) and DRP1 (fission protein) expressions were determined. Mitochondrial degradation by mitophagy was evaluated by transfecting cells with dual pMITO vector. Specific probes were used in order to study cellular production of H_2O_2, ROS, NO and mitochondrial $O_2^{\cdot-}$. Results: Mitochondrial potential was significantly decreased since 30 min (25% compared control cells, $p < 0.01$) in cells treated with CAE. DRP1 expression was increased after 30 min, whereas OPA 1 increased after 60 min of treatment (75% and 100% respectively compared to control cells, $p <0.05$). Early induction of mitophagy was observed, being maximal after 30 min of treatment. It was accompanied by an increase in the expression of Parkin protein. H_2O_2 production was significantly decreased in all CAE-treated cells [30% compared to control, $p <0.05$ (control=0.128±0.010 nmoles H_2O_2/min mg protein)]. Conclusion: Our results show that mitochondrial dysfunction is accompanied by decreased H_2O_2 production in pancreatic acinar cells during PA. In addition, dysfunctional mitochondria are initially degraded by a Parkin-dependent mitophagy, while functional mitochondria would avoid degradation by mitochondrial fusion and elongation.

Keywords: Acute Pancreatitis, Mitophagy, Mitochondrial dysfunction, ROS.

(504) **MODULATION OF THE DIFFERENTIATION PROCESS OF HUMAN INTESTINAL CACO-2 CELLS BY PHOSPHATIDYLCHOLINE**

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In addition to its absorptive function, the intestine represents an important barrier that limits the entry of harmful substances. This capacity is conditioned by an adequate balance between cell proliferation and differentiation, which guarantees the development of both mature enterocytes (transcellular barrier) and tight junctions (paracellular barrier). We demonstrated that phospholipids promote differentiation of neuroblastoma and neural stem cells, but it is unknown whether they regulate the differentiation process at the intestinal level.

Aim: To evaluate the effect of phosphatidylcholine (PtdCho) on the balance proliferation-differentiation in Caco-2 cells, a model of human intestinal epithelium.

Methods: The expression of proliferation/differentiation markers was evaluated by immunoblot between days 4 and 21 of culture. Localization of Zonula occludens-1 (ZO-1), a tight junction protein, was assessed by confocal microscopy.

Results: Cells cultured for 21 days under basal conditions, showed progressive increase in expression of the microvilli structural protein villin with time, consistent with morphological differentiation. By contrast, we found a progressive decrease in expression of PCNA, indicating decreasing proliferation. ZO-1 changed its localization with time, from an intracellular, homogeneous pattern to a peripheral localization, without changes in its total expression. After PtdCho-treatment (40 μM) we observed an increase in villin expression (+33% and +22% at day 14 and 21, $p<0.05$) and a decrease in PCNA expression (-34%, -21% and -9% at day 7, 14 and 21, $p<0.05$) when compared to control cells. The expression of the tumor marker choline kinase also showed a decrease in response to PtdCho (-18% and -29% at day 14 and 21, $p<0.05$), confirming a shift in the proliferation/differentiation balance.

Conclusion: PtdCho accelerated Caco-2 cells differentiation process, suggesting a potential application as a modulator of regenerative and neoplastic processes.

Keywords: phosphatidylcholine; Caco-2; cell proliferation; cell differentiation; intestine

(663) **PRESENCE OF EXOSOMES CONTAINING THE AUTOPHAGY-RELATED PROTEIN VMP1 IN HUMAN SERUM**

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VMP1 is a transmembrane protein that participates of the autophagic process. Overexpression of VMP1 induces autophagy even in full nutrient medium. VMP1 is induced in pancreas diseases such

as pancreatitis and pancreas ductal adenocarcinoma (PDAC). Autophagy has been implicated in the endosome/exosome secretory pathway. Exosomes are small vesicles (40-120 nm) which are excreted by cells and mediate long distance cell communication. The objective of this work was to evaluate the presence of VMP1 in human serum. By western blot, and using three different antibodies, we were able to detect VMP1 in human serum samples* (N=20) from the University Hospital. Since VMP1 possess 6 transmembrane domains and is not a soluble protein, we hypothesize that its presence in serum is as a part of exosomes vesicles. Setting up two exosome purification protocols, by ultracentrifugation and by magnetic beads, we determined the presence of VMP1 in supernatant of different cell lines. Moreover, this presence was confirmed in the exosomal fraction, even under optimum nutrient conditions. Next, we purified exosomes from human serum and evidenced the presence of VMP1. In conclusion, we detected for the first time an autophagic protein in the extracellular compartment and in exosomes from human serum. These results suggest the importance of future research work exploring the relevance of VMP1 as biomarker for pancreas diseases.

*This project has been approved by the Department of Clinical Research of the University Hospital.

Keywords: VMP1, AUTOPHAGY, EXOSOMES.

(480) **THE DEUBIQUITINASE USP9X IS A NEW PLAYER IN THE AUTOPHAGY PATHWAY**

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Intracellular activation of zymogen granules triggers pancreas self-digestion and leads to acute pancreatitis. Most of acute pancreatitis cases are self-limited, suggesting the importance of response mechanisms of acinar cells. VMP1 is an autophagy protein capable of induce autophagy even in non-starved condition. VMP1 is induced by pancreatitis and mediates the zymophagy, a selective type of autophagy-mediated degradation of zymogen granules. Previously we demonstrated that the deubiquitinase USP9x interacts with VMP1 during zymophagy in acute pancreatitis. The aim of this work is to elucidate the role of USP9x in autophagosome formation. We show that shRNA-mediated depletion of USP9x significantly reduces autophagosomes evaluated by LC3-RFP dots per cell in starved HeLa cells (6.8±4.2 vs 33.6±2.4 p<0.05). By immunofluorescence, USP9x presents a cytoplasmic diffuse pattern at basal conditions, and it relocates after 15 min of starvation to a perinuclear area (48.0% ± 1.2 vs 40.5% ± 1.9 p<0.01). In the relocalized pattern, USP9x co-localizes with the early autophagy proteins such as Wipi1, DFCP1, Beclin 1 and VMP1. By western blot, we observed that this response is not accompanied by an increase of USP9x protein levels. Moreover, we demonstrate that VMP1 is necessary for USP9x relocation since USP9x relocation is reduced in cells treated with a shVMP1 (41.3%±2.2 vs 48.0%± 1.2 p<0.01) and it is increased in cells overexpressing VMP1 (47.5%± 2.2 vs 40.5%± 1.0 p<0.01).

In conclusion, we demonstrate for the first time that the deubiquitinase USP9x is an autophagy protein. In response to starvation, USP9x relocates to a perinuclear area with the early autophagic structures. Hierarchically, USP9x acts downstream of VMP1, since VMP1 expression induces USP9x relocation during autophagosome formation. Finally, we suggest that USP9x plays a relevant role in the zymophagy during acute pancreatitis, being part in the early nucleation steps of VMP1-mediated autophagy.

Keywords: AUTOPHAGY, USP9X, VMP1

(238) **ZYMOPHAGY OCCURS IN A CELLULAR MODEL OF PANCREATITIS**

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Pancreatitis is the inflammation of the pancreas due to the intracellular activation of zymogen granules (ZG). VMP1 is an autophagic protein capable of induce autophagy by its mere expression. We have described the zymophagy as a VMP1-mediated type of selective autophagy of activated ZG. The objective of this work was to set up an in vitro model of zymophagy. We used the rat pancreas derived acinar cells line AR42J. These cells were treated with dexamethasone in order to fully differentiate in acinar cells and the presence of ZG were confirmed by amylase immunofluorescence (IF). Caerulein (Cae), a CCK analog, in a final concentration of 1 μM was used to induce the acinar cell damage. We observed a recruitment of ZG to specific spots at 15 min of Cae treatment, which are decorated by the autophagy marker LC3 and VMP1 immunofluorescence. This was coincident with a switch in the levels of protein expression of VMP1, trypsin and amylase by western blot at 15 min. This last finding was accompanied by immunofluorescence colocalization of VMP1, Amylase and Ubiquitin in autophagosomes the cellular model preparations. In conclusion, we were able to reproduce and validate the occurrence of zymophagy process in a cellular model of pancreatitis. Altogether, this model demonstrates that zymophagy is a very early event in the course of pancreatitis.

Keywords: PANCREATITIS, ZYMOPHAGY, VMP1, AUTOPHAGY

(1931) **HEPATOCYTE MITOCHONDRIAL AQUAPORIN-8 CAN MODULATE CHOLESTEROL BIOSYNTHESIS.**

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Hepatocyte mitochondrial aquaporin-8 (mtAQP8) is able to work as a peroxiporin facilitating the release of hydrogen peroxide (H_2O_2). As H_2O_2 signaling is involved in hepatocyte cholesterol synthesis, we studied whether down- or upregulation of mtAQP8 expression is able to modulate cholesterogenesis. Human hepatocyte Huh-7 cells and rat hepatocytes were transfected with species-specific small interfering RNAs (siRNAs) or corresponding scrambled control siRNAs. After 48 h, mtAQP8 protein expression decreased by 50-60% ($P < 0.05$) without affecting cell viability. mtAQP8 knockdown cells showed a decrease in cholesterol synthesis of 25-50% ($P < 0.05$), assessed by following the incorporation of radiolabeled acetate into cholesterol. Cells with 24 h of siRNA transfection did not show either mtAQP8 protein decrease or cholesterol synthesis reduction. When mtAQP8 protein expression was augmented by about 100% ($P < 0.05$) after exposing cells to an AQP8-adenovector, cholesterol synthesis showed a 50% increase ($P < 0.05$). Additionally, mtAQP8-overexpressed cells were treated for 24 h with the mitochondria-targeted antioxidant Mitotempo (50 μM). Under this condition, cholesterol synthesis was reduced about 40% ($P < 0.05$) in control cells and 55% ($P < 0.05$) in cells with mtAQP8 overexpression. None of the experimental groups showed alterations in cell viability. Our data suggest that hepatocyte mtAQP8 can modulate cholesterol synthesis likely facilitating the release of mitochondria-generated H_2O_2.

Keywords: MITOCHONDRIAL AQUAPORIN-8; CHOLESTEROL BIOGENESIS; HIDROGEN PEROXYDE; HEPATOCYTE.

PHARMACOLOGY 10

(1371) **ETHANOLIC EXTRACT FROM THE LEAVES OF *Smallanthus sonchifolius*: A POSSIBLE PHYTOTHERAPEUTIC AGENT WITH ANTI *T. cruzi* ACTIVITY**

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Nowadays and since more than 40 years, the therapeutic options for Chagas disease are limited to two drugs (benznidazol and nifurtimox) with variable efficacy and serious adverse effects. Recently, we have described the isolation and the trypanocidal activity of different sesquiterpene lactones (STLs) from the species *Smallanthus sonchifolius*. The aim of this work was to determine the trypanocidal activity of the ethanolic extract of this plant in order to evaluate its potentiality as a phytotherapeutic agent (herbal medicine). Trypano-

cidal activity was evaluated in different forms of T. cruzi. Parasites were incubated in the presence of the extract (0-100 μg/ml) and the IC50 value was estimated. Citotoxicity was analyzed by MTT in Vero cells. In order to study the mode of action of the extract, epimastigotes were stained with acridine orange and observed in a fluorescence microscope. As positive control parasites were treated with H2O2. The chemical composition of the extract was analyzed by chromatographic techniques and by infrared spectroscopy. Preliminary results, showed the presence of terpenic compounds, possibly STLs. For *T. cruzi* epimastigotes (72h, 28°C) and trypomastigotes (24h, 37°C), IC50 values of 7.4 μg/ml and 16.5 μg/ml were obtained, respectively. In a trial simulating blood banks, the IC50 value for tripomastigotes (24h, 4°C) was 8.5 μg/ml MTT assay showed a value of CC50 of 448.3 μg/ml. Regarding the mechanism of action studied with acridine orange, we observed that in high concentrations (IC90) the extract cause a type of cell death "apoptosis-like", whereas when the parasites were treated with the IC50 concentration, morphologic changes, such as the formation of acidic bodies and some death, were observed. These results showed that the ethanolic extract of *S. sonchifolius* is active against the different forms of *T. cruzi*. Further studies are being carried out in order to identify the compounds responsible of the observed activity.

(1098) **EVALUATION OF THE ANTI-PARASITIC ACTIVITY OF CINCHONA ALKALOIDS AND BILE ACIDS COMPOUNDS AGAINST DIFFERENT DTUS *TRYPANOSOMA CRUZI***

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Current pharmacological treatment (i.e. Benznidazol and Nifurtimox) against *Trypanosoma cruzi*, the etiological agent of Chagas´ Disease, showed to be highly toxic and not effective in the chronic stage of the disease. With the objective to find alternative treatments against the disease, numerous works have demonstrated that quinolone derivatives compounds have cytotoxic activity against parasites. Our data showed that compounds of a library of 16 hybrids of Cinchona alkaloids (Quinine, Quinidine, Cinchonine y Cinchonidine) and bile acids (lithocholic and chenodeoxycholic acids) have an anti-parasitic activity against epimastigotes and trypomastigotes of the CL Brener strain. Because of the high genetic variability that this parasite presents, we aimed to extend our study to another strain from a different discrete typing unit (DTU). With this aim, epimastigotes of the Y strain were treated with the same compounds than the CL Brener strain, in concentrations ranging from three to four times the IC_{50} for CL Brener strain. Our data showed that only three compounds presented anti-parasitic activity ($p<0.05$). When compounds were tested as trypanocide, Y strain parasites showed more resistant than trypomastigotes from the CL Brener strain IC_{50} ($p< 0.05$). Compounds were also tested in invasion assays and a significant reduction ($p<0.01$) in infectivity and in the number of parasites/infected cell ratio were observed. In view of our results, we hypothesize that the genetic differences between DTUs play an important role in the susceptibility to drugs and this observation must have to be taken into account when searching for new drugs against the parasite.

(1441) **MODE OF ACTION OF TWO NATURAL SESQUITERPENE LACTONES ESTAFIETIN AND EUPATORIOPICRIN ON Trypanosoma cruzi.**

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Chagas disease is an endemic disease in Latin America. The drugs available for treatment have limitations because of their adverse effects, toxicity and lack of efficacy in chronic chagasic patients. Sesquieterpene lactones (STLs) arise as promising compounds because of their anti-trypanosomal activity. Knowing that the main mechanism of action of these compounds is the alkylation of the thiol groups of biological molecules, the aim of this study was to evaluate the in vitro interaction of two STLs, estafietin (EF) and eupatoriopicrin (EP), with low molecular weight thiols and the induction of oxidative stress on T. cruzi epimastigotes treated with both STLs. ES and EP have been isolated from *Stevia alpina* Griseb.and *Stevia maimarensis* Hieron. (both Asteraceae), respectively. The purity of both compounds was confirmed by HPLC analysis. The content of free thiol groups was determined by measuring the change in absorbance at 410 nm occurring when SH-groups reduce DTNB. The intracellular oxidative stress was assessed by flow cytometry using the oxidant-sensitive fluorescent probe H_2DCFDA.Both STLs showed a high interaction with sulphydryl reagents such as cysteine, beta-mercaptoethanol and glutathione. The affinity of EP for three compounds was higher than that of ES. The fact that EP possesses two potentially active sites at which Michael-type additions could occur, might explain the difference in affinity with ES, which only has one site. Similar behavior was observed when epimastigotes of T.cruzi were treated with EP or ES during 2-7 hours. Between 2 and 4 hours, EP showed a higher reduction (40 – 60%) in the free thiols levels than that ES (30 – 50%), reaching similar values at 7 hours of treatment (about 65%). This reduction on levels of thiols groups could lead to an oxidative stress within the parasite, which it showed after 7 hour of treatment. Both drugs could exert it antiparasitic effect by interaction with intracellular low weight thiols.

Keywords: Sesquiterpene lactones, Trypanosoma cruzi

(770) **REPELLENT ACTIVITY OF EXTRACT FROM FRUITS OF *Melia azedarach* AGAINST *Triatoma infestans***

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Triatoma infestans is the principal vector of Chagas´s Disease in Argentina. Increasing resistance to pyrethroids used for its control encourages the search for new alternatives. The use of natural products for biological control arises as a new strategy for controlling the vector. Aim of this work was to evaluate the repellent activities of methanolic and acetonic extracts from fruits of *Melia azedarach* (chinaberry tree) by means of the area preference method of fifth and first nymph stages of TA. Qualitative filter papers were divided into two halves, one half treated with methanolic (ME) or acetonic (AC) extracts and the other without treatment and then put inside Petri dishes and multiplewell plates (6 wells). Controls papers were impregnated half with methanol o acetone and half without the solvents. Both treated and control papers were dried at room temperature until complete evaporation of the solvents. One nymph was located in each Petri or well. All the assays were performed in three different days (six times). The phytochemical analysis of each extract was performed as well. Distribution of insects was recorded after 1, 12, 24 and 48 hours. Repellence percentage was determined according to $PR = [(Nc – Nt)/(Nc + Nt)] \times 100$ (Nc: number of insects in the control area; Nt: number of insects in the treated area). Acetonic extract demonstrated high repellent activity after one hour of assay, being 100% for both stages, whereas ME only showed 10-21% repellence. Acetonic extract showed higher concentration of carbohydrates, alkaloids and triterpenes when compared to ME. Considering these results, next assays will involve different AC concentrations in order to determine the cut point for the repellent activity of *Melia azedarach* extract, an ornamental tree that could be useful in the future as an natural alternative for the biological control of *T. infestans*.

(1568) **A NANOCRYSTALS-BASED FORMULATION IMPROVES THE PHARMACOKINETIC PERFORMANCE AND THERAPEUTIC RESPONSE OF ALBENDAZOLE IN DOGS**

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Introduction. Albendazole (ABZ) is broad spectrum antiparasitic agent with a poor aqueous solubility which often leads to erratic bioavailability and therapeutic failures. For poorly soluble drugs, a diminution in particle size could lead to enhanced bioavailability and clinical efficacy. Nanocrystals (NC) are defined as nanoparticles composed of practically 100% drug, being generally stabilized by surfactants or polymeric steric agents and with a mean particle size is below 1 μm. Here we aimed to assess the pharmacokinetic performance and therapeutic response (anthelmintic efficacy) of an ABZ nano-sized formulation in dogs.

Methodology. In the pharmacokinetic study, ABZ self-dispersible nanocrystals (SDNCS) and a control formulation were administered orally to healthy dogs using a cross over design (n=6). The concentrations of the sulphoxide metabolite in plasma were determined by High Performance Liquid Cromatography. For the anthelmintic efficacy trial, SDNCS and a commercially available formulation of ABZ were given to naturally parasitized dogs at 3 dose levels: 6.25, 12.5 and 25 mg/kg. The number of Ancylostoma caninum eggs in the feces up to 30 days was determined using the McMaster technique.

Key findings. The area under the curve, Tmax and Cmax for the SDNCS were improved significantly compared to the control ($p<0.05$ Mann-Whitney). The efficacy study showed no statistical differences between the SDNCS and the commercial formulation at the doses of 25 and 12.5 mg/kg. However, significant differences ($p<0.05$ Kruskal-Wallis) between the treatments were found at 6.25 mg/kg (a quarter of the reference dose) with a reduction in the fecal nematode egg counts of 62.0±21.1 % and 100±0 % for the control and SDNCS respectively.

Conclusions. The improved pharmacokinetic performance observed for the novel formulation of ABZ correlated with an improved in vivo therapeutic response against a model intestinal nematode parasite in dogs.

Keywords**:** Albendazole, Nanocrystals, Pharmacokinetics, Bioavailability, *Ancylostoma caninum.*

(738) **EVALUATION OF IMIQUIMOD LOADED ARCHAEOSOMES AS POTENTIAL IMMUNOTHERAPY FOR CHAGAS DISEASE**

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Archaeosomes prepared with ether lipids of Archaeobacteria constitute a novel family of liposome. They show high adjuvant activity and can be used as delivery system. We evaluate the immunotherapeutic effect of imiquimod loaded archaeosomes (IMQ-ARC) against experimental *T. cruzi* infection. C3H/HeN mice were injected ip with trypomastigotes RA and were administered with free IMQ (1mg/kg), IMQ-ARC, empty ARC or PBS. Treatment was carried out sc on the back at day 0 and 7 pi. Other group received BZ (100mg/kg/day) by gavage as reference therapy for 14 consecutive days. Parasitemia, body weight and mortality were registered. Survival mice were euthanized at 60 dpi and serum samples were obtained to assess the serological profile. Fragments of heart and skeletal muscle were collected for histopathological analysis. The results showed that treatment with IMQ-ARC provoked a significant decrease ($p<0.05$) in parasitemia levels compared with those recorded in PBS controls. Administration of an equivalent amount of empty ARC or free IMQ had no effect on the parasitemia. All mice receiving immunotherapy lost weigh near máximum parasitemia. However, IMQ-ARC group showed lower weight loss compared with IMQ, ARC and PBS groups and at the end of experimental period reached weight values comparable to BZ group. A significant difference in mortality rates ($p<0.05$) between IMQ-ARC group and controls was seen. While all animals treated with IMQ-ARC survived until the end of the experiment, animals receiving free IMQ, empty ARC or PBS succumbed between days 20 and 33 pi. Mice receiving BZ survived without developing detectable parasitemia. The analysis of IgG isotype profiles revealed a mixed IgG1/IgG2a antibody pattern. Ongoing histopathological assays will help to evaluate the inflammatory process and tissue parasitism. In conclusion, IMQ-ARC may represent a potential adjuvant therapy to reduce the toxicity and side effects associated with the treatment of Chagas disease

(374) **SYNTHESIS AND ACTIVITY OF 2-ARYLVINYL-4-QUINOLINE-CARBOXYLIC ACIDS AND THEIR REDUCED ANALOGUES AGAINST *Trypanosoma cruzi* AND *Leishmania amazonensis***

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Chagas disease and leishmaniasis caused by the protozoan parasites *Trypanosoma cruzi* and *Leishmania spp.*, respectively, present a significant burden across the developing world. About 6-7 million people are estimated to be infected with *T. cruzi* while leishmaniasis takes 30,000 deaths annually worldwide. Existing therapeutics suffer from severe side effects, toxicity, complex and prolonged dosing regimens and emerging resistance. Therefore, alternative treatments are intensely searched in order to achieve better results, with less side effects and higher patient adherence. We have reported the synthesis and antiparasitic activity of 2-aryl-4-quinoline-carboxylic acids, some of them were moderately active against *Leishmania* spp., *Plasmodium falciparum* and *T. cruzi*. Moreover, 2-substituted quinolines and especially 2-arylvinyl (or styryl) derivatives isolated from plants or prepared by synthesis, exhibited a wide spectrum of biological activities such as leishmanicidal and trypanocidal. With the goal to increase the activity we have prepared two series of 2-arilvinyl-4-quinolinecarboxylic acids and their corresponding reduced analogues 2-(2-arylethyl)-4-quinolinecarboxylic acids. The first series was synthesized from the microwave-assisted condensation reaction of 2-methylquinoline-4-carboxylic acid with a variety of arylaldehydes under acid catalysis to give the 2-styryl derivatives. The second one was obtained after the catalytic hydrogenation of each product.

At present, all the evaluated 2-styrylquinoline derivatives and their reduced analogues showed no activity against *T. cruzi* (epimastigotes) with IC_{50} values between 16.6 and 157.4 μM and the reference drug benznidazole exhibited 5.8 μM. However, all the reduced compounds were active against *L. amazoniensis* (promastigotes), with IC_{50} values between 4.3 and 6.1 μM, meanwhile the reference drug miltefosine was IC_{50} 28.9 μM. These compounds are promising hits to be assayed in amastigote forms of the parasite.

Keywords: Neglected Diseases, Antileishmanial Activity, Synthesis, Styrylquinolines

(1361) **SYNTHETIC DERIVATIVES OF THE INDOLE ALKALOID TETRAHYDRO-β-CARBOLINE FOR THE TREATMENT OF CHAGAS DISEASE AND LEISHMANIASIS AND ITS POSSIBLE MODE OF ACTION**

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Chagas disease and Leishmaniasis are a major public health issue in our country, mainly in the north where the endemic areas overlap. The efficacy of treatments is variable depending on the infecting species and the stage of infection, in addition to presenting adverse effects and increasing resistance of the parasites. Natural products, such as tetrahydro-β-carbolines (βC), indole-type alkaloids with a common tricyclic nucleus, have shown a variety of pharmacological activities including anti-trypanosomatids effects. We studied 12 alkaloid derivatives *in vitro* in order to find molecules for the design of new alternatives to treatment. Four of them were presented significant activity against T. cruzi (IC_{50} = 4-23μM) and 3 of them behaved similarly in *Leishmania spp*. βC showed to be active also in the *in vivo* murine model of acute *T. cruzi* infection. We evaluated possible mechanisms of action of the βC determining the DNA intercalating power by spectrometry: calf thymus DNA was incubated with the βC and intercalating drugs were used as positive control (C). The binding percentage (B%) was determined from the area under the curve of absorbance vs wavelength. To view morphological and physiological changes, *T. cruzi* epimastigotes and *L. amazonensis* promastigotes ($2x10^6$) were treated for 48h with IC_{50} of the βC, stained with acridine orange (AO) and observed under a fluorescence microscope. Positive C was performed with 4mM H_2O_2 and untreated parasites were used as negative C. βC showed similar % of DNA binding than the C drugs except for one, which has a p-chlorophenyl rest, that presented a greater B%. OA staining showed that the treatment with IC_{50} of the βC causes epimastigotes and promastigotes to round up, increasing the amount of acidic vesicles in the cytoplasm and changes in chromatin. Further studies are required in order to evaluate underlying mechanisms.

INFECTOLOGY 11

(838) **PRELIMINARY STUDIES FOR THE EXPRESSION AND PURIFICATION OF TWO PROTEINS FROM *Candidatus Liberibacter asiaticus***

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Huanglongbing (HLB), disease caused by bacteria of the genus *Candidatus Liberibacter* (CaLib), is having devastating effects on the citrus industry worldwide. However, despite its economic importance, little is known about the biology and pathogenesis mechanisms of these bacteria. The main hurdle in the study of CaLib is that only one specie is culturable *in vitro*, *Liberibacter crescens*, which is not described as pathogen. Of all the HLB-causing species, *Candidatus Liberibacter asiaticus* (CaLas) is the most widespread and studied. Several genomes of CaLas have been sequenced.

By analyzing both the sequenced genomes and the recent publications on the subject, we have selected two proteins that could potentially be involved in the pathogenesis of this bacterium: CLIBASIA_04040 (4040), an uncharacterized putative protein, and CLIBASIA_04260, the CaLas OmpA homologue (OmpA).

We amplified the sequences corresponding to the 4040 protein (without the N-terminal signal peptide) and the N-terminal soluble domain of OmpA. Both open reading frames were cloned into the expression vector pET28a(+). Then, we overexpressed 4040 and OmpA in *Escherichia coli* BL21(DE3) and *E. coli* Rosetta-gami 2, respectively, both with a hexahistidine-coding sequence. Using differential centrifugation, we detected that the 4040 protein was localized in the soluble fraction (cytoplasm or periplasm), while OmpA was localized in the insoluble fraction (inclusion bodies). OmpA was solubilized by heating the insoluble fraction at to 60 °C for 20 minutes. Our next step is to purify these proteins by affinity chromatography to analyze them *in vitro*.

Keywords: *Candidatus Liberibacter asiaticus,* Huanglongbing, HLB

(699) **A NOVEL pSym-BORNE TYPE I PROTEIN SECRETION SYSTEM FROM *Rhizobium leguminosarum* IS RESPONSIBLE FOR SECRETION OF A RTX PROTEIN AND A BACTERIOCIN ACTIVITY.**

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Effective symbiosis between *Rhizobium leguminosarum* (*R.l.*) and the legume requires the exchange of signal molecules that induce the expression of bacterial genes encoded in the symbiotic megaplasmid (pSym). The identification of secretion systems (SS) pSym-associated could contribute to generate future genetic tools for regulating symbiosis and the bacterial lifecycle. *R.l.*bv. *viciae* 248 strain carries the pRL1JI which confers the capability to nodulate pea and vicia plants. A pRL1JI-derivated cosmid library was screened using a polyclonal antiserum against an extracellular protein (EP) whose presence in rhizobial cultures was pSym-dependent. One cosmid was able to restore the secretion of the EP in *R.l.* pRL1JI-cured strain. By random Tn*5*-mutagenesis of the cosmid we isolate a clone that lost the ability to secrete the EP. Sequence analysis of the Tn*5*-clone corroborated that the transposon was inserted in a putative ABC component of a pSym -Type I SS (T1SS). In the same orientation and contiguous to the ABC transporter, genes encoding a MFP component and two ORFs of hypothetical target proteins were identified. Both ORFs belong to a family of calcium binding-proteins called RTX (**R**epeated in t**ox**in) that form pores in target membranes; thus, we named them RTX-1 and RTX-2. Based on the amino-terminal sequence of the EP, we corroborate that the RTX-1 correspond to EP. Bacteriocin´s activity assays performed with *R.l.* carrying either the RTX-SS or the ABC::Tn*5* derivated cosmids showed that the T1SS is required to inhibit the growth of other rhizobial strains. To dilucidate the identity of the bacteriocin activity, deletion mutants affected in *rtx-1* and *rtx-2* were generated. Surprisingly both mutants were able to inhibit the growth of *M.* strains suggesting that the RTX-proteins together or another extracellular factor secreted by the T1SS contribute with the rizobiocine activity.

KEYWORDS: PROTEIN SECRETION SYSTEM, SYMBIOSIS, RHIZOBIUM

(274) **CULTURE SUPERNATANT OF *Lactobacillus plantarum* CRL759 ANTI-INFLAMMATORY EFECT ON LPS-CHALLENGED HUMAN RETINAL PIGMENT EPITHELIUM CELLS**

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Soluble factors produced by probiotic lactic acid bacteria (LAB) such as lactobacilli can reduce inflammatory and oxidative damage. Previous studies in our laboratory showed the immunomodulatory capacity of *L. plantarum* strains on ARPE-19 cells (human retinal pigment epithelium cell line). The aims of this study were to determine whether *L. plantarum* CRL759 supernatant (LplS) was able to modulate the inflammatory response and cells migration triggered by LPS on ARPE-19 cells.

L. plantarum was cultured in DMEM medium at 37ºC, 5% CO_2; LplS was obtained by filtration on 0.22μm membranes. The cytotoxicity of the LplS was determined by MTT assay. A sub- cytotoxic dose of LplS or dexamethasone were pre-incubated with $2.5x10^5$ cells before LPS (50 μ/ml) challenge. Cytokines and nitric oxide (NO) produced by ARPE-19 cells were measured by flow cytometry and Griess assay respectively. Thiobarbituric acid reactive substances (TBARS) were determined as the measure of lipid peroxidation. Migration ability was evaluated using the assay of perpendicular scratch to the cell layer by ImageJ programe. Dexamethasone was used as anti-inflammatory control in all assays.

A dilution of 1:2 of the LplS did not exert toxic effects on ARPE-19 cells after 24h of incubation. LPS increased pro-inflammatory mediators produced by cells. Results showed that levels of IL-6, IL-8, NO and TBARS were significantly lower (50, 71, 93 and 98% respectively) than the LPS stimulated cells. Anti-inflammatory effects of LplS

were comparable to the one achieved by dexamethasone. Migration of LPS-stimulated cells with dexamethasone treatment showed significant decrease at 24h, but cells treated with LplS significantly increased migration at the same time. This is the first report about a probiotic effect on ARPE-19 inflammatory model.

Key words: probiotic; inflammation; ARPE-19

(887) **ENTEROBACTIN SIDEROPHORE IS INVOLVED IN *E. coli* COPPER DETOXIFICATION**

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Copper is a metal that catalyzes the formation of highly reactive oxygen species (ROS) in cell through Fenton-like reaction, leading to an oxidative damage in lipids, DNA and proteins. Previous reports indicate that catecholate-containing molecules such as *E. coli* enterobactin siderophore, can react with Cu^{2+} and reduce it to Cu^{+}. Copper reduction by enterobactin would result toxic because lead to facilitate Cu^{+} uptake and increased ROS production into cells. We previously reported that enterobactin confers protection against various sources of oxidative stress such us Pseudomonad siderophore pyochelin, H_2O_2 and paraquat, independently of its ability to facilitate iron uptake. In this work, we analyzed the role of enterobactin in copper toxicity. To test this, we evaluated the sensitivity of different *E. coli* strains mutant in enterobactin metabolism to copper. We observed that strains impaired in enterobactin production, uptake and hydrolysis were more susceptible to copper damage compared to wild-type strain. Incubation in anaerobic condition or addition of catalase enzyme to the media protected cells lacking enterobactin from copper toxicity. We also found that spent medium from strain mutants showing increased catechol production, exhibited high copper reduction activity. Nevertheless, these strains had the same copper sensitivity than those mutants that do not produce catechols and, in consequence, have no copper reduction activity. Besides, we observed that *entE*, *fepG* and *fes* mutants showed increased ROS levels when they grew in presence of copper compared to wild-type strain. Enterobactin supplementation reduced copper sensitivity and ROS levels in the *entE* mutant but did not have any protective effect on the *fepG* and *fes* mutants. Results suggest that enterobactin plays a role in copper cell detoxification and indicate that enterobactin must to be internalized and hydrolyzed to confer a full protection, possibly by reducing the metal-induced oxidative stress.

Palabras clave: enterobactin – copper - ROS – cell protection

(1776) **A *Mycobacterium smegmatis* MUTANT DEFICIENT IN UNSATURATED FATTY ACID BIOSYNTHESIS AS A TOOL TO DIFFERENTIATE ANTI-MYCOBACTERIAL DRUG ACTION.**

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The goal of stopping human tuberculosis led to a strong commitment for the development of anti-tubercular drugs, leaving aside the need to develop new drugs against Non Tuberculous Mycobacteria (NTM) which are increasingly causing hard to treat infections. Isoxil, a thiourea is active on *Mycobacterium tuberculosis*, inhibiting the synthesis of mycolic acids and oleic acid. This pro-drug is inactive on Non Tuberculous Mycobacteria (NTM) however the reasons are not yet clear. Our recent description of the presence of two pathways carrying on the synthesis of unsaturated fatty acids (named as DesA3 and DesBCT) in the NTM *Mycobacterium smegmatis*, led us to hypothesize that this genetic back-ground could potentially be involved in the lack of activity of Isoxyl. We constructed a *M. smegmatis* double mutant auxotroph for unsaturated fatty acids, which allowed us to show that Isoxyl is active when DesA3 is missing. This result suggests a possible role of oleic acid biosynthesis as a target obscured by the presence of the DesBCT pathway. Based on this result, we assayed the activity of a novel group of di-arylureas, which anti-tubercular activity has recently been published, finding that some compounds displayed inhibitory activity on NTM, although at concentrations higher than for *M. tuberculosis*. This set of compounds was also tested on our *M. smegmatis* mutants deficient in unsaturated fatty acid synthesis in which the activity correlated with the inhibition of mycolic acid biosynthesis. As a whole, we concluded that the utilization of the described mutants allowed us to differentiate the inhibitory activity on mycolic acid biosynthesis from the one inhibiting unsaturated fatty acid biosynthesis; therefore helping to determine the Structure-Activity Relationship of novel compounds targeting those pathways. Finally, since DesBCT is present in most of the NTM, the described mutants will allow for a screening of new inhibitors.

Kewords: Mycobacterium tuberculosis; non tuberculous mycobacteria; unsaturated fatty acid synthesis inhibitors; mycolic acid synthesis inhibitors

(1301) **REGULATION OF LIPID BIOSYNTHESIS IN GRAM-POSITIVE BACTERIA: ROLE OF THE ASP23 FAMILY PROTEIN YQHY AND THE END PRODUCT OF THE PATHWAY ACYL-ACP**

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Bacteria strictly control the synthesis of their membrane phospholipids in response to different nutritional conditions, but the underlying regulatory mechanisms are not fully known yet. The acetyl-CoA carboxylase (ACC) produces malonyl-CoA, a metabolite that is not only a substrate of FASII but also a key regulator of this biosynthetic pathway as it controls the activity of the global repressor FapR and hence the expression of the *fap* regulon in Firmicutes. In this work, we focused on YqhY, which belongs to the family of proteins without known function Asp23. It has been recently reported that a *Bacillus subtilis* Δ*yqhY* mutant rapidly acquire suppressor mutations that affect any of the subunits of the ACC, suggesting that YqhY could be capable of modulating lipid synthesis. In this work, we analyzed [^{14}C]-acetate incorporation into the lipid fraction of a *B. subtilis* Δ*yqhY* mutant and observed a 220% increase with respect to the isogenic wild type strain ($p<0.001$). In addition, we determined that YqhY is necessary for *B. subtilis* normal growth on solid sporulation medium (SM) and solid Spizizen minimal medium (SPI). Moreover, during stationary phase in liquid SM, 3/4 of Δ*yqhY* cells lyse when compared to the isogenic wild type strain ($p<0.05$) indicating a possible role of YqhY during sporulation. On the other hand, we investigated if long chain acyl-ACPs feed-back inhibit fatty acid synthesis. Previous evidence from our group indicated that the absence of the phosphate acyltransferase PlsX produces an arrest of fatty acid synthesis, possibly triggered by acyl-ACPs accumulation. We determined that expression of 'TesA, which degrades acyl-ACPs, in a *B. subtilis plsX* conditional mutant resulted in a 260% increase in acetate incorporation ($p<0.05$), supporting this hypothesis. Our results contribute to understanding the role of YqhY and acyl-ACPs in fatty acid synthesis regulation, shedding light into the control of an essential process in Gram-positive bacteria.

Keywords: YqhY, Acyl-ACP, lipid synthesis

(290) **RELATION OF CELLULAR ENVELOPES CHARACTERISTICS OF *LACTOBACILLUS* SPECIES TO THE PRESENCE OR ABSENCE OF S-LAYER PROTEIN**

Sandra Ruzal

To understand the envelopes organization regarding their relationship with the presence or not of the S-layer protein, we analyzed *L. acidophilus,* a S-layer bearing species compared to *L. casei* a non-S-layer-producing species by analyzing sensitivity to peptidoglycan (PG) hydrolytic enzymes, sensitivity to antibiotics with target cell wall (CW) metabolism, microbial adhesion to solvents (MATS) and Electron microscopy (TEM).

CW and PG sensitivity to Lysozyme or Mutanolysin are increased in *L. acidophilus*, being *L. casei* highly refractory to lyses. Evidence of structural differences in the CW was found by decreased minimal inhibitory concentration (MIC) of hydrolytic enzymes and antibiotics with target PG synthesis for *L. acidophilus.* This suggested that

PG was less cross-linked and/or that fewer layers are present. TEM showed no difference in PG layers thickness although a decrease ratio of thickness to radius is observed for *L. acidophilus*. This ratio correlates with the decrease yield of CW-SDS extraction for *L. acidophilus* compared to that obtained from *L. casei*. For analyzing peptidoglycan composition the fluorophore-assisted carbohydrate electrophoresis (FACE) methods was used and showed that peptidoglycan structure of *L acidophilus* was less cross-linked than that of *L.casei*.

An increase in hydrophobicity of *L. acidophilus* was also observed when compared to the highly hydrophilic *L. casei*. This was related to the protein content, as it was directly decreased after LiCl treatment.

The need of the external highly compact of S-layer protein in some species could be a consequence of the fragility of the cell wall, that show difference in the cell-wall structure (PG cross-linking) and envelope components. Studies on other S-layer-containing and non-containing species is the aim of our future work to corroborate the role of the S-layer in maintaining cell wall stability due to the fragility associated with the structure of peptidoglycan layers.

(1645) **ROLE OF A NEW LOCUS (*mapB*) IN THE BIOGENESIS OF *Brucella* CELL ENVELOPE**

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Brucella species are gram negative bacteria responsible of a zoonosis called brucellosis. They exhibit unique surface properties which make them furtive pathogens and more resistant to several host defence compounds. We have identified a *locus* of *Brucella suis -mapAB-* encoding the TAM machinery, that is proposed to participate in the translocation of autotransporters (ATs) through the outer membrane (OM) in gammaproteobacteria. Δ*mapB* strain showed enhanced sensitivity to lysozyme, Triton X-100 and polymyxin B, indicating that the cell envelope integrity is compromised. This effect was not due to major differences in the LPS structure or to altered total fatty acid composition. To explore the substrates of TAM in *Brucella suis,* label free proteomic analysis was performed. Some proteins related to cell division were absent in *ΔmapB* whole membranes fractions and OMP 25/31 porins were diminished. Regarding to ATs, there was an increase in the expression of 3xFlagBmaB monomeric autotransporter in the Δ*mapB* strain by western blot. However, BmaB was localized in the OM in both wt and Δ*mapB* strains. By SEM we found anomalies in the bacterial morphology of Δ*mapB*, such as more elongated and Y shaped bacteria. *In vivo* approaches indicated that the number of bacteria recovered from macrophages during the initial stages of infection was reduced in the mutant and it showed an attenuated phenotype in mice. Taken together, these results suggest that MapA/MapB are required for cell envelope biogenesis, probably assisting in the correct insertion of a broader subset of protein substrates, which are important for OM stability, virulence and cell division.

Key words: *Brucella*, TAM, membrane biogenesis, autotransporters.

(1025) **THE ROLE OF PEPTIDOGLYCAN HYDROLASES AND THEIR INHIBITORS ON THE CELL WALL HOMEOSTASIS**

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Peptidoglycan hydrolases are widely distributed among Gram-negative bacteria. These enzymes can hydrolyze bonds in the cell wall peptidoglycan and have been proposed to play an important role in the homeostasis of the periplasm, including cleavage for the generation of pores for assembling secretion systems, sites for the insertion of flagella, and lysis during cell division. Given the essential function of the bacterial cell wall, a fine-tuned balance must exist between synthesis and degradation of the peptidoglycan, because uncontrolled hydrolytic activity could result in autolysis of the bacterium. Although the contribution of peptidoglycan hydrolases in cell wall metabolism is well established, the mechanisms of their regulation remain mostly unknown.

Analysis of the genome of zoonotic pathogen *Brucella abortus* allowed us to identify a gene coding for a peptidoglycan hydrolase, which was named *sagA* (for Secretion Activator Gene A) because it is necessary for the secretion of a set of virulence proteins through the outer membrane. In order to understand the molecular role of SagA we developed a new assay to determine, *in vivo*, its peptidoglycanase activity. Additionally, we report the identification and characterization of a gene (Bab1_0102) encoding a periplasmic lysozyme inhibitor and demonstrate that the product of this gene is able to inhibit the activity of SagA in *Brucella abortus*. This new gene was named PhiA for Peptidoglycan Hydrolase Inhibitor A.

Our current hypothesis is that the physiological function of PhiA could be to control the autolytic activity of SagA and probably other peptidoglycan hydrolases within the periplasmic space of *Brucella abortus* in order to down modulate their potential harmful activities.

Keywords: Peptidoglycan hydrolases, lysozyme inhibitor, *Brucella*

(1606) **DYNAMIC RESPONSE OF THE *SINORHIZOBIUM MELILOTI* MEMBRANE DURING CYCLIC TEMPERATURE CHANGES**

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Sinorhizobium meliloti establish simbiosis with *Medicago sativa*. The production and storage of inoculants involve suboptimal conditions. Adaptation and survival in many harmful environmental conditions implies changes in the dynamics and plasticity of the bacterial membrane but may affect the symbiotic capacity.

In this work, we evaluate how cyclic changes in temperature affect the viability and phospholipids (PL) composition of *Sinorhizobium meliloti* and how these modifications affect the adhesion to alfalfa roots.

Culture was grown at 28°C until late exponential phase (control), then it was exposed to: (a) 3 h at 10°C, (b) 3 h at 40°C and (c) 3 h at 10°C. At each stage, an aliquot was taken to assess: the viability (CFU / ml), PL composition, the phase transition temperature (Tm) of the membrane lipid extract (by differential scanning calorimetry) and bacterial adhesion to alfalfa roots (CFU/ml).

The viability decreases after stage-(a), increases after stage-(b) and remain constant after stage-(c). The main PLs were phosphatidylcholine (PC), phosphatidylglycerol (PG), phosphatidylethanolamine (PE) and cardiolipin (CL). Exposure (a) resulted in a decrease in PC (34%) and PG (51%) while PE and CL increased (140% and 238%). The same cells submitted to (b) exhibited an inverse response (increase of PC and PG and decrease of PE and CL) and after stage-(c), the PL composition was slightly modified. The vesicles prepared with lipids extracted from control and from cells at the different stages of the T cycle differed in their Tm (14.8ºC; 9.8ºC;14.2ºC;11.8ºC).

At the end of stage-(a) a reduction in bacterial adhesion (80%) with respect to the control (100%) was observed. After stage-(b) the adhesion increased 150%, remaining constant after returning to 3h at 10°C (stage-(c)).

The successive thermal changes on the same bacterial population modifies the PL composition of the cellular envelope and affect its capacity of early interaction with alfalfa.

Keywords: *Sinorhizobium meliloti*, Thermal changes, Phospholipids, Root adhesion

INFECTOLOGY 12

(486) **THE *Chlamydia trachomatis* INSULINASE/PROTEASE CTL0175 IS REQUIRED FOR EFFICIENT RECOVERY UPON IFNg-INDUCED STRESS.**

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Chlamydia trachomatis (CT) is the most common sexually transmitted bacterial pathogen in humans. CT frequently causes asymptomatic, persistent infections leading to serious complications, particularly in young women. CT is an obligate intracellular bacterium and alternates between two developmental forms: the infectious elementary body (EB) and the replicative reticulate body (RB). In presence of stressors such as interferon-γ (IFNγ) or penicillin, CT enters a viable but non-cultivable, "persistent" state, allowing immune evasion for long periods of time. Once the stressors are removed, CT resumes replication and continues to propagate. CT has been considered a genetically intractable pathogen until very recently, thus the bacterial factors regulating chlamydial persistence remain poorly elucidated. Aiming to identify the genetic determinants involved in chlamydial persistence, we carried out a high throughput screening using a collection of ~800 fully sequenced CT mutants obtained by chemical mutagenesis and identified 8 mutants with defects in recovery upon IFNg- and/or penicillin-induced stress. We focused our study on a mutant strain (M275) carrying 4 point mutations. M275 shows a significant decrease in progeny generation after IFNγ-induced stress compared to the Wt. We then used lateral gene transfer to map the mutation/s responsible for the persistence defect. Recombinant strains that have cleaned the mutations in *bioF* and *ptr* genes show Wt recovery linking the defect to either *ptr* or *bioF* mutations. In parallel, we disrupted *ptr* in CT L2 by insertional gene inactivation with an intron carrying a spectinomycin resistance cassette (aadA). The *ptr::aadA* strain reproduced the defect in progeny-generation after IFNg-induced stress, linking *ptr* to chlamydial persistence. Our results strongly suggest that CTL0175, a non-characterized protease encoded by *ptr*, plays a role in chlamydial persistence upon IFNg-induced stress.

Keywords: Chlamydia trachomatis, persistence, IFNg, pathogenesis.

(499) **CHARACTERIZATION OF *Chlamydia trachomatis PMP* MUTANT STRAINS.**

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Chlamydia trachomatis (CT) is an obligate intracellular pathogen and the leading cause of sexually transmitted bacterial infections globally. CT resides inside a vacuole or "inclusion" and undergoes a life cycle involving the infectious elementary body and the replicating reticulate body. Polymorphic membrane proteins (PMPs) are a family of *Chlamydia*-specific autotransporter proteins with proposed roles in adhesion and antigenic variation. However, due to technical limitations in genetic manipulation of this bacterium, the role of PMPs in CT biology remains poorly elucidated. By chemical mutagenesis coupled with whole genome sequence analysis, we obtained 3 mutants with nonsense mutations in *pmpA/B/C*. Due to the presence of extra mutations/genome (D*pmpA*: 6; D*pmpB*: 17; D*pmpC*: 3) we backcrossed these mutants with a Wt strain to obtain recombinants carrying only the mutations in *pmp* genes. We obtained cleaned recombinants for pmpA/C. For pmpB mutant we were able to clean 14 of 17 mutations. We carried out IFU (Infectious Forming Units) assays in HeLa cells and observed that the generation of infectious progeny in the recombinants carrying nonsense mutations for pmpA/B/C was significantly impaired compared to Wt. These data point out that PMPs are important for efficient propagation of CT in epithelial cells. Additionally, by confocal microscopy and electron microscopy, D*pmpC* strain showed an aggregation phenotype that could not be trans-complemented with the Wt strain in coinfection assays, suggesting a role for PmpC in preventing aggregation of individual bacteria inside the chlamydial inclusion. We are currently using a recently developed technology for gene deletion in CT by insertion of a group II intron in order to obtain pmpA, pmpB and pmpC null mutants to evaluate the role of PMPs in CT pathogenesis in a mice model of infection.

Keywords: Chlamydia trachomatis, Polymorphic membrane proteins.

(1628) **A TWO-COMPONENT SYSTEM CONTROLS H_2O_2 PRODUCTION THAT AFFECTS INTRACELLULAR SURVIVAL OF *STREPTOCOCCUS PNEUMONIAE***

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Streptococcus pneumoniae is a main bacterial pathogen that usually colonizes the upper respiratory tract, but the transition mechanism from colonization to invasion of epithelial cells is not yet completely understood. Previously, we described that intracellular survival and acidic stress-induced lysis are controlled by the ComE response regulator, which also regulates competence development. Here, we demonstrated that a phosphomimetic form of ComE increased its DNA-binding affinity, while non-phosphorylatable ComE mutant displayed a lower DNA affinity. By molecular dynamic simulation, we predicted that ComE phosphorylation induces conformational changes in the DNA-binding domain of this regulator. To identify the ComE-regulated genes under acidic conditions, we performed a comparative transcriptomic analysis between the non-phosphorylatable (*np*) mutant and wild type (*wt*) forms of ComE. We detected a differential expression of 104 genes involved in different cellular processes suggesting that this pathway induces global changes in response to acidic stress. In the *np* mutant, the repression of *spxB* (which encodes for pyruvate oxidase) correlated with a decreased H_2O_2 production, whereas the overexpression of *murN* (which encodes for A peptidoglycan branched peptide synthesis protein) correlated with an increased resistance to antibiotic-induced lysis induced by cell wall antibiotics, with this phenotype being compatible with cell wall alterations. Furthermore, we found that the *np* mutant displayed a blocked acidic stress-induced lysis, while the acid tolerance response was augmented compared with *wt*. We think that these phenotypes might account for the increased survival in pneumocytes of the *np* mutant. We propose that the ComE pathway controls the stress response, thus affecting the intracellular survival of *S. pneumoniae* in pneumocytes, one of the first barriers that this pathogen must cross to establish an infection.

Key words: *Streptococcus pneumoniae*; two-component systems; pneumocytes; intracellular survival

(992) ***SHIGELLA SPP*. FORMER OF FLUVIAL BIOFILMS, WHICH HAVE MEDICAL IMPORTANCE, LIVING IN THE WÁTER OF SAN JUAN RIVER, COMING FROM A SPECIFIC SPRING**

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The microbiological importance of the water of San Juan river is vital to predict the impact that will have on public health. Biofilms constitute a protective mode of growth and development that help microorganisms survive in hostile environment, giving them advantages in colonizating of surfaces, for example, in the fluvial bank course. The river San Juan receives the support of the stream Los Tapones which is submit to an anthropic intervention such as pouring out sewage effluent coming from Bajo Segura´s water treatment plant. Previous studies made by UNSJ, have shown the presence of Salmonella spp. In this work, It was investigated the bacteriological quality of the water, in particular with respect to biofilms forming species, in the water of river San Juan specially in the stretch of Pinar (mouth of the dam Ignacio de la Roza)-stretch of the stream Los Tapones. Some river samples were taken in two points: S1- at the begging of this stretch and S2- at the end of it. These samples were incubated in devices designed for the development of biofilm for 5 years, which wereb removed and dispersed in buffered peptone water, it was incubated 24hs at 28°C. With the suspension were sowing in the selective EMB and SS. Biochemiscal tests were done in order to do the type of level identification. The 25% of the developed ufc selected randomly was isolated, generating the following results: S1: *E.coli*: 100%, S2: *E.coli*: 20%; *Shigella spp.*: 80%. In S1, it was just found *E.coli* while in S2, a population of E.coli , similar to S1,

was isolated and a population of *Shigella* almost 4 times bigger, this proof a epidemiological and ecological risk of the waters as it is a type with not only a high infective capacity but also for the pathology that it produce. These results can guide the governmental organisms about the politics steps require in order to do a sustainable management of the sanitary quality of the hydrological resource.

Keywords: Biofilms, Bacteria, Pathogenicity

(1083) **PAPRIKA EXTRACTS (*CAPSICUM ANNUUM*) FROM THE NORTH OF ARGENTINE INHIBITS THE VIRULENCE OF *ESCHERICHIA COLI* STRAINS**

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The production of protective biofilm (B) and the ability to move due swarming motility, suggest to *Escherichia coli* as an important concern in the food industry. Nowadays, the controls of food spoilage involve chemical preservatives. However, these compounds are potentially toxic. For this reason, spices, generally recognized as safe products, are promising alternatives. The aim of this work was to determine the anti-virulence properties of extracts (E) of paprika (*Capsicum annuum*) against two strains of *E. coli* ATCC 35218 and a food isolate LHICA S58. Their inhibition of B was determined by the crystal violet staining and its metabolism using the MTT assay. The effect of E against the bacterial swarming was investigated using the swarm agar method. Five E was obtained: hexane (EH), chloroform (EC), ethyl acetate (EEA), methanol (EM) and total methanol (ETM). The *E. coli* ATCC 35218 biofilm formation at 24 h was inhibited 86% by the presence of 100 μg/ml of EH. However, nearly all the extracts were able to inhibit the biofilm formation of *E. coli* LHICA S58, at a lower concentration (10 μg / ml). Concerning to the metabolic activity of B cells, for *E. coli* ATCC, at 10 μg/ml, EH and EC showed significant inhibition ($p<0.05$) and at 100 μg/ml all the extracts had significant activities, above all EH 52% and EC 47%. Against *E. coli* LHICA all the E at both concentrations showed significant inhibitions ($p<0.05$) higher than 20%, mainly at 10 μg/ml the EC 40%, EH 36%, and ETM 35%. In addition, the motility of the bacteria was 44% diminished by 200 μg/ml of EH in the culture collection strain as well as, 11% and 12% by EC and EM, respectively, in the isolate from food. Paprika used as a seasoning and flavoring in several foods is able to control virulence *E. coli* and could be considered as a promising natural preservative and food industry sanitizer.

(1103) **GALECTIN-1 ENHANCES CHLAMYDIAL INFECTION BY INCREASING N-GLYCAN-DEPENDENT INVASION OF HOST CELLS**

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Chlamydia trachomatis (Ct) constitutes the most prevalent sexually-transmitted bacterium worldwide for which no effective vaccine exists. Ct is an obligate intracellular pathogen that causes a broad range of acute and chronic genital pathologies in both, men and women. Chronic infections are responsible for severe reproductive tissue damage and lead to female tubal obstruction and infertility. In this work, we evaluate if Galectin-1 (Gal-1), a soluble carbohydrate-binding protein, participates in the recognition and attachment of Ct to human cervical epithelial cells. By lectin blot and flow cytometry, we described the glycan profile of the chlamydial cell wall and the epithelial host cell. We found that Gal-1 increases Ct binding to HeLa cells in a doses dependent manner, assessed by flow cytometry and confocal microscopy. Likely, Gal-1 promotes chlamydial infection by bridging bacterial N-glycans to eukaryotic membrane glycoproteins. Electron microscopy images suggest that Gal-1 could tie together bacterial N-glycans, enhancing bacteria-bacteria interactions and the invasion of host cells by 2-3 grouped Ct, thus, increasing even more chlamydial infection. In agreement, Gal1 increased chlamydial infection *in vivo* in an animal model of genital infection. Taken together, our results showed that Gal1 binds to both bacterial and host *N*-glycans, favoring the recognition, adhesion and internalization of this widespread obligate intracellular pathogen to human cervical cells. Unveiling the mechanisms used by Ct to invade host cells could help to find new therapeutic targets for controlling this highly frequent sexually-transmitted infection.

(1304) **ENHANCED SOLUBILITY AND ANTIBIOFILM ACTIVITY OF RIFAMPICIN BY COMPLEXATION WITH β-CYCLODEXTRIN AND ARGININE**

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Biofilms are highly organized bacterial communities with functional heterogeneity that are formed on biotic and abiotic surfaces. They protect bacteria from the harsh external environment via self produced matrices of extracellular polymeric substances. They are also more resistant to antimicrobial agents than the same bacteria growing in a free swimming state. Rifampicin (RIF) is an antibiofilm antibiotic, which is able to attack the *Staphylococci* in biofilm, but the effectiveness of this drug is hampered by its limited solubility at neutral pH and variable bioavailability. The objective of this study was to improve the solubility and antibiofilm activity of RIF by multicomponent complex (MC) formation with β-cyclodextrin (β-CD) and Arginine (ARG).

The inclusion ratio and binding constant were estimated from the phase-solubility studies (PSS). Complexes were prepared by the freeze-drying or physical mixture methods, and then characterized by infrared spectrometry (IR), thermal analysis (TA), powder X-ray diffraction (XRD) and scanning electron microscopy (SEM). The minimum inhibitory concentration (MIC) was evaluated by the macrodilution method according to CLSI indications. The effects of complexation against biofilms of *Staphylococcus aureus* resistant and sensible to methicillin (SAMR, SAMS) were assessed through the XTT reduction assay.

The PSS demonstrated that the presence of β-CD and ARG produced a significantly increase in RIF solubility. The TA and IR spectra of MC confirmed the molecular interactions between the components. SEM and XRD study showed that the MC was an amorphous solid. The MIC to RIF were 1 and 0.3 μg/ml to SAMR and SAMS, respectively. In biofilms, the MC caused a very significant reduction in cellular metabolic activity in both strains compared to free RIF.

The MC developed in this work might be a promising system for oral drug delivery to treat bacterial infections.

Keywords: rifampicin, multicomponent complex, antibiofilm activity, solubility

(1404) **EVALUATION OF MICROVESICLES CONTAINING SHIGA TOXIN TYPE 2 IN A RAT MODEL OF HEMOLYTIC UREMIC SYNDROME**

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Typical Hemolytic Uremic Syndrome (HUS) is a systemic complication after Shiga toxin producing *Escherichia coli* gastrointestinal infection. HUS mainly affects children under 5 years old and it is characterized by kidney and brain damage. Up today no specific clinical marker of this disease has been identified. The aim of the present work was to evaluate the presence of circulating microvesicles bound to Shiga toxin type 2 (MVs-Stx2) in a rat model of sublethal HUS, in attempt to detect a new clinical maker for HUS early diagnostic. For that, Sprague Dawely female rats (180-250 g) were intraperitoneally injected with Stx2 (0.5 ng/g, n=10) or with vehicle: PBS for Controls (n=8). Animals were daily weighted and blood was collected from the tail vein at 0, 48, 72 and 96 h after treatment. Plasma was obtained for creatinine and urea analysis. Plasma

samples were sequentially ultracentrifuged in order to obtain the MVs-enriched suspension. Then, MVs were labeled with Annexin V-FITC and MVs-Stx2 were detected with a mouse monoclonal anti-Stx2 antibody and a secondary antibody labeled with Alexa Fluor 555. MVs were analyzed by flow cytometry. In addition, two days after treatment rats were housed for 24 h in metabolic cages for urine collection and the creatinine clearance (CrCl) was determined. Rats injected with Stx2 had a significant body weight loss at 72 h and 96 h, compared to Controls ($P<0.01$ at 72 h and $P<0.001$ at 96 h). Both creatinine and urea were elevated in sera from Stx2 injected rats at 72 h and 96 h after injection ($P<0.05$). These results together with a decrease in the CrCl evidence a renal dysfunction. MVs-Stx2 were detected in Stx2 treated rats at 72 and 96 h after injection. However no linear correlation was detected between creatinine plasma levels and MVS-Stx2 ($P=0.5738$). These results indicate that MVs-Stx2 could be a clinical maker for HUS. Experiments are in process to determine if MVs-Stx2 could be a predictor for severity of the disease.

Keywords: Hemolytic Uremic Syndrome, Microvesicles, Shiga toxin, kidney

(1637) **IMPROVED ANTIBIOFILM ACTIVITY OF CIPROFLOXACIN LOADED IN EUDRAGIT E100 AQUEOUS DISPERSIONS AGAINST FLUOROQUINOLONE-RESISTANT *Pseudomonas aeruginosa*.**

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Rising antibiotic resistance and declining development and approval of new drugs motivates approaches to increase the efficacy of existing antibiotics. Previously we reported that fluoroquinolones-containing Eudragit E100 aqueous dispersions exhibit enhanced bactericidal effect against multidrug-resistant *P.aeruginosa* in planktonic state. However, *P.aeruginosa* is an opportunistic pathogen with ability to form biofilms, which are inherently resistant to both antimicrobial agents and host defenses, causing serious problems in the chemical, medical and pharmaceutical industries. Therefore, there is a significant need to develop alternatives to prevent biofilm formation and, more importantly, to disperse or eradicate preattached biofilms.

The aim of this work was to evaluate the effect of Ciprofloxacin-containing Eudragit E100 aqueous dispersions (EuCl-CIP) on biofilms of *P.aeruginosa* formed in Mueller-Hinton broth with standardized bacterial inoculum (10^6 CFU/mL) and incubated at 37°C for 48h. The remaining metabolic activity after 24 h of treatment exposure was quantified (490 nm) using the tetrazolium salt XTT reduction assay. For comparative purposes, the effect of free-drug polymer aqueous dispersions (EuCl) and some concentrations of ciprofloxacin (CIP) were evaluated simultaneously.

Both EuCl and CIP showed partial reduction of cell viability in a concentration-dependent manner. However, the effect was increased when mature biofilms were treated with EuCl-CIP.

The biofilm matrix of *P.aeruginosa* contains a high proportion of alginates, among other components. The positive charges present on Eudragit E100 would be interacting with such components causing alterations in matrix integrity and making CIP more accessible into biofilm.

Using the Eudragit E100 cationic polymer as carrier for antimicrobials is an effective strategy to enhance the efficacy of the drugs on bacterial biofilms, in addition to their effect on planktonic cells.

Keywords: Biofilms, Eudragit E100, *Pseudomonas aeruginosa*, Ciprofloxacin

(1669) ***Pseudomonas aeruginosa* INTERNALIZATION THROUGH EFFEROCYTOSIS UPON BINDING TO APOPTOTIC CELLS: IMMUNE RESPONSE AND BACTERIAL CLEARANCE**

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Instituto de Investigaciones Biotecnológicas Dr. Rodolfo A. Ugalde (IIB-INTECH), UNSAM- CONICET

Pseudomonas aeruginosa (*PA*) is an opportunistic pathogen that infects vulnerable patients, such as those with cystic fibrosis (CF). An advance towards understanding infections caused by *PA* would be to elucidate the mechanisms that operate in the bacteria-host interplay. We have shown that *PA* exhibits a remarkable tropism towards dead cells. As bacteria interact with a polarized epithelium, they attach and aggregate almost exclusively on extruded apoptotic cells, while the rest of the surface seems reluctant to bacterial adhesion. We further showed that *PA* is internalized by epithelial cells surrounding the infected apoptotic cell through efferocytosis, a process in which apopotic cells are engulfed and disposed of by other cells. Bacteria are then eliminated intracellularly. Here we show that two previously characterized *PA* cystic fibrosis isolates (mucoid/non-mucoid) also showed adhesion to extruded apoptotic cells, internalization of bacteria through efferocytosis and a similar intracellular survival pattern. The small GTPase Rac1 has been shown to be a key regulator of apoptotic cell engulfment. We used a MDCK cell line that expressed a dominant-negative version of Rac1. These cells not only showed impaired uptake of apoptotic cells but also diminished *PA* internalization. We speculate that bacteria-laden apoptotic cells are targets for professional phagocytes affecting thus both the innate and the adaptive immune responses. We have now extended our analysis to macrophages (J774 macrophage-like) and dendritic cells (from mouse bone marrow). We have seen that professional phagocytes are able to internalize bacteria-laden apoptotic material. We are on our way to evaluate how this affects cytokine's production, antigen presentation pathway and bacterial killing. Our studies may help to understand why contexts such as CF, which is characterized by chronic lung inflammation, unusual production of apoptotic cells and impaired efferocytosis, favor *PA* colonization.

Keywords: efferocytosis, *Pseudomonas aeruginosa*.

ONCOLOGY-ONCOIMMUNOLOGY 19

(1481) **ANTITUMOR EFFECT OF RAC1 INHIBITOR 1A116 IN COMBINATION WITH TEMOZOLOMIDE IN GLIOBLASTOMA MULTIFORME**

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Rho GTPases represent a family of small GTP-binding proteins involved in cell cytoskeleton organization, migration and proliferation. The aberrant activation of Rac1 GTPase is involved in tumor progression, invasion and chemoresistance in brain tumors. Rac1 is hyperactivated in glioblastoma cells and Rac1 appears to be a suitable target for the development of novel anticancer therapies. Rac1 inhibitors as monotherapy or in combinational settings with traditional chemotherapy such as Temozolomide (TMZ) could have a profound effect on the disease progression.

We analysed the gene expression profiles from Gene Expression Omnibus (GEO) database and found a high correlation between Rac1 expression and poor patient outcome in 80 glioblastoma patient samples. We also examined MGMT expression, the major biomarker for this tumor type, but it did not correlate with patient outcome in our analysis. We tested the effect of 1A116 *in vitro*, a novel Rac1 inhibitor developed by our group, on a panel of neural tumor cell lines.1A116 showed IC_{50} values between 9 μM and 30 μM. We also tested TMZ effect on the same cell panel, showing IC50 values between 100 μM and over 500 μM depending on MGMT status. We combined both drugs and found an interesting cooperative effect. These results show the potential use of Rac1 inhibitor 1A116 as therapeutic agent in combination with TMZ for glioblastoma treatment.

Keywords: Rac1, GBM, TMZ, MGMT

(1273) **COMBINATION OF SUICIDE GENES FOR CANINE MELANOMA TREATMENT: IN VITRO STUDIES**

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Abstract: Looking for better effects of suicide genes on spontaneous canine melanoma, we studied the responses to herpes simplex virus thymidine kinase (HSVtk) and yeast *Saccharomyces cereviseae* cytosine deaminase/uracil phosphoribosyl transferase fusion protein (Ycd::Yuprt). HSVtk phosphorylates ganciclovir (GCV) that finally stops DNA replication. Ycd catalizes the passage from 5-fluocytosine (5-FC) to 5-fluorouracil (5-FU) a thymidine analogue that interferes with DNA replication while Yuprt drives to the synthesis of 5-FUTP that inhibits rRNA and mRNA processing. Monolayers of Bk canine melanoma cells were lipofected with plasmids carrying the fusion gene and subsequently the different concentrations of pro-drugs were added. As evidenced by a fused green fluorescent protein/HSVtk, fluorescent cells could be detected for more than 6 days after lipofection and treatment with GCV. The Bgal-lipofection efficiency for Bk cells was 3.0 ± 0.8 %, as determined by counting the X-gal blue stained cells. The 50% inhibitory concentrations (IC50) for the assayed suicide genes were 0.25 ± 0.10 mg/ml for GCV and 0.63 ± 0.22 μM for 5-FC. We found that serial dilutions (up to 10% of the initial concentrations) of the therapeutic genes with the non-therapeutic *Escherichia coli* B-galactosidase did not correlate with loss of cytotoxicity, suggesting that we were in the presence of a strong bystander effect. Furthermore, the best cytotoxic effects on Bk melanoma cells were found when both HSVtk and Ycd::Yuprt were co-lipofected and both pro-drugs were simultaneously added as compared to either single treatments (HSVtk/GCV or Ycd::Yuprt/5-FC vs. HSVtk/GCV + Ycd::Yuprt/5-FC; $p<0.0001$). Our encouraging results support further research on the combination of suicide genes for local treatment of melanoma tumors in companion animals.

Keywords: lipofection, bystander, thymidine kinase, cytosine deaminase.

(1020) **CYTOTOXIC EFFECTS OF *ALLOYSIA POLYSTACHYA* EXTRACT ON THE TUMOR CELLS**

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Alloysia Polystachya (AP) is an aromatic native plant of Verbenaceae family which is widely distributed in subtropical regions of South America and the North of Argentine. Therapeutic uses of this and other species of *Alloysia* include febrifuge, sedative, stomachic, diuretic, and antispasmodic activities. Moreover, previous studies have demonstrated the anti-metastasic effects of thujone (part of its chemical composition) in melanoma. We have previously demonstrated that cells overexpressing RAC3 are more resistant to cell death, autophagy and senescence induced by several stimuli. Being this molecule overexpressed in several tumors, the aim of this work was to investigate the putative anti-tumoral effects of AP extract in cell lines that naturally (tumoral) or by transfection (non tumoral) overexpress RAC3. For this purpose, we prepared AP hydro-alcoholic extracts (APE) that were assayed over different cell lines. We found that APE (dilutions 1/30 to 1/2000) induced a significant inhibition of cell number after 24h of treatment in human tumor cell lines (HeLa, HCT116, T47D) and the non-tumoral HEK293 overexpressing RAC3, as determined by crystal violet staining ($p<0.05$). This effect involved at least apoptosis induction, as determined by Acridine Orange/Ethidium Bromide staining (more that 50% of apoptotic cells at 30h for all cell lines) and correlated with Caspase 3 activation, as determined by western blot assay. In addition, through bioinformatics studies (DockingServer, SwissDock and Target Prediction softwares) we identified thujone as a possible ligand that could bind RAC3 acetylase domain with high affinity, suggesting perhaps this APE component as a possible mediator of cell death induction in cells overexpressing RAC3, although this and other mechanisms remains to be investigated. Our results suggest that autochthonous plant derivatives could be attractive tools to be investigated as future oncologic therapeutics.

Keywords: *Alloysia Polystachya*, apoptosis, RAC3, tumors

(1501) **DOWNREGULATION OF HTERT AND DECREASE IN TELOMERASE ACTIVITY MEDIATED BY INHIBITION OF RAC1 IN A GLIOBLASTOME MULTIFORME MODEL.**

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Abstract: The telomerase holoenzyme is a specialized ribonucleoprotein reverse transcriptase that directs the telomeric DNA synthesis. This enzyme is repressed in normal human somatic cells, but is activated in most cancers. Little is known concerning how telomerase activity is activated and maintained in cancer cells. Rho family GTPases are critical regulators of cellular functions that play important roles in tumoral progression. Aberrant activity of Rho small G-proteins, particularly Rac1 and their regulators, is a hallmark of cancer, and contributes to the tumorigenic and metastatic phenotypes of cancer cells. In this work we investigated the effects of inhibiting the activation of Rac1, by a novel inhibitor 1A-116, on the regulation of telomerase activity of glioma cell lines. Treatment of LN229 and U87-MG cell lines with 1A-116 5 μM for 24 hs produced a significant decrease in telomerase activity. This regulation was mediated by P53. The inhibition of Rac1 increased the levels of P53 expression, possibly, by preventing the degradation of this protein in the proteosome. The increase in p53 levels triggers a negative regulation in the expression of hTERT mediated by the upregulation of p21 expression. These results describe a new way to modulate hTERT expression, and a direct link between the Rac1 activation state and telomerase.

Keywords: Rac1, Telomerase, Inhibition, hTERT, p53

(472) **NATURAL PRODUCTS AS A SOURCE OF NOVEL SYNTHETIC LETHALITY INDUCERS IN BRCA-DEFICIENT CANCER CELLS**

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Induction of synthetic lethality (SL) is a precision strategy which takes advantage of tumor mutations to induce selective tumor death. A high incidence type of breast and ovarian cancers are characterized by *BRCA1/2* germline mutations, which can thus be used as a genetic background to screen for SL inducers.

Natural products provide a vast source of compounds for drug development due to their great chemical diversity. Thus, the aim of this work was to search for synthetic lethality inducers in a library of plant extracts and pure compounds from Argentina. We used a cell-based screening platform that compares *BRCA1/2 deficient with proficient cells*. In this platform the three cells populations are co-cultured and simultaneously exposed to the plant extracts and pure compounds. Cell survival is calculated considering the ratio of *BRCA*-proficient and *BRCA*-deficient populations at the end of the experiment.

We found one family of pure compounds that triggers SL in BRCA*2*-defficient cells and one active alkaline extract with SL activity against *BRCA1*-defficient cells. To identify the active/s compound/s in this extract we perform bio-guided fractionation using VLC and Sephadex chromatography. We found 3 members of the furoquinoline family of alkaloids as the most active compounds.

Keywords: Synthetic lethality, natural products, breast cancer, BRCA1/2, cell-based screening

(34) **PHOTOTOXIC ACTION OF A LIPOPHILIC Zn(II) PHTHALOCYANINE IN TUMOR SPHEROIDS AND A MURINE COLON CARCINOMA *IN VIVO* MODEL**

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Phthalocyanines have been found to be optimal photosensitizers for photodynamic therapy. In a previous work, we demonstrated the cytotoxic action of a lipophilic Zn(II) phthalocyanine (Pc9) encapsulated into poloxamine polymeric micelles (T1107) in two-dimensional cultures of CT26 cells (murine colon carcinoma, IC_{50} = 10 ± 2 nM). After localizing mainly in lysosomes and endoplasmic reticulum, Pc9-T1107 formulation induced an apoptotic cell death mediated by the generation of reactive oxygen species. In this study, in order to explore the *in vivo* efficacy of Pc9-T1017, we tested two experimental models: three-dimensional CT26 cell cultures and a syngeneic model of Balb/c mice bearing CT26 tumors. When different concentrations of Pc9-T1107 were incubated with CT26 spheroids, no effect on cell survival was evident in the dark, while a significant decrease was shown after irradiation, being the IC_{50} value of 370 ± 11 nM. Thus, after incubating spheroids for different time periods, the volume of Pc9-treated spheroids was smaller than non-treated spheroids. Furthermore, spheroid growth kinetics was dependent on Pc9 concentration. An apoptotic mechanism of cell death was also confirmed in Pc9-exposed spheroids by the observation of highly condensed nuclei stained with Höechst 33258 and by detecting a significant decrease in procaspase-3 expression levels. *In vivo* assays revealed that the administration of 0,074 mg/kg of Pc9-T1107 reduced 46% tumor volume, without affecting body weight of treated-mice. Histological analyses of control and treated tumor slices showed an 11% and 51% of necrotic areas, respectively, after hematoxylin–eosin staining. Furthermore, an increase in the expression levels of Bax and a decrease in Bcl-2/Bcl-X_L/Bid levels were determined after WB of tumor lysates. In conclusion, our results indicated that Pc9 behaves as an efficient photosensitizer inducing an apoptotic cell death both in 3D cultures as well as in a colon carcinoma *in vivo* model.

(110) **DEVELOPMENT OF A NEW BREAST CANCER MOUSE MODEL TO STUDY MECHANISMS INVOLVED IN THE *IN SITU* TO INVASIVE TRANSITION**

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The transition of ductal carcinoma in situ (DCIS) to invasive breast carcinoma requires tumor cells to cross the basement membrane (BM). However, mechanisms underlying BM transmigration are poorly understood. Although there are currently several mouse models with different characteristics, none have been entirely successful in understanding tumor progression in particular in the early stages. The cellular model LM38 consists in three cell lines: LM38-LP (luminal and myoepithelial), LM38-HP (luminal) and the LM38-D2 (myoepithelial).

The development of a tumor model of intraductal growth using the LM38 cell lines is proposed, evaluating the behavior regarding the degree of invasion and the interactions between each of the cellular components that form the tumor.

8- to 10-week old virgin female BALB/c mice were anesthetized, nipples of both inguinal glands #4 were snipped and 2 µl of cell suspension containing $2x10^3$ cells/µl were injected in each gland. After intraductal injection, whole-mount and histology staining revealed that in glands injected with HP and D2 lines, no tumor was observed at either three or six weeks after inoculation (HP: 0 positive glands out of 16 and D2: 0 positive glands out of 20). Inoculation of the bi-cellular LP cell line developed *in situ* tumors, 3 weeks post-intraductal injection which were surrounded by a dense belt of collagen fibers and mioepitelial cells (SMA-*alpha* positive stained by Immunofluoresce). DCIS tumors further progressed to invasive lesions by 5 weeks with a disrupted stromal collagen organization (LP: 21 positive glands out of 46; $p<0,001$ X^2 test vs HP and D2 injected glands).

In this work, a murine model that mimics human pathology in terms of tumor progression was successfully obtained. Besides, the fact that only the LM38-LP model was able to develop tumors indicates that the interaction between the two populations (luminal and myoepithelial cells) confers an advantage for tumor growth.

(1148) **ANTIPROLIFERATIVE ACTIVITY OF BLUEBERRY EXTRACT AGAINST A DIVERSE PANEL OF TUMOR CELL LINES**

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Abstract: Blueberries have been considered to be one of the fruits with the highest antioxidant potentials. Among the most prevalent phytochemicals in berries are the anthocyanin, which are responsible for much of their antioxidant activity and have been reported to have positive impact on health and cancer prevention.

We previously showed that a hydroalcoholic blueberry extract inhibits the proliferation of CT26 and H125 tumor cells. Both, migration and invasion of CT26 were also modulated by the extract. In this work, we reported the production of a new blueberry extract using a different extraction protocol. Frozen blueberries (samples of 1 Kg) were first homogenized in 0,5 L of ethanol (96%) using a domestic blender. Anthocyanins and other phenolic compounds in the homogenates were then extracted at room temperature during 96 h, protected from light. The homogenization step was introduced to achieve a more efficient extraction of anthocyanins. This protocol rendered an extract with a high anthocyanins concentration (505,9 mg/L), determined by the pH differential method as cyanidin-3-glucoside equivalents. The antiproliferative activity of the new blueberry extract was evaluated against a diverse panel of tumor cell lines of different origin, including Colo 205, CT26, MDA-MB-231, H125, PC-3 and SN12C. For all cell lines, the blueberry extract showed a dose dependent inhibitory effect on cell proliferation, with IC_{50} concentration ranging from 6,1 to 17,6 mg/L (cyanidin-3-glucoside equivalents). These results provide a direction for further researches about the antitumoral potential of blueberries.

Keywords: Blueberries, Anthocyanin, Antiproliferative, Cancer

(61) **TARGETING POST-PRENYLATION PROCESSING IN CANCER: STUDY OF THE ROLE OF ICMT IN TUMOR PROGRESSION**

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Most human cancers inactivate the tumor suppressor p53 through missense mutations, leading to the abundant expression of full-length point mutant proteins. p53 mutants not only lose the tumor suppressor function but also may acquire novel activities that promote tumor progression. Despite evidences showing that mutant p53 enhances tumor aggressiveness, the mechanisms underlying this activity are still poorly understood. Even if the mutation abrogates the ability to act as a transcription factor, point mutants alter gene expression through mechanisms that are not well characterized. In order to characterize those mechanisms we studied the effects of mutant p53 on gene expression. Basing on data from microarray analysis on MDA-MB-231 Triple Negative Breast Cancer cells we selected candidate genes and we confirmed the effect of mutant p53 in qRT-PCR assays. We focused on ICMT, which regulates subcellular localization and function of proteins with a C-terminal CaaX motif, including members of the RHO family, by catalyzing carboxymethylation after prenylation. To characterize the effect of p53 family members on transcription we generated reporters containing the ICMT promoter. We found that ICMT expression is regulated by complex interactions between p53 family members. We extended the characterization by deletion mapping and chromatin chIP. Our results also showed that ICMT enhances human tumor cell clonogenic potential *in vitro* and tumor formation *in vivo* in nude mice. By using three dimensional cultures we found that ICMT is able to disrupt epithelial architecture, suggesting a role in the acquisition of aggressive traits. The analysis of public databases also supports our findings on the role of ICMT as an enhancer of tumor progression and on the link with the p53 pathway. Collectively, our

data shows that ICMT deregulation cooperates with the acquisition of aggressive phenotypes and suggests that the interplay with the p53 family may affect tumor progression.

Keywords: Cancer, Mutant p53, ICMT.

BIOPHISICS 9

(1423) **MICROTUBULE BUCKLING IN LIVING CELLS: A COMPUTATIONAL AND EXPERIMENTAL ANALYSIS**

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Microtubules (MTs) are filamentous biopolymers involved in essential biological processes. They form key structures in eukaryotic cells, thus it is very important to determine the mechanisms involved in the formation and maintenance of the microtubule network. Microtubule bucklings are transient and localized events commonly observed in living cells and characterized by a fast bending and its posterior relaxation. These events present characteristic sizes of a few microns and occur naturally in living cells. Active forces provided by molecular motors have been indicated as responsible for most of these rapid deformations. We explore the temporal evolution of MT bucklings and observe that while buckling sizes and durations may vary amongst events, they can be described by the same mechanical approach. In order to obtain an in-depth understanding of these episodes we performed numerical simulations of filaments in a cytoplasmatic-like environment under different load scenarios and were able to associate force application on the MTs with buckling amplitude progressions. Using a two color fluorescence technique we study the interplay between transported vesicles (fm4-64 endosomes) and MTs, in particular we focused on cases were the transported load seemingly caused a buckling event. With these experimental examples we drew a final feedback with the numerical simulations and observed the buckling evolutions in cases were the force acts parallel o perpendicular to the filament.

Keywords: microtubules, buckling, active forces, living cells, fluorescence microscopy.

(785) **APPLICATION OF HIGUCH'S FRACTAL ALGORITHM IN CENTRAL BLOOD PRESSURE PULSE WAVE AND ITS CORRELATION WITH CARDIAC OUTPUT, IN HYPERTENSIVE PATIENTS.**

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Central blood pressure pulse wave (CBPPW) shape is determined by the pattern of ventricular ejection and arterial elasticity, and it represents the load to which the heart is submitted. Fractal algorithm application in CBPPW provides a mathematical model which could predict its behaviour. The conservation of fractal features could estimate the adaptability of the system, while the loss of this features would imply an inability to adapt. It has been proposed to study the utility of Higuchi's Algorithm (HA) for the analysis of the behaviour of CBPPW and the posible relation with the cardiac output (CO). 130 waves from non-treated hypertensive patients, males and females, between 40 and 60 years have been considered. The waves were obteined by Mobil-O-Graph equipment. The fractal dimension (FD), and the Coefficient of regression (R^2) (below 0.8 would indicate the loss of fractal features) of the systolic peak (sp) and the diastolic valley (dv) were determined by the application of HA, and were later correlated with with the CO. Statistical analysis of DF and R^2 was expressed as mean (M) and standard deviation (±). Pearson's coefficient of determination was estimated between DF and R^2 obtained with CO. InfoStat/L software was required. Following results were obtained: DF(ps): M=0,05 ±0,12; R^2(sp): M=0,58 ±0,12; DF(dv): M=0,11 ±0,19; R^2(vd): M= 0,53 ±0,34. Pearson's coefficient of determination between DF (sp) versus CO = 0,11 p< 0,21; between R^2 (sp) versus CO = 0,15 p<0,0094; between DF (dv) versus CO = 0,29 p<0,009; between R^2(dv) versus CO = 0,009 p<0,3. It has been concluded that HA application could reveal the loss of fractal properties in CBPPW from hypertensive patients. The variation of fractal parameters of the of CBPPW can be correlated with determining hemodynamic variables of arterial pressure such as CO.

(822) **ASSESSING THE ROLE OF PHOSPHOLIPID-PROTEIN INTERACTIONS IN CALCIUM PUMP DYNAMICS: INSIGHTS FROM *IN SILICO* STUDIES.**

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Sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA) is a P-type ion transporter from skeletal muscle whose function consists in pumping Ca^{2+} from the cytoplasm into the reticulum with the consequent muscle relaxation. This active transport has been previously described by means of X-ray experiments involving large-scale rearrangements of both cytoplasmic and transmembrane domains. Here, with the object of studying the structural and dynamic behavior of SERCA we performed molecular dynamics simulations at a coarse grained (CG) level. This kind of simplified representations allow the study of large size simulation boxes reaching multimicrosecond timescales. As a preliminary step, we developed a set of CG topologies for phospholipids compatible with the SIRAH force field (http://www.sirahff.com). Simulations were performed using GROMACS 4.6.7. Starting configurations were generated embedding the SERCA reaction state E1-Ca^{2+} (PDB:5XA7) in planar lipid bilayers of different composition and simulating them by triplicate through 3ms at a temperature of 310K. Specific phospholipid-protein interactions reported from crystallographic structures were monitored during the simulations. Basic residues and lipid phosphate groups formed salt bridges showing two classes of interactions: i) side chains emerging from the hydrophobic region and fixing particular phospholipids (i.e Lys262) and ii) side chains approaching the polar head groups from the cytoplasm and changing interacting partners (i.e. Arg110). Along the simulations the transmembrane domain tilted 35º, as observed in experimental data. This behavior was governed by a Trp belt distributed in the membrane-water interface. Combined with experimental data, this in silico approach contributes to better understand the dynamic behavior of SERCA being also a promising strategy to study other transmembrane proteins.

Keywords: Ca^{2+} pump, lipid-protein interactions, molecular dynamics, coarse grain models.

(1668) **BIOINFORMATICS ANALYSES OF TWO DEFENSIN-LIKE PROTEINS FROM *Silybum marianum* FLOWERS**

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Plant defensins and defensin-like proteins (DEFL) are small, basic, cysteine-rich proteins of the innate defence system with a conserved tertiary structure —the cysteine stabilized α/β motif— encompassing a loop named γ-core connecting two β-strands linked to antifungal activity. We cloned and predicted the tertiary structure of two DEFL from *Silybum marianum* flowers (DefSm1D and DefSm2), we also compared both DEFL at sequence and structure level as well as performed a functional analysis based on the predicted structures. Both DEFL are 54 aminoacid proteins with 63% sequence identity, estimated masses *ca.* 6 kDa and pI above 8.5 (Compute pI/Mw tool). Analysis of both γ-core sequences revealed the presence of 3 and 4 positive residues, respectively, probably related to interaction with negative charges on target membrane and/or pathogen cell wall. Structural models of DefSm1D and DefSm2 were built using MOD-

ELLER v9.14 based on Artv1 (PDB: 2KPY) and Ah-AMP1 (PDB: 1BK8) crystal structure; templates were found with HHPred fold assignment method. Comparative analysis of structural models (RCSB PDB Protein Comparison Tool) revealed that sequence variation between DEFL had an effect on the overall structure of these peptides with a global RMSD (root-mean-square deviation) difference of 2.33 Å. Structural models functional analysis (ProFunc server) showed that both defensins share significant results for extracellular component; defence, stress, and biotic stimulus response; killing of cells from another organism and antifungal response. Structural models electrostatic surface potential calculated solving the Poisson-Boltzmann equation (PBEQ-Solver) show a surface charge distribution that endows peptides with amphipathicity, which would allow them to interact with the pathogen's cell membrane. Both DEFL share a well conserved tertiary structure but variability in primary amino acid sequence that can change the activity spectrum exhibited by these closely related proteins.

Keywords: plant defensin, thistle flowers, antifungal protein

(402) **CELL SURFACE RECEPTORS IMPROVE THEIR SIGNALING PROPERTIES WHEN WORKING FAR FROM STEADY-STATE: A MATHEMATICAL/COMPUTATIONAL STUDY**

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Sensing extracellular changes initiates signal transduction and is the first stage of cellular decision-making. Ligand binding to cell membrane receptors is a key event in those sensing stages. It is rarely certain whether cellular responses are related to initial changes in receptor binding or to the level of receptor binding achieved at some later time, but it is likely that the dynamics of receptor/ligand binding contributes significantly to the dynamics of the response. Particularly, certain properties of the sensing steps are usually characterized in equilibrium, like the value of half-maximal effective concentration, the dynamic range, and the Hill coefficient. However, if the time constant of downstream signal transduction steps is shorter than that of ligand-receptor binding, those properties should be evaluated in pre-equilibrium.

Using a simple monovalent binding model, a two-state binding model, and two limiting cases of this last one in which only one receptor form can bind or release ligand, we studied the mentioned properties in pre-equilibrium. We combined analytical tools when possible, with computational modeling and parameter space exploration.

Our results imply that pre-equilibrium sensing is possible depending on the relation of binding and activation rates. When binding rates are slower than activation rates, the system can sense high dose concentrations on pre-equilibrium. Conversely, when binding is faster than activation, pre-equilibrium sensing properties remains similar than steady state properties. Moreover, when the time scales are similar, pre-equilibrium sensing is possible but with certain limitations, depending on the time constant and the ligand concentration involved on the downstream process.

Keywords: Signaling, Dynamic Range, Computational Biology

(745) **FACTORS AFFECTING THE COMPUTATION, AT THE DFT-LEVEL OF THEORY, OF THE ONE-BOND C^{α}-H SPIN-SPIN COUPLING CONSTANTS IN PROTEINS. TEST ON UBIQUITIN.**

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Interest centers here on the analysis of the different factors that affect the computation, at DFT-level of theory, of the one-bond spin-spin coupling constant (SSCC), $^1J_{C\alpha H}$, in proteins. For this purpose, the agreement between observed and computed $^1J_{C\alpha H}$ SSCC, from a large ensemble of NMR- and X-ray derived conformations of the 76-residue α/β protein ubiquitin, was explored by using a combination of approaches that include, among others, treating each amino acid **X** in the protein sequence as a terminally-blocked tripeptide with the sequence Ac-G**X**G-NMe. Despite small differences, arising from the dielectric constant value, the functional and the basis set chosen, the results of our benchmarks indicate that the accuracy of the computed $^1J_{C\alpha H}$ SSCC critically depends on the molecular geometry, namely on the ϕ, ψ, and c1 torsional angles; with a dominant influence of the ψ torsional angle. This work contributes to our general understanding of the main factors affecting the computation of the $^1J_{C\alpha H}$ in proteins, at DFT-level of theory, and provides critical insight about the limitations to reproduce the observed $^1J_{C\alpha H}$ SSCC values.

(550) ***NOVEL MYCOBACTERIUM TUBERCULOSIS* PKNG INHIBITORS. A COMPUTATIONAL-EXPERIMENTAL STUDY**

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Tuberculosis is a chronic disease caused by the bacillus Mycobacterium tuberculosis (Mtb) that remains a leading cause of mortality worldwide. The search for new protein targets and their inhibitory compounds became a priority due to the emergence of multidrug and extremely drug resistant strains. Pkns are the main kinase family in Mtb, consisting of 11 members including pknG, a protein that plays a central role in energy metabolism and the infection process making it an excellent target for drug design. In the present work, we have used docking and molecular dynamics simulations to search for a competitive inhibitor of the ATP binding site of pknG. Three different libraries of small compounds: 1) a standard purchasable compound library of 2.7 million compounds; 2) a kinase focused set from GSK consisting of 360 compounds, and 3) a small fragment based library of 600 compounds. The docking was performed with the rDock program using a pharmacophoric restraint on the kinase hinge interactions. Docking top rank compounds were subject to a protocol of dynamic undocking for further evaluation. Finally, the best 12 compounds from the first set, all the fragments, and the best 10 GSK compounds were tested for inhibition of in vitro kinase activity. We only found actives in the fragment set, which will be further evolved into a full-size drug like compounds.

Keywords: Tuberculosis, Drug Discovery, Docking, Molecular Dynamics, pknG

(419) **HIERARCHY OF FEEDBACKS IN BIOLOGICAL OSCILLATORS: ITS IMPACT IN THE CHARACTERISTICS OF THE OSCILLATIONS**

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In a wide variety of cellular processes biological oscillations occur, like in genetic regulatory networks associated with circadian rhythms. It is of central importance to understand the impact of perturbations in the characteristics of the oscillations. Recently, we have studied a relaxation oscillator built from a combination of non-traditional positive and negative feedbacks, with the positive feedback built from the compartmentalization of a simple cell signaling pathway, like a covalent modification cycle, and the negative feedback built from a sequestration mechanism with a substrate as a downstream target of the active protein. Here, we aimed to characterize a group of relaxation oscillators generated from combining slow negative feedbacks with fast bistable systems of different nature. We explored the bistability caused by (1) an explicit positive feedback, (2) compartmentalization, (3) double phosphorylation or (4) shared enzymes. For each, we compared the amplitude and period sensitivities. To

do this, we randomly generated many parameter sets, measuring the amplitude (A) and period (T) for each oscillatory set. Then, we increased each parameter by 2%, measuring A and T for each increase and constructing in this way a sensitivity plot. For the substrate in the negative feedback module, we found that the median of the sensitivities for A and T had comparable values. On the other hand, for the active protein upstream of the substrate, the sensitivity for A was considerably lower. Also, this sensitivity increases with the parameter that determines the frequency (f). This is in lockstep with the analysis of the A vs. f curves. By doing a sweep of the parameter that controls f, we found a near constant amplitude for the oscillations of the active protein over a wide range of frequencies. While this is expected in oscillators that combine traditional positive and negative feedbacks, our findings show that the same can happen for non-traditional feedbacks.

Keywords: cellular oscillations, feedbacks, sensitivity

(379) **EXPLORING BINDING INTERACTIONS IN LIVING CELLS THROUGH ADVANCED FLUORESCENCE MICROSCOPY AND NUMERICAL SIMULATIONS**

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Fluorescence microscopy is widely used to extract information on intracellular dynamics in living cells. Unfortunately, dynamical processes do not allow following simple rules. The combination of the experimental analysis with Monte Carlo simulations could help with the interpretation of the data and interrogate on the limitations and advantages of the used microscopy technique.

We have previously studied the nuclear, dynamical distribution of the glucocorticoid receptor (GR) and one coactivator in living cells by fluorescence correlation methods. To get insight into these experimental observations, we used the Fluorescence Emission Recipes and Numerical Routines Toolkit to analyze whether fluorescence cross-correlation spectroscopy allows recovering information on the sequentiality of the interactions of GR and the coactivator with the same fixed targets.

We also compared the performance of fluorescence correlation spectroscopy (FCS) and single molecule tracking (SMT) to explore GR-DNA binding interactions. The time-scale of these interactions obtained in SMT experiments are one order of magnitude higher than those obtained by FCS under similar conditions. Our simulations show that SMT may overestimate the residence time of GR on DNA when this is shorter than 1 s, even with a sampling time of 0.2 s. This is due to a preferential tracking of slow moving or fixed molecules in SMT. On the other hand, FCS may sample preferentially frequent, fast binding events and it is limited by the temporal window in which the autocorrelation functions are calculated.

This work stresses the relevance of simulations to help to interpret experimental data from fluorescence microscopy.

Keywords: fluorescence correlation spectroscopy; single molecule tracking; glucocorticoid receptor; simulations

(231) **EXPLANATION OF PETO'S PARADOX: EMERGENT SUPRESSION MECHANISM OF STEM CELLS DYNAMICS.**

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The paradox was pointed out by Richard Peto in 1977 and states that at the species level, the probability of carcinogenesis does not seem to increase with the animal size. This is in principle counterintuitive because the more cells compose an organism, the higher should be the chance of the ocurrence of at least one cancerous cell. For instante, massive animals as elephants and some species of whales show a lower risk of cancer than our specie, despite having 100 or 1000 times more cells than us. In addition, massive animals tend to live longer, making the paradox even more intriguing.

In a recently published article (*M. Gaudiano, Mathematical Biosciences, Vol. 282, págs. 174-180, 2016*), it is presented a computational model able to provide an explanation to the paradox, which is based on the following idea: by pure chance, the descendents of a cancerous cell sometimes neither proliferate enough nor reach the thereshold needed to kill the organism. Even an animal can just die of old age, before the cancerous cells that it bears, start to cause health problems. Obviously, this may not have a strong incidence on the human carcinogenesis probability, which is not very low. But, how does this effect depend on the specie?

MOLECULAR MODELING 1

(250) **A COMPUTATIONAL STUDY OF THE BINDING MODES OF LIGANDS AT BENZODIAZEPINES' BINDING SITE ON GABA$_A$ RECEPTORS**

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The GABA$_A$Rs, γ-Aminobutyric acid type A receptors, belong to the family of pentameric ligand gated ion channels and are the main mediators of fast inhibitory transmission in the mammalian CNS. They are the target of a variety of compounds such as GABA, benzodiazepines (BZDs), anaesthetics, β-carbolines and neurosteroids, and have a fundamental role in neurological health.

In this work we aim to elucidate the binding modes of relevant ligands of the BZDs' binding site, which include classical BZDs, imidazo-BZDs, zolpidem and eszopiclone. We applied computational biophysics methods, namely molecular docking and molecular dynamics (MD) simulations, to undertake this study. We employed a previously developed homology model of the $\alpha 1\beta 2\gamma 2$ subtype (based on a β3 homopentamer as a template, PDBID: 4COF) in a closed desensitized state.

We approached molecular docking through different methods: we used HADDOCK which introduces experimental data as ambiguous interaction restraints and AutoDock Vina which runs a local optimization from random starting conformations. While the highest ranked binding modes were not necessarily the best predictions, some of the docking poses obtained could be related to the experimental data.

In order to assess the stability of the complexes, we performed 100ns MD simulations of the systems with GROMACS (using gromos53a6 force field and SPC water model). The receptor was embedded in a POPC membrane. RMSD calculations of the backbone show that the receptors did not suffer major global changes, although the presence of the ligands and relaxation of the side chains caused local modifications.

With the aid of experimental information we could predict the binding modes for ligands of the BZDs' site in GABA$_A$Rs. These compounds remained bound in the cavity during all MD simulations and improved their contacts with the receptor. Taking these results into account, we can confirm that our model can be used for further investigation of ligand binding.

Keywords: GABAARs, molecular docking, benzodiazepines, molecular dynamics.

(1045) **APLICATION OF COMPUTER-AIDED DRUG REPURPOSING IN THE SEARCH OF NEW THYPANOTHIONE SYNTETHASE INHIBITORS FOR THE TREATMENT OF CHAGAS DISEASE.**

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Chagas disease is an endemic parasitic disease that mainly affects Latin America. The currently available medication display a high incidence of adverse effects and low efficacy in the chronic

phase of infection in adults. Thus, it is very important to find new therapies with higher clinical efficacy and safety profiles.

Computer-aided drug repositioning may contribute to the systematic identification of new pharmacological applications for existing drugs, thus allowing the development of innovative therapeutic solutions in a cost- and time-efficient manner.

Here, we report the development and validation of ligand-based *in silico* models aimed at the identification of trypanothione sintethase (*TryS*) inhibitors. Such enzyme is essential for the biosynthesis of trypanothione, a key metabolite for the maintenance of the redox balance and defense against oxidative stress in *the parasite*. The models were inferred and validated from the molecular structures of 109 compounds previously assayed against *TryS*.

We built 1000 individual classificatory models capable of differentiating molecules with and without inhibitory effect against *TryS*. For model validation, a pilot virtual screening *in silico* campaign was performed against a drug library containing a small proportion of known inhibitors spread among decoys generated through the enhanced Directory of Useful Decoys. Based on the results, we resort to ensemble learning to obtain a 10-model combination which was later applied in the *in silico* screening of DrugBank 3.0 and Sweetlead. Twenty-one hits were classified as potential *TryS* inhibitors. 10 of them were acquired and assayed against *T. cruzi* trypomastigotes.

(1563) **CHARGE DENSITY AS A MOLECULAR DESCRIPTOR TO UNRAVEL STRUCTURE-ACTIVITY RELATIONSHIPS OF CRUZAIN INHIBITORS**

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Chagas disease is endemic to South and Central America caused by the parasite *Tripanosoma cruzi.* Actually just two drugs like nifurtimox and benznidazole are available, however they are highly toxic and drug resistance has been reported. Cruzain (Cz), the major cysteine protease of T. cruzi, is one attractive drug target; since, it is required for all the major proteolytic activities of the parasite life circle.

Cysteine protease inhibitors containing a vinyl sulfone warhead can exhibit good selectivity and a favorable in vivo safety profile despite the irreversible nature of inhibition.

K-777, a vinyl sulfone inhibitor of Cz has shown to be safe and efficacious in animal models of acute and chronic Chagas disease but the project was stopped due to tolerability issues at low dose in primates and dogs.

Jaishankar et al. synthesized and determined the inhibition constant of a series of vinyl sulfone analogs closely related to K-777 with substitutions at P2 and P3. Unfortunately, 3-D structures of these complexes are not available yet. However, there are several solved structures of Cz complexed with vinyl sulfone analogs available in the PDB. Moreover, all these irreversible inhibitors mimic the well known binding mode of classical substrate-like peptidic inhibitors. This structural information allowed us to make a reasonably good initial guess of the binding mode of these K-777 analogues at the Cz active site. Complexes were then subjected to MD simulations. Reduced model systems comprising inhibitor and residues from the Cz binding pocket were constructed from the MD simulation. Charge density topological analysis based in QTAIM was performed on these reduced models to evaluate the inhibitor/Cz interactions.

By carefully inspecting the electron density values at the interactions bond critical points, we found out what are the key interactions that explain the activity differences (Ki values) among K-777 analogues.

Keywords: QTAIM, MD, K-777

(740) **HUMAN TELOMERASE PROTEIN/RNA/DNA COMPLEX: THEORETICAL STUDY OF KEY CONSERVED RESIDUES BY MOLECULAR DYNAMICS SIMULATIONS**

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Telomerase adds several nucleotides to the 3' terminal of a DNA strand. It is a protein/RNA complex, formed by the Telomerase protein and a RNA strand. Inside the protein, in a Reverse Transcriptase (RT) catalytic pocket, there are several conserved residues: a triad of aspartates (D712, D868, D869, for the human case) whose crucial role in the telomerase activity is quite well understood, as well as other residues (K626, R631, K902) the two latter, also bringing the catalytic activity to zero when mutated. But a molecular model description is still lacking. This work aims to study, using molecular dynamics simulations, the role of all these residues in the activity of the human telomerase protein at atomic level. A chimeric model of the complex, constituted by the RT domain of the Telomerase protein, a double strand DNA/RNA, an incoming dNTP and two Mg^{2+} ions was studied in two configurations: i) before the catalytic reaction, with the dNTP at the reaction site, and ii) after the catalytic reaction, were the dNTP was broken in a nucleotide and an inorganic pyrophophate (PPi). Additionally to the wild type (WT) protein, 5 mutants (D712A, D868A, D869A, K902N and R631Q) were also studied.

The results for the WT protein, highlights the role of the ASP residues in the correct positioning of the incoming dNTP into the reaction center. Additionally, the K626, R631 and K902 residues further stabilize the dNTP before the reaction take place and accompany the PPi out of the catalytic site after the reaction. The results for the mutants, indicates different mechanisms through which the ASP residues participate during the reaction and let us to understand that the crucial role of the R631 and K902 residues is accomplished after the reaction is completed. When mutated, they are incapable to accompany the PPi and as a result the PPi stays at the catalytic pocket, stopping the catalytic activity of the telomerase.

Keywords: Molecular Dynamics, Telomerase, Reverse Transcriptase

(382) **IDENTIFICATION OF NEW TRYPANOCIDAL COMPOUNDS THAT INHIBIT PUTRESCINE UPTAKE THROUGH IN SILICO SCREENING AND *IN VITRO* ASSAYS**

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The polyamines putrescine and spermidine are crucial biomolecules for *Trypanosoma cruzi*. In contrast with human cells, *T. cruzi* is incapable of synthesizing polyamines *de novo* and incorporates them from the extracellular medium. The putrescine permease *Tc*PAT12 has been directly linked to the proliferative ability of *T. cruzi*, and has been proposed as potential target for the discovery of novel trypanocidal compounds.

The aim of this study was the identification of inhibitors of putrescine uptake in *T. cruzi.*

Using ligand-based approximations, in conjunction with a homology model of *Tc*PAT12, we have performed an *in silico* screening to identify *Tc*PAT12 potential inhibitors among existing drugs, a strategy known as *drug repositioning*, which allows time- and cost-efficient development of new medications.

We compile a database of 256 polyamine analogs that had previously been assayed against *T. cruzi*. From this dataset, using Dragon molecular descriptors and linear discriminant analysis, we inferred 1000 computational models able to discriminate between inhibitors and non-inhibitors of putrescine transport. The 8 best-performing models were combined through ensemble learning and applied in the virtual screening of Drug Bank 3.0 database. The probability of being active for the selected compounds was assessed through *Positive Predictive Value* (PPV) surfaces analysis. Hits with PPV ≥30% we submitted to molecular *docking* using an *in-house* homology model of *Tc*PAT12. Two hits were selected for *in vitro* eval-

uations: the antiemetic cinnarizine (PPV=50.6%) and the antibiotic clofazimine (PPV=30.0%). Both drugs inhibited putrescine uptake in *T. cruzi* epimastigotes and interfered with their proliferation with an EC_{50} of 6.0μM for cinnarizine and 10.6 μM for clofazimine (previously reported). Moreover, both compounds affected trypomastigotes viability.

The applied strategy allows identification of potential new drugs with considerable saving of time and material resources.

Keywords: Chagas disease, *Trypanosoma cruzi*, polyamines, drug repositioning, *in silico* screening

(1531) **MOLECULAR DYNAMICS AND MM/PBSA ANALYSIS TO IDENTIFY NEW THIOSEMICARBAZONES AS INHIBITORS OF BVDV RdRp**

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In the search for new compounds with antiviral activity, we have found two thiosemicarbazones (**2** and **15**) that inhibit the replication of bovine viral diarrhea virus (BVDV). A cross-resistance assay against the BVDV RNA-dependent RNA polymerase (RdRp) wt and the N264D, A392E mutant indicated that compound **15** lost their inhibitory activity meanwhile the activity of compound **2** was maintained upon mutations.

In this regard, we carried out molecular dynamics simulation and binding free energy calculations via MM/PBSA method to complement the experimental data of cross-resistance assays and to provide insights into the probable mode of action of compounds **2** and **15**. To this end, the structures of the compounds were optimized by HF/6-31G(d) and the structure of the BVDV RdRp was obtained from Protein Data Bank. First, molecular docking study was performed between the ligands and the enzyme in order to obtain the best binding mode, which was used as the starting structure for molecular dynamics. Simulations were achieved with NAMD software and the binding free energies were estimated via the widely used MM/PBSA method.

The obtained results showed that the complex of **15** with wt-BVDV had a favorable binding energy whereas the complex with the mutated enzyme caused a detrimental energetic change. On the other hand, both complexes of compound **2** displayed lower binding energies and, interestingly, with similar energies between them. Comparisons of the free energies components demonstrated that vdW and electrostatic interactions were the main driving forces involved in the binding of **15**.

In conclusion, the values of binding energies for compound **15** were in good agreement with the cross-resistance assay and prove that **15** is highly likely to act as a non-nucleoside inhibitor of the BVDV RdRp. In the case of compound **2**, results suggest a different binding site or target.

Keywords: Thiosemicarbazones, Bovine viral diarrhea virus (BVDV), Molecular dynamics, MM/PBSA.

(879) **MOLECULAR DYNNAMICS AND DOCKING OF DELTAMETHRINE TO TRIATOMA INFESTANS VOLTAGE-GATED SODIUM CHANNEL (VGSC) ALPHA 1 SUBUNIT COMPARED TO HOMOLOGOUS MODELED PROTEINS FROM OTHER INSECTS.**

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Chagas' disease affects 10 million people in Central and South America; the main insect vectors are Triatoma infestans and Rhodnius prolixus. Vector control using pyrethroid insecticide has failed. Persistence of T. infestans due to resistance to pyrethroids has been detected in specific geographic regions. Pyrethroids modify the normal function of voltage-gated sodium channels in the membranes of excitable cells. Knockdown resistance (kdr) is the reduction in the sensitivity to pyrethroids caused by point mutations in the sodium channel gene. Little is known about interactions of insecticides with the channel. This work presents initial results of in silico characterization of deltamethrin docking to α1 subunit of T.infestans vgsc compared to C.pipiens quinquefasciatus vgsc. AGW21772.1 from T.infestans and BN001089.2 from C.pipiens quinquefasciatus were homology modelled on SwissModel Workspace (templates 5x0m.1.A, 5xsy.1.A, 3lut.1.B). The models were subjected to blind docking on SwissDock platform, without solvent. Mosquito protein rendered the most favorable interactions in the region of kdr mutations with scarce alternatives around the P segment, T.infestans resulting favored structures were mainly around the P segment (Δ G -7.073 and –7.81 Kcal/mol respectively). These results did not support the hypothesis that kdr mutations found in T.infestans were as relevant to the insecticide docking as they are in mosquito vgsc. AGW21772.1 wild type lacks a portion of the S6 helix which could explain the difference. An in silico hybrid patched from BN001089.2 was assayed for docking, with Δ G for the kdr region of –7.31 and for the P segment –7.47 Kcal/mol respectively. Similar results were obtained with CHarMM suite. Concludding, kdr mutations on S4-S5 segments may be of less structural relevance for T. infestans than for other species, considering that previous studies on mosquito deliberately restricted the docking with a rigid cage in the kdr region.

(1265) **PROTEIN-LIGAND STRUCTURE AND BINDING FREE ENERGY PREDICTION USING MOLECULAR DYNAMICS IN MIXED SOLVENTS**

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Small water miscible molecules (or cosolvents), that mimic specific types of ligand molecular interactions, have been proven useful to reveal protein–ligand interaction hotspots and thus are a relevant source of information for drug development projects. In the present work we performed molecular dynamics simulations in mixed solvents for 18 different proteins to determine *solvent sites*, i.e. space regions adjacent to the protein surface where the probability of finding a probe solvent atom is higher than that in the bulk solvent. The solvents used were water mixtures of ethanol, acetamide, acetonitrile and methylammonium acetate, as well as pure water. When analyzing the probe capacity to reveal known protein-ligand interactions, results showed remarkable sensitivity for water (0.64) and specificity for ethanol-OH (0.56). These values were further improved when we only considered the ligand-based pharmacophoric points (0.73 and 0.61, respectively). Hydrophobic probes, such as methyl ends of ethanol and acetonitrile, also displayed great performance. Therefore, ethanol specificity makes it a great probe to use as a pharmacophoric bias in docking experiments, with ethanol–OH sites guiding the position of ligand groups with hydrogen bonding capabilities and ethanol-CH_3 sites guiding the location of ligand aromatic hydrophobic rings. Biased redocking and cross docking experiments showed 70% vs. 20% success when compared with the conventional docking method. Furthermore, for targets extracted from the DUD-E data set, ligand enrichment factors were also increased (e.g. EF1% from 2.1% to 14.6% for AmpC β-lactamase). Finally, accurate predictions of binding free energies were obtained by adding the ΔG contribution of solvent sites replaced by crystallized ligand groups capable of establishing the same type of interactions. These results highlight the relevancy of the method as a tool for protein-ligand binding studies.

Keywords: Molecular Dynamics, Cosolvents, Docking, Free Energy.

(1117) **STUDY OF THE AUREIN 1.2 AND MACULATIN 1.1 ANTIMICROBIAL ACTION OVER COMPLEX BILAYERS.**

A MULTIDISCIPLINARY APPROACH USING BOTH COMPUTATIONAL AND EXPERIMENTAL METHODS.

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The antimicrobial peptides (AMPs) are molecules that exert their action mainly against bacteria, among other pathogens. It was suggested in the literature that AMPs act by increasing the permeability of the membrane after an early interaction with the membrane. Among AMPs, two peptides obtained from australian frogs, the aurein 1.2 and the maculatin 1.1 exhibit structural features representative of α- helical AMPs and a well-proven lytic activity. This behavior was captured, both from experiments and computer simulations.

Lactobacillus delbrueckii subsp. lactis (strain CIDCA133) and L. delbrueckii subsp. bulgaricus (strain CIDCA331) showed different susceptibility to the action of human AMPs (β-defensines). Since the membrane composition of each strain is substancially different, we studied here the effect of both AMPs: aurein and maculatin on liposomes made with lipids extracted from both strains, carrying out several liposome content leakage experiments. By the other hand, aiming to understand the molecular mechanism of aurein and maculatin interaction with membranes, we carried out extensive Molecular Dynamics (MD) simulations. In order to mimic the membrane lipid composition of both strains, and due to the size of the bilayer systems and the time scales required, we used the MARTINI coarsegrain force field.

Our results show significant differences in the interaction of the studied AMPs with each strain: both experimental and MD studies let us propose differential models in each case. While maculatin can destabilize the membrane inducing membrane curvature, aurein exhibits surfactant properties, acting as an amphiphilic molecule, and favoring a pore formation. Moreover, we found significant differences respect the susceptibility of strains CIDCA133 and CIDCA331. In the present work, we combined computational methods complemented with experimental techniques to get insights on the particular AMPs ways of action and the resistance of certain membranes against them.

Keywords: antimicrobial peptides, molecular dynamics, coarsegrain, probiotics, complex membranes

(710) **THEORETICAL INSIGHTS INTO THE REACTION AND INHIBITION MECHANISM OF METAL-INDEPENDENT RETAINING GLYCOSYLTRANSFERASE RESPONSIBLE FOR MYCOTHIOL BIOSYNTHESIS**

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Understanding enzymatic reactions with atomic resolution has proven in recent years to be of tremendous interest for biochemical research, and thus, the use of QM/MM methods for the study of reaction mechanisms is experiencing a continuous growth. Glycosyltransferases (GTs) catalyze the formation of glycosidic bonds, and are important for many biotechnological purposes, including drug targeting. Their reaction product may result with only one of the two possible stereochemical outcomes for the reacting anomeric center, and therefore, they are classified as either inverting or retaining GTs. While the inverting GT reaction mechanism has been widely studied, the retaining GT mechanism has always been controversial and several questions remain open to this day. In this work, we take advantage of our recent GPU implementation of a pure QM(DFT-PBE)/MM approach to explore the reaction and inhibition mechanism of MshA, a key retaining GT responsible for the first step of mycothiol biosynthesis, a low weight thiol compound found in pathogens like Mycobacterium tuberculosis that is essential for its survival under oxidative stress conditions. Our results show that the reaction proceeds via a front-side SNi-like concerted reaction mechanism (D_NA_N in IUPAC nomenclature) and has a 17.5 kcal/mol free energy barrier, which is in remarkable agreement with experimental data. Detailed analysis shows that the key reaction step is the diphosphate leaving group dissociation, leading to an oxocarbenium-ion-like transition state. In contrast, fluorinated substrate analogues increase the reaction barrier significantly, rendering the enzyme effectively inactive. Detailed analysis of the electronic structure along the reaction suggests that this particular inhibition mechanism is associated with fluorine's high electronegative nature, which hinders phosphate release and proper stabilization of the transition state.

Keywords: glycosyltransferase; retaining; reaction; mechanism; QM/MM

ONCOLOGY-ONCOIMMUNOLOGY 20

(1345) **SIGNALS THROUGH TCR STIMULATION TRIGGER CD39 EXPRESSION ON CD8⁺ T CELLS FROM LYMPH NODE FROM B16F10- OVA TUMOR-BEARING MICE.**

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CD39 and CD73 play strategic roles in tumor microenvironment. We demonstrated that exhausted CD8⁺ T cells with high expression of CD39 infiltrate tumors, however, they are absent in lymphoid organs. We aimed to evaluate signals that may influence CD39 up-regulation on CD8⁺ T cells. Purified CD8⁺ T cells from draining lymph node of B16 OVA-tumor bearing mice were stimulated with tumor cells culture supernatant or anti-CD3/CD28 in the presence of IL-6 or IL-27 (cytokines that promote exhaustion). Tumor supernatants or TCR stimulation alone for 72hs did not modify CD39 expression observed *ex vivo*, however, IL-27 drove CD39 expression on anti-CD3/antiCD28 stimulated cells (p≤0,05) especially when combined with IL-6 (p≤0,0001) compared to anti-CD3/antiCD28 alone. CD39⁺ cells did not express CD73, but they showed increment of PD-1 (p≤0, 05) and slight increase of Tim-3 upon culture with IL-27. In this same condition, stimulated CD8⁺ CD39+ T cells showed lower frequency of IFNγ⁺ and TNF⁺ cells than CD8⁺ T cells stimulated through TCR (p≤0,05 and p≤0,01). Longer stimulation with anti-CD3/CD28 (48hs, resting 4 days in IL-2 and 24hs of re-stimulation) triggered high CD39, PD-1 and Tim-3 expression on CD8⁺ T cells respect to unstimulated controls. Stimulation of PBMCs from breast cancer patients through TCR (72hs) induced CD39 expression on CD8⁺ T cells (p≤0,05) compared to non-stimulated cells, however purified human CD8⁺ T cells stimulated with anti-CD3/anti-CD28 exhibited lower percentage of CD39⁺ cells compared to CD8⁺ T cells from total PBMCs (8,5%vs52,3%), but this percentage increased in the presence of IL-27 or the combination of IL-6 and IL-27 (17% and 24% respectively). After TCR stimulation in presence of IL-27 plus IL-6, human CD8⁺CD39⁺ T cells exhibited higher expression of PD-1 than CD8⁺ T cells stimulated with anti-CD3/anti-CD28 alone. Altogether these results demonstrated that signal through TCR and cytokines involved in exhaustion trigger CD39 upregulation.

Keywords: CD8, Cancer, CD39, exhaustion

(1320) **TUMOR-INFILTRATING CD39^{HIGH}CD8⁺ T CELLS EXHIBIT A POOR EFFECTOR RESPONSE AND ARE ASSOCIATED TO IMMUNOGENIC TUMORS AND HYPOXIC ENVIRONMENTS**

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Previously we described that upregulation of the immunomodulatory ecto-enzyme CD39 on tumor-infiltrating $CD8^+$ T lymphocytes ($CD8^+$ TILs) is associated with an exhausted phenotype. In this work we aimed to better characterize the effector phenotype of $CD39^+CD8^+$ TILs and their specific response against tumors. Using B16F10-OVA mouse cancer model, we observed by FACS that $CD39^{high}CD8^+$ TILs exhibited a higher frequency of $KLRG\text{-}1^+CD127^-$ cells, a phenotype of short-live effector cells, than $CD39^{low}CD8^+$ TILs ($p \leq 0,05$). They also showed lower % of Ki-67^+ cells ($p \leq 0.05$) and lower phosphorylation of mTOR and S6 ($p \leq 0.05$ for both) than $CD39^{low}CD8^+$ TILs, when re-stimulated *in vitro.* A high proportion of $CD39^{high}CD8^+$ TILs were $CD11a^+CD49d^+$, a phenotype associated with antigenic stimulation. In accordance, when comparing the frequency of $CD39^{high}CD8^+$ TILs between B16F10 and B16F10-OVA tumors we observed a significant higher percentage of these cells in the latter ($p \leq 0.01$), indicating that higher immunogenicity promotes CD39 expression on $CD8^+$ TILs. Studying OVA-specific $CD8^+$ TILs we observed that around 75% of these cells were $CD39^{high}$. OVA-specific $CD39^{high}CD8^+$ TILs showed lower frequency of TNF^+ and IL-2^+ cells, lower proliferative potential and higher inhibitory receptors expression than OVA-specific $CD39^{low}CD8^+$ TILs ($p \leq 0.05$ for all). Immunohistofluorescence of tumoral tissue showed that $CD39^+CD45^+$ cells are accumulated in hypoxic foci. Moreover, $CD39^{high}CD8^+$ TILs showed higher binding to pimonidazole, a hypoxia marker, than $CD39^{low}CD8^+$ TILs. Our results demonstrate that CD39 expression on $CD8^+$ TILs is related to tumor immunogenicity. Moreover, high CD39 expression is associated to a dysfunctional state of antigen-specific $CD8^+$ TILs. Interestingly, hypoxic environments within tumors could promote CD39 expression. Considering the modulatory role of CD39, it emerges as a target for treatments aimed to restore $CD8^+$ T cells anti-tumor immunity.

Keywords: $CD8^+$ T cells, cancer, CD39, exhaustion, hypoxia.

(1786) **CYTOTOXIC EFFECT OF ANTI-HER2 DRUGS IN A 3D MODEL OF HUMAN BREAST CANCER CELLS**

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The HER2 receptor is overexpressed in 20% invasive breast tumors and correlates with low free disease survival. Trastuzumab (Tz), Pertuzumab (Pz) and Trastuzumab-emtansine (T-DM1) are therapeutic monoclonal antibodies that bind HER2. Tumor spheroids are a 3D cell growth model that mimics the structure of *in vivo* small avascular tumors. The aim of this study was to analyze the relevance of 3D architecture on the cytotoxic effect of anti-HER2 clinical therapies.

First, we studied the effect of increasing concentrations of anti-HER2 drugs on the human breast cancer cell line BT474 (HER2+) and Tz resistant BT474-R, JIMT-1 and MCF7 (HER2-) cell lines cultured as monolayers. T-DM1 induced a concentration-dependent response, was more potent than Tz on BT474 (IC50: 0,93 vs 15,56 ug/ml) and also decreased the viability of the resistant BT474-R, JIMT-1 and MCF7 cells (IC50: 3,65; 4,8; 40,25 ug/ml). Pz did not show cytotoxicity.

BT474 cells formed spheroids that reached a diameter of 850 um at day 15 without treatment. We evaluated cytotoxicity on 3D cultures by concentration/response curves and observed that Tz exerted a cytostatic effect inhibiting spheroids growth by 66% compared to control (IgG, $p<0.0001$), while Pz induced a 28% inhibition contrarily to its lack of effect on monolayers. T-DM1 was highly cytotoxic not only inhibiting growth but also reducing spheroid size by 44%, compared to its initial size ($p<0.001$).

The cell subpopulations developed inside the spheroids showed a concentric organization of proliferative (Ki67+), quiescent (Ki67-/Bcl-2+) and dead cells (CAS+) from the periphery to the hypoxic core (HIF-1+) by hematoxylin-eosin staining, immunohistochemistry and flow cytometry. T-DM1 decreased the hypoxic regions, providing higher efficacy than the other drugs.

BT474 spheroids may provide a promising alternative model for studying the anti-HER2 drugs through modulation of the cell subpopulations developed inside the spheroids.

Key Words: HER2, Breast cancer, spheroids, cytotoxicity, resistance

(1791) **GALECTIN 1 PROMOTES TUMOR CELL MIGRATION BY ENHANCING NA^+/H^+ EXCHANGER ISOFORM 1 (NHE1) ACTIVITY**

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Tumor cells evade immune responses and shape local and systemic microenvironments establishing a distinctive cellular phenotype. Galectin 1 (Gal1),a glycan-binding protein, controls tumor progression by modulating tumor immunity, angiogenesis and migration through binding to specific glycan structures of cell surface receptors. In solid tumors,the Na^+/H^+ exchanger isoform 1 (NHE1) favors cancer progression through pH modulation.We aimed to elucidate the role of Gal1 on NHE1 regulation in murine melanoma cells and its possible role in tumor cell migration.To determine NHE1 activity we used a BCECF-AM flow cytometry kinetics assay. We observed increased NHE1 activity in melanoma cells in comparison to a non-tumorigenic cell line.To evaluate whether Gal1 could be involved in NHE1 hyperactivity, cells were treated with NHE1 inhibitor (Eipa), recombinant Gal1 (rGal1) or were silenced for Gal1 (shGal1).We observed diminished NHE1 activity after Eipa treatment with similar results obtained after shGal1 silencing ($p<0.01$). Notably, rGal1 treatment augmented NHE1 activity, reverting the Eipa-dependent inhibition of NHE1 ($p<0.005$). Moreover, NHE1 upregulation was observed in response to exogenous administration of rh-Gal1 and silencing of Gal1 expression in melanoma cells decreased NHE1 protein levels.Accordingly, Gal1 immunoprecipitated with NHE1, suggesting that Gal1 could interact with NHEI and control pH-dependent regulation by this antiporter.To further address the biological relevance of NHE1/Gal1 interaction, we evaluated migration of shGal1 melanoma cells. Both Gal1 signaling or NHE-1 inhibition impaired B16 cell migration. In contrast, exposure to rGal1 restored tumor migration under both conditions ($p<0.005$). Taken together, these results suggest a possible role for Gal1 in modulating tumor cell behavior via NHE1 activation. Understanding the mechanisms through which the acidic microenvironment could shape tumor cell phenotype could help improve anticancer therapies.

Key words: Galectin 1-Na+/H+ exchanger-NHE1

(344) **IDENTIFICATION OF REFRACTORY AND RELAPSED HODGKIN LYMPHOMA PREDICTIVE BIOMARKERS AND THEIR MODULATION AS POTENTIAL DIRECTED-THERAPY TARGETS**

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Hodgkin lymphoma (HL) is a lymphatic system malignancy. The neoplastic cell, Hodgkin-Reed Sternberg (HRS), derive from a late stage of B cell maturation through their transit in germinal center. Despite 90% of early and 60% of late stage chemosensitive patients can achieve a long disease free survival (DFS) with current therapy, refractory and relapsed (RR) HL patients remain a challenge. There are no predictive molecular markers to identify RR patients at diagnosis nor alternative treatment more than high risk toxicity-related rescue chemo schemes.

We have previously reported that HL relies on the constitutive non-canonical NFkB pathway activation mediated by RelB/NIK. This lead to high BCL2 levels and sustained survival. Our molecular studies suggested this could explain the lack of response in RR HL patients.
We aimed to analyze if mediators of this pathway could be used as predictive markers to identify RR HL patients as well as potential targetable factors.
We analyzed the citoplasmic NIK and BCL2 expression in HRS cells of lymph node biopsies in 96 HL patients by inmunohistochemistry [50 female Md age (range) 59 (6-82) and 46 male 42 (9-78)]. The univariate analysis showed no correlation among NIK or BCL2 expression and the prognosis clinical and pathologic parameters. As previously determined by our in vitro experiments a direct correlation was found between NIK and BCL2 expression in the biopsies studied (p=0.01). The analysis of survival, applying the Kaplan-Meier Curves, showed >60% NIK positive HRS cells was associated with shorter DFS [Log Rank Test p=0.008]. >60% BCL2 positive HRS cells correlated with poor prognosis in terms of overall survival [Log Rank Test p=0.002]. Also the Cox Regression Analysis showed that BCL2 expression predicted survival (p=0.01).
NIK and BCL2 are useful predictive markers to identify RR HL patients. Furthermore, they represent attractive potential therapeutic targets, since BCL2 inhibitos have been aproved in other lymphomas.
Keywords**:** Hodgkin Lymphoma, NIK, BCL2, Predictive Biomarker, Directed-therapy.

(1341) **INTERFERON REGULATORY FACTOR 8: A NEW BIOMARKER IN BREAST CANCER?**

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The interferon regulatory factor 8 (IRF8) is crucial for regulating the antitumor immune response and acts as a tumor suppressor gene. Tumors silence the expression of IRF8, and disruption of its function leads to acquiring resistance to apoptosis, decreasing the activation of antitumor immune response and increasing the incidence of metastasis. However, there is little information about IRF8 expression in breast cancer. Our previous results showed that IRF8 expression correlates inversely with the metastatic phenotype in breast cancer, and transcriptional silencing of IRF8 correlates with DNA methylation. These results suggest that IRF8 may have clinical value as potential prognostic marker in breast cancer. The main goal of this work was to evaluate whether expression of IRF8 would allow a more accurate prognosis in the molecular subtype of breast cancer. We performed analysis using a statistical mining tool of published annotated genomic data. High expression of IRF8 was associated with relatively high survival in HER2-enriched (p=0,0006) and Basal-like/TNBC (p=0,0004) breast cancer compared to those patients con low expression of IRF8. In addition, we have found a positive correlation between antitumor immune response related-genes (IFNG, GZMB, IL15, CXCL10, p<0,001) and IRF8 expression. We have also explored different therapeutic strategies using 5-aza-2¶-deoxycytidine (5AZA), DNA methyltransferase inhibitor, to activate the endogenous IRF8 gene in a breast tumor model. Significant inhibition of tumor growth and tumor volume was observed after local injection of 5AZA alone (p<0,05) or in combination with poly I:C (p<0,01) compared to control. In conclusion, these results demonstrate that the inclusion of IRF8 expression as a new biomarker allow a more accurate prognosis in patients with tumor characterized as HER2-enriched or Basal-like/TNBC, and opens opportunities to explore new therapeutic strategies in breast cancer by using DNA demethylation agents.

Keywords: breast cancer, predictive value, survival, oncology, IRF8

(1220) **LSP1-DEFICIENT MICE HAVE AN IMPAIRED CONTROL OF MELANOMA TUMOR GROWTH**

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Leukocyte-specific protein 1 (LSP1) is a 52kDa cytoplasmic F-actin binding phosphoprotein expressed in all human and murine leukocytes as well as in endothelial cells. LSP1 in known as an important regulator of actin cytoskeleton remodeling. Our group has previously shown that *Lsp1*$^{-/-}$ mice have an impaired CTL response after antigen exposure, with *Lsp1*$^{-/-}$ dendritic cells (DCs) failing to induce a strong CTL response in vivo, to migrate to lymphoid tissues and to properly present antigens. To study the role of LSP1 in antitumor immune response, we employed the MO5 melanoma model. WT and *Lsp1*$^{-/-}$ mice were injected subcutaneously with 10^5 MO5 cells and followed-up until day 26. Tumors in *Lsp1*$^{-/-}$ mice grew significantly faster and bigger than in WT mice (p<0.001). Leukocyte populations were assessed in tumor, draining lymph node (dLN) and spleen by flow cytometry. In spleen of *Lsp1*$^{-/-}$ mice we found an increased frequency of CD8α^+ DCs, CD103$^+$ DCs, CD103$^+$CD8α^- DCs and inflammatory monocytes, a decreased frequency of CD8α^- DCs and B cells and no difference of T cells and neutrophils. No significant changes were observed in the frequencies of the same cell populations in dLN. No differences were observed in the frequency of tumor infiltrating leukocytes between *Lsp1*$^{-/-}$ vs. WT mice. Histologic assessment of tumors in *Lsp1*$^{-/-}$ mice showed a much smaller intratumoral necrosis as well as lower polymorphonuclear leukocyte infiltration and higher mononuclear cell infiltration than WT mice. Multivariate statistical analysis of all available data clearly showed that distribution of leukocyte populations from *Lsp1*$^{-/-}$ mice is different to the observed in *Lsp1*$^{+/+}$ mice after melanoma implantation. Our hypothesis is that LSP1 deficiency prevents generation of effective antitumor immune response in the early moments after MO5 cell implantation. Functional characterization of tumor infiltrating cells is under study.

Keywords: LSP1, tumor, melanoma, DCs.

(266) **GALECTIN 7 PROMOTES CHEMICAL SKIN CARCINOGENESIS THROUGH INDUCTION OF MYELOID REGULATORY CELLS IN MICE**

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Skin immunity is finely regulated by a broad network of cytokines and growth factors present in the epidermis and dermis to maintain tissue homeostasis. Disruption of this cellular and molecular balance may trigger different skin inflammatory diseases including psoriasis and dermatitis or neoplastic transformations like squamous cell carcinoma. Galectins (Gals), a family of beta-galactoside proteins that signal via glycosylated receptors, have emerged as key regulators of immune cell homeostasis. Galectin-7 (Gal7) is abundant in keratinocytes and is tightly regulated in response to skin environmental stress, suggesting that its altered expression may contribute to skin disease. The aim of this study was to evaluate the role of Gal7 during skin carcinogenesis. Using bioinformatic analysis (Enrichr resource and Cytoscape´s plugins ClueGo/CluePedia) we found that several oncogenic drivers (SOX2, NRAS, FOS, CD44 and RAC1 among others) were up-regulated in transgenic mice (Tg46) over-expressing Gal7 under the K14 promoter as, compared to wild type (WT) and Gal7-deficient (Lgals7-/-) mice. This expression profile positively correlated with a higher number of skin papillomas developed in the skin of Tg46 mice compared to WT or Lgals7-/- animals. No-

tably, these mice were more susceptible to two-stage induced-carcinogenesis and developed papillomas at day 45, whereas WT and Lgals7-/- animals developed skin lesions at day 53 and 60 respectively. Interestingly, Tg46 mice overexpressing Gal7 in keratinocytes exhibited a higher percentage of CD11b+Ly6G-Ly6C+ myeloid-derived suppressor cells (MDSCs,) in the spleen compared to their WT counterpart. MDSCs purified from Tg46 mice showed enhanced immunosuppressive activity as compared to WT and Lgals7-/- MDSCs in in vitro lymphoproliferation assay. This enhanced immunosuppressive effect may account for increased tumor susceptibility in vivo. In conclusion, altered expression of Gal7 may contribute to skin carcinogenesis by favoring dysregulation of oncogenic drivers and promoting expansion of immunosuppressive MDSCs.

(63) **IL-15 PROMOTION OF ANTITUMOR IMMUNE RESPONSE AGAINST BREAST CANCER IN COMBINATION WITH CETUXIMAB *IN VITRO* AND *EX VIVO***

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EGFR is a potential target for Breast Cancer (BC) therapy, especially for Triple Negative BC (TNBC) subtype. Cetuximab, an IgG1 anti-EGFR mAb, was not effective in clinical trials in combination with chemotherapy. We propose that Cetuximab exerts antitumor effects mediated by the immune system that need combination with proinflammatory cytokines due to the immunosuppressive nature of tumor microenvironment. The aim of this study was to investigate *in vitro* the role of IL-15 on dendritic cell (DC) and Natural Killer (NK) cell interaction promoted by Cetuximab-coated TNBC cells; and *ex vivo* the effects of IL-15 on phenotype and function of lymphocytes, particularly NK cells, that infiltrate BC tumors.

First, human monocyte-derived DCs were co-cultured with autologous isolated NK cells, TNBC cells, Cetuximab and/or IL-15 *in vitro* for 24hs. IL-15 enhanced NK cell activation in the presence of Cetuximab coated TNBC cells showing an increased CD25 expression and IFN-y production ($p<0.05$). As we have previously shown, DC maturation was promoted by Cetuximab-coated TNBC in the presence of NK cells, and this maturation was further enhanced by IL-15 ($p<0.05$ for CD83 and CD86 expression).

For *ex vivo* studies, tumors infiltrating lymph nodes from BC patients were dissociated using an enzymatic/mechanic protocol. IL-15 treatment for 24hs promoted CD69, NKG2D, NKp30 and perforin expression on NK cells. IL-15 also augmented NK and T cell proliferation after a 5-day treatment. Cetuximab alone did not stimulate lymphocyte proliferation but, in the presence of IL-15, it further enhanced NK and T cell proliferation in some of the tumors.

Our results suggest that IL-15 is an interesting cytokine for its combination with Cetuximab since it recovers NK cell expression of activating receptors in an immunosuppressive microenvironment, and might promote NK-DC cross-talk and subsequent cross-presentation to CD8 T cells.

Key words: Breast Cancer, Cetuximab, IL-15, NK cells, Dendritic cells

(1167) **THE GLYCAN-GALECTIN AXIS MODULATES TUMOR DEVELOPMENT AND IMMUNE RESPONSE IN A MURINE MODEL OF COLITIS-ASSOCIATED COLORECTAL CANCER**

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Inflammatory Bowel Diseases (IBD) have been associated with an increase in the incidence of colorectal cancer (CRC), the third more frequent cancer worldwide. It has been previously reported that the intestinal inflammatory microenvironment favors neoplastic development, although the underlying molecular mechanisms are not yet completely understood. During the last decades, aberrant cell surface glycosylation has been considered an important hallmark of cancer and tumor progression. Glycosylation changes trigger different biological processes *via* interaction with glycan-binding proteins such as galectins. We aimed to study the effect of the inflammatory microenvironment over the galectin-glycan interactions and CRC progression. We focused our research on the association between intestinal inflammation and neoplastic development with special emphasis in the glycome and its interaction with Galectin-1 and Galectin-4, as they exert important and opposite roles in CRC progression and its metastatic potential. With this purpose, we have established a mouse model of colitis-associated colorectal cancer (CACRC) based on the administration of azoxymethane (AOM) and dextran sulfate sodium (DSS). Wild-type and Galectin-1 KO mice were injected with AOM and then exposed to DSS-containing drinking water. Weight loss, stool consistency and blood were monitored and, after sacrifice, we analyzed number of tumors and tumor size, studied the cellular immune infiltrate and performed immunohistochemical analyses for selected molecules. We found that Galectin-1-lacking mice develop an improved immune response against the tumors, with an increase in activated $CD4^+$ ($p=0,03$) and $CD8^+$ ($p=0,03$) T cells. These changes are not related to DSS-induced inflammation, as its administration without AOM does not alter any of these parameters. We also carried out a meta-analysis of transcriptomic data for mouse models of sporadic CRC (sCRC) and CACRC. Metadata analysis showed differential glycogene expression profiles between sCRC and CACRC, indicating a potential influence of intestinal inflammation in aberrant glycosylation profiles.

Keywords: Colorectal cancer, colitis, IBD, galectin, glycans.

ONCOLOGY-ONCOIMMUNOLOGY 20

(779) **THE MYELOID DIFFERENTIATION PRIMARY RESPONSE PROTEIN 88 (MYD88) IS INVOLVED IN THE PREVENTION OF LIVER CANCER DEVELOPMENT**

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MyD88 is involved in the Toll-like receptor and IL-1 receptor signaling pathway in the innate immune response. It is known that inflammation is an important component of tumorigenesis. We state that the signaling through MyD88 has a key role in liver cancer development in mice. Adult C57BL/6 wild-type (WT) and $MyD88^{-/-}$ mice (23-25 g) were subject to a model of early liver cancer development. This was induced by administration of 2 i.p. doses of diethylnitrosamine (75 mg/kg bw) 2 weeks apart. One week after the last injection, mice received 20 mg/kg bw of 2-acetylaminofluorene by gastric probe 3 days a week for 3 weeks. All studies were performed before the initiation of the treatment and showed no difference between genotypes. After the hepatocarcinogenic treatment, $MyD88^{-/-}$ mice showed lower body weight but higher liver weight than WT mice. We confirmed the complete absence of liver MyD88 protein expression by immunoblotting, as well as MyD88 mRNA expression, when evaluated by qPCR. Liver histology analysis showed scatter alterations on hepatocyte architecture, with accumulation of cytosolic lipid droplets (+23%) and increased inflammatory infiltration (+45%) in $MyD88^{-/-}$ mice compared to WT mice. Hepatic enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were slightly increased (+15%, and +12%, respectively) in plasma of $MyD88^{-/-}$ mice compared to WT mice, indicating a mild liver damage. Then, we evaluated proliferation and apoptosis by immunoblotting. We found that $MyD88^{-/-}$ mice presented with increased protein expression of proliferation cell nuclear antigen (PCNA) (+35%) and decreased levels of caspase 3 (-31%) in total liver homogenates. These studies represent the first steps in the evaluation of the role of MyD88 in liver cancer development, and demonstrate that MyD88 is participating in preventing chemical hepatocarcinogenesis; exposing, once again, the tight relationship between the immune system and the de development of cancer.

Keywords: inflammation, HCC, chemical hepatocarcinogenesis, apoptosis, proliferation

(1923) **TETRAIODOTHYROACETIC ACID (TETRAC) IN COMBINATION WITH CETUXIMAB INHIBITS CELL PROLIFERATION IN COLORECTAL CANCER CELLS**

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Thyroid hormones induce cancer cell proliferation via a cell surface receptor on integrin αvβ3. The deaminated metabolite of L-thyroxine (T4), tetraiodothyroacetic acid (tetrac), and its nanoparticulate formulation, Nano-diamino-tetrac (NDAT), act at the thyroid hormone receptor on αvβ3 to inhibit tumor cell proliferation in various in vitro and in vivo models. We evaluated the effect of T4 on proliferation of human colorectal cancer cell lines, using mRNA and protein markers. The effect of tetrac/NDAT was also studied in combination with cetuximab, a monoclonal antibody to the epidermal growth factor receptor (EGFR), on cell proliferation, gene expression profiling and cell counts. T4 increased PCNA, cyclin D1 and c-Myc levels in HT-29 cells and in both wild-type K-ras mutant HCT-116 colon cancer cells. In wild-type HCT-116 cells, the combination of NDAT and cetuximab inhibited expression of the same proliferative genes whose expression was induced by T4 and did so comparably to cetuximab, alone. In the K-ras mutant HCT-116 cells that are cetuximab-resistant, however, the combination of NDAT and cetuximab potentiated inhibition of T4-induced cell proliferation, compared to cetuximab, alone, restoring and enhancing tumor cell response to cetuximab. In addition to these findings, we will report the ability of NDAT, endowed with pro-apoptotic and anti-angiogenic properties expressed at αvβ3, to inhibit the PD-1 (programmed death-1)/PD-L1 (programmed death ligand-1) checkpoint that protects tumor cells against immune attack and is highly expressed in tumor cells.

Key words: Tetraiodothyroacetic Acid, thyroid hormone, integrin αvβ3, colorectal cancer

(170) **EFFECT OF THE COMBINED TREATMENT OF INTERFERON ALFA-2B (IFN) AND VITAMIN E (VITE) ON THE DEVELOPMENT OF LIVER CANCER**

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IFN has been used for the treatment of patients with hepatitis B or C, and VitE was shown to have inhibitory effects on liver cancer due to its antiangiogenic, antioxidant and antiproliferative activities. We aimed to evaluate if the combined therapy of IFN with VitE has a synergistic inhibitory effect on liver cancer development, cell proliferation and apoptosis compared to each separate therapy. Adult male Wistar rats were subjected to a two-phase model of liver cancer (initiated-promoted, IP group). Initiation: 2 i.p. doses of diethylnitrosamine (150 mg/kg bw) 2 weeks apart. One week after the last injection, rats received 20 mg/kg bw of 2-acetylaminofluorene by gastric probe 4 days per week for 3 weeks. IP rats also received IFN $6{,}5x10^5$ U/kg bw (IFN group); α-Tocopherol, an isomer of VitE, 3 mg/kg bw (E group); and both drugs in a combined therapy (IFN-E group). After treatment and euthanasia, the livers were removed and altered hepatic foci (AHF) were determined by immunohistochemistry (rGST-P). To determine fibrosis, collagen was analyzed by Direct Red 80 staining. Also, we analyzed oxidative stress (OS) by TBARS, and proliferation (proliferating cell nuclear antigen, PCNA) and apoptosis (cit. c release) by immunolotting. Results: IFN-group: apoptosis (+162%*), OS (+75%*), PCNA protein levels (-68%*), number of AHF per liver (-35%*), percentage of liver occupied by foci (-83%*) and percentage of fibrosis (-39%*). E-group: apoptosis (+127%*), OS (-40%˙), with no changes in PCNA, in the number and percentage of AHF and fibrosis. IFN-E group: apoptosis (-60%#), OS (-120%#), PCNA (+102%#), number and percentage of AHF (+35%# and +80%#) and percentage of fibrosis (+29%#) (*p<0.05vsIP; #p<0.05vsIFN). These results did not show a useful effect of VitE against the development of liver cancer. What is more striking, though, is that VitE seems to block the beneficial effect of IFN, possibly due to its antioxidant power.

Keywords: preneoplasic liver, proliferation, apoptosis, fibrosis, oxidative stress

(85) **MOLECULAR CHARACTERIZATION OF LEUKEMIC STEM CELLS IN CHRONIC MYELOID LEUKEMIA BY SMALL RNA SEQUENCING**

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Molecular relapse after treatment discontinuation in chronic myeloid leukemia (CML) has been attributed to the persistence of leukemic stem cells (LSCs), which share multiple features with their normal counterparts (hematopoietic stem cells, HSCs). In order to contribute to their molecular characterization, we performed next-generation sequencing (NGS) of small RNAs comparing LSCs vs. HSCs in CML patients. Materials and methods: bone marrow or peripheral blood $CD34^+$ cells from CML patients at diagnosis were sorted into a LSC-enriched ($CD34^+CD38^-CD26^+$) and a HSC-enriched ($CD34^+CD38^-CD26^-$) fraction. Purity was assessed by *BCR-ABL1* RT-qPCR in colony-forming unit (CFU) assays. Total RNA preserving the small RNA fraction was directly isolated from sorted cells. Small RNA NGS libraries were sequenced using the Illumina HiSeq 2500 platform. MicroRNA counts and differential expression values were obtained by Chimira and GFOLD algorithms. *In silico* predictions of targets and pathways were performed with miRPath. Results: The protocol for cell sorting and RNA extraction from highly pure, low-abundance fractions was optimized and performed in 7 CML patients and 5 healthy donors. Given the low yield of RNA, we pooled samples from different patients or donors. LSC- vs. HSC-enriched fractions resulted in 100% vs. 8.3% of *BCR-ABL1*$^+$ CFUs, respectively (mean %*BCR-ABL1/ABL1*: 99.7% vs. 0.95%, n=2). One hundred and twenty-three microRNAs were differentially expressed in LSC- vs. HSC-enriched fractions from CML patients. Comparison against healthy donors resulted in 19 microRNAs that clustered in potentially relevant pathways such as ECM-receptor interaction, regulation of pluripotency of stem cells, and proteoglicans in cancer. Conclusions: As far as we are concerned, this is the first report of the miRNome of highly enriched LSC and HSC fractions in CML patients by NGS, providing valuable data for the search of LSC-specific therapeutic targets.

Keywords: microRNAs, leukemic stem cells, chronic myeloid leukemia

(156) **IS HYALURONAN A TUMOR PROMOTER? DIFFERENT ACTION ON ANGIOGENIC BEHAVIOR OF MACROPHAGES IN BREAST AND COLON CANCER HUMAN MODEL.**

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Hyaluronan(HA) is a glycosaminoglycan present in the extracellular matrix and acts as a modulator of immune and angiogenic responses. At homeostasis, high-molecular weight (HMW) HA is predominant whereas the low-molecular weight (LMW) form is present in inflammation. HA in tumor microenvironment is signal for recruiting tumor associated macrophages, that could regulate angiogenesis.

Aim: To evaluate the effect of exogenous HA (HMW, LMW) on human monocytes/macrophages (MO) and their angiogenic behavior in breast and colon carcinoma.

Methods: MDA-MB 231 or LoVo from human breast and colorectal carcinoma tumor lysates (TL) were prepared by freeze–thaw cycles. MO from PBMCs were pulsed with TL plus HA(20ug/ml) LMW(≅1,5x10^{6}Da) or HMW(≅2x10^{5}Da) for 24h. MO were characterized with CD14, HLA, CD80 and CD206 by flow cytometry. VEGF level was evaluated by ELISA assay. For the *in vivo* xenograft mouse model MDA-MB-231 or LoVo cells were inoculated in the flank of Nu/nu mice. After 9 days, MO pulsed or not with HA were inoculated sc next to the tumor. Tumor volume was measured 3 times/week. Animals were euthanatized, tumors were fixed and stained with Lectin GSLI-FITC and DAPI for vasculature detection.

Results: The HA treatments did not modify the expression of MO cell surface markers. VEGF biosynthesis levels increased when MO were treated with MDA TL plus HA HMW in comparison to MDA TL alone and plus HA LMW. However, VEGF levels showed no significative difference in LoVo TL treatments. In the MDA model, mice inoculated with MO plus HA HMW increased tumor volume and its vasculature. While in the LoVo model no differences were found between groups.

Conclusion: HA HMW modulates MO angiogenic behavior in breast carcinoma. However, HMW HA is unable to do the same action in colon carcinoma context. Our results provide evidence that HA modulation of this cells depends not only of its molecular weight but also of the tumor context.

Keywords: hyaluronan, monocytes, tumor, angiogenesis

(122) **CORRELATION BETWEEN HLA-G AND VEGF EXPRESSION IN CLEAR CELL RENAL CELL CARCINOMA**

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Clear cell renal cell carcinoma (ccRCC) is the most common type of renal cancer that occurs in adults. It has the worst prognosis among common renal epithelial tumors. Vascular endothelial growth factor (VEGF) family is important in tumor grow because it regulates vasculogenesis, angiogenesis and lymphangiogenesis. The VEGF-A was the first purified, and the most frequently found. The antiangiogenic treatments have allowed an improvement in patient survival but despite recent promising advances, the effectiveness of this approach has been limited in ccRCC. An explanation could be the great intra-tumor heterogeneity and the presence of different isoforms. This was recently observed with the non-classical class I molecule human leukocyte antigen G (*HLA-G*), which is a new immune checkpoint expressed in ccRCC that allows tumor escape from immunovigilance. In this context, the aim of the study is to identify the different VEGF isoforms expressed in ccRCC and determine whether there is a significant relationship between VEGF and HLA-G isoforms expression. Our cohort is composed of surgically operated ccRCC patients at the urology department of Saint-Louis Hospital (Paris, France) and at the Hospital «Evita Pueblo», Berazategui, Buenos Aires, Argentina. To apprehend the intratumoral heterogeneity, 4 to 10 sections were isolated from each tumor and studied by RT-PCR. Our preliminary results reveal that patients with the strongest HLA-G expression have also strong VEGF expression in all the studied sections. However, the VEGF expression heterogeneity is much lower than that of HLA-G transcripts. This is particularly interesting considering recent investigations demonstrating that VEGF is the downstream effector of ILT4, one of the main receptors for HLA-G.

Key words: Human Carcinoma, HLA-G, VEGF, Kidney.

(773) **HUMAN PAPILLOMAVIRUS INFECTION IN LUNG SQUAMOUS CELL CARCINOMA AND CORRELATION TO P16^{INK4A} EXPRESSION AMONG ARGENTINE POPULATION**

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Background: Lung cancer is the leading cause of cancer death worldwide. In 1979, Syrjänen suggested a role for human papillomavirus (HPV) infection in bronchial carcinoma. Many studies have found HPV on lung carcinoma, predominantly in squamous cell carcinoma (SCC). There seems to be a geographical factor determining prevalence rates. In Latin America, only 2 studies, altogether including 51 cases of lung SCC, examined this association. However, data from Argentina is lacking.

Objective: -To assess the incidence of HPV infection in lung SCC of Argentine population and correlate with p16^{INK4a} expression.

Material and Methods: The study was approved by CEMIC's Ethics Committee. Informed consent was obtained. Materials consisted of formalin-fixed paraffin-embedded (FFPE) tissue from 29 surgically excised and 11 transbronchial biopsies of primary lung SCC evaluated between 2006-2016.

HPV Genotyping: Wide-spectrum HPVs' DNA (L1-ORF) was amplified by PCR. Positive specimens were genotyped by PCR for types 16 and 18.

Immunohistochemistry: All p16 staining's were performed on VENTANA BenchMark GX. Staining patterns were interpreted on a binary way (positive or negative). Only cases with diffusely intense cytoplasmic and/or nucleic staining on tumor cell (TC) were considered positive.

Result: HPV was isolated in 10/40 cases (25%). Twenty-eight cases (70%) were both p16 and HPV negative. Three cases were HPV 16 positive, 5 cases HPV 18, and 2 were coinfected with HPV 16 and 18.

p16 was positive on TC in 5 out of 40 cases (13%). On 10 cases, p16 was positive in the bronchial epithelium, although negative on TC.

Conclusion: We detected an HPV infection rate of 25%. HPV18 was the common genotype. On 7 cases, normal bronchial epithelium was both p16 and HPV positive, suggesting that adjacent tumor tissue may harbor HPV. p16 should not be used as a surrogate marker for HPV infection, since it is only positive on 60% of cases.

Keywords: human papillomavirus, lung carcinoma, squamous cell carcinoma, p16.

(526) **EFFECT OF EP2014/064243 PEPTIDE ON THE GROWTH OF A MAMMARY ADENOCARCINOMA: PRELIMINARY STUDY.**

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Breast cancer show, as many malignant tissues, several pathways that regulates cell's cycle, tumor duplication and cell apoptosis. One

of the best known pathways is the Ras signal transduction for apoptosis regulation. In order to demonstrate the activity of these peptides against aggressive breast cancer cells, we performed the present study in a murine breast cancer line developed in our laboratory (Facultad de Ciencias Médicas, UNLP). We compare the overall survival of mice affected by this type of malignancy receiving either placebo or peptide EP2014/064243 that induce apoptosis and cell cycle arrest. We used adult male C3H/S-strain mice. The animals were divided into 2 groups: group 1 (control): injected with placebo and group 2 injected with the peptide. Conditions concerning animal management fully respected the policy and mandates of the Guide for the Care and Use of Laboratory Animal Research of the National Research Council. After an appropriate period of synchronization, the C3H/S-histocompatible breast carcinoma was grafted into the subcutaneous tissue of each animal's flank. We measure the tumor growth, on alternate days, since the moment they become visible macroscopically. The data were statistically analyzed using Anova. The tumor growth were registered until an average volume of 4 cm3. We observed tumor growth in both groups, in group 2 the latency time of tumor onset was greater than controls and they have a delay to reach their maximum development size. However, we couldn´t observe statistically significant differences between the groups. In this preliminary study we concluded that the peptide could inhibit or delay tumor growth. Thus, we need further studies to confirm that this peptide pathway could be considered as a promising target for anticancer therapy.

Keywords: tumor, mice, peptide.

(1866) **CLINICAL RELEVANCE OF PDCD4, A NUCLEAR ERBB-2 TARGET GENE, IN DIFFERENT BREAST CANCER SUBTYPES**

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PDCD4 (programmed cell death 4) is a tumor suppressor gene involved in metastasis in breast cancer (BC). We previously reported that ErbB-2 nuclear function as a transcription factor (TF) and also as a coactivator of TF Stat3, leads to increased microRNA-21 levels, therefore decreasing PDCD4 expression favouring metastasis. To explore PDCD4 clinical relevance in BC, we analyzed PDCD4 expression by immunohistochemistry in 541 patients with primary invasive BC. We found that 34% of patients showed PDCD4 expression and was associated with good prognosis (p=0.009). Surrogate BC subtypes include Luminal A (Lum A, estrogen and progesterone receptor positive, ER+/PR+), Luminal B (Lum B, ER+/PR-), Lum B-ErbB-2 positive (+), ErbB-2+-non luminal, and triple negative BC (TNBC, ER-/PR-/ErbB2-). PDCD4 expression was associated with a higher overall survival probability in patients with Lum A, Lum B, and ErbB-2+ subtypes, but not in TNBC (p=0.044, p=0.016, p=0.05, and p=0.40, respectively). Correlation of PDCD4 mRNA expression and patient outcome using publicly available KM plotter database, showed similar results. Interestingly, we found that 80% (84/104) of TNBC patients lacked PDCD4 expression. Western blot showed that PDCD4 levels were decreased in TNBC (MDA-MB-453, -231 and -468) compared to Lum A cells. As our previous reports indicated that TNBC cells express NErbB-2 which modulates PDCD4 levels, we explored the effects of NErbB-2 blockade in PDCD4 expression by using ErbB-2ΔNLS mutant which acts as a dominant negative inhibitor of endogenous NErbB-2. ErbB-2ΔNLS increased PDCD4 levels in TNBC cells. We also found that in PDCD4 negative TNBC patients NErbB-2 presence was associated with poor prognosis (p=0.031). Our findings stress the association in TNBC between high levels of NErbB-2, lack of PDCD4 expression, tumor metastasis and poor prognosis. We also reveal PDCD4 expression as a marker of better clinical outcome in Lum A, Lum B and ErbB-2+ BC subtypes.

Keywords**:** Breast Cancer Subtypes, PDCD4, Clinical Biomarker, Nuclear ErbB-2, Triple Negative Breast Cancer

Our findings stress the association in TNBC between high levels of NErbB-2, lack of PDCD4 expression, tumor metastasis and poor prognosis.

(1028) **MRP4 AS AN ONCOGENE IN PANCREATIC CANCER: OVEREXPRESSION OF MRP4 INDUCES CELL PROLIFERATION, TUMOR GROWTH AND CHEMORESISTANCE.**

Ana Sahores.

Pancreatic ductal adenocarcinoma (PDAC) is the fourth cause of cancer-related deaths worldwide; due to its late diagnosis, high resistance to chemotherapy and early onset of metastasis. Even after the emergence of new targeted agents and the possibility of therapeutic combinations, no differences in overall survival are actually seen. Thus, there is an urgent need of novel therapeutic strategies to target PDAC. MRP4 transports endogenous and exogenous substances and its deregulation has been reported in numerous types of cancers. Recent evidence from our lab shows that the activity of MRP4, mainly the efflux of AMPc, is critical for PDAC cell proliferation and that its inhibition causes a clear decrease in malignancy and invasive capacity, suggesting MRP4 as an attractive therapeutic target. The aim of this study was to investigate the role of MRP4 in pancreatic cancer progression and chemoresistance. We used the BxPC-3 pancreatic cell line to establish two clones which overexpress MRP4 significantly (G6 and H7) compared to the control cell line (C4). Western blot and qPCR assays confirmed higher MRP4 protein and mRNA levels, respectively. MRP4 overexpression confers a higher proliferative rate and lower duplication time ($p<0.001$) in the BxPC-3 clones. This proliferative advantage was maintained in *in vivo* xenografts ($p<0.01$). Treatment with two MRP4 inhibitors, MK571 and Ceefourin1, impaired cell proliferation of control as well as MRP4-overexpressing clones ($p<0.01$; 50 μM). Interestingly, although MRP4 does not transport 5-Fluoruracil (5-FU), H7 cells display a higher tolerance to this agent compared to control cells ($p<0.05$). Our results suggest that the up-regulation of MRP4 could represent an adaptive advantage associated to poor prognosis, evidenced by higher cell proliferation and resistance to 5-FU treatment in a pancreatic cancer model. We propose MRP4 as a possible biomarker to predict resistance and that its modulation could improve chemotherapy response.

Keywords**:** Pancreatic ductal adenocarcinoma, MRP4, tumor progression

GENETICS AND MOLECULAR BIOLOGY 21

(922) **TEMPORAL REGULATION OF IMMUNE CELLS PRODUCTION IN *DROSOPHILA MELANOGASTER* DURING DEVELOPMENT AND INFECTION**

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Fundación Instituto Leloir

Abstract: In the fruit fly *Drosophila melanogaster* hematopoiesis takes place in the lymph gland, which proliferates and differentiates throughout the larval period. The production of mature hemocytes needs to be temporally coordinated to adjust to developmental times and to respond to stimuli such as infection. The balance between self-renewal and differentiation of progenitor cells is critical for these responses and is affected in diseases including leukemia. It has been shown previously that a peak of the steroid hormone Ecdysone promotes hemocyte production at three successive steps: proliferation, liberation and activation. Here we study the regulation of hemocyte production at the level of differentiation of progenitor cells in the lymph gland. We propose that Ecdysone plays a role in maintaining tissue homeostasis and triggers cell differentiation both in the context of infection and normal development.

Key words: Hematopoiesis, Ecdysone, Cell differentiation, Lymph gland, Drosophila

(1163) **TOOLS FOR SINGLE-CELL ANALYSIS OF *DROSOPHILA* METABOLISM**

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It is a well stablished fact that tumor cells suffer a metabolic reprograming that makes them unable to fully oxidize carbon chains through mitochondrial catabolism, turning glycolysis into their main ATP source. Recently, the idea that this metabolic switch (called Warburg effect) could be a feature of cell proliferation and differentiation during normal development has started to be worked out. We have developed several tools to test this idea in *Drosophila melanogaster* development. We generated three different FRET sensors, each of them reporting intracellular levels of a different key metabolite. Pyronic senses pyruvate, Laconic senses lactate, whilst OGSOR senses 2-oxoglutarate (2-OG). The 2-OG sensor OGSOR was originally developed in a prokaryotic system, so we are in the process of analyzing its behavior in *Drosophila* tissues throughout development. Its emission spectrum, as well as its dynamic response to increasing levels of extracellular 2-OG, were stablished after driving its expression in several larval organs. We observed an increase in intracellular levels of 2-OG elicited by both oxygen and nutrient deprivation, as well as in cells in which mitochondrial 2-OG transporters have been genetically downregulated. This implies that most of the cytosolic 2-OG arises from glutamate deamination rather than from the Krebs cycle. In addition, we engineered in a *Drosophila* fly line a transcriptional reporter of the Pyruvate Dehydrogenase Kinase (PDHK), one of the central regulators of the metabolic switch. The PDHK transcriptional reporter revealed very specific tissues and cell types in which this enzyme is highly expressed, suggesting that these zones might undergo strong glycolytic catabolism. Analysis of these particular expression territories with the remaining tools (FRET sensors) that we have developed will light on the metabolic profile of differentiating cells during *Drosophila* development.

Keywords: Warburg – Glycolysis – Metabolism – FRET sensor – *Drosophila*

(1190) **ROLE OF EIGER/TNF-ALPHA IN COORDINATING TISSUE GROWTH IN *DROSOPHILA*.**

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How growth of tissues and organs are coordinated during animal development to give rise to well-proportioned structures, are unsolved questions in biology and are important to understand the origin of human pathologies. Our previous work revealed a novel role of the tumor suppressor protein Dp53 in coordinating growth between adjacent cell populations within a developing tissue. By reducing the growth rates of defined territories in the wing primordium of *Drosophila*, we showed that the tissue responds as a whole and adjacent cell populations decrease their growth and proliferation rates. Dp53 plays a crucial role in regulating non-autonomously the reduction of tissue size and cell cycle proliferation and in its absence, the organ loses its normal proportions. By combining gene expression analysis and *in vivo* loss-of-function experiments, we identified *eiger*, the *Drosophila* homologue of mammalian TNF-alpha, as a putative signaling molecules downstream of Dp53. Interestingly, *eiger* is induced in the growth-depleted territory in a p53-dependent manner and is required to coordinate growth. Eiger appears to act through its receptor Grindelwald and the JNK signaling pathway. These new findings indicate that Eiger might act as a tissue local signal, emerging as an important player in growth coordination between adjacent cell populations.

Keywords: Drosophila, growth coordination, Dp53, Eiger/TNF-alpha

(1552) **EIF4E AND 4E-BP MODULATE CALCIUM HANDLING IN DROSOPHILA HEART VIA DIRECT INTERACTION BETWEEN EIF4E-4 AND SERCA PUMP**

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We have studied the effect of genetic up and downregulation of eIF4E and 4E-BP on cardiac calcium handling using *Drosophila melanogaster* as genetic model. We assessed the intracellular calcium level by registering the fluorescent signal of a cardiac reporter system (TinC-Gal4; UAS-GCaMP3) in semi-intact preparation of 7 days-old flies. Overexpression of 4EBP incremented the Ca^{2+}-transient amplitude and relaxation, and the sarcoplasmic reticulum (SR) calcium load. These effects were linked to a higher SR Ca^{2+} reuptake through the Ca^{+2}-ATPase pump (SERCA) and consequently release of Ca^{2+} by ryanodine receptors (RyR) was higher than control.

Through bioinformatic analysis we found that SERCA possesses within its amino acid sequence the domain of interaction with eIF4E present in 4EBP (YXXXXLϕ). This suggest that the results observed by the functional experiments could be due to an interaction between the eIF4E and SERCA proteins, affected probably by 4E-BP, whose eIF4E binding site is the same as that of SERCA.

Using a two hybrid assay we assessed the interaction of *Drosophila*'s seven eIF4E isoforms with SERCA. We found that only eIF4E-4 interacts directly with SERCA. These results suggest that the effects in the activity of SERCA observed in the transgenic lines could be due to the direct interaction proved between SERCA and eIF4E-4.

Our results provide evidence of the effects of modulating 4EBP in the heart of *Drosophila* and that these effects might be due to an interplay between 4EBP, eIF4E, more precisely its isoform 4 and SERCA.

Keywords: Drosophila, heart, calcium, eIF4E, 4EBP, SERCA

(1223) ***ORSAI*, A NOVEL NUCLEAR REGULATOR OF CELL METABOLISM**

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orsai (*osi*), a novel gene we are characterizing in the lab, is considered to be part of the LYR domain containing protein family. This family includes proteins involved in the assembly or activity of the mitochondrial complex I as a structural component, accessory protein, co-factor or part of the assemble machinery of iron-sulfur clusters. The human orthologue of *osi*, recently characterized, is involved in acute encephalopathy, seizures, mild spasticity and neuroradiological features when mutated. The main goal of this work is to shed light on *osi´s* function. As a first step we analyzed its cellular and subcellular localization, as well as the phenotypes triggered by its dysfunction.

In *Drosophila*, while control larvae feed until they reach the critical weight, *osi* mutants stop feeding and die early on, as stage 1 larvae. We monitored feeding, locomotion, and olfactory responses in well characterized behavioral paradigms and performed growth and survival curves to examine larval phenotypes in depth; in addition, we performed biochemical experiments to assess mitochondrial function; immunostaining to define localization of the protein and electron microscopy to register morphological phenotypes. Results were analyzed using t-test or ANOVA whenever necessary. Behavioral assays suggest that *osi* mutants respond normally to olfactory cues and are capable of feeding; however, *osi* depleted larvae barely grow in size. At the molecular level, this phenotype correlates with a clear impairment in basal metabolism, characterized by reduced oxygen consumption, decreased mitochondrial ATP along with increased ROS levels. As a result, mitochondria are smaller and fragmented. In addition, reduced OSI levels trigger a defective cell size and shape. Surprisingly, in most tissues OSI was not found in mitochondria but in the nucleus.

From all data we propose that OSI is a nuclear regulator of cellular metabolism.

Keywords: *Drosophila*, mitochondrial complex I, developmental arrest

(1807) **UNDERSTANDING EISOSOMES MEMBRANE DOMAINS´ ROLE IN AGING**

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The plasma membrane (PM) of *Saccharomyces cerevisiae*, contains at least a dozen of different nanodomains. Particularly, eisosomes are reservoirs of more than 25 proteins including transporters, signaling molecules, proteins reported to be involved in cellular aging and proteins of unknown function. Eisosomes are structured in PM invaginations by scaffolds composed mainly by two cytoplasmic proteins Pil1 and Lsp1. In knockout strains of *PIL1* domain´s organization is lost. A model of post-mitotic cellular aging (chronological aging) was developed in *S. cerevisiae.* Cells are induced to enter a non-dividing state and the viability of the population is measured for weeks. Performing Chronological Longevity Survival (CLS) assays we found that the Δ*pil1* mutant strain has an extended longevity phenotype. Lifespan extension was observed in two different *S. cerevisiae* genetic backgrounds and by different viability measurement methods. Besides, loss of Pil1 increases lifespan additively with calorie restriction (CR) and does not influence glucose depletion. These results indicate that simulation of CR is not the underlying mechanism for Δpil1-dependet increase in lifespan. We also used alternative approaches to determine if Δpil1 lifespan extension was independent of the nutritional environment or the acidification of the media. CLS assays were performed switching cell´s populations to water (extreme CR) and in the standard medium buffered to alkaline pH. Aging signal transduction pathways also sense amino acids availability. Since eisosomes colocalize with sites of tryptophan uptake, we analyzed if incorporation of this amino acid is affected in a Δpil1 mutant strain. Understanding eisosomes´ role in aging will likely contribute to further describing *S. cerevisiae* aging process and domains function.

(363) **IDENTIFICATION OF NON-CONVENTIONAL WINE YEAST SPECIES IN *VITIS* ECOSYSTEMS OTHER THAN *V. VINIFERA***

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The surface of grapes lodges the microbiota responsible for spontaneous fermentation of grape must. Little is known about the communities of yeast present on grapes from *Vitis* species other than *V. vinifera*. We studied the identity and dynamics of the indigenous yeast population present during the spontaneous fermentation of must from Isabella (*V. labrusca* L.) grapes, harvested at the province of Córdoba (Argentina). Non-*Saccharomyces* and *S. cerevisiae* yeast species were isolated and identified using selective growth media and DNA-based molecular methods. Changes in the yeast population during spontaneous fermentation of Isabella grapes followed a pattern similar to those described for *V. vinifera* grapes. *S. bacillaris*, a yeast species ubiquitously present in oenological ecosystems, was the most preponderant yeast (~70%) at early stages of spontaneous fermentation of *V. labrusca* grape must. Isolated *S. bacillaris* strains show phenotypic differences with *S. bacillaris* strains isolated from *V. vinifera* ecosystems. *C. azymoides*, *C. californica* and *P. cecembensis*, yeast species not previously found on *V. vinifera* ecosystems, were recognized on Isabella fermenting grape must. Interestingly, these yeast species have recently been isolated from *V. labrusca* grapes harvested from vineyards in the Azores Archipelago. Moreover, these yeast species were not isolated from *V. vinifera* ecosystems at both Cordoba and the Azores Archipelago. Thus, specific *Vitis*-yeast species associations may be formed independently of the geographic location of the vineyards. We suggest that specific biological interactions between grapevines and yeast species may underlie the assembly of differential *Vitis*-microbial communities. Based on these results, we propose that *Vitis* species other than *V. vinifera* constitute poorly explored oenological ecosystems, being a potential rich reservoir of wine yeast with unique genetic and phenotypic properties.

Keywords: Vitis, grapes, microbiota, yeast, spontaneous fermentation

(954) **THE IMPORTANCE OF THE GENETIC BACKGROUND AND THE NITROGEN SOURCE IN YEAST AGING**

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Aging is a complex process accompanied by progressive, deleterious changes that eventually lead to death. Caloric restriction (CR) is one of the best non-genetic means that can extend lifespan in almost every organism studied. CR consists in the mere reduction of caloric intake without malnutrition. The aim of this work was to analyse if the effect of CR, a well-known intervention to delay aging, is influenced by the yeast genetic background and by the nitrogen source used. Chronological lifespan (CLS) was measured using the colony forming unit spot assay in yeast cells derived from two different genetic backgrounds grown in 0.5% (CR condition) or 2% (control condition) glucose as carbon source and in proline (10 mM) or ammonium sulphate (0.5 %) as nitrogen source. We found that CR had opposite effect on CLS of the BY4741 and 23344c background strains (auxotrophic and prototrophic strains, respectively) grown on ammonium sulphate. In BY4741 cells grown in 0.5% glucose lifespan extension was observed whereas 23344c cells were more long lived in 2% than in 0.5% glucose. When proline was used as nitrogen source, aging of both strains was delayed and was similar in both carbon conditions. Tolerance to oxidative stress was determined during aging, being BY4741 cells grown on CR condition more resistant independently of the nitrogen source. On the other hand, viability, stress resistance, cellular respiratory activity, autophagy and the activity of the UPR pathway were determined in cells during the first 72 hours of growth before cells were submitted to the aging process. Several differences and similarities were found in cells of both genetic backgrounds grown in those growth conditions. Findings presented here demonstrate how profoundly genetic background and nitrogen source can modify lifespan extension of cells. Moreover, results obtained during the first 72 hours of growth will contribute to establish relationships between the way cells live and their longevity.

Key words: aging, genetic background, caloric restriction, yeast

(1001) **DISCOVERING NEW FUNCTIONS OF GABA-RESPONSIVE TRANSCRIPTION FACTORS IN *S. CEREVISIAE***

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S cerevisiae cells can adapt to a great variety of nutrients and the use of these nutrients must be highly coordinated to enable cells to get the major profits of them. For this purpose, not only general transcriptional and translational mechanisms are triggered, but also specialized transcription factors (TFs) are activated generating activation or repression of specific sets of genes. Amino acids can be metabolized and used as nitrogen and carbon sources or as building blocks for protein biosynthesis. When several amino acids are available, yeast cells use them in a well-defined order as a consequence of a coordinated gene regulation. The γ-aminobutyric acid (GABA) can be used by yeast cells as nitrogen source. *UGA* genes encode the enzymes involved in this process and the TFs Uga3 and Dal81 induce these genes in the presence of GABA. While Dal81 is a pleiotropic factor, Uga3 has been described as a transcription factor specific for GABA metabolism. The analysis of the growth phenotype of mutants deficient in Dal81 or Uga3 under different conditions, suggested to us that these TFs might participate in more pathways than the described for them. We also found by coIP/WB that Uga3 and Dal81 interact even in the absence of GABA. We analysed the

expression of several genes involved in the use of different amino acids in strains deficient in different TFs. We used the reporter gene method. We found that Uga3 regulate the induction by leucine of *BAP2* gene, a gene involved in the branched amino acid catabolism. However, *AGP1* and *BAP3*, involved in the same pathway are not regulated by Uga3. We also found that Dal81 is essential for the expression of genes of the Unfolded Protein Response (UPR) pathway. The main goal of the UPR is to restore the equilibrium between protein load and folding capacity of the endoplasmic reticulum; it clearly has both survival and cell death effects. So, Dal81 has an important physiological role, besides the regulation of amino acids catabolism.

Palabras clave: S. cerevisiae, factores de transcripción, regulación génica

(1208) **REGULATION OF SEX TYPE IDENTITY IN SACCHAROMYCES CEREVISIAE**

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IFIBYNE

Sex type determination in the yeast *Saccharomyces cerevisiae* depends on the combinatorial expression of different transcription factors, encoded by two alleles of the MAT locus. Two factors per sex (MATa1 and MATa2 in MATa cells; MATα1 and MATα2 in MATα cells), together with Mcm1, are necessary and sufficient to establish and maintain the correct cell types. Among the genes that they activate and repress differentially, there are those that code for the pheromones needed for mating and their specific receptors. Previous high throughput studies suggested that other transcription factors could be important for the maintenance of sex type identity, since their deletion resulted in some cases in the induction of opposite-sex specific genes upon stimulation with pheromone. To test this hypothesis, we deleted these transcription factors in a MATa strain that also contained fluorescent reporters for two MATα specific genes MF(ALPHA)2 and SAG1, and determined reporter abundance by quantitative fluorescence microscopy. Upon α factor pheromone stimulation, some of these strains showed strong reporter induction compared to wild type, in which there was none. To corroborate this result, we employed a complementary strategy, in which we replaced the endogenous promoter with that of GAL1, repressed in glucose medium. In MATa cells thus modified we observed induction of the MATα reporters in glucose but not in galactose, again indicating that some of these factors prevent ectopic expression of opposite sex genes.
These findings, taken together, suggest that specific mechanism exist to maintain the sex type identity during the mating response, and we are currently exploring the underlying mechanisms.

Keywords: MATING RESPONSE, YEAST, FLUORESCENCE MICROSCOPY

GENETICS AND MOLECULAR BIOLOGY 22

(751) **MOLECULAR AND BIOINFORMATIC CHARACTERIZATION OF TWO NOVEL UNSTABLE HEMOGLOBIN VARIANTS LEADING TO DOMINANT β-THALASSEMIA**

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Abstract: Although tahalassemic syndromes are typically inherited in a Mendelian recessive manner, dominant forms of β-thalassemia have been described, due to mutations that affect both the structure as well as and the quantity of the synthesized β-globin chains. In this report we present 2 novel small deletions that cause the loss of 2 and 3 amino acids, respectively, leading to dominant unstable variants in 2 pediatric patients. The complete *HBB* gene of both patients and their parents was cloned in the pGEM®-T Easy Vector Systems and sequenced. The altered amino acid sequences were studied analyzing their physicochemical properties, sequence conservation and secondary and tertiary structure predictions. The first patient presented the deletion *HBB*:c.29_37delCTGCCGTTA (p.Ala10_Thr12del) in heterozygote state. The new variant was given the name Hb JC Paz. As a result of this mutation, an Alanine, a Valine and a Threonine of the "A" α-helix of the β-globin chain are deleted. These deleted residues are closed to N-term and at least one α-helix turn is expected to be lost. Moreover this alteration could affect the polar interactions of V1 and H2 with the allosteric modulator 2,3-diphosphoglycerate. The second proband presented the mutation *HBB*:c.182_187delTGAAGG (p.Val60_Lys61del) in heterozygote state, resulting in the variant named Hb Tavapy. Consequence of this deletion, the Valine and Lysine of codons 60 and 61 are lost, altering the globular structure of the modified chain. Provean sequence based prediction of p.Val60_Lys61del indicates a deleterious effect with a strong score. Furthermore, deleted residues are close to the distal histidine (H63) in the heme pocket. Both deletions arose as *de novo* mutations. The predicted structural models provided a feasable explanation for the clinical outcomes observed in the patients and offered additional insights to the importance of key amino acids in the β-globin chain.

Keywords: Unstable hemoglobin variants; *HBB* gene; Dominant beta-thalassemia; Bioinformatics

(819) **FILIPIN STAINING TEST FOR THE DIAGNOSIS OF NIEMANN-PICK TYPE C (NPC) IN A CHILDREN`S HOSPITAL IN BUENOS AIRES, ARGENTINA**

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Abstract: Niemann-Pick disease type C (NPC) is an atypical lysosomal storage disorder resulting from mutations in either *NPC1* or *NPC2* gene, currently conceived as a lipid trafficking disorder. Symptoms of suspicion are grouped into visceral (neonatal cholestatic jaundice, splenomegaly, fetal edema), neurological (vertical supranuclear visual paralysis, cataplexy, ataxia, dysphagia, dystonia) and non-specific psychiatric disorders. The age of presentation goes from perinatal period (mainly systemic) until adolescence or adulthood (mainly neurological). This heterogeneity of symptoms can overlap with other diseases, generating a delay between the initial presentation of symptoms and the accurate diagnosis. Impaired output of cholesterol from the late endosomal/lysosomal compartment is a key element of the pathogenesis and can be visualized by fluorescence microscopy after staining with filipin. The "filipin test" performed on cultured fibroblasts, is the historical gold standard method to confirm the diagnosis.

The aim of this study was to develop the filipin staining as a diagnostic test for NPC to perform in a children`s hospital.

For this purpose, we carried out a descriptive, transversal study in a pediatric population, between 2015 and 2017. Cultured skin fibroblasts from 15 patients with presumptive diagnosis of NPC were studied. The performance of the test was compared to filipin staining performed in fibroblast cell culture from 6 patients with other pathologies unrelated with cholesterol metabolism (Control group). Of the total of 15 NPC suspected patients, filipin staining showed a classical positive pattern in 4 patients. All patients of control group showed a filipin staining negative pattern. The values of the performance of this method were 0.27 for sensibility and 0.92 for specificity. In conclusion, we have been developed a useful method to perform NPC diagnosis in a national pediatric reference centre.

Keywords: Niemann Pick disease, type C, Filipin, Cholesterol, Fibroblast.

(932) **LARGE-SCALE MOLECULAR STUDY OF HEREDITARY HEARING LOSS GENES IN DEAF PATIENTS: WIDE RANGE OF POSSIBILITIES**

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Hereditary Hearing Loss (HHL) is a common trait affecting 1 in 2000 new born children. The genotype heterogeinity and the presence of over 100 different genes involved in HHL lead us to go on board with Whole Exome Sequencing (WES) in order to search for a putative correlation between hearing loss and sequence variations.
We designed a flowchart in order to exclude all the probably spurious variations obtained and target for few candidates. To approach this, we filtered results and candidate variations were segregated throughout family members looking for relationship with phenotype. Variations positively selected, were analyzed using different bioinformatics predictors in order to study amino acid change impact in proteins and looking for pathogenicity. Additionally, conservation studies, structure and functional domain analysis in proteins, collection of information from databases, prediction of functionality and functional analysis in vivo were performed.
Using this strategy we identified causative mutations in four families with syndromic and non-syndromic hearing loss. Analysis and filtering process of more than 100 genes were successful and we identified two missense variants that had been previously reported, and two missense and frameshift mutations that resulted novel. In one family no causative mutations were identified in the 100 candidate genes so a Trio WES analysis is still in process in order to propose a new candidate gene possibly related to the pathology.
Functional studies of some of the identified mutations, using Zebra fish models, are under way.
We show in the present study some clearcut results using WES analysis as a successful strategy for hearing loss study. We prove that our flowchart is advantageous and noteworthy for large-scale molecular analysis. These findings clearly highlight the importance of genetic studies followed by in sílico and in-vivo validation to better understand the genetic basis of Hereditary Hearing loss.
Keywords: Hearing loss; mutations, whole exome sequencing, filtering process, functional studies.

(948) **HIGH FREQUENCY OF UNDIAGNOSED EHLERS-DANLOS SYNDROME (EDS) IN CONGENITAL ADRENAL HYPERPLASIA (CAH) PATIENTS DUE TO *CYP21A2* GENE DELETIONS.**

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The contiguous gene deletion syndrome, CAH-X, was reported in an 8.5% of CAH patients with a TNXA/TNXB chimera resulting in deletions of *CYP21A2*, encoding 21-hydroxylase necessary for cortisol biosynthesis, and *TNXB*, encoding the extracellular matrix glycoprotein tenascin-X (TNX). There are three *TNXA/TNXB* chimeras (CH1, CH2, CH3) that differ in the junction site, resulting in *TNXB* haploinsufficiency or dominant negative effect and an EDS phenotype. Recently, it has been described a biallelic form of CAH-X syndrome.

Objective: To analyze copy number variations and genetic status of *TNXB* gene in 62 CAH patients due to *CYP21A2* deletion to determine the frequency of EDS in our population.

A total of 62 unrelated CAH patients carriers of *CYP21A2* gene deletion were screened for *TNXB* defects. All the patients were analyzed for the presence of CH1 MLPA analysis evidenced by a 120 bp deletion in *TNXB* exon 35, and confirmed by exon 35 sequencing analysis. Patients carriers of CH1 were screened for other *TNXB* alterations related to CH2 and CH3 by exon 40, 41 and 43 Sanger sequencing.

The presence of *TNXB* deletion (CH1) was found in 28/66 alleles carriers of *CYP21A2* gene deletion (42%). Haploinsufficiency of *TNXB* was found in 24 patients (CH1), one patient was homozygous for CH1 (biallelic form) and two patients were compound heterozygous for CH1 and CH2 (biallelic form). Moreover, one patient was found to harbour a *CYP21A2* and *TNXB* deletion allele inherited from his father. MLPA and sequencing revealed the presence of a mosaicism in the patient´s father blood leukocytes.

A high frequency of *TNXB* haploinsufficiency was found in deletion carrier alleles in our population. MLPA and Sanger sequencing techniques resulted useful to characterize *TNXB* deletion. Clinical evaluation for connective tissue dysplasia including cardiologic investigation should be routinely performed in CAH patients, particularly those harboring a *CYP21A2* deletion.

Key words: *CYP21A2; TNXB;* Ehlers-Danlos; CAH

(1037) **MOLECULAR APPROACHES FOR DIAGNOSIS OF SEVERE HAEMOPHILIA A AFFECTED PATIENTS IN ARGENTINA: A COST-EFFECTIVE SCHEME**

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Background: Haemophilia A (HA), the commonest X-linked coagulopathy, is caused by defects in the factor VIII gene (*F8*). Due to *F8* size and complexity and mutational heterogeneity, gene testing in HA still represents a technical challenge. A half of severe HA (sHA) is caused by recurrent inversions disrupting *F8* at IVS22 (Inv22) and IVS1 (Inv1).

The rest of sHAs are mostly caused by family-specific large or small del/ins and point mutations.

Aims: Present a cost-effective gene testing algorithm for families with sHA suitable for developing countries.

Methods: Leukocyte-extracted genomic DNA from patients are subjected to sequential *F8* genotyping protocols: Inv22/Inv1 diagnosis by inverse shifting PCR; PCR amplification targeting all relevant *F8* sequences in 38 products to detect large deletions in inversion negative cases (designing specific gap-PCR or qPCR approaches for carrier diagnosis); and conformation sensitive gel electrophoresis (CSGE) screening for small mutations on the *F8* amplimers in multiplex and characterisation of the anomalous CSGE product by Sanger sequencing. Genotype/Phenotype assignment of the observed variant is achieved by applying internationally accepted criteria.

Results: We characterised the causative mutation in 325 families with sHA, whereas 13 families remain uncharacterised (3.8%). We found 153 cases with the Inv22 (45.3%) (82% Inv22 type 1 and 18%, type 2), 4 Inv1 (1.2%), 18 large deletions (5.3%), 58 small ins/del (17.2%), 48 missense (14.2%), 32 nonsense (9.5%), and 12 splicing defects (3.6%).

Conclusions: The presented algorithm allowed characterisation of the sHA causative mutation in 96.2% of families in a relatively rapid and cost-effective way. The remnant 4% may be due to the intrinsic limitation of the CSGE screening (≈95%), the theoretical extent of the *F8* PCR amplification scheme to detect deep intronic splicing defects and other rare mutations, and the failure of gene dosage analyses to detect duplications.

Keywords: *F8*, haemophilia, cost-effective diagnosis

(1443) **GENETIC DIAGNOSIS OF DYSTROPHINOPATHIES USING NEXT-GENERATION SEQUENCING. CHARACTERIZATION OF TWO NOVEL SPLICING MUTATIONS IN *DMD* GENE**

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Dystrophinopathies constitute a group of neuromuscular diseases caused by pathogenic variants in *DMD* gene. Large deletions/duplications account for 70% of mutations and are detected by MLPA. In the remaining 30% of patients, small mutations are observed. *DMD* gene is composed by 79 exons; therefore sequencing with Sanger method is costly, time-consuming and laborious. Next-generation sequencing (NGS) enables detection of small mutations in a more efficient and cost-effective approach. Objective: to detect small mutations in *DMD* gene using NGS technology. Methods: whole-exome sequencing was performed by Macrogen service in 15 male patients with clinical/biopsy suspicion of dystrophinopathies in whom deletions/duplications had been previously rule out. Quality control, mapping and alignment of the sequences were reviewed in our laboratory. Only variants in the *DMD* gene were analysed. In order to classify the identified variants dbSNP, 1000 Genomes, Exac, ClinVar, Leiden and HGMD databases were consulted. To predict the effect of the variants, SIFT, PolyPhen-2, Mutation Taster and Human Splicing Finder software were used. Total RNA was isolated from muscle biopsy to analyse the effect of two novel splicing mutations. Results: Pathogenic variants were identified in 14 of the 15 patients analysed: 5 nonsense mutations, 5 small deletions, 3 splicing mutations and 1 duplication. mRNA analysis of two novel splice site mutations was performed. Mutation c.3786+5G>C (exon 27) revealed three different transcripts: wild-type, skipping of exon 27 and skipping of exon 27 to 29. Mutation c.9650-1delG (exon 67), produce one transcript skipping exon 67 and two other transcripts with different cryptic acceptor sites in exon 67. Conclusion: the NGS approach used proved to be a highly sensitive tool to detect small mutations in *DMD* gene. This study shows the importance of transcript analysis to determine the consequences of splicing mutations.

Keywords: *DMD* gene, next-generation sequencing, splicing mutation, dystrophinopathies.

(1630) **MOLECULAR DIAGNOSIS OF THE 21-HYDROXYLASE DEFICIENCY**

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Congenital adrenal hyperplasia, the most frequent inborn metabolism error, is caused in 90-95% of the cases by mutations in the 21-hydroxylase gene (*CYP21A2*). The deficiency can present as severe or classical form (C), and nonclassical (NC). About 70% of haplotypes have a bimodular arrangement, with one module carrying *CYP21A2* and the other the homologous pseudogene *CYP21A1P*. Due to the high sequence identity, most of the disease causing mutations are the consequence of non-homologous recombination or gene conversion events between the gene and its pseudogene. Nevertheless, an increasing number of mutations have been found in the last years. The aim of our group is to study the genetic defects that contribute to the phenotypic expression of the pathology. In the present work, we describe our molecular diagnostic algorithm and an update of the mutations found in our population. A total of 1253 samples were studied: 675 patients (517 NC and 158 C) diagnosed as 21-hydroxylase deficient, 411 relatives and 167 partners. Disease causing mutations were analyzed by allele-specific/RFLP PCRs and/or by direct sequencing. When necessary, gene dosage was analyzed by MLPA and the regulatory regions of the gene were sequenced. At least 120 different genotypes were identified in our cohort, including 19 rare or less frequent and 11 novel mutations. The frequency of all mutations was determined. Among the 411 relatives, 387 presented at least one mutated allele. 28/167 partners were classified as carriers. *CYP21A2* gene is located in one of the most complex regions of the genome. The application of different methodologies is necessary for the complete study of the gene. Our current molecular diagnostic algorithm allows us to fully genotype patients, in order to complete genetic counseling.

Keywords: 21-hydroxylase deficiency, *CYP21A2* gene, molecular diagnosis

(1837) **MOLECULAR DIAGNOSIS OF MCADD PATIENTS: CHARACTERIZATION OF THREE NOVEL MUTATIONS IN THE *ACADM* GENE**

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Medium-chain acyl-CoA dehydrogenase deficiency (MCADD) is the most frequent inherited defect of fatty acid oxidation. It is characterized by hypoglycemic crisis under fasting or stress conditions, leading to lethargy, seizures and even death. MCADD is recessively inherited, caused by mutations in the *ACADM* gene. The most common mutation is c.985A>G. In cases detected by newborn screening, the frequency of the prevalent mutation is lower than that observed in clinically affected patients, and variants that have never seen before in those patients, are identified. The aim of this study was to describe the molecular characterization of *ACADM* gene in MCADD patients and newborns with positive screening result. DNA was extracted from peripheral blood or dried blood spot from 12 patients. c.985A>G mutation was studied by PCR-RFLP. Samples negative or heterozygous for c.985A>G were sequenced by analysis of the 12 exons and the exon-intron boundaries of the *ACADM* gene. Taking into account the 9 unrelated patients, c.985A>G appeared in 5/8 alleles of the 4 symptomatic patients and in 4/10 alleles of the 5 screening-positive newborns. 8 different sequence variations were identified, including 3 novel: c.119-12A>G was detected in two siblings with c.985A>G. The older brother presented with hypoglycemia and seizures, his sister was asymptomatic. *In silico* tools predicted a possible alteration of splicing; c.608T>C (p.L203S) was found with c.985A>G in a patient presented with hypoglycemia and fever. Several *in silico* tools predicted this variant as deleterious; c.1012C>T (p.Q338X) was detected with c.985A>G in a newborn with an abnormal screening result. This variant is predicted as deleterious as it is a nonsense mutation. In this cohort, mutational spectrum differed between clinically diagnosed patients and those detected by newborn screening, as previously reported in other studies. Molecular and *in silico* analysis proved to be important to confirm the MCADD diagnosis.

(1838) **ALGORITHM FOR MOLECULAR DIAGNOSIS OF NEUROFIBROMATOSIS NF1, NF2 AND NF3 (SCHWANNOMATOSIS)**

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Neuropathological evaluation of CNS tumors is increasingly dependent on molecular genetic tests for proper classification, pre-

diction of biological behavior and patient management. The neurofibromatoses (NFs) consist of at least three autosomal dominant inherited disorders: neurofibromatosis type 1 (NF1), type 2 (NF2), and schwannomatosis (NF3). The molecular diagnosis is still difficult due to: 1) absence of hotspots in *NF1/NF2* genes, 2) ≥50% of sporadic cases for NF1/NF2, 3) *NF1* gene large size and the existence of several pseudogenes. NF3 the newly recognized form is poorly understood: 1) only 15% of cases are inherited, 2) is caused by concomitant loss of several tumor suppressor genes by a single mutational event, 3) the 2 predisposition genes (*SMARCB1* and *LZTR1*) identified do not explain all cases. Our aim is to show the diagnostic algorithms for molecular genetic testing for the NFs. We have used segregation analysis of STRs, mutational screening by DNA sequencing and exome sequencing (WES).The analysis of a family with NF1 numerous patients revealed the at-risk haplotype in one on the unaffected probands and a recombination event in two individuals (one affected and one asymptomatic). In four out of 11 NF2 patients three small novel germinal mutations (2 frameshift and 1 splice-site) and one partial deletion of the maternal *NF2* copy were identified, as well as a loss of heterozygosity (LOH) in the fifth patient. Molecular analysis of four patients with NF3 showed no mutations in *SMARCB1* gene. One of the patients with family history studied by WES, did not show alterations in the predisposing genes**.** Analysis of four of this patient´s tumors did not display the frequently observed LOH. Evaluation of abnormalities in these genes was performed using a diagnostic algorithm which depends on the type of NF, family history and sample availability.

Keywords: Neurofibromatosis 1, Neurofibromatosis 2, Schwannomatosis/NF3, complex TSGs inactivation, exome sequencing

IMMUNOLOGY (INNATE IMMUNITY) 7

(159) **PROBIOTICS INCREASE ANTIMICROBIAL ACTIVITY OF PANETH CELLS IN ELDER MICE.**

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The number of bacteria is very low in the small intestinal tract. This seems to be, at least in part, to the abundant constitutive antimicrobial peptides (AMPs) which are expressed by crypt Paneth cells. Our aim, using mice as experimental model, was to explore whether probiotics can strengthen intestinal barrier through the life of the animals, by increasing the microbicidal activity.

BALB/c mice from 21, 28, 35, 42, 54, 61 and 180 days old, received *L. casei* CRL 431 (Lc 431), *L. paracasei* CNCM I-1518 (Lp 1518) or water (control), upon 7 and 5 days, respectively. After, mice were sacrificed and intestinal fluids, small and large intestine sections were taken.

Forty two days old mice, fed with Lc 431 or Lp 1518, increased the number of positive Paneth cells (64.29 ± 3.58 and 61.80± 5.31%, respectively), respect to control (40.10 ± 3.9%) (Mean ±SEM). Weaning mice (21 days old) that received Lc 431 or Lp 1518 did not show a significant microbicidal activity in the intestinal fluids. In contrast, an important reduction in the CFU of *S. aureus* and S.Typhimurium were observed at 35, 42, 54 and 61 days old ($p<0.05$) as well as in elder mice (180 days old) fed with the lactobacilli. By electron microscopy (EM), pathogens co-incubated with the intestinal fluids from probiotics fed mice, displayed severe disruption of the walls cells and fragmentation.

Interestingly, intestinal fluids of mice from different ages fed with the probiotics showed microbicidal activity against Lc 431 and Lp 1518. These results show the regular consumption of probiotics, do not induce overgrown of them, nor cause adverse effect on the microbiota. Moreover, oral administration of Lc431 or Lp 1518 did not influence the population of total anaerobic bacteria, lactobacilli, and enterobacteria in the colon, at any of age analyzed.

Probiotics appear as effective tools, in young as well as in elder mice, to enhance AMPs in order to face pathogens, without deregulate commensal bacteria and gut homeostasis.

Keywords: Probiotics, antimicrobial peptides, Paneth cells.

(370) **MYELOID-DERIVED SUPPRESSOR CELLS EXPANDED IN INTESTINAL MUCOSA AND ESPLEEN AFTER ORAL INFECTION WITH YERSINIA ENTEROCOLITICA: ROLE OF VIRULENCE FACTORS**

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Myeloid-derived suppressor cells (MDSCs) are a heterogeneous population of immature and immunesupressive myeloid cells. These cells are characterized by expression of CD11b and Gr-1, and are divided into two major subsets: granulocytic and monocytic MDSCs. *Yersinia enterocolitica* (Ye) are Gram-negative bacteria that cause food-borne acute or chronic gastrointestinal diseases. The role of MDSCs in Ye infection has not been determined. The purpose was to elucidate whether oral Ye infection induces the expansion of MDSCs and to define the role of these cells in this infection. Therefore, C57BL/6 mice were infected with Ye WAP-314 serotype O:8. On days 5 post-infection (p.i), cell infiltration in mesenteric lymph nodes (MLN), Peyer´s patches (PP) and spleen was analyzed. Suppressive activity was determined by MTT assay. Moreover, the role of Ye outer protein P (YopP) or H (YopH) in MDSC expansion was explored using for infection Ye deficient in YopP or YopH, respectively. We found that Ye-infected mice presented an increase in the frequencies of $CD11b^{+}Gr\text{-}1^{+}$ cells in PP, MLN and spleen on days 5 p.i, compared to uninfected mice ($p<0.05$). In PP and spleen, granulocytic subset was expanded while in MLN both granulocytic and monocytic subsets were detected. In addition, splenocytes and MLN cells obtained from MDSC-depleted mice and stimulated with specific antigen showed increased proliferation compared to non-depleted mice ($p<0.01$). Moreover, we observed that Ye deficient in YopP induced an increase of MDSCs frequency in intestinal mucosa compared to mice infected with Ye WAP ($p<0.01$). Interestingly, MDSC depletion induced a significant increase in the bacterial load in PP, MLN and spleen in comparison with non-depleted mice ($p<0.05$). We conclude that oral Ye infection induces expansion of MDSCs, which could directly or indirectly promote the elimination of bacteria. MDSC accumulation in intestinal mucosa may be modulated by YopP.

Keywords: *Yersinia enterocolitica*; Infection; MDSCs

(473) **NEW PROTEOME SCREANING METHODS USEFUL TO SENSITIVILY MONITOR NOVELSPECIFIC BIOMARKERS OF TLR-SPECIFIC SIGNALING WITH POTENTIAL IN DIAGNOSTIC AND DRUG TARGETING OF INFECTIOUS DISEASES.**

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Abstract: There is a worrying paucity of research for the accurate identification of intracellular protein biomarkers specific for the ligation of an individual receptor involved in immune and/or inflammatory responses. Finding them is complicated and failed so far due to the intense crosstalk between the different inflammatory or immune receptor pathways which share a plethora of adapter, scaffold and signalling proteins, at multiple levels in several cell types. They also share PTMs between proteins. Thus, we developed novel, potent procedures to sensitively and systematically screen for proteome alterations in human macrophages infected with different bacteria or stimulated with specific TLR2/4 ligands. We found many cytosolic proteins affected in their levels. One was a novel post-translationally generated, anionic form of a chaperone. It was always up-regulated ($p=0.01$, $n=33$) in the treatments and by 9-fold ($p=0.05$) in the peaking time-point. Its level and temporal profile were reproducible after bacterial infection or specific ligation of one TLR type but not the others. The results strongly suggest that its increment is not influenced by the ligand structure but only by the ligation act and by the ligand(s) concentration/half life. It was coreceptor-independent. Inter-

estingly, its late-phase increment was sustained in time, still at day 4 (7-fold). We propose models concerning novel PTM(s) accounting for its reproducible pI change (p=0.01) and its spatio-temporal behaviour. The results suggest a role in a cytosolic complex associated specifically with its TLR TIR and rheostatically integrating the sustained responses (receptor trafficking, tolerance, degradation and autophagy). Its properties make this unique chaperone form an ideal specific biomarker suitable for future translational research oriented to diagnostics and receptor targeting in inflammatory, infectious and immune diseases and also to vaccine development or validation. *Keywords:* TLR, proteome

(614) **PLATELETS DIRECTLY INTERACT WITH *Brucella abortus* AND MODULATE INFECTED HUMAN MONOCYTES TOWARDS A PRO-INFLAMMATORY PROFILE**

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Brucellosis is an infectious disease elicited by bacteria of the genus *Brucella*. Platelets have been described as mediators of hemostasis. However, they have recently got involved in the modulation of innate and adaptive immune responses. We have previously reported that platelets modulate *B. abortus*-mediated infection of human monocytes. The first aim of this study was to characterize the interaction between platelets and *B. abortus*. For this, human platelets were co-incubated with *B. abortus*-GFP for 4 h and the interaction was quantified by confocal microscopy and flow cytometry. Our results showed that platelets are able to interact with bacteria in a dose-dependent fashion. Moreover, this interaction stimulated platelet activation, measured as fibrinogen binding and P-selectin expression ($p<0.001$). The next aim was to further analyze the ability of platelets to modulate functional aspects of infected monocytes. To address this, THP-1 cells were infected with *B. abortus* in presence or absence of platelets for different times. The supernatants from infected cells were collected and quantified by ELISA. The presence of platelets during monocytes/macrophages infection stimulated IL-1β, TNF-α, IL-8 and MCP-1 secretion ($p<0.001$) while it inhibited the secretion of IL-10 ($p<0.01$). We have previously demonstrated that platelets stimulated the expression of ICAM-1 (CD54) in monocytes surface. In order to investigate this mechanism, monocytes were stimulated with supernatants from *B. abortus*-infected platelets and surface CD54 was measured by flow cytometry. Our results show that CD54 induction is due to soluble factors released by infected platelets ($p<0.001$) and sCD40L and PAF are two of the mediators involved. Overall, our results indicate that platelets can directly sense and react to *B. abortus* presence. Moreover, they modulate the *B. abortus*-infected monocytes increasing their pro-inflammatory capacity, which could promote the resolution of the infection.

Keywords: human monocytes; human macrophages; *Brucella abortus*; Platelets

(958) **NEUTROPHILS ACTIVATED BY IMMUNOCOMPLEXES MODULATE CD4 T CELL RESPONSE IN LYMPH NODES**

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Previously we demonstrated that immunocomplexes (IC) generated by injecting OVA in the footpad of immunized mice that have anti-OVA antibodies, induce the migration of OVA^+ neutrophil to draining popliteal lymph nodes (D-poLNs). In the present study we evaluate the influence of neutrophils activated by IC on CD4 T cell response in lymph nodes.

C57BL/6 mice were immunized with OVA emulsified in Freund's complete adjuvant and 15 days later boosted with OVA emulsified in Freund's incomplete adjuvant. Ten days after last immunization they were injected with OVA-FITC in the footpad and D-poLNs were obtained 6 to 48 h later; saline solution was injected on footpad corresponding to control non-draining popliteal lymph nodes (ND-poLNs).

OVA^+ neutrophils migrated to D-poLNs 6 h after OVA injection ($p<0.001$) and at 12 h they were no longer detected. At longer times, we observed that total number of D-poLNs cells increase ($p<0.01$). Particularly, a greater number of CD4 T cells were detected at 48 h in D-poLNs compared to ND-poLNs ($p<0.001$). Naïve ($p<0.05$), effector memory ($p<0.01$) and central memory ($p<0.001$) subtypes were increased. Interesting, CD4 T cells exhibited higher expression of CD69 ($p<0.001$) and Ki67 ($p<0.001$) and higher production of IFNγ ($p<0.05$) and IL17 ($p<0.05$) when compared to CD4 T cells in ND-poLNs. Moreover, D-poLNs showed an increase in $Foxp3^+CD25^+$ Treg population ($p<0.001$).

In order to confirm the influence of neutrophils in CD4 T cells response, we treated mice with anti-Ly6G to deplete neutrophils. We observed a lower number of total poLNs cells ($p<0.001$), CD4 T cells ($p<0.01$) and Treg cells ($p<0.01$) in D-poLNs from mice depleted of neutrophils compare to D-poLNs from isotype treated mice. Besides, CD4 T cells and Tregs showed lower levels of Ki67 ($p<0.05$) when mice were treated with anti-Ly6G.

These findings indicate that OVA^+ neutrophils in D-poLNs promote CD4 T cells proliferation and activation, as well as the development of Tregs.

Keywords: Neutrophils, immunocomplexes, Lymph nodes, CD4 T cells, Tregs.

(1040) ***MINTHOSTACHYS VERTICILLATA* ESSENTIAL OIL MODULATES THE INNATE IMMUNE RESPONSE IN A MOUSE MASTITIS MODEL**

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The aim of this study was to characterize the immunomodulatory effect of *M. verticillata* essential oil (EO) in a mouse mastitis model. Balb/c lactating mice were inoculated in mammary glands (MG) as follows: Group 1 (control): 100 μl of PBS/DMSO (0.05%); Group 2: 100 μl of *Enterococcus faecium* ($1x10^8$CFU/ml); Group 3: EO (100μg/ml) administered twice with an interval of 3 days; Group 4: pretreatment with EO and a challenge with *E. faecium*. Animals were sacrificed at 24, 48, 72, 96 and 360 h post-inoculation (p.i). Bacterial count and histopathology of MG were determined. Gene expression levels of TLR2, TNF-α and IL-1β in the samples were evaluated by quantitative real time-PCR. Group 2 showed intense infiltrations of PMNs since 24 h to 96 h p.i. Bacterial count was low before 72 h and increased from 72 to 360 h p.i. A low expression level of TLR2 was observed whereas an increase expression level of pro-inflammatory cytokines was detected at 96 h p.i regarding the gene expression level of group 1 ($p<0.001$). In MG of group 3, few infiltrations of PMNs were observed. Low expression levels of TLR2 and high expression levels of TNF-α and IL-1β were observed respect to the control group ($p<0.01$; $p<0.001$; $p<0.05$) these expression levels were significantly lower than those found in MG of group 2 ($p<0.001$). MG of group 4, showed few or no infiltration of PMNs at all times assayed and the bacterial count decreased respect to group 2 since 72 h ($p<0.001$). In this group low expression levels of TLR2 were detected and TNF-α and IL-1β expression level were lower than those detected in the MG of group 2 ($p<0.001$). The results showed that EO enhanced mechanisms of innate immunity in the MG by the synthesis of pro-inflammatory cytokines, promoting the recruitment of PMNs to the site of infection and reducing the bacterial load. These results provides new information and serve as base for the possible use of EO as therapeutic strategy in the prevention or control of bovine mastitis.

Key words: Minthostachys verticillata, essential oil, immunomodulatory effect, innate immune response, *Enterococcus faecium* infection.

(1092) **MODULATION OF PURINERGIC SIGNALING HAS A TISSUE-DEPENDENT IMPACT ON THE IMMUNE RE-**

SPONSE AGAINST *TRYPANOSOMA CRUZI* INFECTION

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Adenosine (ADO), an immune-regulatory metabolite, is produced by hydrolysis of ATP that accumulates during tissue injury and inflammation. ADO generated through CD73 activity is an extracellular signaling molecule that is involved in anti-inflammatory mechanisms and provides a feedback to control tissue damage mediated by the host immune response to several infections. We hypothesize that the balance between ATP and ADO is crucial in the development of Chagas disease. The aim of this study was to determine the balance of purinergic signals and its impact in the host immune response developed in different *T. cruzi* target tissues. The kinetic of cardiac macrophage (Ma) subsets showed a predominant inflammatory/M1 profile throughout the acute infection ($p<0.001$) with higher frequency of IL-12+ and iNOS+ M1 Ma ($p<0.05$) and augmented cardiac NO levels ($p<0.001$) in CD73-deficient (KO) compared to C57BL/6 (WT) mice. Moreover, KO mice exhibited increased frequency of cardiac IFN-γ+ and CD107a+ CD8 T lymphocytes ($p<0.05$) and a consequent lower cardiac parasite load ($p<0.05$). Nevertheless, these mice had higher parasitemia ($p<0.05$) associated to lower plasmatic NO levels. Strikingly, in visceral adipose tissue (VAT), parasite load was increased ($p<0.01$) in KO mice, this may be due to an increased basal VAT/body weight ratio ($p<0.001$) compared to uninfected WT mice, generating an important niche for parasite growth. As in VAT, parasite load in KO liver was augmented compared to WT ($p<0.05$). Furthermore, CD73 abrogation significantly decreased extracellular release of ADO in infected heart and VAT but not in liver. These findings could be explained by the different purinergic signaling impact in the target tissues evidenced by about 12-fold increase in ATP/ADO ratio in KO/WT heart compared to a 5 and 1 in VAT and liver respectively. In conclusion, purinergic system differentially modulates the host immune response against *T. cruzi* infection in a target tissue-dependent manner.

Keywords: Cd73 – Adenosine – Visceral Adipose Tissue - Hepatic Tissue – Cardiac Tissue

(1382) **MODULATING EFFECTS OF 5,5-DIMETHYL-1-PYRROLINE *N*-OXIDE ON THE TRANSCRIPTOME OF LIPOPOLYSACCHARIDE-PRIMED MACROPHAGES**

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The search for mechanism-based therapeutics against chronic inflammation (CI) is a highly desired focus of pharmaceutical industries. The switch of macrophages phenotype from a normal M2 towards an inflammatory M1 phenotype is known to play an important pathogenic role in a number of CI diseases, including adipose tissue inflammation in obesity. 5,5-dimethyl-1-pirroline *N*-oxide (DMPO) is a nitrone spin trap originally synthesized as a spin trap for study of free radicals. Previously we found that DMPO can also produce anti-inflammatory effects in a mouse model of diet-induced obesity. However information regarding transcriptional effects of this spin trap remains unknown. Herein, we hypothesize that DMPO by itself can prevent LPS-induced M1-like activation of macrophages by changing its transcriptome. To test this hypothesis we incubated RAW 264.7 cells with 1 ng/ml LPS in the presence or absence of 50 mM DMPO for 6h. RNA was extracted and used for transcriptomics analyses using microarray technology (Illumina). Functional data analysis showed 79 differentially expressed genes (DEGs) in DMPO vs Control comparison (ONE-way ANOVA with a FDR = 0.05). We used DAVID databases for identifying enriched Gene onthology terms and Ingenuity Pathway Analysis (IPA) for functional analysis. We found that DMPO DEGs were related to immune system process and negative regulation of innate immune response among others. Functional analysis indicated that IRF7 and TLRs were related (predicted inhibitions) to the observed transcriptomic effects of DMPO. Functional data analyses were consistent with DMPO dampening LPS-induced inflammation in RAW 264.7, these effects were confirmed using Nanostring technology. Taking together our data surprisingly indicates that DMPO by itself affects gene expression related to regulation of immune system. This idea suggests DMPO can serve as a structural platform for the design of novel compounds to reduce macrophage activation at inflammation sites.

Keywords: macrophage, lypopolysaccharide, phenotypic switch, DMPO, transcriptomics

(1418) **NON-NEURONAL CHOLINERGIC SYSTEM MODULATE THE CROSS-TALK BETWEEN THE IMMUNE SYSTEM AND GLIOBLASTOMA CELLS**

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Glioblastoma multiforme (GBM) is the deadliest and most common type of human primary brain tumor. This tumor is defined by the hallmark features of uncontrolled cellular proliferation, diffuse infiltration, robust angiogenesis, resistance to apoptosis and genomic instability. Acetylcholine is a neurotransmitter which can also modulates cell survival, proliferation and differentiation in neuronal and non-neuronal cells such as immune cells, which has been referred to as a “non-neuronal cholinergic system”. The aim of this work was to elucidate the relevance of the non-neuronal cholinergic system in the interaction between immune and GBM cells. We first evaluated the expression of acetylcholine receptors in human GBM cell lines by fluorescence microscopy. We found that both U251 and U373 human GBM cells express acetylcholine muscarinic receptors M1 and M3. In order to evaluate whether the cholinergic system affects the cross-talk with immune cells, human U251 cells were co-cultured with human dendritic cells (DC) in the presence of cholinergic agonists (carbachol and muscarine). Mononuclear cells were isolated from buffy coats of healthy adult nonsmoker volunteer and CD14+ cells were then isolated by positive selection and then were cultured with GM-CSF and IL-4. The co-cultures were incubated in the presence of carbachol 10^{-8} M) and muscarine (10^{-8}M). We found that U251 cells upregulated the expression of CD86 in DCs as assessed by flow cytometry in presence of carbachol and muscarine with respect to control co-cultures ($p<0.05$). Human U251 and U373 GBM cells were cultured in presence of a cholinergic agonist (carbachol) to evaluate their expression of the coactivation marker ligand OX40 ligand (OX40L) as assessed by flow cytometry, but no differences were observed between the cells treated with carbachol and controls. Conclusions: our findings suggest that the non-neuronal cholinergic system is present in GBM cells and could modulate their cross-talk with the immune system.

(1464) **mTOR INHIBITION IN *trypanosoma cruzi* INFECTED MACROPHAGES ACTIVES NLRP3 AND INDUCES MITOCHONDRIAL ROS PRODUCTION THAT REGULATE PARASITE SURVIVAL.**

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We have previously shown that *Trypanosoma cruzi* infection in macrophages (Mo) activates mTOR pathway and its inhibition de-

creased parasite replication. However, in rapamycin (Rap) pretreated and *T. cruzi* infected Mo, we observed reduced nitric oxide production and iNOS expression compared to control cells. Therefore, the aim of this work was to determine alternative activated mechanisms involved in controlling parasite during mTOR inhibition. In this sense, we found that cytoplasmic ROS (cROS) as well as IDO activity were not involved as inflammatory mechanisms. Consequently, to study possible mediators involved in parasite killing, we obtained bone marrow derived Mo (BMDM) from different KO mice pretreated them with Rap and then infected. Parasite load was evaluated 72h post infection (pi) by immunofluorescencia (IF). The results showed a significant increase in parasite load in BMDM from IL-6-KO, TNFa-R-KO and NLRP3-KO compared to WT BMDM. However, parasite number stands out in BMDM from NLRP3 KO ($p<0.05$). Taking into account that NLRP3 is a key component of the inflammasome, our next aim was to determine whether this pathway is involved. We observed that Rap-pretreated and infected BMDM showed a significant increase in NLRP3 and IL-1β expression at 6h pi ($p<0.05$). Besides, TNF-a, IL-6, and IL12 expression were also increase at 12, 18 and 24h pi ($p<0.05$). It has been shown that inflammasome uses the mitochondria as platform of assembly. We found a significantly increase in mitochondrial ROS (mtROS) production at 3 and 6h pi ($p<0.05$). To evaluate the relevance of mtROS, BMDM were treated with DPI (NADPH oxidase inhibitor), then infected and parasite load was studied by IF. We observed that Rap+DPI pretreated BMDM had significantly higher parasite load compared to Rap pretreated BMDM ($p<0.05$). Our findings strongly support that mTOR inhibition during *T. cruzi* infection in Mo induced inflammasome activation that leads to mtROS production controlling parasite survival.

METABOLISM AND NUTRITION 5

(1502) **CYTOKINE-INDUCED ENDOPLASMIC RETICULUM STRESS IN β-CELLS IS AMELIORATED BY COMPOUND A**

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Type 1 diabetes (T1D) is a T cell-mediated autoimmune disease that selectively destroys insulin producing β-cells. ER stress and subsequent insulin secretory deficiency in β-cells precede the onset of autoimmune diabetes. Pro-inflammatory cytokines (IL-1β+IFN-γ; CYT) signaling leads to activation of ER stress in β-cells. We reported that Compound A (2-(4-acetoxyphenyl)-2-chloro-N-methyl-ethylammonium chloride; CpdA), a dissociative glucocorticoid receptor (GR)-ligand, is an effective modulator of T and dendritic cells. The aim of this study was to explore the beneficial effects of CpdA on CYT-induced β-cell ER stress; the classic GR-ligand dexamethasone was used for comparison. CpdA significantly reduced CYT-induced NO secretion by the insulinoma INS-1E cells ($p<0.01$). CpdA treatment inhibited CYT-triggered IκBα phosphorylation protecting its degradation and hampering, in consequence, NF-κB nuclear translocation in INS-1E cells ($p<0.05$). CpdA treatment impaired eIF2α phosphorylation enhancement and the increase of ER stress protein markers expression, such as ATF4 and CHOP, in CYT-challenged INS-1E cells ($p<0.05$). The expression of chaperones involved in protein folding and processing (PDI, ORP150) was enhanced in the presence of CpdA ($p<0.05$). CpdA administration (i.p.) to NODscid mice adoptively transferred with diabetogenic splenocytes (from diabetic NOD mice) led to a delay of disease onset ($p<0.001$ vs control). CpdA-treated mice showed a reduction in islet leukocytes infiltration and preserved insulin expression in comparison with veh-treated group, assessed by immunohistochemistry. In summary, we demonstrate that CpdA directly improves the UPR attenuating ER stress in β-cells challenged by CYT. The latter together with our previous reports on immune cells modulation, might warrant the administration of CpdA as a novel therapeutic strategy with dual activity on autoimmune diabetes.

Keywords: autoimmunity, glucocorticoids, islets of Langerhans.

(179) **DIETARY SOY PROTEIN IMPROVES THE ALTERED INTRA-MYOCARDIAL GLUCOSE METABOLISM IN A HYPERTENSIVE, DYSLIPEMIC, INSULIN RESISTANT RAT MODEL.**

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The aim of the present work was to investigate whether the replacement of dietary casein by soy protein isolated (SPI) could be able to improve and/or even reverse the underlying mechanisms involved in the impaired glucose metabolism in the heart of hypertensive, dyslipemic, insulin resistant rats fed a sucrose rich diet (SRD). Methodology: Wistar rats were fed SRD for 4 months. From month 4 to 8, half the animals continued with the SRD and the other half were fed a SRD in which the source of protein, casein, was substituted by SPI. The control group received a diet with maize starch as a source of carbohydrate. In the heart muscle were determined: a. Enzymatic activities of hexokinase (HK) and pyruvate dehydrogenase complex (PDHc), b. Protein mass levels of Glut-4 at basal conditions and under insulin stimulation (euglycemic-hyperinsulemic clamp), AMPK, pAMPK, UCP2 and PPARα (Western Blot). Besides blood pressure, and plasma levels of glucose, insulin, triglycerides and free fatty acids (FFA) were analyzed. Results: Compared with the SRD-fed group the results showed that SPI significantly increased ($P<0.05$) Glut-4 protein mass level under insulin stimulation and normalized glucose phosphorylation and oxidation -estimated by HK and PDHc activities- respectively ($P<0.05$). Besides, a reduction of pAMPK protein mass level was recorded ($P<0.05$) without changes in UCP2 and PPARα. Blood pressure, dyslipidemia, glucose homeostasis and insulin sensitivity were normalized by SPI ($P<0.05$). This study provides new information showing the beneficial effects of soy protein upon the altered pathways of glucose metabolism in the heart muscle of dyslipemic insulin-resistant rat model.

Keywords: soy protein, heart muscle, glucose metabolism, sucrose-rich diet

(1550) **EFFECT OF C-TYPE NATRIURETIC PEPTIDE ON OXIDATIVE STRESS AND ADIPOSE TISSUE**

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C-type natriuretic peptide (CNP) was described as an anorexigenic peptide at central level. Previously, we showed that chronic CNP administration enhances endothelial nitric oxide (NO) system and decreases vascular oxidative stress (OS). The aim of the present study was to evaluate the effects of chronic CNP treatment on systemic OS and adipose tissue (AT) content in normotensive and spontaneously hypertensive rats (SHR).

Design and method: 12-week-old male Wistar (W) and SHR were infused with CNP (0.75 μg/hr) or saline (S) (osmotic pumps, 14 days). Animals were fed standard rat chow and tap water ad libitum. Systolic blood pressure (SBP, mmHg) was recorded (tail-cuff). After treatment, plasma samples were collected to determine the content of: thiobarbituric acid reactive species (TBARS, nmol MDA/mL) and gluthatione (GSH, μmol/L). Urine samples were collected to measure excretion of nitrites and nitrates (NOx, nmol/min.100g body weight (BW)). Animals were decapitated and epididymal (E) AT were removed and weighted (g/100g BW), and adipocyte area (AA, μ^2) was measured in hematoxylin-eosin stained slices. Statistics: 2-way ANOVA, Bonferroni test ad hoc. n=4 rats/group.

Results: SHR showed higher SBP and plasmatic OS (SBP:W-S=119±5, W-CNP=121±4, SHR-S=187±3*, SHR-CNP=158±6#; TBARS:W-S=1.3±0.2, W-CNP=1.4±0.1, SHR-S=13.2±1.0*, SHR-CNP=11.7±1.3; GSH:W-S=129±17, W-CNP=256±34*, SHR-S=100±15, SHR-CNP=197±28#; NOx-:W-S=1.1±0.1, W-CNP=1.6±0.1*, SHR-S=1.6±0.1*, SHR-CNP=1.7±0.1; *p<0.01vs.W-S; #p<0.01vs.SHR-S). CNP decreased SBP, but did not modify the content of TBARS in SHR. In addition, CNP did not modify BW and EAT in both groups. However, CNP administration decreased EAA (W-S=4663±388, W-CNP=3260±310*, SHR-S=5960±431*, SHR-CNP=4855±266#; *p<0.01vs.W-S; #p<0.01vs.SHR-S).

Conclusions: Our results show that chronic CNP treatment enhanced antioxidant species and decreased EAA in both groups. In addition to these beneficial effects, CNP decreased SBP in this model of hypertension.

Keywords: Natriuretic peptides, adipose tissue, metabolism, oxidative stress, blood pressure.

(999) **EFFECT OF GLUCOSE DEPRIVATION ON SERTOLI CELL LIPID METABOLISM**

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Abstract: Sertoli cells (SC) possess a unique metabolism based on its role as nutritional support for germ cells. SC actively metabolize glucose into lactate to provide energy to cells located in the adluminal compartment of seminiferous tubules. Additionally, it has been demonstrated that SC can oxidize fatty acids (FA) and also store them as triacylglycerides (TAG) within lipid droplets (LD). We have previously demonstrated that SC are able to survive in culture for at least 48 h in the absence of glucose suggesting that, under this adverse condition, SC lipid metabolism would become essential for survival. However, no experiments regarding this matter were performed. The aim of the study was to investigate the effect of glucose deprivation on the regulation of lipid catabolism in SC. For this purpose, SC isolated from 20-day-old rats were cultured for 48 h in media containing different concentrations of glucose: 8 mM (concentration commonly used in the culture media of SC) and 0 mM. FA oxidation (measured as ^3H_2O production from [9,10-^{3}H(N)]-palmitic acid), carnitine palmitoyltransferase 1A (CPT1A) expression (RT-qPCR), TAG levels (spectrophotometric method) and LD content (Red Oil O staining) were evaluated. Results are shown as means±S.D. of triplicate incubations in one representative experiment out of three. (*P<0.05 **P<0.01 ***P<0.001 vs.8 mM). Results obtained show that glucose deprivation in SC culture medium induced an increase in FA oxidation (8 mM: 2.5±0.6, 0 mM: 5.2±0.5** pmol Ox Palm/h/μg DNA) without changes in CPT1A mRNA levels. Additionally, they show that glucose deprivation led to a decrease in TAG content and in LD number *(TAG* 8 mM: 1.4±0.1, 0 mM: 0.9±0.1* μg TAG/ μg DNA; *LD* 8mM: 0.8±0.3, 0mM: 0.2±0.1*** ratio LD/ cell).

Altogether, these results suggest that SC are able to adapt and survive under conditions of glucose deprivation by increasing FA utilization to ensure their own energetic requirements.

Keywords: Sertoli Cell, Glucose deprivation, Fatty Acid, Lipid metabolism

(1545) **EFFECTS OF DIETARY ZINC RESTRICTION DURING PRENATAL AND POSTNATAL LIFE IN METABOLIC PROFILE AND ADIPOSE TISSUE OF ADULT MALE RATS**

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Zinc deficiency during fetal life and lactation induces cardiovascular and renal alterations in adult male rats. There is a relationship between adipose tissue (AT) dysfunction and the development of cardiovascular and metabolic diseases.

Objective: To evaluate the metabolic profile, as well as, morphology and oxidative state of retroperitoneal AT (rAT) in male rats subjected to moderate zinc deficiency during fetal life, lactation and/or growth.

Method: Female Wistar rats received during pregnancy until weaning control (C,30ppm) or zinc deficient (B,8ppm) diet. At weaning, C male offspring continued on C diet (CC) and B offspring received B or C diets for 60 days (BB, BC). At day 74 of life, glucose tolerance curve (min.mg/dL) and serum triglyceride concentration (TG, mg/dl) were determined. At day 81, rats were sacrificed to evaluate in rAT: adipocyte area (μm^2), lipid peroxidation (TBARS, nmol MDA/mg.prot) and the activity of antioxidant enzymes (catalase (CAT), pmol/mg.prot; gluthatione peroxidase (GPx), μmol/min.mg.prot and superoxide dismutase (SOD), USOD/mg.Prot).

Results: Area under the glucose tolerance curve (CC:27855±592; BB:26370±891, BC:28668±1013);TG (CC:85±4; BB:112±6*§, BC:80±5); Adipocyte area (CC:6231±974; BB:6828±1132, BC:6427±1910);TBARS (CC:0.35±0.04, BB:0.55±0.03*§, BC:0.82±0.04*);CAT (CC:1.44±0.09, BB:1.03±0.09*, BC:1.20±0.04*); GPx (CC:89±7, BB:58±3*, BC:67±7*); SOD (CC:2.4±0.2, BB:3.0±0.2, BC:2.3±0.2). One way ANOVA, Bonferroni post-hoc test (n=6/group, *p<0.05 vs CC, §p<0.05 vs BC).

Conclusion: BB rats showed increased TG. Although rAT from all experimental groups showed similar adipocyte area, this tissue exhibited a pro-oxidant state and a decreased in antioxidant defenses. Adequate zinc content after weaning reversed the changes in lipid profile but not in the oxidative state of rAT. Zinc deficiency predisposes to the development of cardiovascular risk factors by programming alterations of metabolic profile and oxidative state of rAT.

Key words: prenatal and postnatal life, zinc deficiency, lipid and glucose metabolism, adipose tissue, oxidative stress

(865) **EFFECTS OF MILK PRODUCTION IN LIVER LIPID METABOLISM OF DAIRY CATTLE DURING THE TRANSITION PERIOD**

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The transition period (TP) represents a conflictive scenario for the lactation of dairy cows, in which the liver attempts to fix the important fat mobilization as a result of a negative energy balance. The aim of this study was to analyze the impact of milk production in some hepatic lipid metabolism pathways of dairy cattle during the TP. Blood samples and liver biopsies from cows (n=18) were taken at 28 and 14 days prepartum and at 4, 14, 28 and 60 days postpartum. The animals were divided into high production (HP) group and low production (LP) group considering the average of the dairy production up to 120 days. Plasmatic concentrations of non-esterified fatty acids (NEFAs) and beta-hydroxybutyrate (BHB), aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT), glucose, triglycerides and insulin along with liver triglycerides (LT) content were measured. Moreover, the mRNA expression of carnitine palmitoyltransferase-1 (CPT1), acyl-CoA oxidase-1 (ACOX1) and diacylglycerol acyltransferase-1 (DGAT1) was determined by real time-PCR, and the protein expression of ACOX1 and PPAR-a by western blot assays. The AST, GGT and insulin concentrations were similar in both groups (p>0.05). While the glycemia and triglyceridemia were lower (p<0.05), the concentrations of BHB, NEFAs and LT content were higher at 4 and 14 days postpartum (p<0.05) in the HP group. Furthermore in this group, while the mRNA expression of CPT1 and ACOX1 were higher along the postpartum period, the protein expression of PPAR-a was higher on day 60. Although the ACOX1 protein expression was similar in both groups, the HP group

showed a tendency to increase (p<0.076). The mRNA expression of DGAT1 was similar in both groups (p>0.05). Significant differences were registered in liver lipid metabolism according the milk production. These results suggest that HP have a major capacity of fatty acids oxidation trying to resolve the increased fat mobilization during the TP.

Keywords: milk production, liver lipid metabolism, transition period, dairy cattle.

(424) **EFFECTS OF OLIGODEOXYNUCLEOTIDE IMT504 ON CELL VIABILITY AND GENE EXPRESION IN A MURINE BETA CELL LINE (MIN6B1)**

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We have previously demonstrated that treatment with IMT504 promotes significant improvement in the diabetic condition in diverse animal models. We have also shown effects on gene expression on freshly isolated islets from diabetic IMT504-treated animals. Based on these results, here we evaluated if the effects of IMT504 observed *in vivo* were due to direct effects on beta cell function. In particular we studied cell viability and gene expression. A murine beta cell line (MIN6B1, kindly donated by Dr. P. Halban) was used. Cells were cultured in DMEM with 20 mM glucose, 15% SFB, 71 uM βmercaptoethanol. Cell viability was analized by MTS while gene expression of Pdx-1, INS2, INS1 and MafA was analized by qPCR, using cyclophilin as housekeeping gene. To study cell viability, cells were stimulated for 24 or 48 h with 0 (C) 0,4 (IMT0,4), 2,2 (IMT2,2) and 4 ug/ul (IMT4) of IMT504 in DMEM, 20 mM glucose, 0,5% BSA, 71 uM βmercaptoethanol. For gene expression, cells were stimulated for 12, 24 and 48 h with the same stimuli. No differences in cell viability were observed at the time points studied (ANOVA for repeated measures: NS; n=5). Expression of Pdx-1 and INS2 was significantly increased by 48 h stimulation with IMT504 [ANOVA for repeated measures: Pdx-1 (A.U.): C=0,47±0,03; IMT0,4=0,40±0,03; IMT2,2=0,52±0,04; IMT4=0,68±0,05 p<0,05, IMT4 different from C and IMT0,4; INS2 (A.U.): C=1,76±0,08; IMT0,4=1,86±0,11; IMT2,2=2,00±0,11; IMT4=02,69±0,14 p<0,05, IMT4 different from C and IMT2,2; n=7], while no changes in INS1 and MafA were observed (NS; n=4). After 12 or 24 h of stimulation with IMT504, no significant differences were observed in either Pdx-1 or INS2 (NS; n = 3). Our results demonstrate a direct effect of IMT504 on gene expression in beta cells, i.e. stimulation of Pdx-1 and INS2. Further studies must be done to dilucidate their implications on beta cell function recovery in diabetic animals.

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Keywords: MIN6B1;IMT504; Pdx-1; INS2; cell viability.

(431) **ENHANCED LIPOPROTEIN LIPASE ACTIVITY IN EPICARDIAL ADIPOSE TISSUE FROM PATIENTS WITH CORONARY ARTERY DISEASE: ITS BEHAVIOR IN INSULIN RESISTANCE**

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Epicardial adipose tissue (EAT) is a visceral AT surrounding and infiltrating myocardium and coronary arteries, which volume is directly associated with an increased risk of coronary artery disease (CAD). Lipoprotein lipase (LPL) is an enzyme involved in lipolysis of Triglycerides (TG)-rich lipoproteins promoting fatty acid deposition in AT. In EAT little is known about LPL behavior and regulation in insulin-resistance (IR) states. Aim: to evaluate LPL activity and its regulators ANGPTL4 and PPARγ, in EAT from CAD patients, and their behavior according to IR states. Patients and methods: in EAT and subcutaneous AT (SAT) from patients undergoing coronary artery bypass graft (CAD, n=29) or valve replacement (No CAD, n=19) LPL activity was evaluated, as well as ANGPTL4 and PPARγ expression. Serum lipid profile was assessed, and TG/HDL cholesterol (HDL-C) index was calculated, as a surrogate marker of IR. The study was approved by the Ethic Committee of the Hospital de Clínicas, UBA. Results: EAT LPL activity was higher in CAD than No CAD (p=0.02), without differences in SAT LPL activity between groups. ANGPTL4 expression was decreased in EAT from CAD (p=0.005), without differences in PPARγ expression between groups. TG/HDL-C tend to be higher in CAD than No CAD (p=0.07), negatively associated with EAT LPL activity in CAD (p=0.008). When dividing CAD patients in tertiles of TG/HDL-C, EAT LPL activity was lower in the higher TG/HDL-C tertiles (T1 vs T2, p=0.05; T1 vs T3, p=0.004), with increased expression of ANGPTL4 in T3 (p=0.06 vs T1). No differences were observed in PPARγ expression between tertiles of TG/HDL-C. Conclusions: Although EAT LPL activity decrease with IR in CAD patients, it remained increase compared to No CAD. In accordance, ANGPTL4 presented an antagonist behavior, supporting its role as a negative regulator of LPL. These findings suggest that other mechanisms beyond IR, could be involved in the enzyme regulation in EAT.

Epicardial adipose tissue; Coronary artery disease; Lipoprotein lipase; Angiopoietin-like 4; Peroxisome proliferator activated receptor γ; Insulin resistance

(545) **EVALUATION OF LIVER NON-PARENCHYMAL CELLS (NPC) POPULATIONS DURING HIGH FAT DIET (HFD)-DERIVED HEPATIC INFLAMMATORY RESPONSE. ROLE OF TUMOR NECROSIS FACTOR-ALFA RECEPTOR 1 (TNFR1)**

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Obesity is a complex metabolic disorder, characterized by chronic low-grade inflammation, increased pro-inflammatory circulating cytokines as TNF-α and IL-1β, and immune cell infiltration in the liver. Hepatic non-parenchymal cells, as resident macrophages (Kupffer cells) as well as recruited macrophages, play a significant role in liver inflammation. In this work, we aim to evaluate the role of TNFR1 in hepatic inflammation in a model of obesity induced by HFD. Six-week-old C57BL/6 wild type (WT) and and knockout C57BL/6-Tnfrsf1atm1Imx/J (TNFR1-KO) mice were fed with regular chow diet (C-WT; C-KO) or a 40% high fat diet for 16 weeks (HFD-WT; HFD-KO). Histological evaluation of hepatic tissue stained with hematoxylin and eosin showed mild inflammation in HFD-WT and moderate to severe inflammation in HFD-KO. To characterize immune cells populations, NPC were isolated from livers by *collagenase perfusion* procedure, differential centrifugation and were analyzed by flow cytometry. Macrophages content ($CD45^+F4/80^+$ cells) was higher in KO mice (HFD-WT:36% vs HFD-KO:51%), in both, resident ($CD45^+F4/80^{HIGH}$ cells in HFD-WT:6% vs HFD-KO:10%) and recruited populations ($CD45^+F4/80^{LOW}$ cells in HFD-WT:30% vs HFD-KO:40% or $CD11b^+CCR2^+$ cells in HFD-WT:2.3% vs HFD-KO:9.3%) (p<0.05). In addition, macrophages isolated from KO mice showed a classical pro-inflammatory activation state (M1), assessed by a 136% increase in $CD45^+F4/80^+CD11c^+$ cells when compared to HFD-WT (p<0.05), and by higher expressions of iNOS (HFD-WT:141%; HFD-KO: 361%) and COX-2 (HFD-WT:155%; HFD-KO:312%; p<0.05) analyzed by western blot in NPC. Evaluation by RT-qPCR of M2 (alternatively activated) polarization markers, as

Arginase-1 and *Hemeoxigenase-1,* evidenced lower levels in HFD KO group (-91% and -64% respectively). Based on these results, we suggest that the lack of TNFR1 increases hepatic content of M1 macrophages that contribute to HFD-derived hepatic inflammation.

Keywords: Non Parenchymal Cells (NPC), High Fat Diet (HFD), TNFR1, Hepatic Inflammation

PLANT BIOLOGY 6

(128) PRODUCTION OF NUCLEOPROTEIN FROM CANINE DISTEMPER VIRUS IN TRANSPLASTOMIC TOBACCO PLANTS

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The use of plants as bioreactors for the expression of pathogen antigens is an attractive alternative to produce cost-effective subunits vaccines. Chloroplast transplastomic technology offers many advantages over other plant expression systems, including stable high-level of recombinant protein expression and transgene containment. In this work, we evaluated the production of the nucleoprotein (NP) from an Argentina strain of canine distemper virus (CDV) in transplastomic tobacco plants. The NP sequence was fused to the endogenous promoter and 5′-untranslated region of the *psbA* gene and with a C-terminal 6xHis-tag. The transgene was targeted to the transcriptionally active spacer region between the *trnI* and *trnA* genes within the ribosomal operon. Finally, the construction was introduced into *Nicotiana tabacum* cv. Petite Havana chloroplasts by biolistic transformation. Stable integration of NP transgene and homoplasmy of the transformed plants were confirmed by Southern blot analysis. NP transplastomic plants exhibited normal growth and morphology. When total protein content from transplastomic leaf extracts was separated by SDS-PAGE, a 60-kDa band of the expected size for NP could be visualized upon staining with Coomassie blue. This band was also detected by Western blot using an anti-NP monoclonal antibody. In order to evaluate the solubility of NP, total soluble and insoluble proteins were extracted from developed mature leaves. The specific 60-kDa band was observed mainly in the insoluble fraction by Coomassie blue staining and Western blot. The chloroplast-derived NP will be used to evaluate the immune responses elicited in mice after subcutaneous and oral administration.

Keywords: plant-based vaccine; Canine distemper virus; Chloroplast transformation; recombinant protein; *Nicotiana tabacum*

(1384) ROOT GROWTH ANALYSIS IN PSARK::IPT WHEAT TRANSGENIC PLANTS UPON CADMIUM EXPOSURE

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Adverse environmental conditions are closely associated to plant growth inhibition. Plant hormones play an important role in the adaptation to a stressful environment. In this context, engineering cytokinin (CK) metabolism is described as a prospective way to improve crop plants adaptation to adverse growth conditions. Transgenic plants expressing the isopentenyltransferase (IPT) gene, a key enzyme in cytokinin biosynthesis, placed under the control of PSARK promoter have resulted promising for maintaining steady state CK level higher than normal concentration during stress, but not high enough to impinge in developmental processes. Herein, we studied the postembryonic wheat root response to Cd stress in wild type (PIF) and in Psark:IPT transgenic (TR4) *Triticum aestivum* L plants. Twenty seeds were placed in flasks with 30 mL of distilled (control) or containing 10 μM $CdCl_2$ (PIFCd and TR4Cd) and incubated at 24 ± 2°C in darkness for 72 h. Despite Cd treatment significantly decreased root length in both genotypes, statistically significant interaction was detected between genotypes and Cd treatment. This result was supported by root biomass determination where PIF plants were more affected by Cd than TR4 ones. The metal decreased transcripts accumulation of genes related to the cell cycle G1/S transition (*rdr*, *mcm2*), but up-regulated genes related to cell expansion (TaExpA8, TaExpB8, TaExpA6 and TaExpB10) in the root apical meristem (RAM). Interestingly statistical analysis showed that the effect in expansin transcripts level was genotype-dependent, regardless of the treatment. Cadmium hastened cells displacement from the cell division zone to elongation/differentiation zone, resulting in a shortened meristem in PIF plants. These results indicated that Psark:IPT plants have a greater ability to sustain embryo root elongation under Cd stress. More studies are necessary to evaluate the importance of the RAM transition zone as an oscillatory zone that connects root growth and development with environmental cues.

Keys words: WHEAT, CADMIUM, ABIOTIC STRESS, CYTOKININ

(1006) ROOT METABOLIC ADJUSTMENTS IN *Ilex paraguariensis* IN RESPONSE TO DROUGHT

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Yerba mate (*Ilex paraguariensis* A. St.-Hil) is a subtropical tree species of great economic importance for the Northeast of Argentine. It has been determined that an important factor in the productivity of this crop is its ability to acclimate to the processes of water scarcity. With the aim to dilucidated the biochemical mechanisms involved in plant root response to dehydration, three plants from a drought tolerant cultivar were subjected to progressive soil water deficit until Ψ_{soil} = -2MPa, under controlled environmental conditions. Three well-watered plants were included as controls. Metabolites were extracted from lyophilized root tissue, 16 compounds were determined using enzymatic and colorimetric assays, whereas 44 by GC-MS. The data were coupled to RNA-Seq information (Illumina HiSeq 1500) obtained from another assay performed under the same experimental conditions. The metabolites that behaved differentially (*t* test, $p<0.05$) between control and stressed plants mainly corresponded to compounds belonging to the family of carbohydrates, amino acids and organic acids (malate, 2-oxoglutaric acid). Even more, several transcripts related to the metabolic pathways involved were identified and analyzed. Adequate evaluation of metabolomic data in combination with transcriptomic data may contribute to the identification of biological markers of tolerance to dehydration in *Ilex paraguariensis* and selection of candidate genes to transfer superior characters to this or another crop.

Key words: Ilex paraguariensis, metabolome, transcriptome, roots, drought

(585) ROOTING *EX VITRO* AND ACCLIMATIZATION OF MICROPROPAGATED SHOOTS OF *Eucalyptus grandis* THROUGH A HYDROPONIC SYSTEM

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Plant micropropagation can be critical in certain species for multiplication of interesting cultivars and breeding programs. *Eucalyptus grandis* is a very important wood crop in Argentina and its micropropagation is still an extensive process that requires long periods (six months). The objective of this work was to develop a faster mi-

cropropagation protocol adapted to *E. grandis* in order to properly satisfy local demand. To address this challenge, we rooted *ex vitro* and acclimated micropropagated shoots in a hydroponics system. We used nodal segments as initial explants to obtain the micropropagated shoots. The initial protocol for explant sterilization (24 g l^{-1} sodium hypochlorite during 5 min followed by three washes with sterile water of 5 min) was sufficient (sterilization rate 95.0 ± 1.8%). The sterile nodal segments were cultured in a MS medium supplemented with 1 mg l^{-1} butiric acid, sucrose 30 g l^{-1} and agar 0.6 g l^{-1}, and incubated during four weeks at 25 ± 2°C under 16 h day photoperiod. Active carbon (1 g l^{-1}) was added to MS medium to absorb the phenols produced by the eucalyptus explants. A 73 ± 8.7% of nodal segments grew generating 1.73 ± 1.03 micropropagated shoots per explant with a length of 0.76 ± 0.44 cm. Micropropagated shoots were transferred to a hydroponic system to allow *ex vitro* rooting and acclimatization (nutritional solution: Hoagland supplemented with 2 mg l^{-1} indol butyric acid). After four weeks the 11 ± 5.8% of micropropagated shoots had developed roots. All plantlets obtained were recultured to continue its growth in a new hydroponic system without plant growth regulators, after four weeks the plants were ready to transfer to soil. The protocol allowed to obtain micropropagated plants in 12 weeks. Although additional assays are necessary to improve the *ex vitro* rooting rate of the micropropagated shoots, our results show that traditional micropropagation *Eucalyptus* protocols can be substantially reduced to half of the time.

Keywords: Rooting *ex vitro*, acclimatization, *Eucalyptus grandis,* micropropagation, hydroponics.

(1309) ***SENECIO SELLOI* SPRENG (ASTERACEAE) FLOWER EXTRACT EFFECTS ON THE PEROXIDATION OF MICROSOMES AND MITOCHONDRIA MEMBRANES FROM RAT LIVER**

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Senecio selloi Spreng ("margarita de campo", Asteraceae, SS) is a 40-80 cm height bush with sticky leaves and yellow daisy-like flowers. Pyrrolizidine hepatotoxic alkaloids (PAs) have been identified in the plant and have caused poisoning when eaten by cattle. Despite this, many *Senecio* spp. are used by native people from Latin America as grounded leaves or flowers mixed with creams or as decoctions to heal skin injuries. Considering the latter, a methanolic extract from dried flowers of SS was studied for its phytochemistry and peroxidation activity on microsome and mitochondria membranes of hepatocytes from Wistar AH/HOK rats. A series of tests were performed on the extract to determine flavonoids, phenolic compound, lipids, carbohydrates and alkaloids, among others. Chemiluminicense and peroxidation were initiated by adding ascorbate to both microsomes and mitochondria. Both microsomal (1 mg) and mitochondrial (0.5 mg) protein with the addition of SS extract (0.025, 0.10, 0.40 and 0.80 μg) were incubated at 37 °C for 180 minutes in 0.01 M phosphate buffer pH 7.4 and 0.4 mM ascorbate. Controls with ascorbate but without extract were used. Phytochemistry demonstrated that flavonoids and phenolic compounds were the more concentrated. Regarding peroxidation, counts per minute (cpm) originated from the light emission from mitochondria incubated with SS was statistically lower (concentration dependent) when compared to controls, demonstrating protection against peroxidation. On the contrary, incubation of microsomes with SS did not cause inhibition of peroxidation. These results could be due to the different chemical membrane structure of the organelles used for this study. Some *Senecio* species are toxic when ingested by both humans and animals due to liver bioactivation of PAs. However, when applied to the skin, the phenolic and flavonoids present in the flowers may exert antioxidant properties; this could be related to the folk use of some *Senecio* species.

Key words: *Senecio selloi*, peroxidation, antioxidant activity.

(581) **SYNTHESIS AND EVALUATION OF A SUPERABSORBENT-FERTILIZER COMPOSITE FOR MAXIMIZING THE NUTRIENT AND WATER USE EFFICIENCY IN FORESTRY PLANTATIONS**

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Reducing fertilizer use is a priority in the quest for sustainable agroforest systems. In short rotation *Eucalyptus* plantations, NPK pellets are routinely added to the seedling's top soil layer at planting, potentially leading to increased seedling mortality, nutrient loss and environmental degradation. To address this triple challenge, the development of efficient fertilization practices is essential. In the present work, we synthesized a crosslinked acrylic-cellulosic superabsorbent composite (SAPH-BAL) containing small amounts of specific nutrients integrated in the polymer matrix. We analyzed the composite's chemical and rheological properties, and assessed the viability of *Eucalyptus* plantations supplied with it at planting. Physiological measurements confirmed the suitability of SAPH-BAL in greenhouse grown, potted seedlings subjected to different growth conditions, showing that it efficiently delivers nutrients while protecting seedlings from drought stress. Field experiments carried out at ten South American locations covering an ample range of environmental conditions confirmed the beneficial effect of SAPH-BAL on growth (>20% on average) and survival (>90%) in comparison to the conventional fertilization scheme (superabsorbent + 75g NPK). Furthermore, it was found that plants treated with SAPH-BAL were less affected by the differences in rainfall regimes during the experiments compared to those fertilized conventionally. To the best of our knowledge this is the first report describing the successful use of superabsorbents for root targeted delivery of fertilizers in large scale forestry operations.

(1513) **THE NITROALKENE NITRO OLEIC ACID (NO_2OA) REGULATES STOMATAL CLOSURE AND ROOT DEVELOPMENT IN ARABIDOPSIS**

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The free radical nitric oxide (NO) has been recognized as a second messenger in stomatal closure. NO belongs to a family of reactive nitrogen species (RNS) that mediates signaling process at low and localized concentrations. In mammals, NO-derived reactive species nitrated unsaturated fatty acids, yielding electrophilic nitroalkene derivatives (NO_2-fatty acid), which can *per se* act as a signaling molecule. The presence of these molecules in plants it was recently reported in olive and Arabidopsis, however the mechanism of action is still unknown. In this work we demonstrate the effect of NO_2-OA in two different physiologic processes, the stomatal closure and root development.

Plants regulate the balance of water via the modulation of stomatal aperture. We studied the physiological effect of NO_2-OA in the regulation of stomatal closure on Arabidopsis isolated epidermal peels. NO2-OA induces stomatal closure in a dose and time dependent manner. On the other hand, NO_2-OA inhibits primary root development. We further analyzed reactive oxygen species (ROS) production using the fluorescent probe H_2DCFDA or DAB and we observed ROS only in a few scattered root cortex cells within the elongation zone.

These preliminary results demonstrate the novel role of nitroalkenes in the regulation of different physiological processes in plants.

(1161) **UNRAVELING MANNITOL METABOLISM IN CELERY PLANTS**

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Sugar alcohols (like mannitol and glucitol) are major photosynthetic products in a significant number of agronomically important species. These metabolites also play a key role as compatible solutes under different abiotic stress conditions (including salinity, drought and cold). In celery (*Apium graveolens*) almost 80% of newly fixed CO_2 is equally partitioned between sucrose and mannitol, while only a small part is allocated to starch. Carbon partitioning in these plants is more complex than in other species and must be orchestrated accordingly. However, the molecular mechanisms regulating mannitol synthesis, storage and transport are far from being well understood. In this work, we grew celery plants under long (16 h), normal (12 h) and short (8 h) photoperiods. Leaves were harvested every 2 h for a complete light/dark cycle to measure the levels of soluble sugars and starch. Additionally, we produced and characterized mannose 6-phosphate reductase and mannitol dehydrogenase, the key enzymes for mannitol synthesis and degradation, respectively. These proteins were also used to quantify mannose 6-phosphate and mannitol in samples from celery plants grown under different conditions. We found that accumulation of both starch and soluble sugars is higher in longer photoperiods. Interestingly, the pattern of starch accumulation differs from that observed for *Arabidopsis thaliana*, which accumulates large amounts of starch in leaves during the photoperiod to use it as carbon source during the following night. Our results suggest that carbon allocation is differentially regulated in celery leaves and reveal that caution should be taken when extrapolating results from model plants to other species.

Keywords: Sugar alcohols, carbon metabolism, starch, sucrose, *Apium graveolens*

(904) **IDENTIFICATION AND CHARACTERIZATION OF SEED STORAGE PROTEIN FROM NATIVE QUINOA GERMPLASM FROM NORTHWEST ARGENTINA**

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Quinoa (*Chenopodium quinoa*), a pseudocereal native to the Andean regions, is characterized mainly by the quality and nutritional value of its proteins due to an excellent balance of essential amino acids. However, different quinoa landraces exhibit different nutritional composition and morphological attributes. Therefore, we propose to perform comparative proteomics studies among different ecotypes of quinoa from Northwest Argentina (NWA) region to identify nutritional attributes and classify germplasm accessions. Initially, we optimized a two-dimensional gel electrophoresis (2-DE) protocol using commercial quinoa seeds from the southern Bolivian highlands, one of the most important areas of quinoa production. Two protein extraction methods, phenol/ammonium acetate (phenol method) and trichloroacetic acid/acetone (TCA method), were evaluated to determine their efficiency in separating quinoa proteins by 2-DE. The phenol method showed higher protein resolution and spot intensity of all proteins. Next, high-intensity spots were isolated, digested with trypsin and identified by MALDI-TOF/TOF mass spectrometry followed by in silico analysis. Most of the spots were identified as seed storage proteins and showed a high proportion of essential amino acids. On the other hand, we analyzed seeds amino acids profiles of native quinoa germplasm from NWA. We found differences among quinoa accessions for the amino acids studied. This variation in the amino acid content can be explained by genetic variability and/or environmental growth conditions. Thus, seed storage proteins and amino acid composition play a crucial role in determining the nutritional value of quinoa. Altogether, these results emphasize that the expression of quinoa seed storage proteins containing high proportions of essential amino acids is a potential strategy for the nutritional improvement of food crops.

Keywords: Quinoa, proteome, storage proteins

(229) **ENZIMATIC DEFENSE STRATEGIES IN WHEAT UNDER STRESS BY THREE HEAVY METALS: ASSESSMENT BY MULTIVARIATE ANALYSIS**

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Abstract: The antioxidant response of wheat was analyzed in plants exposed to three different heavy metals. Wheat plants 45 days-old were exposed to Al, As and Cd in soil under two stress condition: Al: 60 mg kg^{-1} soil at pH 7 and 60 mg kg^{-1} soil at pH 4; Cd: 10 mg kg^{-1} soil at pH 7 and 33 mg kg^{-1} soil at pH 7; As: 10 mg kg^{-1} soil at pH 7 and 50 mg kg^{-1} soil at pH 7. Ten days after heavy metal exposition, aerial parts were collected and the following measures were made: lipid peroxidation, catalase, ascorbate peroxidase and guaiacol peroxidase and dry weight. The obtained data matrix was analyzed by principal components analysis (PCA) showing the following responses: increasing of ascorbate peroxidase activity due to Al stress; increasing of catalase activity due to As stress and increasing of guaiacol peroxidase activity due to Cd stress. PCA was an effective chemometric tool to elucidate the antioxidant mechanisms as response to different metals exposures.

Keywords: antioxidant enzymes; heavy metals; stress, multivariate analyses

NEUROSCIENCE 13

(75) **ACTIVATION-INDUCED REGULATION OF THE GABA$_A$ RECEPTOR: MOLECULAR BASES OF THE TOLERANCE PHENOMENON**

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Abstract: Benzodiazepine tolerance induced by chronic administration is the result of adaptive changes in $GABA_A$ receptors which are examples of neuronal plasticity. We have previously demonstrated that prolonged diazepam administration in rats results in tolerance to the sedative and anxiolytic effects that is accompanied with alterations in $GABA_A$ receptors in the cerebral cortex. The aim of this work was to investigate the molecular mechanism of benzodiazepine tolerance in an *in vitro* model of cultured neurons exposed to diazepam. To this end, primary neuronal cultures, prepared from rat cerebral cortices, were exposed to 50 µM diazepam for 48 h. At the end of this incubation cells were collected for binding, western blot or quantitative real-time PCR experiments. The exposure to diazepam induced a 40 % decrease ($p<0.05$, one sample Student´s *t* test) in the potentiation of [^{3}H]flunitrazepam binding by 1 mM GABA, named uncoupling. Uncoupling was prevented in the presence of flumazenil or picrotoxin ($p<0.05$, one-way ANOVA and Tukey´s post hoc tests), suggesting that depends on the binding of the benzodiazepine to its specific recognition site and on the $GABA_A$ receptor activation. Uncoupling was also blocked by nifedipine, an inhibitor of L-type voltage-gated calcium channels (L-VGCC, $p<0.05$, one-way ANOVA and Tukey´s post hoc tests). In addition, diazepam treatment induced a 30 % decrease ($p<0.05$, one sample Student´s *t* test) in $GABA_A$ receptor α1 subunit mRNA and protein levels that is prevented by nifedipine ($p<0.05$, one-way ANOVA and Tukey´s post hoc tests). These results suggest that the mechanism of tolerance is mediated by repression of α1 subunit gene expression induced by Ca^{2+} influx through L-VGCC that would finally result in the uncoupling of $GABA_A$ receptor allosteric interactions. The understanding of the tolerance mechanism will contribute to the design of new drugs that will maintain their efficacies after long treatments.

Keywords: $GABA_A$ receptor, GABA, benzodiazepines, tolerance

(780) **ANTICONVULSANT COMPOUND WITH A SELECTIVE MECHANISM OF ACTION ON NAV1.1 AND NAV1.2 VOLTAGE-GATED SODIUM CHANNEL ISOFORM.**

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Epilepsy is central nervous system (CNS) pathology that affects

over 50 million people in the world. Recent studies have demonstrated that in Latin America and Caribbean occurs more than 70% of death in people with epilepsy respect to the entire American continent (been epilepsy the primary cause of death). Antiepileptic drugs (AEDs) are the treatment of choice. AEDs may act via activation of $GABA_A$ receptor, inhibition of glutamate receptors or changing voltage-gated ionic channels as sodium (Na_v), potassium (K_v) or calcium (Ca_v). We have studied over these years, a new synthetic compound N-butyl-1,2,3-oxathiazolidine-4-ona-2,2-dioxide (NBOD) as anticonvulsant, antidepressant, anxiolityc and antioxidant effects. As anticonvulsant has a potency over 5000 times higher than valproic acid in MES test. After a series of preliminary studies (*in vivo* and *in vitro*) in order to elucidate its mechanism of action; Nav emerged as a promising molecular target. Nav present nine different isoforms, been Nav 1.1, 1.2, 1.4 and 1.6 most prevalent in CNS. Na_v have at least three well determined states: open, closed and inactivated. Some AEDs use in clinical, are able to bind to the inactivate state reducing the available fraction of them associated with a significantly reduction of neural hyperexcitability. Using the patch-clamp technique, the molecular mechanism of NBOD effect was explored on Na_v 1.1 and 1.2 isoforms stably transfected in HEK293 cell line. Preliminary results demonstrated that NBOD presents an isoform selective effect, while it did not affect the Na_v 1.2, it significantly inhibits the Na_v 1.1 mediated Na+ current (56%±10, n=4) and the effect was reversible. Moreover, we observed a left shift of h curve ($V_{1/2}$= -73 mV with NOBD vs. -55 mV (n=4) in control conditions) suggesting that it is able to reduce the available fraction. The results show for the first time an anticonvulsant compound that allow to block selectively the isoform Na_v 1.1 respect to Na_v 1.2 isoform.

*Keywords:*voltage-gated sodium channel, anticonvulsant, HEK 293, pacth-clamp.

(1389) FACILITATION OF ELECTRICALLY-EVOKED ACH SECRETION WHEN ACTIVATING A2A ADENOSINE RECEPTORS AT MAMMALIAN NEUROMUSCULAR JUNCTION (NMJ)

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At mammalian NMJ, ATP is co-released with ACh, and metabolized *via* ecto-nucleotidases to adenosine (AD), which modulates ACh release through presynaptic inhibitory A1 or facilitatory A2A receptors (R). We have found that during high stimulation frequency (50 Hz) enough AD is generated, able to reduce neurosecretion *via* A1R whereas A2AR were not activated. To analyze if more endogenous AD is required to activate A2AR, the action of specific A2A antagonist or agonist (SCH-58261 [SCH, 50 nM] or PSB-0777 [PSB, 20 nM]) was evaluated in phrenic-diaphragm preparations (CF1 mice) when the nerve was stimulated at 100 Hz. Considering that, at frequencies lower than 100 Hz, A2AR are not occupied by AD and that, at high $[K^+]_o$, L-type voltage-gated calcium channels (L-VGCCs) are involved in the mechanism of facilitation, we studied the effect of PSB in the presence of the L-VGCC blocker nitrendipine (NIT, 5 μm) at 0.5 Hz, 5 Hz and 50 Hz (750 pulses each). At 100 Hz, SCH (n= 4) did not change the amplitude of the 1º EPP but reduced mean EPP amplitude ($p<0.01$), last 20 EPPs ($p<0.01$) and last 20 EPPs/1º EPP ratio ($p<0.05$). Conversely, PSB (n=4) significantly increase 1º EPP amplitude ($p<0.01$) but did not modify mean EPP amplitude and last 20 EPPs. Last 20 EPPs/1º EPP ratio was decreased ($p<0.05$). NIT prevented the facilitatory effect of PSB on 1º EPP amplitude, mean EPP amplitude and last 20 EPPs at 0.5 (n=4), 5 (n=4) and 50 Hz (n=6). Last 20 EPPs/1º EPP ratio remained unchanged. These findings suggest that during 100 Hz endogenous AD activates A2AR provoking facilitation after the beginning of the train and that L-VGCCs are related to the modulatory effect of PSB. More experiments are required to elucidate if activation of A2AR induces an elevation of Ca^{2+} influx *via* L-VGCCs or increases gating charge movements of L-VDCC which could cause the opening of the endoplasmic reticulum ryanodine receptors, creating a local Ca^{2+} microdomain responsible for the exocytosis facilitation.

Keywords: A2A adenosine receptors, endogenous adenosine, L-type voltage-gated calcium channels, mammalian neuromuscular junction

(1754) FGD6 EXPRESSION IN FOCAL BENIGN INFANTILE EPILEPSY (FBIE): ITS POTENTIAL ROLE IN EPILEPSY PATHOPHYSIOLOGY

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Epilepsy is the third most common cause of neurological disorders. Focal Benign Infantile Epilepsy (FBIE) is the most prevalent in the paediatric population and belongs to the idiopathic epilepsy group. A recent genetic analysis of familial FBIE determined FGD6 as a potential candidate to be involved in epilepsy pathogenesis. The affected members presented a p.E276G mutation in homozygosis, suggesting mutated FGD6 as a probable cause of epilepsy in this family. FGD6 function is not well established. It belongs to FGD family, in which some of the members have a known function such as Cdc42 GEF (guanine exchange factors) activators. Cdc42 is a Rho GTPase and has a key role in CNS, regulating cytoskeleton, synaptogenesis, spines and dendrite formation and axon guidance. We studied FGD6 expression in several culture models using, wild type, FGD6 heterozygous and FGD6 homozygous cells. Our results show that in a neuroepithelial culture model with induced pluripotent cells (IPS), FGD6 is expressed in neurons in the three experimental groups. In this model, FGD6 is found aggregated in the cytoplasm and plasma membrane when analysed cells from epileptic subjects with a homozygous mutation in FGD6, compared to a homogeneous distribution in wild type cells. Cytoskeleton studies in fibroblasts revealed a decrease in actin filaments, as well as disorganization of the cytoskeleton in epileptic patients. Finally, we studied by qRT-PCR FGD6 expression in a SK-N-SH neuroblastoma cells, and we observed a decrease in gene expression when the cells differentiate to a more mature phenotype ($p \leq 0,05$), followed by a raise in FGD6 mRNA as the cells continue to differentiate. Our results suggest that in neurons FGD6 expression is tightly regulated during cell maturation, and that FGD6 mutation affects cytoskeleton dynamics.

Keywords: epilepsy, FGD6, neurons, cytoskeleton, actin.

(288) GLUTATHIONE GATES $GABA_A\beta3$ RECEPTORS

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Abstract: Glutathione (GSH) is a thiol tripeptide that acts as the major antioxidant and redox buffer in the brain. It can be found at mM concentrations in all the neuronal compartments and the extracellular milieu. GSH exerts a protective role against oxidative stress, in the detoxification of H_2O_2 and lipid peroxides (as cofactor of the GSH peroxidase), scavenging of $OH^{\cdot}$ and regeneration of the most important antioxidants (vitamins C and E). GSH is also involved in the regulation of many synaptic functions and in neuronal plasticity. Furthermore, GSH deficiency has been implicated in neurodegenerative diseases. Previous reports demonstrated that GSH modulates GABAergic neurotransmission, although experimental evidence is limited.

We analyzed the effect of GSH on responses mediated by homomeric $GABA_A\beta3$ receptors. $GABA_A\beta3$ receptors are ionic channels exhibiting spontaneous openings that give place to agonist-independent Cl^- currents, which are sensitive to picrotoxin and Zn^{2+}. $GABA_A\beta3$ receptors can also be activated by mM concentrations of GABA (>3mM). In the present study, human $GABA_A\beta3$ receptors were expressed in *Xenopus laevis* oocytes and Cl^- currents recorded by two-electrode voltage-clamp. Experimental results showed that the concentrations of GSH needed to gate $GABA_A\beta3$ receptors are lower than those of GABA. GSH effects were reversible, dose dependent and voltage independent. GSH also prevented Zn^{2+} in-

hibition of $GABA_A\beta3$ responses. GSSG, the oxidized form of GSH, also activated $GABA_A\beta3$ currents, but in a lesser extent than GSH. Irreversible alkylation of sulphydryl groups within the β3 subunits by N-ethylmaleimide failed to prevent GSH effects. Meanwhile, in preliminary experiments, GSH modulated $GABA_A\alpha4\beta3$ but not $GABA_A\alpha4\beta3\delta$ receptors. Given the molecular arrangement of these receptors, the presence of the β3-β3 interface might be essential for GSH actions. Further experiments are being carried out to elucidate the mechanisms of action underlying GSH effects.

Keywords: $GABA_A$ receptors, glutathione.

(640) **NEW ANTICONVULSANT DRUGS PREVENT P-GLICOPROTEIN (PGP) OVEREXPRESSIÓN IN A PHARMACORESISTANT SEIZURE MODEL IN MICE.**

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About 30% of the patients with epilepsy do not respond to clinically established anticonvulsants, despite achieving effective concentrations in plasma. One of the most accepted hypotheses to explain refractory epilepsy is the overexpression of ABC transporters at blood-brain barrier level, which appears to be induced by glutamate through activation of the COX-2 pathway.

Here, we have applied ligand-based *in silico* screening (based on linear discriminant analysis and conformation-independent descriptors) to select compounds from the DrugBank and Sweetlead databases which, according to our computational models, display combined anticonvulsant and anti-inflammatory activity.

5 of the hits from the *in silico* screening have been assayed in three acute seizure models (MES, PTZ and MP). Those which showed anticonvulsant activity in both MES and PTZ tests without neurotoxic effects but did not in MP test (sebacic acid and γ-decanolactone) were evaluated in a pharmacoresistant seizure model in mice (MP23 model).

The MP23 model consists in inducing daily clonic seizures during 23 consecutive days through 3-mercaptopropionic acid (MP) administration. At the end of the treatment, mice are refractory to phenytoin and phenobarbital effects showing an increased P-glycoprotein brain expression. Sebacic acid and γ-decanolactone were administered 30 min before MP administration every day. On day 24 PHT resistance and P-gp expression were studied.

All compounds selected by *in silico* screening showed anticonvulsant activity in at last one of the acute seizure tests. Sebacic acid and γ-decanolactone administration before MP in the MP23 model showed a higher PHT effect in relation to MP alone ($p<0.01$; $p<0.10$ respectively). In addition, mice treated with sebacic acid showed brain Pgp levels even lower than the control group ($p<0.01$).

These results suggest than sebacic acid might be a promising drug candidate to prevent the development of pharmacoresistance in epilepsy.

Keywords: Refractory Epìlepsy – P-glycoprotein – Anticonvulsant Drugs – Anti-inflammatory – Sebacic acid.

(459) **NEW THERAPEUTIC STRATEGIES IN EPILEPSY: IS IT POSSIBLE TO PREVENT THE EPILPETOGENESIS?**

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Epilepsy affects 1% of the world population and usually requires chronic pharmacological treatment. An important percentage of patients with temporal lobe epilepsy (TLE) refer the antecedent of an initial precipitating event (IPE) usually febrile seizure, during childhood, followed by a latency period (LP) until the onset of the chronic epileptic seizures. Gabapentin (GBP) is an antiepileptic drug useful as a coadjuvant in the treatment of refractory epilepsy and especially in treatment of neuropathic pain. In an experimental model of temporary lobe epilepsy (TLE) achieved by the lithium-pilocarpine administration; we have previously shown that during LP occur neurodegeneration, reactive gliosis and macrophages infiltration in the brain hippocampus and pyriform cortex. Gabapentin treatment successfully reduced neuronal degeneration and reactive gliosis (Rossi et al., 2013, PLOS One). In this work, we determined the GBP effects when subacutely administered for a short period of time (4 days) following the experimental IPE. Male Wistar rats were treated with by lithium-pilocarpine (127 mg/kg and 30 mg/kg respectively) developing an status epilepticus (IPE) that were limited to 20 min by 20 mg/kg diazepam administration. 24 h later animals received GBP (400 mg/kg/d) for 4 days. This treatment reduced the reactive microgliosis and changed the microglial phenotype towards a less pro-inflammatory morphology. When the GBP-treated animals were exposed to repeated pilocarpine subconvulsive doses, 21 days after the IPE, we observed that epileptic threshold was increased compared with vehicle-treated animals. Our results show that an early pharmacological intervention after the IPE is able to alter the neurodegeneration and reactive gliosis that are the biological substrate of epileptogenesis.

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Keywords: microglia; reactive gliosis; Gabapentin; epileptogenesis

(481) **STATUS EPILEPTICUS INDUCES HEART DYSFUNCTION AND P-GLYCOPROTEIN OVEREXPRESSION IN CARDIOMYOCYTES: IMPLICATIONS FOR SUDDEN DEATH SYNDROME IN EPILEPSY.**

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Sudden Unexpected Death in Epilepsy (SUDEP) is commonly observed cause of death after status epilepticus (SE). Refractory epilepsywas associated with P-glycoprotein (P-gp) brain overexpression and high risk of SUDEP by acute fatal heart failure. We have previously described brain/heart P-gp overexpression related with fatal-SEafter repetitive penthylenetetrazole-inducedseizures (Auzmendi et al.2013).In clinical grounds, loss of the ^{99m}Tc-SESTAMIBI retention is used asa biomarkerofhypoxic/ischemia(H/I) heart failure. We here studied cardiac functional parameters and the expression of P-gp in an experimental model of temporal lobe epilepsy (TLE). SE was induced on300 g Wistar rats by lithium chloride plus pilocarpine treatment (127 mg/kg and 30 mg/kg respectively) and stopped with 20 mg/kg diazepam after 20 min of SE. One group of animals received one SE (1xSE) and another group were subjected to repeated SE (once a week; 4 weeks) (4xSE). After 72 h of SE, ^{99m}Tc-SESTAMIBI (37MBq-i.v) was administrated and static images of whole heart were acquired in a gamma camera to evaluate tracer heart retention. By 5 days after SE, animals from the 1xSE group already showed in the ECG analysis both a decreased heart rate and extended QT time. On the other hand, animals from the 4xSE group showed after the first SE an increased expression of P-gp and HIF-1 analyzed by immunohistochemistry in cardiomyocytes, 50% reduced ^{99m}Tc-SESTAMIBI heart retention and increased mortality with each SE. We conclude that SE might act as H/I heart inducer with simultaneous overexpression of HIF-1 and P-gp in cardiomyocytes, severe loss of ^{99m}Tc-SESTAMIBI heart retentions and altered cardiac rhythms, associated with increased mortality. Under these conditions, new stress will start acute heart failure that could to lead

in death, depending on severity of stress. These results could help to explain the mechanisms underlying in SUDEP.

(259) **STOICHIOMETRY AND KINETICS OF ACTIVATION AND POTENTIATION OF NICOTINIC ALPHA7BETA2 HETEROMERIC RECEPTORS**

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The α7 nicotinic receptor (nAChR) is a promising drug target for neurological and inflammatory disorders. It has been considered the homomeric member of the family. The recent discovery of α7β2 receptor in brain led to the urgent need of its functional characterization. Our main goal is to determine the stoichiometry of the heteromeric α7β2 receptor and its activation and potentiation profile. We generated receptors with fixed stoichiometry by two different approaches. One involved the generation of concatemeric α7β2 pentamers of different stoichiometries, and the other involved co-expression of unlinked α7 and β2 subunits, with the α7 subunit carrying a reporter mutation. Receptors were expressed in mammalian cells and function was evaluated by single-channel recordings. We found that α7 can assemble with one, two or three β2 subunits to form functional receptors. As the number of β2 subunits in the pentamer increases, the durations of openings and activation episodes, called bursts, increase progressively whereas channel conductance remains constant. We proposed that the prolonged bursts observed for α7β2 can be used as the signature of the presence of heteromeric receptors in native tissues. The prolonged activation episodes and reduced desensitization of α7β2 may have an important impact on calcium-dependent intracellular signaling and neuronal excitability. By using mutant subunits, we demonstrated that activation of α7β2 occurs through the α7/α7 binding-site interface. Among α7 positive allosteric modulators (PAMs), which emerge as novel therapeutic tools, type I PAMs were more selective for α7 than for α7β2 whereas PNU-120596, a type II PAM, similarly potentiated all α7-containing receptors. Statistically significance differences were established at p-values<0.05.This first single-channel study of α7β2 provides basis for deciphering the role and functional location of this novel receptor and opens doors for the development of selective therapeutic drugs.

Keywords: alpha7beta2 nicotinic receptors, concatemers, patch-clamp.

BIOTECHNOLOGY AND BIOINFORMATICS 7

(320) **IDENTIFICATION AND CHARACTERIZATION OF A NEW CLASS OF C22 STEROL DESATURASE FROM *TETRAHYMENA THERMOPHILA***

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T. thermophila does not require sterols, but synthesizes as a surrogate the triterpenoid "tetrahymanol". If sterols are available, they are avidly incorporated by the ciliate and bioconverted to 7,22-*bis*-dehydrocholestrol, whereas tetrahymanol synthesis is completely inhibited. This bioconversion requires four desaturating activities; three of them were previously characterized by our groups. Here we describe the identification of the last fourth enzyme, the C22 desaturase, instrumental in the development of microbial systems for the synthesis of tailored steroids. All known C22 sterol desaturases belong to the P450 oxygenases, essential enzymes for the synthesis of ergosterol in fungi or phytosterols in plants. *T. thermophila* has 44 putative P450 oxygenases, all of them sharing low similarity to canonical C22 desaturases. The ciliate enzyme was induced by sterols, required NAD(P)H and was inhibited by azide and cyanide, but not by azoles, typical properties of oxygenases belonging to the superfamily fatty-acid desaturases/hydroxylases (FAD/H). Analysis of a differential transcriptome carried out on *T. thermophila*, grown in presence or absence of sterols, allowed us to detect several FAD/H significantly induced by sterols. The two more induced ones, named Des1 and Des2 (sharing 68% similarity) were selected for further characterization. Expression of Des1 or Des2 in a *Saccharomyces cerevisiae* C22 desaturase mutant, both rescued the synthesis of the yeast ergosterol. Additional genetic approaches performed on the ciliate have unambiguously confirmed the C-22 activity of these two isoenzymes. It is the first description of a C22 sterol desaturase which is not a P450 oxygenase. These results also expand the repertoire of FAD/H specificities.

Keywords: sterols, bioconversion, desaturases, ciliates

(1099) **ISOLATION AND CHARACTERIZATION OF STRONG CONSTITUTIVE PROMOTERS ACTIVE IN *Lactococcus lactis* AND *Escherichia coli*.**

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Lactic acid bacteria are an increasingly interesting model for the expression of recombinant proteins of industrial interest because of their GRAS (Generally Recognized As Safe) condition. If the protein to be overproduced is non-toxic to the bacterium, constitutive promoters can be used for expression. The objective of the this work was to isolate strong promoters of constitutive expression from *L. lactis*, also active in *E. coli*. The advantage of these promoters is that they would allow the construction and preliminary analysis of DNA constructs in *E. coli*, a very easy to work bacteria, for later use in lactic acid bacteria. To this end, a library of *L. lactis* genomic DNA fragments was generated by digestion with different restriction enzymes and subsequently, the 400-2000 bp fragments were cloned into a reporter vector for promoter regions (pTLGR). *E. coli* competent cells were transformed with the ligation mixture and the presence of a DNA fragment with strong constitutive promoter activity was revealed by the appearance of red colonies, because of the expression of the red fluorescent protein of the reporter vector. A plasmid purification was carried out from these red colonies and used to transform *L. lactis*competent cells. *L. lactis* colonies showing a rosaceous color were selected, plasmids purified and sequenced. We identified 4 different DNA regions capable of acting as strong constitutive promoter regions in both,*L. lactis* and *E. coli*. The putative promoter regions were identified in the *L. lactis* genome. Moreover, 3 of the 4 regions identified were reported in the literature as strong promoter regions in transcriptomic studies of *L. lactis*. Preliminary analysis showed that the four identified promoter regions would be considerably more active than the most active constitutive promoter identified in *L. lactis* to date.

Keywords: strong constitutive promoter, *Lactococcus lactis*, *Escherichia coli*

(1140) ***BRUCELLA ABORTUS* EXPLOITS HOST CELL ALPHA-ENOLASE VIA THE VIRB EFFECTOR BPE123 TO PROMOTE INTRACELLULAR REPLICATION**

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Brucella abortus, the causative agent of bovine brucellosis, invades and replicates within cells inside a membrane-bound compartment known as the *Brucella* containing vacuole (BCV). *Brucella* Type IV Secretion System (VirB) is a major virulence factor, which translocate effector proteins from the BCV into host cell cytoplasm, thus modulating several signaling pathways to favor bacterial intracellular survival and replication. BPE123 is a *B.abortus* VirB sustrate previusly identified by our group. In an attempt to identify host cell proteins interacting with BPE123, a pull-down assay was performed and human alpha-enolasa (ENO1) was identified as a potential interaction partner of BPE123. This interaction was confirmed in vivo by immunoprecipitation assay and by confocal microscopy analysis of ENO1 redistribution in the presence of BPE123. Aditionally, quantitative analysis of confocal micrographs of macrophages infected with *B. abortus* showed that ENO1 associates to BCVs in a

BPE123-dependent manner, indicating that interaction with translocated BPE123 might also be occurring during the intracellular phase of *B. abortus*. Moreover, down-regulation of the expression of ENO1 in HeLa cells infected with *B. abortus* affected intracellular replication, demostrating a role of ENO1 in *Brucella* intracellular lifestyle. To investigated if the interaction between BPE123 and ENO1 affects the catalityc activity of ENO1, activity assays were performed and demostrated that ENO1 activity is enhanced not only in HeLa cells ectopically expresing BPE123 but also in *B. abortus*-infected THP-1 macrophages. These results suggests that interaction between BPE123 and ENO1 induces structural and/or functional changes accounting for the activation of host cell alpha-enolase during the infection process. Further experiments are underway to study the changes of the kinetic parameters of ENO1 in the presence of BPE123.

Keywords: Brucella abortus; Type IV secretion; intracellular replication; alpha-enolase; BPE123

(1283) **CR(VI) AND LINDANE REMOVAL BY *Streptomyces sp.*M7 IS IMPROVED BY MAIZE ROOT EXUDATE**

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Environmental mixed pollution by both organic and inorganic compounds are detected worldwide. Phytoremediation techniques have been proposed as ecofriendly methods for cleaning up polluted sites. Several studies have demonstrated enhanced dissipation of contaminants at the root-soil interface through an increase in microbial activity caused by the release of plant root exudates (REs). The aim of this study was to evaluate the effectiveness for Cr(VI) and lindane removal by *Streptomyces* M7 cultured in a co-contaminated system in presence of maize REs. In order to evaluate the performance of *Streptomyces* M7 on lindane and chromium removal, flasks with minimal medium (MM) with 1 g L^{-1} glucose were artificially contaminated with 2 mg L^{-1} lindane and/or 25 mg L^{-1} of Cr(VI), and inoculated with *Streptomyces* M7. Then, in order to evaluate the effect of maize REs on lindane and chromium removal by *Streptomyces* M7, flasks with MM (without glucose) were supplemented with maize REs (0.4 gC L^{-1}). Then, MM was contaminated with 2 mg L^{-1} lindane and/or 25 mg L^{-1} of Cr(VI) and inoculated with *Streptomyces* M7 .Flasks were incubated at 30 ºC, 150 rpm, five days. Microbial growth and contaminants concentrations were determined. Our results showed when REs were added to the contaminated MM as the only carbon source, microbial removal of Cr(VI) and lindane increased significantly in comparison to contaminant removal obtained in MM with glucose 1 g L^{-1}. The maximum removal of 91% of lindane and 49% of Cr(VI) were obtained in the co-contaminated system. *Streptomyces* M7 showed plant growth promoting traits which could improve plant performance in contaminated soils. The results presented in this study provide evidence that maize REs improved growth of *Streptomyces* M7 when REs were used as a carbon source in comparison to glucose. Lindane and Cr(VI) removal was considerably enhanced making evident the phytoremediation potential of the actinobacteria-plant partnership.

Keywords: *Streptomyces*, Root exudates, Bio/phytoremediation, Co-contamination

(1681) **SEQUENCE ANALYSIS OF FLAVONOL SYNTHASE, KEY IN THE POLYPHENOL METABOLISM OF *LIGARIA CUNEIFOLIA***

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Ligaria cuneifolia (R. et P.) Tiegh (Santalales, Loranthaceae) is an hemiparasitic plant with several pharmacological activities (antioxidant, antiproliferative, antitumor, hypolipidemic and antimicrobial) attributed to its flavonoids. However, there is no information about the sequences of the enzymes involved in the synthesis of these metabolites in *L. cuneifolia*. Hence, our goal is to elucidate the sequence of the Flavonol synthase protein (FLS), a key enzyme for the production of flavonols.

For this purpose, we have made an exhaustive search for the FLS sequences of the Div. Magnoliophyta in the databases. We have selected a total of 15 sequences from orders close to Santalales (according to phylogenetic tree APG3) as there is not a sequence from Santalales in the databases. Then, degenerate primers were designed using the CODEHOP software (COnsensus-DEgenerate Hybrid Oligonucleotide Primer). Those primers come from conserved regions from a multiple alignment of proteins that will be amplified by PCR in order to identify homologous genes. Once the primers were designed, total RNA was extracted from foliar tissue and used as a template to synthesize the first strand of copy DNA (cDNA) using the Superscript II retrotranscriptase. Different combinations of the degenerate primers were used to amplify the cDNA by PCR. The PCR product (338 bp) was cloned into the pGEM-T vector and sequenced. With the obtained fragment a search was made in the database Nucleotide collection (nr / nt) of GenBank using the program Blastn. Only FLS alignments of vascular plants were obtained. In following experiments, the RACE technique will be used to obtain the complete enzyme sequence. The knowledge generated in this work would be of relevance for future applications in metabolic engineering of *L. cuneifolia* flavonoids.

Key words: *Ligaria cuneifolia*, flavonol synthase, flavonoid, CODEHOP.

(1738) **BIO-INFORMATIC ANALYSIS OF BACTERIOPHAGES ACTIVE ON *Staphylococcus aureus* ISOLATED FROM HUMAN NOSTRILS.**

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The rise of Multi-Drug resistant bacteria has triggered worldwide concern. Novel antibiotic molecules are urgently needed to fight those patogens. An alternative strategy lays in the utilization of bacteriophages which are natural bacterial predators, this strategy was put forward but left aside due to the availability of natural and synthetic antibiotics.

Staphylococcus aureus causes several mild to life threatening pathologies in humans and animals; circulating in health centers as well as in the community. Our group has studied *S. aureus* nasal carriage by health staff at a major public hospital; as part of that project we screened by nasal swabs for bacteriophages active against this pathogen. During this process we isolated 23 temperate bacteriophages whose description in terms of genomic sequence, bioinformatic analysis and host range is reported.

The genomes annotation, function prediction and domain search was carried out using BLASTP, pfam and Interproscan. Our results yielded a bacteriophage (named Mat_T), displaying a broad host range comparable to that of the most active lytic bacteriophage reported, bacteriophage K (87% and 85% of activity against 43 *S.aureus* local isolates respectivelly). In spite of a detailed analysis of integrase and anti-repressor genes encoded in the isolated bacteriophages, we were not able to find an explanation for the high killing activity of bacteriophage Mat_T.

Keywords: *Staphylococcus aureus*; temperate bacteriophages; bacteriophage integrases and excissionases.

(1887) **CHARACTERIZATION OF THE INTERACTION OF KELCH DOMAIN FORM KEAP-1 WITH PEPTIDES DERIVED FROM NRF2 TRANSCRIPTION FACTOR.**

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Nrf2 is formed by 605 protein residues, and is a major transcription factor involved in the regulation of cytoprotective genes. The N-terminal domain (termed Neh2) is involved in the negative regulation of Nrf2 activity. The C-terminal domain is a basic region-leucine zipper (bZIP), required for DNA binding and nuclear import and export. Keap-1 is the principal negative regulator of cytoprotective gene expression. In solution it is found as a dimer, with 624 protein residues for each monomer. Kelch domain of Keap-1 binds to the Neh2 domain, sequesters Nrf2 to the cytoplasm and mediates proteasomal degradation through polyubiquitination.

In this study the interaction of Kelch domain of Keap-1 with several peptides derived from Neh2 domain of Nrf2 was analyzed, in order to determine which are the main residues contributing to the binding. The interactions of each peptide with Kelch domain were characterized with NMR, by comparing the $^{1}H,^{15}N$-HSQC spectra (heteronuclear single quantum coherence) obtained for each one.

To get insights with atomistic detail into every important interactions between Kelch domain residues and Neh2 derived peptides, we used Molecular Dynamics Simulations (MD) with a Simulated Annealing (SA) protocol. The crystallographic structure used as a starting point corresponds to a complex of Kelch domain of human Keap-1 with a 16-mer peptide of human Nrf2 (PDB ID: 2FLU).

The results allowed us to establish the binding mode for each peptide, and determine the smaller peptide that still maintains a high binding affinity. Furthermore, we were able to identify the most important interactions contributing for the binding in each case. Finally, the outcome of this study could facilitate the screening and design of novel small molecules to be used as a therapeutic compounds, blocking the binding of Keap-1 to Nrf2.

(1914) **CRISPR-Cas SYSTEMS IN ENTEROCOCCAL STRAINS OF DAIRY ORIGIN**

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The CRISPR-Cas modules are adaptive immune systems that restrict the acquisition of foreign mobile elements. They can be found in many bacteria and most archaea, and consist of a CRISPR array, comprising conserved short direct repeats (DR) separated by short variable DNA sequences (spacers), preceded by a 5'-leader sequence; and of *cas* genes that encode proteins performing various, only partially characterized functions essential for the system's activity. Particularly spacers, represent remnants of genetic code from past invaders (protospacers) and work as guides to specifically target and cleave the nucleic acids of cognate viruses or plasmids. Based on the evidence indicating that enterococci possessing one or more complete CRISPR-Cas cassettes are less parasitized by phage and other mobile elements, which include antibiotic resistance genes, the presence of these immune systems in the genomes of 14 enterococcal strains isolated from milk or dairy products was evaluated. For this purpose, the CRISPRFinder webserver was used. As a result, it was observed that 7 strains, belonging to 4 different species, included at least one CRISPR array with three motifs (DR + spacer) and a minimum of two identical DRs; and that almost all strains had 1 or more spacers, with the exception of *Enterococcus* (*E.*) *mundtii* CRL1656 and ATCC 882 with no CRISPR. Then, in order to identify and label spacers origin, the CRISPRTarget program was run setting Genbank-Phage and Ref-Seq-Plasmid as databases. Thus, from a total of 64 spacers that were present in both confirmed and questionable CRISPR, only 12 protospacers could be identified with a final score ≥ 25 and 1 or no mismatches. Of these, 9, from a same or different strains, occur in a region codifying a replication protein RepA of several *E. faecium* and *E. hirae* plasmids. All in all, the evaluation of the potential of food related strains to acquire foreign DNA that may encode unwanted features is important in terms of food safety.

Keywords: Enterococcus, dairy origin, CRISPR-Cas system, spacers

(1920) **PROTMISCUITY: PROMISCUOS PROTEINS DATABASE**

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We have developed a database to study the relationship between conformational diversity and protein promiscuity. The database, called ProtMiscuity, was developed through the implementation of web-scraping and text mining techniques. As a result, we obtained 99 entries related to a total of 66 promiscuous proteins with one or more than one characterized promiscuous activities. Catalysed reactions, both promiscuous and canonical, were characterized with all the information available in the literature: substrates, products, Km, Kcat, active site composition and 3D arrangement and reaction conditions. The Protmiscuity is linked with other databases providing additional information such as conformational extension (CoDNaS) and protein function (KEGG, Uniprot , Atlas Catalytic Site, QuickGo) as well as structural details (PDB) for each protein. In addition, substrates and products were related to the information available in PDB Ligand Expo to obtain possible ligands with chemical similarity. A total of 68 different promiscuous reactions were characterized and a final amount of 220 molecules (substrates or products) were related with corresponding reactions. Interestingly, the 32.31% of the proteins in the database have more than one promiscuous associated activity, being the esterase activity the most common promiscuous activity. Protmiscuity could allow users to browse well-curated experimental information that could be used to increase our understanding the underlying mechanisms of protein promiscuity as well to design experiments in industrial, biotechnological and drug design applications.

METABOLISM AND NUTRITION 6

(1752) **HYPOTHALAMIC POMC PROTECTS AGAINST GLUCOSE INTOLERANCE BY IMPROVING INSULIN SENSITIVITY**

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Elucidating the central nervous system mechanisms that participate in the control of glucose homeostasis could lead to new therapies for diabetes. The hypothalamus is a main regulator of metabolism in all vertebrates. In particular, hypothalamic arcuate proopiomelanocortin (POMC) neurons sense the energy status of the organism by the integration of peripheral signals such as leptin, glucose and insulin. POMC derived peptides such as β-endorphin and α-MSH, are involved in many physiological functions including reproduction, energy homeostasis and reward. POMC deficient patients and mice are hiperfagic, obese and diabetic. The role of POMC *neurons* in glucose homeostasis has been well established, but it remains to be elucidated if hypothalamic POMC *peptide* is directly involved, especially considering that these neurons may also co-secrete other neuropeptides and neurotransmitters. To address this question we used a mouse line that bears a reversible mutation that prevents arcuate *Pomc* expression (arc*Pomc*$^{-/-}$). To determine the role of central POMC in maintaining glucose homeostasis independently of body weight, we subjected arc*Pomc*$^{-/-}$ weanling mice (before overweight development), to a glucose tolerance test. Our results show that mutant mice have reduced glucose tolerance compared to their wild type siblings ($p<0.05$). Furthermore, mutant mice are less sensitive to insulin than control mice when subjected to an insulin tolerance test ($p<0.05$). Notably, *Pomc* reactivation leads to

complete glucose tolerance normalization as well as insulin sensitivity restoration. These results indicate that hypothalamic POMC expression protects against glucose intolerance, at least in part, by enhancing insulin sensitivity. Furthermore, since *Pomc* reactivation completely restores the diabetogenic phenotype, arcuate *Pomc* could be a potential target for diabetes therapy.

Keywords: POMC, hypothalamus, glucose, insulin, diabetes

(1509) **MCAM KNOCKDOWN INHIBITS PPAR GAMMA EXPRESSION IN THE LATE STAGES OF 3T3-L1 FIBROBLASTS DIFFERENTIATION TO ADIPOCYTES**

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Obesity is a chronic disease characterized by an excessive expansion of the adipose tissue which is associated with an increase in the number and size of adipocytes. Therefore, the knowledge on the development and function of the adipose tissue may help to design new approaches for the treatment of this disease. We have previously observed that the expression of Melanoma Cell Adhesion Molecule (MCAM), a transmembrane protein of the immunoglobulin superfamily, increases during 3T3-L1 fibroblasts differentiation to adipocytes, subsequent to the activation of the master regulator of adipogenesis, PPARγ, and this increase is maintained in the mature adipocytes. On the other hand, we have also found that shRNA mediated MCAM downregulation impaired 3T3-L1 cells differentiation and induction of PPARγ as well as expression of genes activated by PPARγ. To further evaluate the effects of knocking down MCAM expression, we investigated the events that precede and are necessary for early PPARγ activation such as C/EBPβ induction, β-catenin downregulation and ERK activation. We found that these early events of the differentiation process were not affected in the MCAM knockdown cells. In keeping with this, the early increase in PPARγ mRNA observed 24 h after induction of differentiation and that precedes MCAM induction, was not altered in the knockdown cells. However, PPARγ expression was inhibited after day 3 of differentiation in these cells, when a positive feedback loop has been described to be necessary to maintain PPARγ upregulation. In conclusion, our findings suggest that MCAM is a gene upregulated and necessary to maintain PPARγ induction in the late but not in the early stages of 3T3-L1 fibroblasts adipogenesis.

Keywords: MCAM; adipogenesis; PPAR gamma; 3T3-L1 fibroblasts

(191) **MICROSOMAL TRIACYLGLYCEROL TRANSFER PROTEIN (MTP) PARTICIPATES IN THE DYNAMICS OF FORMATION OF HEPATIC CYTOSOLIC LIPID DROPLETS (LDS)**

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In eukaryotic cells, lipids are stored in lipid droplets (LDs), organelles composed of a hydrophobic core covered by a monolayer of surface phospholipids. MTP locates in the lumen of the endoplasmic reticulum (ER) and participates in the secretion of lipids from the liver as very low density lipoproteins (VLDL). There is evidence that MTP could be associated with cytosolic LDs. However, no studies were performed in hepatic LDs. The objective of this work was to study the subcellular distribution of MTP and its interaction with LDs in hepatocytes. First, we obtained hepatic LDs from mice and evaluated the associated proteins by mass spectrometry followed by immunoblotting. Upon checking fraction purity with the appropriate antibodies, we found that amongst the 325 proteins associated to the LDs, several were from the ER, such as calnexin, carboxylesterase Ces3/Tgh, and, interestingly, also MTP. Then, we worked with primary rat hepatocytes stimulated with 0,4mM oleic acid complexed with bovine serum albumin (OA/BSA) to produce LDs. Using confocal microscopy, we observed that MTP localized in the ER under basal conditions, but located around the LDs after incubation with OA/BSA. Then, we analyzed the distribution of MTP in microsomal and LDs fractions by subcellular fractionation followed by immunoblotting. We found that MTP protein expression increased in LD fractions in a time-dependent manner upon OA/BSA incubation, with a concomitant decrease in the microsomal fraction. Finally, we generated a stable clone expressing the MTP-FLAG construct using Huh7 cells. So far, we found that the stable cell line is functional, increasing VLDL secretion compared to control cells. Conclusion: our results demonstrated the role of MTP as a multifunctional protein. We believe that through its interaction with cytosolic LDs, MTP would be participating not only in the secretion of VLDL, but also in LDs metabolism.

Keywords: steatosis, lipid metabolism, VLDL secretion, liver, endoplasmic reticulum

(1809) **NEGATIVE IMPACT OF CETP ACTIVITY ON HDL APOLIPOPROTEIN A-I CONTENT AND PARAOXONASE 1 ACTIVITY IN CHILDREN AND ADOLESCENTS.**

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Background: High density lipoprotein (HDL) antiatherogenic capacity is mainly dependent on apo A-I content and on the activities of the antioxidant enzyme paraoxonase 1 (PON1) and the cholesteryl ester transfer protein (CETP).

Objective: To evaluate the effect of CETP activity on apo A-I concentration and PON1 status.

Methods: 280 apparently healthy children and adolescents between 6 and 16 years old were recruited. Anthropometric parameters were registered and z-body mass index (z-BMI) was calculated. Lipid profile was evaluated by automatized methods, apo A-I and apo B levels by immunoturbidimetry, CETP activity by a radiometric assay and PON1 activity employing paraoxon as substrate.

Results: In univariate correlation analysis, CETP activity was associated with triglycerides (TG; r=0.33; p<0.01), HDL-C (r=-0.40; p<0.01), and apo A-I (r=-0.37; p<0.01), while PON activity with HDL-C (r=0.27; p<0.01) and apo A-I (r=0.18; p<0.01). In multiple linear regression analysis, adjusting by sex, age and z-BMI, we found a negative association between CETP activity and PON activity (r^2=0.18; beta=-0.15; p<0.05). When apo A-I was included in the model, this association lost significance and CETP only remained negatively associated with apo A-I (r^2=0.17; beta=-0.29; p<0.001). A similar analysis carried out considering PON as dependent variable, again this activity resulted to be negatively associated with CETP activity (r^2=0.18; beta=-0.13; p<0.05). When apo A-I was included in the model, this association also lost significance and PON activity remained positively associated with apo A-I (r^2=0.19; beta=0.15; p<0.05).

Conclusion: CETP activity would lead to altered PON antioxidant function, as reflected by CETP negative association with PON activity. Furthermore, CETP would affect PON through alterations in apo A-I levels, as reflected by apo A-I negative association with CETP activity and the lack of association between CETP and PON activities when apo A-I was included in the model.

Keywords: Cholesteryl ester transfer protein, Paraoxonase, High density lipoprotein, Apolipoprotein A-I, Triglycerides.

(925) **OXIDATIVE STRESS IN LIVER IN A SIX MONTH VITAMIN A DEFICIENCY MODEL. RELATION WITH RETINOIC ACID LEVELS FROM SERUM AND LIVER.**

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Vitamin A (VA) plays an important role in growth, development and

cellular differentiation. By the way, preview works in our laboratory have shown that VA deficiency (VAD) modifies oxidative parameters in liver, heart and aorta. The objective of this study was to determine the effect of VAD on hepatic expression of pro-oxidant and antioxidant genes, and their relation with hepatic and serum levels of retinoic acid (RA). Methodology: Virgin female rats of the Wistar strain were separated at weaning into 3 groups: fed with VA sufficient diet for 6 months (A+), VA deficient diet for 6 months (A-) and VA deficient diet for 150 days followed by refeeding with VA sufficient for 30 days (R). Then, serum and liver were extracted for RA assessment by Neeld and Pearson modified technique. The expressions of endothelial and inducible isoforms of nitric oxide synthetase (eNOS and iNOS, respectively), super oxide dismutase (SOD) and catalase (CAT) were measured by RT-PCR. Statistical analyzes applied: One-way ANOVA analysis, Tukey post Test, Correlation and Regression Analysis with Graphpad Prism, $p<0.05$ was used. Results: eNOS, iNOS and SOD decreased in A- with respect to A+ group (eNOS, $p<0.05$; iNOS, $p<0.0001$; SOD, $p<0,005$). Only iNOS levels were recovered with VA refeeding. No changes were observed in CAT expression. The regression analysis resulted in a positive correlation between hepatic RA and eNOS ($R^2= 0.8758$; $p<0.005$), iNOS ($R^2= 0.8209$; $p<0.0001$) and SOD ($R^2= 0.3597$; $p<0.05$). In case of serum RA, a positive correlation was found with eNOS ($R^2= 0.8570$; $p=0.0028$) and iNOS ($R^2= 0.7439$; $p=0.0003$). We conclude that long term VAD affects the levels of pro-oxidant (eNOS/iNOS) and antioxidant (SOD) genes, being only iNOS levels recovered by the refeeding. It's demonstrated the high relation between the hepatic and serum RA levels and the expression of this genes, and for the first time an antioxidant effect of long term VAD by depleting eNOS and iNOS in liver.

(652) PRENATAL STRESS INDUCES METABOLIC DISORDERS IN BALB/C MICE.

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Prenatal exposure to maternal stress may program the fetal HPA axis, potentially leading to altered metabolism in later life, associated with obesity and diabetes. This effects could be worsen in adulthood if the individual doesn't eat healthy. In the present work, we studied the effect of prenatal stress (PS) on metabolism and the impact of high fat diet (HFD). For this purpose pregnant BALB/c female mice were stressed by movement restriction during the last week of pregnancy leaving a control group (NPS). After weaning, mice were fed with HFD or a standard diet (SD).

After 8 weeks of diet, PS+HFD females showed a higher body weight (BW) than the other groups ($p<0.001$ vs NPS+HFD and PS+SD). PS males had a greater BW throughout the experiment ($p<0.01$ PS+HFD vs NPS+HFD and $p<0.01$ PS+SD vs NSP+SD). These animals did not present alterations in the glucose tolerance test. When we performed an insulin tolerance test, in both genders, we observed an altered response linked to HFD rather than PS. Total cholesterol was higher in HFD female groups ($p<0.001$ vs NPS+SD and $p<0.05$ vs PS+SD). In contrast, PS males had higher values independently of the diet ($p<0.001$ vs NPS+SD and NPS+HFD). Similar results were found when we measure plasmatic triglycerides ($p<0.001$ vs NPS+SD and NPS+HFD). While triglycerides levels in females increased with HFD ($p<0.001$ NPS+HFD vs. PS+HFD). In summary: Females presented alterations due to PS but only observable under HFD intake, such as an increase in body weight and elevated triglycerides. On the other hand, males showed metabolic disorders mainly due to PS like an increased BW, total cholesterol and triglycerides. In conclusion, PS alters male metabolism in adulthood, regardless of the diet consumed. In females, the metabolic disorders are evident with the intake of HFD. Finally, it's possible report that PS generates sex and diet-dependent metabolic alterations in mice of the BALB/c strain.

Keywords: prenatal stress (PS), high fat diet (HFD), metabolic disorder, fetal programming.

(1439) REGULATION OF FATTY ACIDS IN ADIPOSE TISSUE OF RATS FED FUNCTIONAL MILK FAT AT HIGH FAT LEVELS

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Rumenic acid and vaccenic acid, naturally present on dairy products, showed to induce health benefits on lipid metabolism, therefore some efforts have been made to increase their levels in the milk fat (MF) and obtain a functional milk fat (FMF). Since adipose tissue (AT) might play an important role on plasma triacylglyceride modulation, the aim of this study was to investigate the regulation of fatty acid (FA) in adipose tissue of animals fed a FMF at high fat levels. Male Wistar rats were fed (60-d) with S7 (soybean oil, 7%), S30 (soybean oil, 30%), MF30 (soybean oil, 3% + MF, 27%) or FMF30 (soybean oil, 3% + FMF, 27%) diets. In AT was assessed: weight, lipoprotein lipase (LPL) activity by fluorometric method, lipogenic and oxidative expression of enzymes by RT-PCR and FA profile by Gas chromatography. Statistical differences ($p<0.05$) were tested by ANOVA (1x3) and Student t' Test. MF30 vs S7 increased the AT weight (+30%), and FMF prevent this effect. Compared with S7, the S30 and MF30 diets increased the AT-LPL activity (+39% and +32%, respectively), and the mRNA levels of CPT-1 (+110% and +85%), without changes in those of DGAT. The FMF30 group (vs S7) also showed an increased AT-LPL activity (+100%), but comparing with MF the elevation of CPT-I gene expression was higher (163%) and, additionally, a lower lipogenesis reflected by reduced DGAT gene expression (-63%) and SCD1 index (c9-16:1/16:0 ratio) (-48%) was observed. Compared to MF30, the FMF30 diet increased the levels of α-linolenic acid (40%), rumenic acid (183%) and vaccenic acid (+169%). In conclusion, the FMF prevented the AT accretion by a higher b-oxidation/lipogénesis ratio, and these balance could be associated with a significant incorporation of rumenic and vaccenic acids in this tissue.

Keywords: rumenic acid, vaccenic acid, functional milk fat, fatty acid regulation.

(1351) **SERUM GHRELIN LEVELS, APPETITE SENSATION AND BODY MASS INDEX BEFORE AND AFTER *Helicobacter pylori* ERADICATION**

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We have previously demonstrated an association between *Helicobacter pylori* gastric infection and lower circulating levels of ghrelin, an orexigenic hormone mainly produced in the stomach. The objective of this study was to evaluate the effect of *H. pylori* eradication on serum ghrelin concentration, appetite sensation and body mass index (BMI). Dyspeptic adults referred for an upper gastrointestinal endoscopy were included in this longitudinal study. Weight and height were measured for BMI calculation, and appetite sensation was evaluated using a validated questionnaire (SNAQ). Serum ghrelin levels were determined by ELISA, and gastric biopsies were obtained during endoscopy for histopathological analysis. Infected patients received eradication therapy and returned after 12 weeks for control, in which the same variables were assessed. Statistical analysis was performed using the Wilcoxon Signed Rank Test. 117 adults (43.2±12.8y) were included, 66.7% women (CI95%; 57.7-74.5). Prevalence of *H. pylori* infection was 69.0% (CI95%; 60.0-76.6%). From the 80 patients who received eradication therapy,

31 returned for control until now, with an eradication rate of 58.1%. BMI of the patients did not differ significantly before and after *H. pylori* eradication 27.5 (IQR; 22.0-31.8) vs 28.0 (IQR; 22.0-31.1), (*P*=0.76). Appetite sensation SNAQ scores were 16.0 (IQR; 12.5-17.0) pre- vs 16.0 (IQR; 15.0-17.0) post-eradication, without a statistically significant difference (*P*=0.93). Median ghrelin concentrations were 639.8pg/mL (IQR; 363.5-772.8) before and 525.5pg/mL (IQR; 323.6-565.0) after eradication, which differed significantly (*P*=0.03). A decrease of the severity of gastric histopathology was observed after eradication. In conclusion, *H. pylori* eradication affects circulating ghrelin levels. However, the completion of this protocol would be needed to evaluate whether BMI and appetite sensation are also affected after *H. pylori* eradication.

Keywords: *H. pylori*, ghrelin, appetite, BMI

(918) **VITAMIN A DEFICIENCY IN LONG TERM ALTERS HDLCOL LEVELS IN LIVER. RELATION BETWEEN HEPATIC HDLCOL, GLUCOSE AND LIPIDS OF SERUM.**

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Vitamin A (VA) plays an important role in development and homeostasis of energy metabolism, adipocyte differentiation and lipid metabolism in many organs such as liver, heart and aorta. The objective of this study was to determine the effect of VA deficiency (VAD) on hepatic HDL cholesterol (hHDLcol) and its relation with the serum levels of glucose (GL) and lipids. Metodology: Wistar female virgin rats were separated at weaning into 3 groups: fed with VA sufficient diet for 6 months (A+), VA deficient diet for 6 months (A-) and VA deficient diet for 150 days followed by refeeding with VA sufficient for 30 days (R). After this time, serum and liver were extracted for lipid assessment. Hepatic lipid extraction was carried out followed by determination of hHDLcol by enzymatic assay. In serum were determined GL, total cholesterol (TC), triglycerides (TG), HDLcol and LDL cholesterol (LDLcol) by enzymatic assays. VLDLcol levels were determined by Friedewald equation. Statistical analyzes applied: One-way ANOVA analysis, Tukey post Test, Correlation and Regression Analysis with Graphpad Prism, p<0,05 was used. Results: hHDLcol decreased in A- (p<0.0254) and R (p<0.0082) groups with respect to A+ group. CT, HDLcol, VLDLcol and TG of serum decreased in A- group with respect to A+ and R groups (p <0.01); GL decreased in A- with respect to A+ and R (p <0.005) groups. The regression analysis results in a positive correlation between hHDLcol and GL (R^2= 0,6151; p=0,0212), TG (R^2= 0,7221; p=0,0075), VLDLcol (R^2= 0,7221; p=0,0075) and LDLcol+VLDLcol index (R2= 0,6095; p=0,0222). We conclude that long term VAD significantly affects the levels of hHDLcol, circulating lipids and GL. A one-month feed with VA sufficient diet seems enough to recover normal values except for the case of hHDLcol levels that are not recovered by refeeding. The maintenance of hHDLcol levels could be particularly important for the homeostasis and maintenance of GL, TG and VLDLcol in serum.

REPRODUCTION AND FERTILITY 10

(903) **EFFECT OF PRENATAL HYPERANDROGENIZATIONON UTERINE TISSUE**

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Abstract: Polycystic Ovarian Syndrome (PCOS) is the most common endocrinopathy in women at reproductive age. PCOS is characterized by oligo or anovulation, hyperandrogenism and ovarian cysts. PCOS involves several health complications, such as insulin resistance and diabetes, obesity, infertility and endometrial cancer. Prenatal hyperandrogenism (PH) is hypothesized as one of the main factors contributing to PCOS. We aimed to investigate which parameters are altered by PHin the uterine tissue. Pregnant Sprague Dawley rats were injected with testosterone (1mg) during days 16 and 19 of pregnancy. A control group (C) wasgenerated by vehicle injection (vegetable oil). We defined female offspring phenotype as PH ovulatory (PHov) or anovulatory (PHanov). All animals were sacrificed at 90 days of life. The glandular density of the sub-epithelial and stromal compartments was evaluated in histological sections upon H&E staining. The oxidative status was studied by lipid peroxidation and GSH. Androgen receptor (AR) and phosphorylated Akt-Ser473 were assessed by western blot. AR was studied by immunohistochemistry. Data were analyzed by One-way ANOVA or Student *t*-test.Our results showed that PH increased the glandular density of the sub-epithelial and stromal uterine compartments. Lipid peroxidation was significantly increased in the PHanov group when compared with C and PHov groups (p<0.05). The antioxidant system reversed the oxidation in the PHanov group by increasing GSH levels (p<0.05). PH induced increased expression of AR (p<0.05), which was found located in the cytoplasm of luminal and glandular epithelial cells in the PHanov group, when compared to C and PHov groups (HSCORE)(p<0.05).Significant increased protein levels of pAkt-Ser473 were found in the PHanov group when compared to C and PHov groups (p<0.05).In conclusion, PH altered the proliferative and oxidative state in the uterine tissue.

Keywords: polycystic ovarian syndrome, hyperandrogenism, uterus, androgen excess.

(1259) **AQP3 IS REQUIRED FOR EXTRAVILLOUS TROPHOBLAST MIGRATION DURING PLACENTATION**

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Development of the human placenta is critical for a successful pregnancy. It requires that the extravillous cytotrophoblast cells (EVTs) migrate, invade and transform maternal spiral arteries. It was recently reported that aquaporin 3 (AQP3) is expressed in trophoblast cells during early gestation and its expression decreased dramatically in preeclamptic placentas. Although, AQP3 is involved in the migration of different cell types, its role in human placenta is still unknown.

Objective: To evaluate the role of AQP3 in the migration and the invasion of EVT.

Methods: Trophoblast cells (Swan 71 cell line) were cultured in complete DMEM-F12. AQP3 expression was examined by Western blot, RT-PCR and immunofluorescence. Cells were also treated with 100 μM $CuSO_4$ (AQP3 specific blocker) or SiRNA-AQP3 to inhibit AQP3. Migration was assessed by wound healing assay. Transwell invasion assay was also performed. Metalloproteinase activities were evaluated by gelatin gel zymography.

Results: AQP3 expression was found in Swan-71. Wound healing assay: the closure of the gap expressed as Control vs $CuSO_4$ (n=5) at each time point was: 8 h (20.77 ±1.23 vs 14.44± 1.21, p<0.05), 18 h (47.50 ± 1.82 vs 39.65 ± 2.46, p<0.05), 24 h (56.34 ± 1.22 vs 49.75 ± 2.38, p<0.05), and Control vs. SiRNA-AQP3 (n=3) was: 8 h (21.61 ±1.22 vs 15.99± 1.23, p<0.05), 18 h (41,74 ± 1,12 vs 33.80 ± 1.53, p<0.01), 24 h (57.10 ± 2.60 vs 46.33 ± 3.24, p<0.05). Transwell invasion and zymography assays: No differences were observed between control and treated cells.

Conclusion: Our results show that the blocking of AQP3 significantly attenuates migration (but no invasion) in Swan 71 cells, suggesting a role of AQP3 during the early stages of placental development. Our work provides new evidence that an altered expression of placental AQP3 may produce failures in placentation, a feature of preeclamptic placentas.

Key word: Extravillous cytotrophoblast, AQP3, Cell migration.

(319) **EFFECTS OF A DIETARY TREATMENT WITH OLIVE OIL IN PLACENTAL mTOR PATHWAY IN A RAT MODEL OF GESTATIONAL DIABETES MELLITUS**

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We previously found intrauterine programming of gestational diabetes (GDM) in the offspring of pregestational diabetic rats. Placentas from GDM rats show overactivation of mechanistic target of rapamycin (mTOR) signaling pathways (mTORC1 and mTORC2), involved in fetal overnutrition and overgrowth. Maternal diets supplemented with olive oil have previously shown beneficial metabolic effects in the placenta in pregestational diabetic experimental models.

Aim: to evaluate the effect of a maternal dietary treatment with olive oil in term placentas from GDM rats.

Methods: Offspring from control and pregestational diabetic rats were mated with control males, and fed with a standard diet or a diet enriched in 6% olive oil (OO) from day 1 to 21 of gestation. On day 21 of gestation, metabolic parameters and placental mTOR signaling pathways (Western blot) were evaluated.

Results: During gestation the offspring of diabetic rats develops GDM, which was not prevented by the OO diet. In the placenta, a decrease in the total 4EBP1 expression in the placentas from GDM rats (44%, $p<0.05$) was prevented by the maternal treatment with OO ($p<0.001$), whereas no changes in phosphorylated 4EBP1 levels were observed in the GDM treated and untreated group, suggesting that the OO enriched diet prevents GDM-induced mTORC1 overactivation. GDM- induced increase in SGK1 phosphorylation in the placenta (36%, $p<0.01$) was not prevented by the OO maternal diet, total AKT levels increased (40%, $p<0.05$) and phosphorylated/total PKCα ratio returns to control values in placentas of GDM rats treated with the OO diet, suggesting a regulation of the overactivated mTORC2 pathways in GDM rats.

Conclusions: Although maternal olive oil supplementation did not prevent GDM, it prevented impairments in mTORC1 and mTORC2 pathways in the placenta, possibly favoring fetal nutrition and metabolic regulation in the offspring´s later life.

Gestational Diabetes Mellitus, Placenta, mTOR, Olive oil

(1466) ***FMR1*** **TRANSCRIPT ISOFORMS IN THE RAT OVARY**

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The Fragile X Mental Retardation-1 gene (*FMR1*) could produce at least 20 human mRNAs through alternative splicing of its 17 exons. Individual isoforms may have particular cellular roles, however, most of the studies concerning *FMR1* splicing isoforms were made in neural tissues and little or no information on other tissues is available. We have previously described the expression of at least 4 different isoforms of the protein in different follicular stages of the rat ovary and preliminary results of our group in this tissue showed the expression of *Fmr1* mRNA variants by alternative splicing of exons 12 and 15 but not of exon 17.

The aim of this work was to confirm these results and to analyze the potential inclusion of exon 14 in the *Fmr1* mRNA isoforms in different stages of follicular development in the ovary and other rat tissues.

Ovaries and follicles were obtained from Sprague-Dawley rats of 21-23 days (preantral follicles) and from rats treated with diethylstilbestrol or eCG (early antral or preovulatory follicles). In addition, muscle and brain tissues were obtained, as well as testis from male rats. Total RNA was extracted, and cDNA synthesized. Fluorescent PCR was performed and products visualized by capillary electrophoresis.

We found 6 different isoforms that are transcribed in all the tissues analyzed: 3 splicing sites for exon 15 and one that produces the presence/lack of exon 14. The three isoforms resulting from the use of the three alternative splicing sites of exon 15 retaining exon 14 were the most abundant in all tissues. Also, some differences in the PCR products' peak heights were observed among different tissues, suggesting differential expression of the isoforms.

We conclude that it's unlikely for the differential expression of the mRNA variants observed in ovary and follicles, to be casual, but probably represents a specific role for some of them in the gonad.

Keywords: *FMR1*, splicing, isoforms, rat ovary.

(1322) **EXPRESSION OF AQUAPORIN-3 (AQP3) IN HUMAN PREECLAMPTIC PLACENTA**

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Trophoblast apoptosis is exacerbated in preeclampsia. Recently, we have reported that the blocking of aquaporin 3 (AQP3) abrogates the apoptotic response of the trophoblast. In this context, we hypothesized that changes in placental AQP3 are required to trigger the programmed cell death.

Our aim was to determine the expression of AQP3 in normal and preeclamptic placentas.

This study was approved by the ethics committee of the Hospital Nacional Dr. Prof. A. Posadas.

We performed semiquantitative western blot and PCR and immunohistochemistry to examine mRNA levels, protein expression and localization of AQP3 in normal and preeclamptic placentas.

We demonstrated that AQP3 protein expression was reduced 45% compared to normal placentas *(n =13, *p < 0.05)*, AQP3 mRNA decreased 62% *(n =13, *p < 0.05)*. Regarding AQP3 localization, it was found in apical membrane of syncytiotrophoblast in normal placentas, but it was undetectable in preeclamptic placentas.

Our results show that the expression of AQP3 is dramatically reduced in preeclamptic placentas. At the light of our results we speculate that the decreased expression of AQP3 might be an adaptive response of the placenta to reduce trophoblast apoptosis which is related to the clinical manifestations of preeclampsia.

Key words: AQP3, trophoblast, preeclamptic placentas

(60) **ENRICHED ENVIRONMENT PRODUCES MOLECULLAR AND PHYSIOLOGICAL CHANGES THAT PREVENTS INDUCED PRETERM DELIVERY IN A MICE MODEL**

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Our group developed a murine model of preterm delivery (PTD) induced by the administration of bacterial lipopolysaccharide (LPS) that produces an 85% of PTD. The effects of environmental enrichment (EE), which are mainly positive, seem to be associated with an overall improvement in the psychological and physical wellbeing of animals. The aim of this work was to evaluate the effect of exposition to an EE for reducing PTD rate in our mouse model. EE protocol consisted in housing a group of ten 6-week old BALB/c females in larger cages containing a variety of objects that provide optimal conditions for social interaction, further exploration, visual, cognitive, and voluntary exercise activity. Standard conditions (control environment, CE) consisted of standard laboratory cages housing 4 animals. After 6 weeks females were mated with males in regular cages and then pregnant females returned to EE (or CE) cages till day 15th of pregnancy, when LPS (or saline solution) was administered to evaluate percentage of PTD. We observed that pretreatment with EE felt by 41% LPS-induced PTD and offspring born from these mothers had a normal development. We studied different inflammatory mediators in uterus, and observed an impact of EE in the expression levels of CD14 and TLR-4 that were significantly diminished. Similarly, we observed that EE treatment significantly prevented the increase of NOS activity induced by LPS. Moreover, we analyzed leukocyte infiltration and matrix metalloproteinase 2 and 9 activity in cervical tissue to evaluate signs of cervical ripening, and observed that LPS increased leukocyte infiltration in both groups, but MMP-9 activity was increased only in CE treated mice. Results were analyzed by one or two-way ANOVA as appropriate and comparisons were made by Tukey's test. Collectively, our results showed a protective effect of the enrichment of the environment against an immune challenge

during pregnancy that involves molecular and physiological changes.

Enriched Environment, Preterm Delivery, Cervical Ripening, Leukocyte Infiltration, Matrix Metalloproteinases

(1231) **HYPEROSMOTIC STRESS MODULATES THE EXPRESSION AND FUNCTION OF AQPS IN HUMAN AMNION**

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Amniotic fluid is essential for normal fetal growth and development. The expression of aquaporins (AQP) 1, 3, 8 and 9 has been described in fetal membranes and it was proposed that they could play a key role in amniotic fluid homeostasis. Emerging data correlates the abnormal expression of amnion AQPs with pathological alterations of amniotic fluid volume such as the oligohydramnios or polyhydramnios.

Recently, we demonstrated that AQPs facilitate the water transport across the human amnion, being the AQP1 the most important contributor. However, the regulation of the amnion AQPs by changes in the osmolarity was not studied yet. The aim of this work was to study the effect of hyperosmolar stress on the expression and function of the AQPs present in the human amnion.

This study was approved by the ethics committee of the Hospital Nacional Dr. Prof. Alejandro Posadas. Human amnion explants were cultured in complete DMEM-F12 and different sucrose hyperosmolar solutions were added to generate the hyperosmolar conditions. The expression of AQP 1, 3 and 9 was analyzed by the Western Blot and PCR. Amnion explants were mounted in a modified Ussing chamber, net transepithelial water movement was recorded with and without an osmotic gradient, and osmotic permeability (pOsm) was calculated.

In amnion explants cultured in 400 mOsm (hyperosmolar condition), we observed that AQP1 expression decreased 80.0% ± 4,8%, while AQP3 diminished 42.2% ± 11.6% and AQP9 72.4% ± 1.3% compared to those explants cultured in isoosmolar condition (300 mOsm) *(n=5, p< 0.05).* In agreement with these results, POsm also decreased 50.0% ± 4.7% (n=5, p<0.001).

Our results showed for the first time, that the expression and function of human amnion AQPs are regulated by changes in the osmolarity. Although the etiologies of the oligo and polyhydramnios are still unknown, our work provides new evidence that the altered expression of AQPs may be related to the development of oligo and polyhydramnios

Key Words: Amnion; Water Flux; Hiperosmolarity; Human Placenta

(593) **MULTIPLE PREGNANCIES AND HYPERTENSIVE DISORDERS IN PREGNANCY: HAVE THEY DIFFERENT PATHOGENESIS?**

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Hypertensive disorders in pregnancy constitute a number of different disorders with different unknown etiologies and pathologic characteristics. Among them preeclampsia associated to multiple pregnancies (MPs) is a possible candidate to be studied as a separate group. Our hypothesis is that an insufficient relationship between the placenta and the baby in gestation could give rise to the clinical symptoms even though the placentation mechanism has not been inadequately developed. Objective: To characterize preeclampsia associated to MPs. Methodology: We conducted a retrospective approach in which all patients with MPs attended in the Hospital Posadas during 2010, 2012 and 2014 were studied. We focused on their obstetric history, birth weight and gestational age. Results: We studied 247 MPs. The incidence of MPs was between 1 and 2%, similar to previous reports described in the literature. Primiparous women with MPs have a 3.1-fold increase relative risk (1.31-7.30) of developing preeclampsia compared to those with a simple pregnancy (p=0.08). However, women with previous pregnancies and current MP have the same relative risk of developing preeclampsia compared to women with previous pregnancies and current single pregnancies *(2.54 vs 2.34 p>0.05).* We found no significant differences between the gestational ages, birth weights, intrauterine growth retardation incidence, and neonatal mortality rate between preeclamptic women with MP and normotensive women with single pregnancy. In addition, no significant differences were found in uric acid levels after the 20th week of gestation in both groups, which could rule out endothelial dysfunction *(3.89±1.01 mg/dl vs 4.50±1.73 mg/dl, p>0.05)*. Conclusion: Our results support the hypothesis that preeclampsia in women with MPs may be due to a pathophysiological mechanism different from that proposed for women with simple pregnancy, perhaps related to a placental system that does not meet the fetal requirements.

(1081) **PARTICIPATION OF CANNABINOID RECEPTOR 1 (CB1) ON LIPOPOLYSACCHARIDE (LPS)-INDUCED PRETERM LABOR**

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The endocannabinoid system (eCS) is comprised by a large family of amides, esters and ethers of long chain polyunsaturated fatty acids, most of which are ligands for cannabinoid receptors (CB1 and CB2) and participate in numerous physiological and pathological processes during pregnancy. Anandamide (AEA) is synthesized on demand from membrane lipid precursors by the action of N-acylphosphatidylethanolamine-specific phospholipase D (NAPE-PLD). The biological activity of AEA is terminated by its intracellular degradation by a fatty acid amide hydrolase (FAAH).

Prematurity is the leading cause of mortality and morbidity in neonates. It is well known that premature deliveries are associated with infectious process. We have previously developed a murine model of LPS-induced preterm labor to mimic the pathological process implicated in prematurity due to infections.

The aim of this study is to explore the role of the eCS on LPS-induced preterm labor.

A study of uterine and cervix explants was performed to characterize and also to investigate the eCS role in mediating the effects of LPS on proinflammatory mediators.

We have already shown by western blot the presence of eCS (FAAH, NAPE-PLD, CB1, CB2) in mouse pregnant uterus and in this study we observed that eCS is also expressed in cervix explants. Also uterine FAAH expression was diminished by LPS treatment (p<0.05).

LPS was able to increase uterine nitric oxide (NO) and prostaglandins synthesis and we observed that this was abrogated when the explants were incubated with and antagonist of CB1 receptor (AM251, p<0.05) but not by an antagonist of CB2 receptor (AM 630).

Moreover we found that CB1-KO mice show lower preterm labor rate (83% vs. 13%).

Collectively, our results indicate a participation of CB1 receptors in the effects of LPS. These data suggest that LPS induces a systemic change in eCS signaling ligand production, which in turn regulates the pro-inflammatory response associated with preterm labor.

(1510) **PROBIOTIC *Lactobacillus Kefiri* PREVENTS LIPOPOLYSACCHARIDE-INDUCED PRETERM BIRTH IN MICE**

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Preterm birth (PTB) is a recurrent complication of pregnancy affecting 5-18% of all births worldwide and leading to serious consequences for the mother and the progeny. Although aetiology is not fully determinate, it is known that inflammation is a contributing factor to both infection-mediated and spontaneous PTB. Probiotics were proved to be able to modulate immune responses promoting an anti-inflammatory environment. Hence, they are being widely used in the prevention and treatment of infectious diseases and immunopathologies. The aim of this work was to study the effects of probiotic *Lactobacillus kefiri* on lipopolysaccharide (LPS) induced PTB in pregnant mice. C57BL76 females were administered every 48h by oral gavage during a week with probiotic (PB) (10^8 CFU) (n=5) or vehicle (milk) (n=5). Afterward, animals were mated with BALB/c males. Day of the vaginal plug detection was considered day 0.5 of pregnancy. Treatment continued every 48h during pregnancy, and females were challenged with LPS (10 ug) on gd 16.5 (~3 days before expected on-time birth). Births before day 18.5 were considered PTB. Mice from control group given LPS exhibited 100% of PTB (5/5), with a majority delivering dead pups (18/25, 72%) or live pups (7/25, 28%) prematurely. Remarkably, treatment with *L. kefiri* completely protected pregnant mice to develop LPS-induced PTB (0%, 0/5), with a majority delivering viable term pups (19/26, 73%), ($p < 0.0001$, analysed by $\chi 2$ test). Additionally, while uterus from PB mice looked healthy, uterus from control mice exhibited highly haemorrhagic areas and implantations sites were barely recognized. Pups born from PB treated mice were not only bigger at birth but also gained significantly higher weight during lactation period compared to pups from control mice (one way ANOVA, $p < 0.0001$). Our results clearly demonstrate that probiotic *L. kefiri* prevents LPS-induced PTB *in vivo*, opening new avenues to explore its therapeutic use for preventing PTB in humans.

Key Words: Preterm Birth, Lactobacillus Kefiri, Probiotic.

GENETICS AND MOLECULAR BIOLOGY 23

(255) **DEVELOPMENT TO INFECTIVITY IN *TRYPANOSOMA CRUZI* INDUCED BY AN RNA-BINDING PROTEIN THROUGH TRANSLATIONAL REPRESSION**

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Gene expression regulation is crucial for cell survival and differentiation. Trypanosomes, protozoan parasites of medical importance, rely mainly on posttranscriptional mechanisms to differentiate and replicate in insect vectors and vertebrate hosts. RNA binding proteins (RBPs), which associate to the 3'-UTR of mature mRNAs, are thought to orchestrate master developmental programs for these processes to happen. Yet, the molecular mechanisms by which differentiation occurs remain largely unexplored for these human pathogens. Here, we show that a small increase in the levels of the RBP TcUBP1 can recapitulate parasite differentiation in *Trypanosoma cruzi*, the parasite responsible for Chagas disease in the Americas. Ectopic inducible expression of TcUBP1 in non-infectious stages (epimastigotes) promoted the differentiation into infective metacyclic trypomastigotes. This transformation included repositioning of the kinetoplast, the expression of a virulence factor such as trans-sialidase and growth arrest, without affecting cell viability. Moreover, TcUBP1-derived metacyclic trypomastigotes proved to be infective in cell culture. Using a 3' UTR tethering approach we were able to show that ectopic expression of TcUBP1 promotes translational repression, which was confirmed by a global reduction in translation rates. Furthermore, expression of mutated and truncated forms of TcUBP1 revealed the importance of low complexity (LC) regions for the induced development to infectivity. Collectively, our results show that an RBP has a central role in the differentiation of *T. cruzi* epimastigotes to infective metacyclic trypomastigotes, offering insights of a translational repression mechanism involving LC sequences.

Keywords: Trypanosomes, Rna-Binding Protein, Translation

(494) **TRANSCRIPTOME ANALYSIS OF THE *Triatoma Infestans* (HEMIPTERA: REDUVIIDAE) INTEGUMENT.**

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INIBIOLP

The insect integument, formed by the cuticle and the underlying epidermis, is essential to insect fitness, regulating lipid biosynthesis and storage, insect growth and development. Its participation in insecticide resistance was recently shown in *Triatoma infestans*; one of the major vectors of Chagas disease in South America. Cuticle thickening together with larger amounts of cuticular hydrocarbons contribute to the cuticular resistance factor in insecticide resistant populations,however, genomic data is scarce. In this study, we performed a transcriptome analysis of the nymph integument in order to identify the genes expressed and their potential role. More than 10% of the transcripts encoded proteins putatively involved in the metabolism of fatty acids and related components (fatty acid synthases, elongases, desaturases, fatty alcohol reductases), structural integument proteins, and components of the insecticide detoxification system (cytochrome P450s, esterases and glutathione transferases). Present work showed a differential expression pattern of several genes related to cuticle lipid biosynthesis, cuticle formation and insecticide detoxification in pyrethroid-resistant insects. These results suggest that the overexpression of some integument genes may contribute to insecticide resistance in *T. infestans* through mechanisms not only involving a delayed penetration through the cuticle, but also an increased detoxification of the insecticide. The function of relevant genes potentially involved in insecticide resistance was studied by RNA interference. This is the first transcriptome analysis of a triatomine integument, and together with prior biochemical information, will help further understand the integument role in a wide array of mechanisms.

Keywords: *Triatoma infestans*, transcriptome, integument, cuticle lipid metabolism, insecticide resistance.

(222) **STEADY-STATE LEVELS OF TRANSCRIPTS CODING FOR TRYPOMASTIGOTE SURFACE GLYCOPROTEINS IN *Trypanosoma cruzi* ARE COORDINATELY REGULATED BY *Tc*UBP1**

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Regulation of gene expression in trypanosomatid parasitic protozoa, including *Trypanosoma cruzi*, the causative agent of Chagas disease, is mostly achieved post-transcriptionally. RNA regulons consist of ribonoucleoprotein complexes that respond to exogenous and/or endogenous cell signals to regulate the co-expression of functionally related proteins. In this regards, *Tc*UBP1 (*T. cruzi* U-rich binding protein 1) plays a role in mRNA turnover by binding to transcripts harboring a signature element in their 3′-untranslated regions (UTRs). Among UBP1 targets we found a subset of the *TcS (Trans-sialidase/Trans-sialidase-like)* superfamily, a large group of glycoprotein molecules that are differentially expressed in the mammalian stage of the parasite (trypomastigote). The aim of our work is to analyze the impact of the *Tc*UBP1-*TcS* interaction, and the biological function of this RNA-binding protein as a regulatory factor of this gene family. For this purpose, we first analyzed by qPCR the expression of four representative members in two stages of *T. cruzi* CL Brener wild type parasites, together with a common target by amplifying a conserved region present in most of the *TcS* targets 3′-UTRs. We found an enrichment of >10-fold difference in the in-

fective trypomastigotes over the non-infective epimastigotes in *TcS* transcritps but not in RNA controls (p<0.05, Student t-test). Then, a *Tc*UBP1-GFP construction or GFP, as a control, was cloned into the pTcINDEX tetracycline inducible vector and transfected in transgenic epimastigotes expressing tetR. Overexpression of *Tc*UBP1 in these cells showed an increase from 5 to 20 times of the *TcS* targets comparing to non-induced and GFP controls (p<0.05, Student t-test). Altogether, our results point to a coordinately up-regulation effect of numerous *TcS* transcripts as a specific response to *Tc*UBP1 overexpression, suggesting a role of this RRM protein in regulation of trypomastigote surface glycoproteins synthesis.

Keywords: Post-transcriptional regulon, RNA-binding protein, RNA abundance, surface glycoproteins

(1553) **TbRRM1 HAVE OPPOSITE ROLES IN TRANSCRIPTIONAL REGULATION OF RNA POL II-TRANSCRIBED UNITS IN *Trypanosoma brucei***

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TbRRM1 is an essential RNA binding protein from *T. brucei* which belongs to the SR-related protein family. Previous studies from our lab indicated that TbRRM1 ablation by RNAi in procyclic cells leads to cell-cycle block and cell death by an apoptotic-like mechanism. Recently, TbRRM1 was found associated to numerous mRNAs and to core histones, which suggests a dual role in both transcriptional and post-transcriptional regulation.

To determine if TbRRM1 is involved in the *trans*-splicing process, we first evaluated the steady-state level of the SL-RNA by RTqPCR in parasites depleted or not of TbRRM1. The SL-RNA level increased after RNAi induction relative to uninduced parasites, suggesting either a global *trans*-splicing defect or an up-regulation of the SL-RNA by increased transcription. To evaluate the *trans*-splicing efficiency in parasites depleted of TbRRM1, we then determined by RTqPCR, the pre/mature mRNA ratio of TbNOP86 and 60S ribosomal protein L38, known to be down-regulated after *TbRRM1* knockdown. Both pre and mature mRNA levels were reduced after *TbRRM1* silencing indicating a transcriptional impairment induced by *TbRRM1* knockdown, rather than a *trans*-splicing defect, which was not affected.

We then carried out Run-On assays to evaluate if TbRRM1 is involved in gene transcription regulation. We found a global decrease of RNA synthesis after RNAi induction. Moreover, transcription analyses of neighboring RNA Pol II-dependent protein-coding genes showed that TbRRM1 is required for transcription elongation. Interestingly, our results also showed that transcription of the *SL-RNA* was significantly increased in parasites depleted of TbRRM1, which correlated with increased SL-RNA levels.

In summary, our data show that TbRRM1 could be involved in both facilitating transcription elongation of polycistronic transcription units and repressing expression of monocistronic units of the *SL-RNA* genes, both process mediated by RNA Pol II.

Keywords: RNA binding protein, *trans*- splicing, transcription elongation, epigenetic regulation, chromatin remodeling.

(1856) **ABC TRANSPORTER TRANSCRIPTION REGULATION BY *Staphylococcus aureus* SAOUHSC01313-01314 TWO COMPONENT SYSTEM**

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Staphylococcus aureus, a G+ bacterium, is a frequent component of human microbiota that can turn into a dangerous pathogen. The pathogenicity of this bacterium is caused by expression of a myriad of virulence factors. Since two component systems (TCSs) are the principal mechanism involved in stress response, we studied two contiguous genes located in strain NCTC 8325, SAOUHSC01313-01314, coding for a membrane-histidine kinase (HK) and a response regulator, respectively. These genes showed homology with *Bacillus subtilis* DesKR, which regulates transcription of its sole desaturase in response to changes in membrane fluidity. The TCSsa is located downstream of genes encoding a putative ABC transporter (SAOUHSC01311-SAOUHSC01312) that carries in its promoter a box very similar to the regulatory region of *des*. This TCS/ABC link resembles the prototype detoxification module found in *Firmicutes*.

In order to study the transcriptional activation of the ABC transporter by the TCS we constructed a transcriptional fusion of SAOUHSC01311 promoter to *lac*Z, in plasmid pTCVlac. This plasmid, pARD22, was transformed in wild type strains RN4220 and SH1000, and the derivatives ΔSAOUHSC01313-01314, strains ARD10 and ARD11, respectively. β-gal assays of strains carrying pARD22 were conducted at different temperatures and media composition. We found that the TCSsa regulates the transcription of the genes encoding the ABC transporter in a temperature dependent fashion. However, in contrast to the *des gene,* transcription was induced at 37°C rather than at low temperature. We also found that expression of the ABC transporter occurs in stationary phase, and requires minerals and sugars in the culture medium.

These results show that, despite the similarity between DesK and SAOUHSC01313, the HKsa responds to different stimuli, and shed light on the possible role of this HK in the membrane of *S aureus* exposed to environment cues very different from those that *B subtilis* faces in its environment.

Keywords: *S. aureus*, TCS, transcriptional regulation, transporter

(723) **INTERACTION OF CARBON AND NITROGEN METABOLISM IN MYCOBACTERIA: A STUDY OF MSMEG_0432, A PLEIOTROPIC TRANSCRIPTIONAL REGULATOR**

Julián Agustín Bulssico

NlpR encodes a transcriptional regulator involved in the carbon and nitrogen metabolism in *Rhodococcus jostii*. It was demonstrated in this organism that in nitrogen starvation, NlpR acts as a transcriptional activator of several genes associated to triacyclglycerol accumulation and nitrogen assimilation, particularly in the nitrite uptake and its reduction to ammonium. In this context, we aimed to assess the importance of this regulator in the closely related organism *Mycobacterium smegmatis*. In this species, MSMEG_0432 encodes a putative ortholog of rhodococcus NlpR. A knock out mutant was constructed by in-frame deletion using a suicide vector. All recombination events were checked by PCR. Once the mutant strain was confirmed, it was complemented with a plasmid carrying a wild type copy of MSMEG_0432 under the control of a promoter inducible by anhydrotetracycline (ATc). The ability to grow in different minimal media containing different sources and amounts of nitrogen sources was analyzed in the wild type, knock out mutant and complemented strain. Different ATc concentrations were evaluated in different culture medium for the complemented strain. The analysis of the growth curves showed that, while all strains were able to grow in minimal medium with ammonium as the only nitrogen source, neither the mutant nor the complemented strain were able to grow with nitrate as the sole nitrogen source in the absence of ATc. In the complemented strain, growth in minimal medium with nitrate was only observed when ATc was added. Thus, by adding increasing concentrations of ATc, the wild type phenotype was restored up to the wild type levels when using nitrate as nitrogen source. These results suggest the implication MSMEG_0432 in the activation of several genes involved in the nitrite reduction in *Mycobacterium*. Further studies are being performed to evaluate the expression levels of genes related to nitrate reduction and the TAG synthesis in different growth phases.

Keywords: Mycobacterium smegmatis, nitrogen metabolism, transcriptional regulation.

(962) **REGULATORY ROLE OF THE LIMPET TRANSCRIPTION FACTOR IN *TRIATOMA INFESTANS* IMMUNE RESPONSE**

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INIBIOLP

As a part of the innate humoral response to microbial attack, insects activate the expression of antimicrobial peptides (AMPs). Understanding the regulatory mechanisms of this response in the Chagas disease vector *Triatoma infestans*, the main insect vector of *Tripanosoma cruzi* in the southern cone of South America, is important since biological control strategies against pyrethroid-resistant insect populations were recently addressed by using the entomopathogenic fungus *Beauveria bassiana*. In this study, we started the molecular and functional characterization of the LIMPET transcription factor, potentially involved in the regulation of AMPs expression. Primers were designed to complete its sequence and alignments with related species were made to determine its sequence homology. Functional characterization was performed through dsRNA silencing and qPCR assessment of the LIMPET gene but also a group of AMPs called defensins. Multiple reference genes method and Student *t*-test were used to analyze the data. After a 94% silencing of the *limpet* mRNA after injection, an initial decrease in expression in defensins was detected at 24h (control 16.4 ± 7.1 and injected 0.08 ± 0.12, p=0.012), but an increase of expression and even overexpression was observed at 72h (control 0.22 ± 0.09 and injected 1.22 ± 0.59, p=0.03). We concluded that LIMPET is involved in the molecular regulation pathway of *T. infestans* immune response including AMPs, although might be not the only regulator. Currently, there is ongoing work on this system which includes infection with *B. bassiana* to assess the response of *limpet* and the involved defensins when confronted to microbial attack. This work would lead to a better understanding of how the regulation pathway of *T. infestans* immune response in which *limpet* is involved works.

Keywords: Triatomines, Immunity, *limpet*, defensins

GASTROENTEROLOGY 2

(519) COENZYME Q10 LEVELS IN MATERNAL AND CORD BLOOD IN INTRAHEPATIC CHOLESTASIS OF PREGNANCY

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Abstract: Intrahepatic cholestasis of pregnancy (ICP) is a high-risk medical condition due to the serious consequences it may have on the fetus. ICP is characterized by bile acid accumulation in maternal serum which leads to an imbalance between reactive oxygen species production and antioxidant defenses thus enhancing an oxidative environment for the fetus. In previous studies we reported that women with ICP have decreased plasma coenzyme Q10 (CoQ10) levels. CoQ10 is a redox substance integrated into the mitochondrial respiratory chain which acts as an electron carrier to generate cellular energy. Further, it is recognized as the primary regenerating antioxidant playing an intrinsic role against oxidative insult. In the present study, we measured CoQ10 and bile acids levels in maternal and umbilical cord blood in normal pregnancy and ICP (n= 23 and n=13, respectively). Total bile acids (TBA) were significantly higher in maternal and umbilical cord blood from ICP (p<0.01) and had a hydrophobic profile. In ICP CoQ10 was decreased (p<0.001) in maternal blood samples but that of the umbilical cord showed no changes. However when CoQ10 levels were normalized to cholesterol ($CoQ10_{Chol}$), the level of the coenzyme was significantly reduced in both maternal and umbilical cord blood samples from ICP (p<0.001 and p<0.05, respectively). The $CoQ10_{Chol}$/ TBA ratio was diminished in mothers with ICP and in their respective umbilical cord samples (p<0.01). Present findings support that fetuses from women with ICP are at risk of oxidative stress damage. The recognition of CoQ10 deficiency would allow a possible therapeutic strategy based on CoQ10 supplementation to women with IPC in order to avoid oxidative stress damage to the fetus.

Keywords: Oxidative stress, ubiquinone, bile acids, cord blood, pregnancy cholestasis

(476) EFFECT OF GENISTEIN ON PARAQUAT-INDUCED HEPATOTOXICITY IN RATS

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Background and aims: Previously we found that genistein (GNT) treatment induced P-glycoprotein (P-gp) expression and activity in rat liver. The aim of this study was to evaluate if GNT treatment reduce hepatotoxicity induced by the herbicide paraquat (PQ), a known P-gp substrate.

Methods: Male Wistar rats were randomized in 4 experimental groups (n=3). Control, GNT (5 mg/Kg/day s.c.,4 days), PQ (50 mg/Kg/day i.p., last day) and GNT+PQ (PQ injected the last day of GNT treatment). On the 5th day hepatic lipoperoxidation (LPO) was evaluated by the thiobarbituric acid reactive substances (TBARS) methodology. Serum levels of alanine transaminase (ALT) and aspartate transaminase (AST) were measured with commercial kits. Liver samples were processed for histological analysis. Hepatic glutathione-S-transferase (GST) activity, which is associated with the prevention of PQ toxicity, was determined by UV-spectrophotometry.

Results (% of control)*:* Pretreatment of PQ-intoxicated rats with GNT reduced hepatic LPO as compared with PQ alone (Control: 100 ± 2, GNT: 99 ± 12, PQ: 120 ± 5 *, GNT+PQ: 101 ± 6) (* p<0.05 vs. Control, GNT and GNT+PQ). GST, AST and ALT activities were not modified by PQ and/or GNT treatment. Histological alterations induced by PQ exposure, such as microvesicular steatosis and hepatocyte swelling, were partially prevented by GNT administration.

Conclusion: Our results suggest that GNT pre-treatment ameliorates the markers of PQ-induced hepatotoxicity evaluated. As GST activity is unmodified by GNT treatment, its involvement in liver protection against PQ hepatotoxicity is unlikely. The hepatic P-gp protein and activity induction by GNT could lead to a decrease in PQ intrahepatic content and thus reduces the hepatic injury induced by PQ.

Keywords: Genistein, Paraquat, P-glycoprotein, Liver.

(109) ENZYMES INVOLVED IN EICOSANOID CASCADE ARE DIFFERENTIALLY EXPRESSED DURING LIVER REGENERATION AFTER PARTIAL HEPATECTOMY (PH) IN RATS

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Metabolism of arachidonic acid either through the cyclooxygenase (COX-2 or 1) or the 5-lipoxygenase (5-LOX) pathways leads to the formation of different bioactive eicosanoids: prostanoids (prostaglandins and tromboxanes) and leukotrienes (LT), respectively. While it has been established that prostaglandins are necessary

mediators of liver proliferation during regeneration, the role of LT is not clear. 5-LOX produces LTA4 which is immediately converted to LTB4 by LTA4 hydrolase (LTA4-H) or LTC4 by LTC4 synthase (LTC4-S). Previous studies from our lab showed that treatment with a 5-LOX inhibitor produces a decrease in LT content and a reduction in liver proliferation after PH in rats, indicating that LT are necessary for liver regeneration. Objective: to study the expression of enzymes involved in the synthesis of eicosanoids during liver regeneration, focusing on the enzymes of LT biosynthesis. Methods and results: Adult male Wistar rats were subjected to sham surgery or PH (70 % liver removal). Liver samples were obtained 1 and 24 h post-surgery. Also, non-parenchymal liver cells (SNPs) were isolated by collagenase perfusion since 5-LOX and LTA_4-H are expressed in these cells. qPCR studies showed a decrease (-90%*) on LTC_4-S mRNA with no changes on 5-LOX or LTA4-H mRNA 1 h post-HP. However, immunoblot analysis showed that 5-LOX protein levels 1h post-PH were significantly increased in PH rats (+40%*). mRNA expressions of both 5-LOX and LTC_4-S 24h post-PH were significantly reduced (-75 %* and -90%*, respectively), while COX-2 mRNA was increased (+200 %*) at that time. This decrease of 5-LOX 24h post-PH also caused a reduction in intrahepatic LTB4, determined by ELISA. (*p<0,05 vs. sham). Conclusion: Enzymes involved in eicosanoids synthesis are differentially expressed during the time-course of liver regeneration. While COX-2 products are crucial at longer times (24h) post-PH, LT, and particularly LTB4, seem to be necessary at shorter times (1 h).

Keywords: Liver regeneration, leukotrienes, prostaglandins, 5-lipoxygenase

(275) **HUMAN AQUAPORIN-8 (hAQP8) TRANSDUCTION STIMULATES AMMONIA DETOXIFICATION IN PRIMARY HEPATOCYTE CULTURE**

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The ammonia detoxification is crucial to prevent the development of pathological states as hyperammonemia and encephalopathy. In previous gene silencing studies, our laboratory has demonstrated the critical role that hepatocyte mitochondrial AQP8 plays in the urea cycle, specifically by promoting ammonia uptake. Aim: to study the effect of hAQP8 transduction in hepatocytes on ammonia detoxification via ureagenesis. Methods and Results: We made use of the adenovector AdhAQP8 which was designed by us encoding hAQP8 and containing EGFP as reporter gene. An adenovector encoding for EGFP alone was used as control. Primary culture rat hepatocytes were exposed to AdhAQP8 or control vector. After 48 h, hAQP8 was properly expressed in inner mitochondrial membranes as assessed by subcellular fractionation and immunoblotting. The transduction process did not alter LDH leakage or cell morphology. When urea synthesis was augmented in transduced hepatocytes by incubation with increasing concentrations of NH_4Cl (0, 0.5, 1.0, and 1.5 mM), it was observed that ureagenesis from ammonia significantly increased by about 50% ($P < 0.02$) in hAQP8-transduced cells. For these studies, urea was assessed by a non-enzymatic spectrophotometric assay (Abnova urea assay kit). Nuclear magnetic resonance studies using ^{15}N-labeled ammonia confirmed that ^{15}N-labeled urea synthesis was markedly increased in hAQP8-transduced hepatocytes (+180%, $P < 0.001$). **Conclusion:** The mitochondrial expression of hAQP8 in rat hepatocytes significantly increased ammonia detoxification by ureagenesis. This finding might have potential therapeutic implications for hyperammonemia disorders.

Keywords: Aquaporin-8; Ureagenesis; Ammonia Detoxification; Adenovector

(210) **INDUCTION OF HEMOXYGENASE 1 (HO1) PREVENTS ACUTE HEPATIC CHOLESTASIS PRODUCED BY OXIDATIVE STRESS (OS) IN THE RAT**

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Here, we studied the effect of HO1 induction and consequent increase in endogenous levels of bilirubin (BR) on OS-induced cholestasis. Wistar rats were treated with Hemin (H) and biliary concentrations of BR were determined, finding that it increased 6-8h post i. p. injection of 20 mg/kg H (12.6±2.5 *vs* 5.3±0.6 for vehicle, p<0.001; n=4). Oxidative cholestatic injury was induced by *tert*-butyl hydroperoxide (*t*BOOH,440 µmol/kg, i.p.) and bile flow (µl/min/g liver) was monitored finding that it decreased 4-6h post treatment (p<0.05 *vs* control; n=6). Pretreatment with H completely prevented reduction of bile flow (1.65±0.04 and 1.30±0.03, respectively; p<0.01; n=6). Redox state was evaluated by measuring levels of lipid peroxidation (LP), oxidized glutathione/total glutathione ratio (GSSG/GSHt) and activity of antioxidant enzymes catalase (CAT) and superoxide dismutase (SOD). We found that *t*BOOH caused an increase in LP (0.170±0.022 nmol MDA/mg proteín *vs* C, 0.090±0.005 nmol MDA/mg protein p<0.05; n=6) while pretreatment with H prevented this increase (0.082±0.001 nmol MDA/mg protein 0.05 *vs* *t*BOOH; n=6). GSSG/GSHt ratio increased after treatment with *t*BOOH (0.40±0.09 *vs* C, 0.14±0.12, p<0.05; n=6) while pretreatment with H prevented this increase (0.20±0.01, p<0.05; n=6). CAT and SOD activities were increased in *t*BOOH group (p<0.05 *vs* C, n=3, for both enzymes) while pre-treatment with H completely prevented these increases (p<0.05 *vs* *t*BOOH, n=3). We also studied the function of two key hepatocanalicular transporters, Bsep and Mrp2, by determining biliary excretion of their specific substrates, bile salts (BS) and GSHt, respectively. Biliary excretion of both BS and GSHt decreased after treatment with *t*BOOH, and pretreatment with H prevented these decreases (p<0.05 *vs* *t*BOOH, n=4). We conclude that induction of HO1 and consequent elevation of BR protect the liver from oxidative injury and contribute to limit the progression of cholestatic liver diseases concurring with OS.

Keywords: Oxidative stress, cholestasis, HO1, bilirrubin.

(1058) **MODULATION OF P-GLYCOPROTEIN IN RAT LIVER BY PROLACTIN**

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Prolactin (PRL) regulates multiple physiological functions in mammals and its plasma levels increase in lactating females. Expression of PRL receptor in liver has suggested a role for this hormone in regulating liver function. Previous studies have demonstrated that PRL regulates the expression of different hepatic transporters, such as bile salt export pump (Bsep). However, it remains unknown whether PRL can modulate P-glycoprotein (Pgp) expression, a canalicular transporter responsible for xenobiotic excretion. Here we evaluate the role of PRL in the modulation of hepatic Pgp expression. We performed western blot studies using both *in vivo* and *in vitro* models. First, we found increased hepatic Pgp expression in lactating post-partum rats (PP) (+53% vs virgin female rats, p<0.05, n=4). To further analyze the role of PRL in Pgp up-regulation, we used ovariectomized (OVX) rats in which endogenous PRL secretion was additionally suppressed by bromocriptine (B) (0.2 mg/day/100 g bw; s.c.). PRL administration to these rats (300 µg/day for 7 days at a constant rate via osmotic minipumps, reaching PRL plasma levels similar to those observed in PP rats) increased Pgp levels (+121% vs OVX rats treated with vehicle and B, p<0.05, n=3). Second, we used sandwich-cultured rat hepatocytes (SCRHs) as *in vitro* model consisting in hepatocytes plated between layers of collagen that maintain a 3D shape and distribution of cytoskeletal proteins similar to that observed *in vivo*. Cultures exposed to 0.10 µg/mL of PRL (concentration associated with plasma levels in PP rats) exhibit higher Pgp expression (+205% vs SCRHs exposed to PRL vehicle, p<0.01, n=8) after 48 h treatment. In conclusion, PRL increases hepatic Pgp expression in both models. Moreover, SCRHs is a useful *in vitro* model to study the molecular mechanism of Pgp induction

by PRL. These findings suggest the possibility of altered pharmacokinetics of xenobiotics substrates of Pgp in PP period, including therapeutic agents.

Keywords: Prolactin, P-glycoprotein, liver, hepatic transporter, lactating rats

(1490) **THE ABSENCE OF SPARC (SECRETED PROTEIN ACIDIC AND RICH IN CYSTEINE) ATTENUATES LIVER INFLAMMATION AND FIBROSIS IN NON-ALCOHOLIC STEATOHEPATITIS MICE MODELS**

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Non-alcoholic fatty liver (NAFLD) consists of fat accumulation in hepatocytes. It encompasses a set of clinical conditions ranging from fatty liver, hepatocyte damage and inflammation (steatohepatitis or NASH) and its subsequent complications: liver fibrosis, cirrhosis and hepatocellular carcinoma. SPARC is a matricellular protein associated with inflammatory processes, tissue remodeling, regulation of fibrillar collagen deposits, among other biological functions.

The aim of this project was to study the role of SPARC in the context of two NASH models: 1) the streptozotocin-induced NASH model (STAM); 2) and the diet-induced obesity (DIO) model. For STAM model, 2 days old SPARC$^{-/-}$ and SPARC$^{+/+}$ mice were subcutaneous injected with 200 mg streptozotocin (SZT) and fed with high fat (HF) or control (LF) diet since weaning for 8 weeks. For DIO model, SPARC$^{-/-}$ and SPARC$^{+/+}$ mice were fed for 20 weeks with HF. SPARC and pro-inflammatory cytokines expression were assessed by qPCR. The degree of NASH was measured using the NAS score; and fibrosis was assessed by picrosirius red staining. Triglycerides, cholesterol and serum transaminases were also measured.

Liver SPARC expression was increased in HF-fed mice in both experimental models. In the STAM model weight curves demonstrated that SPARC$^{+/+}$ and SPARC$^{-/-}$ mice, either LF or HF, increased their weight equally; in contrast, DIO model shows weight difference between LF and HF fed-mice. HF-fed SPARC$^{-/-}$ mice in STAM model developed less fibrosis, as well as HF-fed SPARC$^{-/-}$ mice from DIO model. According to NAS score, HF-fed mice from STAM model developed NASH after 8 weeks, whereas in DIO model only HF-fed SPARC$^{-/-}$ mice develop incipient NASH. Inflammatory cytokines expression were increased in HF-fed SPARC$^{+/+}$ mice compared to SPARC$^{-/-}$ mice from both experimental models.

We present novel evidences that demonstrate a role for SPARC in the development of NASH. SPARC could play a key role as a target for prevention of NASH progression.

Keywords: Sparc / Nash / Stam / Dio

(1278) **THE ROLE OF SPARC (SECRETED PROTEIN ACIDIC AND RICH IN CYSTEINE) IN THE LIVER SINUSOIDAL ENDOTHELIUM IN AN EXPERIMENTAL MODEL OF ACUTE LIVER FAILURE.**

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Acute liver failure (ALF) is characterized by a rapid deterioration of liver function. Liver sinusoidal endothelial cells (LSEC) have a key role during ALF. Different pathological agents can target LSEC, disrupting sinusoidal endothelium and facilitating T-cell migration into liver parenchyma. SPARC is a matricellular protein involved in many processes including cell-cell interaction and adhesion. After injury, SPARC is overexpressed in EC inducing different cellular processes. We have observed that SPARC$^{-/-}$ mice are protected from ALF damage. In this work, we study the role of SPARC in sinusoidal endothelial injury. In an *in vivo* approach of ALF, SPARC$^{+/+}$ and SPARC$^{-/-}$ mice were subjected to conA injury and LSEC monolayer morphology was studied by electronic microscopy. LSEC primary cultured from SPARC$^{+/+}$ and SPARC$^{-/-}$ were used to study the effects of conA *in vitro*. SPARC expression, cytoskeletal structure, cell morphology and activation were assessed. Microscopic analysis revealed that LSEC monolayer was well preserved and less activated in conA-treated SPARC$^{-/-}$ mice compared to SPARC$^{+/+}$. SPARC$^{-/-}$ LSEC phenotype and endocytic capacity were conserved. SPARC expression was increased in conA-treated LSEC (7.3±1 vs 1±0.1). In addition, SPARC$^{-/-}$conA-treated LSEC showed a marked decrease in VCAM-1 expression; cell morphology was more preserved and no alterations in actin cytoskeleton organization in SPARC$^{-/-}$ mice; contrarily, SPARC$^{+/+}$LSEC showed a clear disturbance in cell appearance and actin filament architecture. Consistently, qPCR analysis showed that SPARC$^{-/-}$conA-treated LSEC increased their expression of *capzb* (1.7±0.1 vs 1.2±0.1), a regulator of actin filament dynamics, and decreased *tubb2b* expression (0.01±0.1 vs 2.4±0.01), a major component of microtubules, compared with SPARC$^{+/+}$conA-treated LSEC. Our results suggest that SPARC plays an interesting role in LSEC under conA damage. Inhibition of SPARC merits further investigation as a potential therapeutic target.

Liver sinusoidal endothelial cell (LSEC) – SPARC – Acute liver failure (ALF)

(767) **UNRAVELING THE ROLE OF PROHIBITIN-1, A NOVEL LIGAND OF GALECTIN-1 IN HEPATOCELLULAR CARCINOMA CELL VIABILITY**

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Galectin-1 (Gal1) is a glycan-binding protein overexpressed in hepatocellular carcinoma (HCC). We demonstrated that Gal1 upregulation in human HCC cells induces cell proliferation, epithelial-mesenchymal transition, tumor growth and metastasis. By proteomics we have identified prohibitin-1 (PHB) as a new Gal1 ligand in HCC cells. PHB has multiple functions depending on its subcellular localization. Its role in cancer is controversial, it was found downregulated in most human HCC tissues analyzed but in other HCC samples its overexpression was observed. Liver-specific PHB knockout (KO) mice develop HCC spontaneously suggesting that PHB functions as tumor suppressor, although when highly expressed it promotes HCC cell migration *in vitro* showing its involvement in tumor progression. Thus, we aimed to elucidate PHB role in HCC cell viability and to analyze if PHB expression is regulated by Gal1. By immunofluorescence, we observed that PHB localizes in a punctate pattern in human HepG2 and HuH-7 HCC cells, both in nucleus and cytoplasm. By siRNA transfection, we downregulated PHB expression in these cells: HepG2-siPHB 31±15 vs HepG2-siControl 76±19% ($p<0.05$ n=3); HuH7-siPHB 36±9 vs HuH7-siControl 96±19% ($p<0.05$ n=7) at 72 h post-transfection (Western blot, Wb). Cell viability (MTT) at 72 h decreased in HuH7-siPHB cells (85±5%) compared to HuH-7-si-Control cells (100%, $p<0.05$ n=7) whereas the opposite effect was observed in HepG2-siPHB cells (118±9 vs control cells 100% n=3). PHB expression increased in Gal1 overexpressing HepG2-Gal1 cells (181±36% $p<0.05$ n=5) and decreased in HepG2 cells transfected with Gal1 shRNA (13±2% $p<0.01$ n=3) respect to the corresponding controls (Wb). In the liver of 2-week old-Gal1 KO mice PHB expression also decreased (63±7% n=4 vs wild-type mice 107±21% n=3). In conclusion, PHB modulates HCC cell viability in a cell line-dependent manner and Gal1 regulates PHB expression both in normal and tumor hepatocytes. CONICET PIP647-UBA-20020150100005BA.

Key words: Galectin-1, hepatocellular carcinoma, prohibitin-1

PHARMACOLOGY (PHYTOPHARMACOLOGY) 11

(57) ***Acacia aroma*** **HIDORALCOHOLIC LEAF EXTRACT: A PROPHYLACTIC EFFECTIVE TREATMENT FOR ETHANOL-INDUCED GASTRIC LESIONS IN RATS**

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The pathophysiology of peptic ulcers results from an imbalance between some aggressive and defensive factors. *Acacia aroma* Gill ex Hook&Arn (Tusca) is a native northwestern's Argentinian plant; it has been used traditionally to treat ulcers and wounds. The aim of this study was to evaluate the gastric effect of Tusca leaves hidroalcoholic extract (HAE) on an ulcer experimental model. A qualitative and quantitative phytochemical analysis was carried out on 10% HAE (in ethanol 70º) and its antioxidant activity was also studied. Extract's ability to bind gastric mucosa was determined too. Ethanol induced gastric ulcer model using male Wistar rats was carried out. Experimental design (n=6 animals/group) 1. Control group received 0.9% NaCl, 2. Positive control group received Sucralfate (100mg/Kg, orally) 3. Treated group, received HAE 150mg/kg orally. Mucus content was determined by Alcian Blue method. Macroscopic (Number of ulcers, severity and percentage of ulcerated area) and microscopic (histologic and histochemical) studies of stomachs obtained from each group were carried out. The activity of catalase and the levels of reduced glutathione and malondialdehyde were quantified in gastric homogenates. Tusca HAE showed a significant ($p \leq 0.05$) free radical scavenging activity *in vitro* compared to Quercetin and also had the ability to bind gastric mucosa. The animal group treated with the HAE exhibited a decrease in ulceration percentage, and an increase in mucus content compared with the untreated group. The oxidative stress parameters of Tusca treated group approached to normality. In summary, HAE is effective to protect the gastric mucosa from ethanol induced injury by binding to its surface and strengthening mucus layer. This effect is also linked to extract´s antioxidant property. Future studies will be necessary to determine the active compounds and additional mechanisms involved.

Keywords: *Acacia aroma,* gastroprotective.effect, hidroalcoholic extract.

(622) **GASTROPROTECTIVE ACTIVITY OF *Aristolochia argentina*: ROLE OF PROSTAGLANDINS, NITRIC OXIDE AND INTERACTION WITH COX**

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Aristolochia argentina (Aristolochiaceae) is popularly known as "charrúa", "charruga", "patito", "buche de pavo". The roots of this plant are used in folk medicine for the treatment of ulcers. Previously, we have demonstrated that *A. argentina* prevented the gastric ulcer induced by several necrotizing agents (ethanol, HCl, NaOH). Allantoin was isolated from the roots of *A. argentina*. The aim of the study was to evaluate the mechanism of action and the role of sulfhydryl groups, nitric oxide and prostaglandins in the gastroprotection of *A. argentina*, and the structural basis of interaction of the principle active allantoin with cyclooxigenases (COX_1 and COX_2). The effects of *A. argentina* lyophilized extract (AALE) on ethanol-induced gastric ulcer were studied in Wistar rats. The activity of AALE on ethanol-induced lesions continued even after the inhibition of endogenous sulfhydryls following pretreatment with NEM (N-ethymaleimide). Both indomethacin, a prostaglandins synthesis inhibitor, and L-NNA (Nω-nitro-L-arginine), a nitric oxide synthase inhibitor, antagonized AALE gastroprotective activity ($p<0.001$). Moreover, the docking of allantoin into the crystallographic structure of COX_1 and COX_2 was done using AUTODOCK4. The allantoin docking with COX_1 showed that it binds to the protein active site, a long and deep hydrophobic channel, between C6 and C11. The allantoin docking with COX_2 showed that it binds to the protein active site between C8 and C11. Allantoin occupies a similar region as several NSAIDs in the crystal structure of these complexes with COX_2. The interaction of allantoin with COX_2 active site may indicate that has a role in the antiulcer mechanism. Present findings suggest the possible involvement of prostaglandins and nitric oxide, at least in part, in the antiulcer effect of AALE.

KeyWords: *Aristolochia argentina*; ulcer; cyclooxigenase

(1181) **IN VITRO ACTIVITY OF *Jodina rhombifolia* LEAVES AND BARK AQUEOUS EXTRACTS ON ISOLATED RAT THIN INTESTINE**

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The leaves and bark of *Jodina rhombifolia* (Hook. & Arn.) Reissek (Santalaceae) are usually employed in Argentine folk medicine for a great variety of digestive tract affections, however, few studies have evaluated the effect of this specie on gastrointestinal functions. In our previous *in vivo* studies, these aerial parts have demonstrated a reduction of intestinal transit. Therefore, the aim of this work was to evaluate the activities of bark and leaves extracts, by separate, on *in vitro* intestinal contractility of rats. Effects were evaluated through contractile concentration-response curves (CRC) induced by Carbachol and $CaCl_2$ in isolated rat thin intestine segments that were suspended in tissue baths to measure the antispasmodic activity. Leaves and bark aqueous extracts were obtained by infusion and decoction at 10%w/v, respectively, and subsequently concentrated and lyophilized. The effects of CRC in the presence and absence of extracts were statistically compared by two-way ANOVA [treatment (extract dose); Carbachol or $CaCl_2$ concentration]. In dose-dependent manner, extracts reversibly and non-competitively antagonized the contractions provoked by spasmogenic agents (Carbachol and $CaCl_2$); ANOVA indicated a significative effect of treatment ($p<0.001$), as well as Carbachol ($p<0.001$) and $CaCl_2$ concentration ($p<0.001$). The extracts of leaves and bark inhibited the CRC of Carbachol with IC_{50} = 10.57 ± 1.38 and 16.20 ± 1.21 mg/ml, respectively, and the CRC of $CaCl_2$ with IC_{50} = 8.29 ± 1.54 and 8.58 ± 1.52 mg/ml, respectively. The results obtained suggest that leaves extract is more potent than bark extract as intestinal antispasmodics, while both similarly inhibit CRC of Ca^{+2}. This suggests that the non-competitive interference with Ca^{+2} influx to smooth muscle is the cause of the antispasmodic effect of the two fractions. This study provides novel evidence to validate the folk use in digestive pathologies, due to their antispasmodic properties.

(1247) **ORAL ADMINISTRATION OF *Zuccagnia punctata* EXTRACT HAS BENEFICIAL EFFECTS IN HYPERCHOLESTEROLEMIC RABBITS**

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The consumption of flavonoids has been shown to prevent cardiovascular diseases (CD) including atherosclerosis. In turn, extracts from *Zuccagnia punctata* have demonstrated to be rich in flavonoids. **Purpose:** To study the effects of oral treatment with *Z. punctata* extract (ZpE) on clinical parameters: lipid profile, oxidative stress status and vascular function in a rabbit model of hypercholesterolemia. **Methods:** Rabbits were separated in five groups: fed standard chow untreated (CD) or treated with 2.5 mg GAE/day ZpE (CD-ZpE); fed 1% cholesterol-enriched chow untreated (HD), treated with 2.5 mg GAE/day ZpE (HD-ZpE) or with 2.5 mg Rosuvastatin (HD-R), during 6 weeks. Body weights (BW), mean blood pressure (MAP), heart rate (HR), visceral abdominal fat (VAF) were determined. Total cholesterol (TC), triglycerides (TG), fasting glucose (FG), aspartate amino transferase (AST), alanine amino transferase (ALT), bilirubin, creatinine, thiobarbituric acids reactive substances (TBARS) and glutathione reduced/oxidized index were measured in serum. Abdominal aorta was excised and vascular function was assessed by acetylcholine (Ach) relaxation and contractile response to norepinephrine (NE) and angiotensin II (Ang II). **Results:** ZpE reduced

MAP [(mmHg) HD: 73±2; HD-ZpE: 48±2; HD-R: 50±5, p<0.05)], TC [(mg/dl) HD: 950±117; HD-ZpE: 369±101; HD-R: 816±225; p<0.05)], TG [(mg/dl) HD: 175±25; HD-ZpE: 93±19; HD-R: 79±13; p<0.05)] and TBARS and increased glutathione reduced/oxidized index in HD rabbits. No differences were found in AST, ALT, bilirubin or creatinine. ZpE normalized Ach relaxation [(%) CD: 66±5; HD: 27±4; HD-ZpE: 66±8; HD-R: 43±4; p<0.05); reduced Ang II-response [(mg) HD: 5563±399; HD-ZpE: 4026±594; HD-R: 4358±875, p<0.05)] and reduced NE-affinity [(pEC_{50}) HD: 6.21±0.13; HD-ZpE: 5.84±0.12, p<0.05)]. **Conclusion:** Oral administration of ZpE as natural product in the prevention of CD related with hypercholesterolemia and endothelial dysfunction is very promising.

(1690) **PHARMACOLOGYCAL ACTIVITY OF PEPERINA (*Minthostachys veticillata*) ON GASTROINTESTINAL TRACT**

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Inflammatory bowel disease (IBD) is a chronic disorder characterized by pain, cramps and inflammation of the gastrointestinal tract. To date, no complete response has been achieved with conventional therapies, hence the importance of developing new treatment strategies. Peperina (PP) is a medicinal native plant, with a traditional use as a digestive, antispasmodic and antidiarrheic infusion. Although there is no scientific evaluation of its aqueous extract on gastrointestinal tract. Doses of PP (250 and 500 mg/Kg, vo) and reference drug mesalazine (MZ: 300 mg/kg, vo), were tested in colitis model induced by acetic acid. PP induced a reduction in the colon weight/length ratio, expressed in g/2cm (control: 0.14±0.02 colitis: 0.53±0.06, PP 250 mg/Kg:0.36±0.03, PP 500 mg/Kg:0.36±0.05, MZ: 0.31±0.02) and biomarker of oxidative stress, GSSG/GSH ratio (control: 0.03±0.01, colitis: 0.96±0.11, PP 250 mg/Kg: 0.30±0.05, PP 500 mg/Kg:0.17±0.10, MZ:0.06±0.03). Also, PP reduced the severity of microscopic tissue damage induced by acetic acid and caused a recovery in the number of goblet cells. PP, vo significantly reduced the intestinal motility induced by ricin oil (125 mg/Kg: 70.6±4.4, 250 mg/Kg: 63.7±6.9 and 500 mg/Kg: 58.7±11.2) as reference drug, atropine (0.1 mg/kg i.p: 31.4±5.3). Also, PP significantly reduced the response in a concentration-dependent manner induced by Ach ($1x10^{-9}$-$1x10^{-4}$ M) in isolated jejunum (Emax 1 mg/mL: 79.6%; Emax 3 mg/mL: 54.9% and pD2 1.0 mg/mL: 5.4, pD2 3.0mg/mL:4.4), suggesting a spasmolytic effect of the extract. Furthermore, the extract non-competitively inhibited the response-concentration induced by $CaCl_2$ (0.1 mg/mL: 85.3%, 0.3 mg/mL: 49.6%, 1 mg/mL: 27.1 %, 3 mg/mL: 5.4%) and inhibited both low K+ (25mM) and high K+ (80 mM)-induced contractions, suggesting antagonistic effect on calcium channels. Therefore, this study could demonstrate that PP possesses beneficial properties in a preclinical model related to gastrointestinal diseases.

Keywords: experimental colitis, *Minthostachys verticillata*, isolated jejunum

(1605) **YACON FLOUR IMPROVES NON-ALCOHOLIC FATTY LIVER THROUGH TGF-**B **PATHWAY**

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TGF-β pathway regulates several cellular process including regulation of matrix proteins expression such as collagens I and III, and fibronectin. Non-alcoholic fatty liver disease (NAFLD) covers a large spectrum of histological changes, including steatosis, hepatocyte injury (ballooning), inflammatory infiltration, and changes in pericellular matrix proteins. In the present study we investigated the participation of the TGF-β/Smad signaling pathway in the effects of S. sonchifolius (yacón) on the NAFLD in a diet induced model. Adult male Wistar rats maintained with a rat standard chow were randomly separated into a control group (water ad libitum) and a F-group (fructose 10% w/v, ad libitum, F). After 20 weeks F rats, were randomly assigned according to the treatment: F or F+Y (340mg yacon flour/kg bw) for 12 weeks. Dietary supplementation with yacon roots showed a decrease in serum and hepatic triglycerides, cholesterol, VLDL, ALT and AST activities. Yacon also decreased inflammatory cell infiltration in F-livers, improved oxidative state and lipid peroxidation. Yacon supplement significantly down-regulated TGF-β/Smad signaling, reducing the expression of the TGF-β1, TGF-R1, TGF-R2 mRNAs, as well as the TGF-β1, p-smad protein expression. The collagens type I, collagen type III, and fibronectin mRNA expression were also down-regulated in the liver of F+Ygroup. On the other hands F+Y group showed less collagens I and III depositions in liver. These results suggested that yacon roots are able to improve NAFLD modulating TGF-β/Smad signaling pathway.

Key words: TGF-β pathway, yacon, non-alcoholic fatty liver disease, steatosis, extracellular matrix.

(97) **LIGARIA CUNEIFOLIA (L.*Cuneifolia*) PROTOANTHOCYANIDINE FRACTION PRODUCES PLASMA DIMINUTION OF CHOLESTEROL AND TRIGLYCERIDES IMPROVEMENT MORPHOLOGYCAL INDEX IN RATS FED WITH HIGH FAT DIET (HFD)**

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We were demonstrated that *Ligaria cuneifolia (Lc)* crude extract increased blood viscosity and decreased plasma cholesterol in rats. In the present study, we analyzed the *Lc* proanthocyanidin enriched fraction (P*Lc)* effect on cholesterol (Cho), Triglycerides (TG) and blood fluidity in adult male Wistar rats (aged 70 days, n=12) fed with standard chow diet added with 40% bovine meat juice during 28 days (HFD). The rats were administered i.p. each 24hr during 10 days with either physiological solution (controls C, n=6) or P*Lc* 3mg /100g body weight (treated T; n=6), in day 11 they were anesthesized i.p. with Ketamine/Xylazine (100mg/kg/3mg/kg) to obtain blood samples by cardiac puncture. Plasma assays: Cho (enzymatic method with cholesterol oxidase esterase), TG, HDL-Cho, and LDL-Cho. Blood assays: blood viscosity (BV) and plasma viscosity (PV) by rotational viscosimeter. Standardized relative BV (SBV) to a 45% hematocrit [$(BV/PV)^{45/Hto}$]; rigidity index (RI) by filtration method, cell shape was assessed by microscopy and the corresponding morphologycal index (MI) was calculated. Results: (mean ± SD). PlasmaCho(mg%): C: 168.00±6.63,T:100.11±4.91*; ChoHDL: C:32.20±1.46,T: 28.00±2.39(n.s); ChoLDL: C: 24.00±0.63,T:17.78±0.62*; TG: C: 191.80 ± 21.45,T:133.00±9.68*; [(BV/PV) 45/Hto]:C:5.39±0.10,T: 5.20±0.37(n.s.); RI:C:5.44±0.33;T: 5.54±0,32(n.s.); Mean Corpuscular Volume (μm3): C: 64.84±6.76; T: 68.25±3.64 (n.s.); MI: C:-2.4 ± 0.20;T:-1.74± 0.08* (*p<0.05 vs. C, Student's t Test for unpaired data). P*Lc* treatment shows a lipid-lowering effect without alteration of blood viscosity in rats fed with a HFD. We suggest that the diminution of plasma Cho could be caused by an increase of the biliary excretion rate of bile salts (products of hepatic metabolism of Cho) which was obtained in our studies in the treated rats. The decrease in MI indicates an improvement of the erythrocyte form which favours the passage of them in the microcirculation.

Keywords: *Ligaria cuneifolia*, Proanthocyanidin, Morphologycal Index, cholesterol, high fat diet.

(577) **PHENOLIC COMPOSITION AND IN VITRO CYTOTOXIC ACTIVITIES OF TOTAL EXTRACTS AND FRACTIONS FROM ANDEAN POTATOES**

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Andean potatoes (*Solanum tuberosum* L. ssp. *andigena*) are a good source of dietary antioxidant polyphenols. We have previously demonstrated that polyphenol extracts from Andean potato tubers

exerted a dose-dependent cytotoxic effect in human neuroblastoma SH-SY5Y cells, being skin extracts more potent than flesh ones. This study investigated the phenolic composition and the *in vitro* cytotoxic activities of total extracts and fractions of skin and flesh tubers of three Andean potato varieties (Santa María, Waicha and Moradita). Potato total extracts were subjected to liquid-liquid fractionation using the solvent ethyl acetate in organic and aqueous fractions. Both fractions were analyzed by DAD-HPLC and results corroborated the expected composition of each fraction. Organic fractions were rich in phenolic acids (chlorogenic acid the most abundant one) and aqueous fractions contained mainly anthocyanins (except in non-pigmented Waicha and Moradita fleshes). The major anthocyanidins present in red tissues (Santa María skin and flesh, and Waicha skin) were pelargonidin and peonidin, while in Moradita purple skin petunidin and malvidin were the major ones. Fractions containing phenolic acids did not result cytotoxic against SH-SY5Y cells, and indeed some of them increase cellular metabolism compared to controls. Aqueous fractions were cytotoxic and even more potent than their respective total extracts. Treatment with a combination of both fractions showed similar cytotoxic response to the corresponding extract. Since some of the bioactive aqueous fractions lack pigmentation (Waicha and Moradita fleshes) it is tempting to speculate that anthocyanins are not direct responsible and that other compounds, unidentified yet, play a crucial role in inducing cell death. Findings indicate that the activity of Andean potato extracts is a combination of various compounds and contribute to the revalorization of potato as a functional food.

Keywords: cytotoxicity; phenolic compound; *Solanum tuberosum* L.

(1194) **CANNABIS-BASED OIL: EXTRACTION METHODS AND QUANTIFICATION OF ACTIVE PRINCIPLES**

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In the last few years, the use of cannabis-based oil for therapeutical purposes has significantly increased. However, Cannabis is an exception to conventional design, development and testing which generally applies to medicines. Definitely, it was promoted by patients, instead of scientific researchers or physicians. Recently, diverse studies have been demonstrated the pharmacological properties of the different active principles of cannabis for their application in several pathologies. Tetrahydrocannabinolic (THC) and cannabidiolic (CBD) are the two more studied cannabinoids and are found in trichomes of cannabis inflorescence. For therapeutic uses, it is often consumed as oil extracts. Nevertheless, their preparation procedure has not been standardized and the relative composition of active principles is poorly known. The aim of this study was to standardize the method for ethanolic extraction of THC and CBD and measure their relative composition in different varieties of *Cannabis sativa.* High performance liquid chromatography (HPLC) was employed to perform the determinations presented in this study. We analyzed the effect of time incubation on cannabinoids yield. Samples were extracted by homogenization with blender for 2 minutes and incubated for 0, 1, 2, 6, 12, 24 h and then daily along a week. Aliquots from each time did not show differences in THC and CBD yield. These results indicate that it is not necessary to prolong the maceration time to increase the cannabinoids yield when this homogenization method is applied. Relative content of THC and CBD was measured in four different plant varieties. In all cases, THC level was higher than CBD, varying from 3 to 28 times. In summary, the data obtained here represent fundamental information both for the physician, and for an eventual clinical trial, which must take into account all the characteristics of the product under examination.

(260) **ANTI-SNAKE VENOM ACTIVITIES OF *Casearia sylvestris* EXTRACTS**

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Casearia sylvestris Sw. (*burro-kaa, guaçatonga*), has ethnopharmacological antecedents as ophidian antivenom in several regions, inhibiting the hemorrhagic, edematogenic, proteolytic and coagulant activities of *Bothrops* sp venom. In this study, we evaluated the activity of native plants extraxts against *B. diporus* venom in order to verify the ethnopharmacological knowledge from the northeast region of Argentina.

Aerial parts of *C. sylvestris* were collected in Corrientes at two different vegetative stages: autumn (I) and spring (II). The material was dried and powdered in order to prepare aqueous, ethanolic and hexanic extracts. The screening of the extracts antisnake venom activity was performed by SDS-PAGE electrophoresis. The coagulant activity neutralization was evaluated by recalcification time of citrated plasma with a Col1 coagulometer. Inhibition of the indirect hemolytic activity was evaluated through on agarose-blood-phosphatidylcoline gel plates and the inhibition of the proteolytic activity by SDS-PAGE with casein. Phytochemical analysis was performed in order to identify chemical groups of compounds.

The results from SDS-PAGE showed the ethanol extract as the most active against *B. diporus* venom, independently of the vegetative state of the plant. The ethanol extract inhibited 54% (II) and 60% (I) of coagulant activity (venom-extract ratio, 1:30); 50% (II) and 44% (I) of indirect hemolytic activity (1:40) and 100% (I) and (II) of proteolytic activity (1:30) of venom. The demonstrated phenols, anthraquinones, alkaloids, steroids and tannins were found as components of the active extract.

In conclusion, we were able to demonstrate that the ethanolic extracts of *C. sylvestris* leaves possess *in vitro* antisnake activity against *B. diporus* venom supporting its ethnopharmacological antecedents. Further research is required to identify the single components responsible for this activity and, so, the best way for their administration in ophidian accidents.

Key words: alexiteric activity; ethnobotany; *in vitro* activity; *Casearia sylvestris*

INFECTOLOGY 13

(316) **HP_{24}, A NOVEL PEROXISOME PROLIFERATOR ACTIVATED RECEPTOR GAMMA AGONIST: MOLECULAR MODELING AND SIGNALING PATHWAYS INVOLVED IN *T. CRUZI*-INFECTED MACROPHAGES**

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T. cruzi infection induces an intense inflammatory response in diverse host tissues. Upon parasite uptake, macrophages, which are involved in the clearance of infection, increase inflammatory mediators, leading to parasite killing. However, exacerbation of the inflammatory response may lead to tissue damage. PPARγ is a ligand-dependent nuclear transcription factor that exerts important anti-inflammatory effects.

In this work we show for the first time, the mechanism of action of a newly synthesized PPARγ ligand, pyridine carboxylic acid derivative (HP_{24}) on macrophages (MΦ) from *T. cruzi*-infected mice. In order to elucidate how HP_{24} interacts with PPARγ, virtual docking analysis was performed. Then, we evaluated the signaling pathways involved in the action of HP_{24}, using NO release as a functional marker. T0070907 (T007), a specific PPARγ antagonist, partially restored NO release by HP_{24}-treated MΦ ($P<0.05$, HP_{24}-treated MΦ vs. T007- HP_{24}-treated MΦ) (Griess reaction), revealing the participation of the PPARγ-dependent as well as, PPARγ-independent sig-

naling pathways in the effects of HP_{24}. Furthermore, we found that HP_{24} exerts its effect through the PI3K/Akt/mTOR. We observed an increase in Akt and p70 phosphorylation, as evaluated by western blot (Wb) ($P<0.05$). Moreover, we observed that HP_{24} treatment inhibits IKK phosphorylation and increase IKBα cytosolic expression, indicating that the NF-κB pathway cascade is also implicated in the anti-inflammatory action of HP_{24} (Wb, $P<0.05$). To evaluate the activity of HP_{24} against *T. cruzi,* bloodstream trypomastigotes were treated *in vitro*. Viable motile parasites were then counted in a Neubauer chamber. We did not find significant viability differences after treatment ($P>0.05$).

The study of new PPARγ agonists, opens perspectives for them, as coadjuvants of the etiological treatment of infectious diseases, that may contribute to a better outcome, particularly when overt inflammation contributes to severe pathology.

Keywords: *T. cruzi*, New PPARγ ligand, Macrophages, NF-κB pathway, Inflammation

(397) **URINARY TRACT INFECTIONS IN ELDERLY PATIENTS FROM A GENERAL HOSPITAL: ANTIMICROBIAL RESISTANCE IN *Escherichia coli***

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Abstract: Urinary tract infection (UTI) is a common cause of morbidity and mortality in older adults. Antimicrobial resistance in UTI produced by *Escherichia coli* in elderly patients attending to a General Hospital was investigated. During the period July 2011-July 2015, patients over 70 years-old, without urinary catheters and no antimicrobial therapy seven days before sampling and with urinary infection symptoms were included. N: 3,920 urine samples were analyzed. Phenotypic characterization was carried out with conventional tests. *In vitro* qualitative (disk diffusion method) and quantitative (Minimum Inhibitory Concentration, broth dilution) antimicrobial resistance according to Clinical and Laboratory Standards Institutes`s recommendations was performed. Antimicrobials assayed: ampicillin, amoxicillin-clavulanate acid, cephalotin, cefuroxime, cefoxitin, cefotaxime, ceftazidime, cefepime, imipenem, ertapenem, gentamicin, nalidixic acid, ciprofloxacin, trimethoprim-sulfamethoxazole (TMS) and nitrofurantoin. Included *E. coli* isolates expressed resistance to ampicillin (80.5%), nalidixic acid (61.7%), ciprofloxacin (42.8%), TMS (37.6%), amoxicillin-clavulanate (28.6%), cephalotin (21.6%), cefuroxime (20.7%), gentamicin (12.1%), cefotaxime (9.7%), ceftazidime (9.7%), cefepime (8.4%), cefoxitin (3.1%) and nitrofurantoin (2.3%). Resistance to carbapenems was not observed. Production of ESBL was detected (7.6%) in community acquired (96%) and health-care associated (4%) isolates. A high prevalence of resistance to beta-lactams and to other antimicrobials was observed in *E. coli* isolates from patients over 70 years-old with UTI. Detection of antimicrobial multi-resistant isolates highlights the need of a continuous surveillance of antimicrobial resistance in adult patients with UTI produced by *E. coli*.

Keywords: Urinary tract, infections, *E. coli*, antimicrobial resistance, elderly

(506) **INHIBITORY EFFECT OF LACTOBACILLI ON *Candida albicans* VAGINAL INFECTION IN AN EXPERIMENTAL MOUSE MODEL**

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Vulvovaginal candidiasis (VVC) is one of the most frequent infection affecting the life quality of women worldwide, highly associated with *Candida albicans* (C.a.). Healthy vaginal microbiota is dominated by *Lactobacillus*, which form a defense line against pathogens. Beneficial vaginal lactobacilli were previously selected and evaluated in our laboratory. In this work, the effect of different intravaginal (i.va.) doses of *Lactobacillus reuteri* CRL1324 (CRL1324) or *L. rhamnosus* CRL1332 (CRL1332) against vaginal C.a. challenge in a murine experimental model was evaluated. Female BALB/c mice in pseudo-estrous were randomly assigned to five groups: a) Control: C.a. group [7 i.va. saline inoculations, later i.va. C.a. (10^6 CFU) challenge, and again 7 saline inoculations]; b) Preventive: CRL1324 or CRL1332 (7 doses) + C.a. group [7 i.va. CRL1324 or CRL 1332 (10^8 CFU) inoculations, C.a. challenge, and later 7 saline inoculations]; and c) Preventive-Therapeutic: CRL1324 or CRL1332 (7 doses) + C.a. + CRL1324 or CRL1332 (7 doses) group (7 i.va. CRL1324 or CRL 1332 inoculations, C.a. challenge, and 7 i.va. CRL1324 or CRL 1332 inoculations). On days 1, 5 and 7 post-C.a. challenge the following assays were applied: a) number of cultivable C.a., CRL1324 and CRL1332 from vaginal washing, and b) vaginal cytology and histology. Data were analyzed using ANOVA (general linear model). Fourteen doses of both CRL1324 and CRL 1332 were able to significantly reduce ($P<0.05$) the C.a. numbers, while seven doses of the two strains showed no inhibitory effect ($P>0.05$). A significant increase ($P<0.05$) in vaginal CRL1324 and CRL 1332 number was only evidenced after 14 doses. The two CRL1324 treatments reduced the C.a. induced leukocyte influx, while only the preventive-therapeutic CRL1332 treatment decreased it, indicating a strain-dependent behavior. The results indicate that these lactobacilli are promising candidates to be included in probiotic products for protection against VVC in women.

(544) **BEHAVIOR OF BENEFICIAL VAGINAL LACTIC ACID BACTERIA (BVLAB) TO THERMAL CONDITIONS FOR TECHNOLOGICAL APPLICATIONS**

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Lactic Acid Bacteria constitute the main population of healthy vaginal microbiome, being responsible of the maintenance of urogenital tract physiological balance. Our research group have characterized BVLAB for the design of pharmaceuticals products, in which they need to maintain their viability and functional properties during industrial process and shelf life. Drying technological processes are applied, using high or drastic temperature changes that differ from the one required for their optimal growth, affecting the beneficial properties that support the selection of the probiotic strains. The aim of this work was to evaluate the BVALB survival to different high-temperature conditions. The strains studied were *Lactobacillus reuteri* (3 strains), *L. gasseri* (12), *L. rhamnosus* (4), *L. salivarius* (2), *L. johnsonii* (1), *L. paracasei* (2) and *L. delbrueckii* (6). BVLAB cells from the late-exponential growth phase were washed twice with buffer (PBS pH=6.5) and later inoculated (2%) in MRS broth, incubated at different temperatures (37ºC, 42ºC, 50ºC and 55ºC) for 24 hours. The growth was followed by optical density (OD_{560nm}) at 3, 6, 9, 12 and 24 h to calculate growth´s parameters. CFU/ml was determined by plate culture method in some strains. Anova test was used for statistical evaluation. The results indicate that most of the strains showed similar growth patterns at 37 ºC and 42ºC with 95% significance ($p<0.05$). The lactobacilli didn´t grow when incubated for 9 h at 50 ºC or 55 ºC. However, when transferred at 37ºC, they were able to grow showing a slower kinetic. The behavior of the BVLAB evaluated is strain-dependent, not related to the metabolic group, supporting further studies directed to induce thermal resistance in the selected strains with better features. In this way, they could resist the extreme thermal conditions applied throughout the technological process applied, to get viable bacteria in pharmabiotic formulations with long shelf life.

(974) **ACTIVATION OF iNKT CELLS PREVENTS Salmonella-ENTEROCOLITIS AND Salmonella-INDUCED REACTIVE ARTRHITIS BY DOWNREGULATING IL-17-PRODUCING γδT CELLS**

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Background: Reactive arthritis (ReA) is a sterile joint inflamma-

tion as a sequel to *Salmonella* gut infection. We have previously demonstrated that the severity of joint lesions is directly related with the intestinal levels of IL- 17 generated during *S.* Enteritidis infection. γδT lymphocytes are a possible source of IL-17. It has been suggested that γδT responses are modulated by iNKT cells, therefore, here we analyze the involvement of γδT and iNKT cells on *Salmonella*-induced ReA.

Methods: Adult female BALB/c mice received 3-4 x 10^3 colony forming units of *S.* Enteritidis by the gastrointestinal route. Studies were performed 1 and 5 days after infection. Mesenteric lymph node γδT population was achieved by flow cytometry after enterocolitis onset. iNKT cell activation was studied using alpha-galactosylceramide (a- GalCer) and γδT activity was blocked with anti-γδT monoclonal antibody. Then, histological tissue evaluation and mesenteric IL-17 expression by qPCR were assessed.

Results: We found that during *S.* Enteritidis infection the total number of γδT cells is increased in mesenteric lymph nodes. Infected mice treated with a-GalCer showed diminished intestinal and joint lesions concomitantly with a significant decrease in mesenteric IL-17 expression. Animals suffering from enterocolitis treated with anti- γδT antibody presented the same phenomenon. In addition, mesenteric γδT population was decreased in number in a-GalCer treated animals.

Conclusions: Our results indicate that activation of iNKT cells renders protection against *Salmonella* ReA. This beneficial effect of a-GalCer treatment would be related to the decrease in IL-17 produced mainly by γδT cells.

Palabras claves: *Salmonella*, enterocolitis, Reactive Artrhtitis, iNKT cells, γδT cells.

(1406) **INHIBITION OF TRANSCELLULAR WATER ABSORPTION IN HUMAN RENAL MICROVASCULAR ENDOTHELIAL CELLS IN RESPONSE TO SHIGA TOXIN TYPE 2 (STX2).**

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Post-diarrhea hemolytic uremic syndrome (HUS) is the most common cause of acute renal failure in children younger than 5 years old in Argentina. Clinical and histological renal damage has been strongly associated to Shiga toxin type 2 (Stx2) mainly produced by *E. coli* O157:H7. Previously, we have demonstrated an inhibition of the net water transport (Jw) across primary culture of human renal microvascular endothelial cells (HGEC) in response to Stx2 (Alvarez *et al* Plos One 2016). Jw is the result of water transfer across the paracellular and transcellular pathways induced by the hydrostatic and osmotic pressure gradients, respectively. Hence, the purpose of this study was to evaluate the hydrostatic (P_{hidr}) and the osmotic (P_{osm}) permeability and also FITC-Dextran transport in HGEC monolayers to clarify the mechanisms by which Stx2 inhibits the net absorptive Jw. Cells were cultured on Millicells supports (1.13 cm^2) until confluence (TEER: 201.3 ± 4.1 Ω.cm^2, n = 3). For P_{hidr} and P_{osm} assays, Millicells were placed in an Ussing chamber and the Jw, automatically registered across HGEC monolayers, was proportionally modified by applying hydrostatic (3 to 10 cm H_2O) or osmotic (10-25 mOsm PEG 8000) pressure gradients. The P_{hidr} (cm/seg) and P_{osm} (10^{-3} cm/seg) before and after 1 h of treatment with Stx2 (10 ng/ml) were estimated from the slope of the corresponding regression lines. The P_{hidr} was similar before and after Stx2 treatment (3.0 ± 1.5 *vs.* 2.5 ± 0.8, n=3, ns). This result was in agreement with FITC-Dextran transport across HGEC. On the contrary, the P_{osm} showed a significantly decrease in response to Stx2 (4.6 ± 0.3 *vs.* 3.7 ± 0.5, n=3, $p<0.05$). These results demonstrate that Stx2 inhibits the Jw by affecting the transcellular water transfer. Therefore, we can speculate that aquaporins, involved in the water transport in endothelial cells, can be altered by Stx2 and this fact can contribute to the development of the early events of HUS pathogenesis.

Key words: hemolytic uremic syndrome, Shiga toxin type 2, hydrostatic and osmotic permeability.

(1448) **EFFECTS OF A NON-TOXIN PRODUCING *E. coli* O157:H7 AND Stx2 ON A HUMAN MICROVASCULAR ENDOTHELIAL CELL LINE (HMEC-1)**

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Shiga toxin (Stx)-producing *Escherichia coli* (STEC) strains are responsible of bloody diarrhea (hemorrhagic colitis) and hemolytic uremic syndrome (HUS). Hemorrhagic colitis (HC) is a major complication of HUS that sometimes appears prior to the detection of characteristic HUS findings (Rahman et al, 2012). The patients with HC showed severe renal and neurologic disease. It is believed that HC may be the result of compromise of local blood vessels and that could be the cause of the most severe HUS complications. *E. coli* O157:H7 is, by far, the most prevalent serotype associated with HUS and Stx2 is the major virulence factor associated for the more severe symptoms of the infection. Our aim was to study the effects of a non-toxin producing *E. coli* O157:H7 and Stx2 on HMEC-1 (an immortalized human microvascular endothelial cell line) in order to better understand the means by which STEC induce HC. We examined cell viability after 4 h of incubation with purified Stx2, a mutant of *E. coli* O157:H7 lacking *stx2* gene (O157:H7Δstx2), *E. coli* HB101 (an *E. coli* strain lacking adhesive capabilities), and filtered O157:H7Δstx2 supernatant (SN), which was used to assess basal cytotoxicity. Cells were grown in 96-well culture plate and viability was measured by neutral red uptake after 24 h under growth-arrested condition. We have also evaluated bacterial adhesion by colony-forming unit (CFU/ml) assay and Giemsa-staining direct observation. We observed cytotoxic effects induced by Stx2 (CD_{50}:0.01 ug/ml) and by O157:H7Δstx2 (CD_{50}: $2x10^8$ CFU/ml). Moreover, significant bacterial adhesion was found with O157:H7Δstx2 compared with the other strain ($P<0.05$), These results suggest that endothelial infection by STEC could play a central role in the establishment of HC leading to Stx2 systemic damage and the most severe HUS cases.

Keywords: Hemorrhagic colitis, Shiga toxin, STEC, HMEC-1, Human microvascular endothelial cells.

(1559) **A DIGUANILATE CYCLASE REGULATES MOTILITY AND BIOFILM FORMATION IN BORDETELLA BRONCHISEPTICA MEDIATED BY A GGDEF-EAL PROTEIN.**

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Bordetella bronchiseptica is a bacterium that causes respiratory infections in a variety of mammalian hosts. We have already described c-di-GMP (cdG) role in motility and biofilm formation. We previously showed that over expressing BdcA (*Bordetella diguanilate cyclase A*) triggers high cdG levels and significantly impaired movement and enhanced biofilm formation.

In this work we determinate motility and flagelline expression of strains with high cdG leves due to BdcA overexpression. We detected that BdcA not only reduced bacteria motility but also abolished flagellin expression. This inhibition was dependent on diguanilate cyclase (DGC) activity because an inactive version of BdcA (GGDEFxGAAF) did not inhibit motility, neither enhances biofilm formation.

CdG binds to different proteins to trigger specific responses. Based on known cdG binding proteins, we propose proteins that may be involved in BdcA inhibition: LapD, PilZ and BB2109. Individual deletion mutants of each gene were obtained in *B. bronchiseptica* and BdcA was overexpressed on them. All strains were evaluated in motility and biofilm experiments. Deletion of *lapD* or *pilZ* showed no effects. However, BdcA mediated motility inhibition and enhanced

biofilm in *B. bronchiseptica* were abolished only when BB2109 gene was deleted.

We also quantifed intracellular cdG levels in ΔBB2109 strain overexpresing BdcA (*Bb*ΔBB2109p*bdcA*). CdG levels were significantly lower in *Bb*ΔBB2109p*bdcA* than in *Bbp**bdcA* (0.75±0.6 vs 10±2 nM cdG/mg dry weight respectively) suggesting that BdcA DGC activity is dependent on BB2109 presence.

In the present work we described that BdcA DGC activity is necessary for biofilm and motility regulation. Moreover, a GGDEF-EAL protein, BB2109, is necessary for BdcA DGC role in biofilm and motility regulation in *B. bronchiseptica*.

We present a model where both proteins and cdG are involved in motility and biofilm formation probably by interacting between them.

Key Words: *Bordetella;* c-di-GMP; Biofilm; Motility

(1573) **INFLUENCE OF COMMUNICATION BETWEEN HUMAN RENAL ENDOTHELIAL AND EPITHELIAL CELLS ON PRO-INFLAMMATORY MEDIATORS INDUCED BY *Escherichia coli* SHIGA TYPE 2 AND SUBTILASE TOXINS.**

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Hemolytic Uremic Syndrome associated to Shiga toxin (Stx)-producing *E. coli* infection is the most common cause of acute renal failure (ARF) in children in Argentina. Stx type 2 (Stx2) damages human renal microvascular endothelial cells (HGEC) and tubular epithelial cells (HK-2) and induces a broad endothelial inflammatory response that injures the tubules. Subtilase cytotoxin (SubAB) may also contribute to this pathology. We developed HGEC/HK-2 coculture as a model of renal proximal tubule to clarify the involvement of the endothelial-epithelial cross talk in kidney toxins damages. In this work, we evaluated the response of selected pro-inflammatory mediators when HGEC, HK-2 and HGEC/HK-2 were incubated with Stx2 (0.01ng/ml), SubAB (1ng/ml) or Stx2+SubAB [(0.01+1) ng/ml] for 24 h. Culture supernatants were collected and IL-6, IL-8 and TNF-α were quantified by ELISA (Stx2, SubAB, Stx2+SubAB vs Ctrl, n=4). Controls showed a differential release of IL-6, IL-8 and TNF-α between monocultures and cocultures, suggesting that soluble mediator's secretion depends on the cell type and culture conditions ($p<0.05$). In HGEC/HK-2 cocultures; Stx2, SubAB and Stx2+SubAB caused a significant increase on IL-6 (4592 ± 258, 4254± 325, 4395± 317 vs 3448 ± 179 pg/ml, $p<0.05$) and IL-8 release (1730 ± 161, 1746 ± 110, 1830 ± 104 vs 1272 ± 85 pg/ml, $p<0.05$) but they did not modulate TNF-α. Stx2 increased IL-6 (5505 ± 170 vs 4572 ± 101 pg/ml, $p<0.05$), IL-8 (5188 ± 234 vs 3876 ± 99 pg/ml, $p<0.05$) and TNF-α (141 ± 30 vs 45 ± 3 pg/ml, $p<0.05$) on HGEC monocultures. In addition, Stx2+SubAB increased TNF-α levels (85 ± 13 vs 45 ± 3, $p<0.05$). Toxins did not have effect on the release of IL-6 and TNF-α in HK-2, but SubAB and Stx2+SubAB decreased IL-8 levels. These results show that the endothelium-epithelium interaction modulates the inflammatory responses caused by Stx2 and SubAB on renal cells and may contribute to the early events of ARF.

Key words: Hemolytic Uremic Syndrome, Shiga toxin type 2, Subtilase toxin, Cytokines, Chemokines.

(1829) **EVALUATION OF A LATERAL FLOW IMMUNOASSAY DEVICE FOR HUMAN LEPTOSPIROSIS DIAGNOSIS IN ARGENTINE: COMPARATIVE ANALYSIS WITH THE CURRENTLY APPLIED TECHNIQUES.**

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Beyond the key role of the specific laboratory in leptospirosis diagnosis, methods available to date are complex, time consuming and requires some equipment and training staff, causing delay in the leptospirosis diagnosis and affects directly the health of the patients; in consequence new rapid techniques are claimed. The aim of the present work was to evaluate an LFI device for detection of human leptospirosis (Lepto-IFL) developed by our research group. A double-blinded assay was carried out to evaluate the test against a reduced panel of serum samples (n= 70) belonging to patients with suspected leptospirosis. The samples had been previously classified into confirmed cases or discarded cases according to the national diagnostic algorithm, in the National Reference Laboratory of Leptospirosis "INER Dr. Emilio Coni". Samples were grouped depending on days post onset (dpo): 0-3 dpo samples, 4-6 dpo samples, 7-10 dpo samples and >10 dpo samples. Performance of Lepto-IFL device was compared to that of MAT (Microaglutination, gold standar test) and IgM-ELISA (screening test). Lepto-IFL showed best results from 4 dpo onward. Sensitivity (*Se*) was 71.4% for 4-6 dpo samples, raising 100% from day 7. Specificity (*Sp*) varied slightly between 90-100% along the different dpo ranges. Determination of *Kappa* coefficient (*k*) showed that Lepto-IFL best correlated with referential diagnosis, giving a $k= 0.63$ for 4-6 dpo samples and $k=1$ for 7-10 and >10 dpo samples. Lepto-IFL device yielded the best performance compare with the currently used methods for leptospirosis detection. Although it is necessary to continue working on some features of the test, it has been shown to be a promising alternative to incorporate to leptospirosis diagnosis and may further improve the quality of diagnosis.

Keywords: Leptospirosis, diagnosis, Lateral flow immunoassay.

INFECTOLOGY 14

(253) **LACTIC ACID BATERIA: DESIGN AND EVALUATION OF A PRODUCT FOR THE PREVENTION OF BOVINE MASTITIS**

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Abstract: In order to develop a natural product for the prevention of bovine mastitis, the effect of excipients and phytocompounds on the viability of two potential probiotic Lactic Acid Bacteria (LAB) and after inoculation in mammary glands was evaluated. The viability of LAB against several compounds (Asian *Centella*, *Echinacea, Aloe vera*, Vitamins C and D, Fructose and Sepigel) was evaluated by minimal inhibitory concentration and death curves. No inhibition was observed for phytocompounds at any of the tested concentrations and no significant differences ($p<0.05$) were found between death curves at different concentrations and the control. Similar results were observed for Vitamin D and Sepigel. Vitamin C did not inhibit the growth of LAB at 12.5mg/ml and delay significantly ($p>0.05$) the growth of both LAB at higher concentrations (25, 50 and 100mg/ml). Fructose delays the growth of LAB at 300mg/ml and cause an inhibitory effect at 600mg/ml. The effect of selected compounds and concentrations were determined in an experimental inoculation trial in cows by Somatic Cell Count (SCC) and clinical signs and symptoms determinations. For this, healthy cows were inoculated intramammarily with *Echinacea* (6.25mg/ml), Fructose (10mg/ml), Sepigel (1%), LAB ($3x10^6$ cfu/ml), Diluent (*Echinacea*+fructose+Sepigel) or Product (LAB + diluent). None of the inoculated quarters showed adverse reactions and no anomalies were detected either at the local or systemic level. The quarters inoculated with *Equinacea*, LAB and product, showed a statistically significant increase ($p<0.05$) in the SCC respect to the control. In quarters inoculated with fructose, Sepigel and diluent, although an increase in the SCC was also observed, this was not significant ($p>0.05$) respect to the control. The results allow concluding that it was possible to obtain a complete probiotic formulation for the prevention of bovine mastitis and thus helps to reduce the use of antibiotics as a prophylactic measure.

Keywords: Lactic Acid Bacteria, Bovine Mastitis, Probiotics

(597) **GENOMIC CHARACTERIZATION OF A COLLECTION OF ST5 *Staphylococcus aureus* ISOLATES FROM PATIENTS WITH OSTEOMYELITIS.**

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Staphylococcus aureus is a highly prevalent human pathogen that emerges as one of the most prevalent causative agents of osteomyelitis. The *S. aureus* genome carries a vast array of genes coding for virulence and evasion factors. The aim of this study was the characterization of a collection of ST5 *S. aureus* isolates from patients with osteomyelitis through whole genome analysis to detect association between genes and several defined variables. *S. aureus* ST5 isolates from 19 patients with chronic osteomyelitis from 5 different hospitals in Argentina were sequenced with Illumina MiSeq. Reads were assembled d*e novo* with SPAdes. The genomes were annotated with Prokka and pangenome analysis was performed using Roary. Abricate was used to screen virulence factors and antimicrobial resistance genes. Scoary was used to perform the pangenome wide association study. The *S. aureus* genome mean of size was 2,86 Mbp, with an N50 of 1,24 Mbp, with an average of 12 contigs per isolate and a 32,83% GC content. An average of 2.628 CDS per genome and 61 tRNA were predicted. A total of 3.443 different genes were detected and 2.307 of these genes were shared by all isolates (core genome). Every isolate contained from 65 to 69 characterized virulence genes. Eight of 19 isolates carried genes coding for resistance to aminoglycosides, 18/19 to fluoroquinolones, 9/19 to erythromycin, 1/19 to chloramphenicol and 12/19 were MRSA (*mec*A gene). The pangenome wide association study revealed a beta-lactamase associated only one Hospital. No gene was associated with the other 4 hospitals investigated. No gene was associated with chronic or acute infection. In conclusion, the sequence analysis of closely related *S. aureus* isolates revealed a conserved core genome. We found a beta-lactamase gene associated with isolates from only one Hospital. The present methodology is a suitable tool to investigate related isolates and identify genes involved in virulence or antibiotic resistance.

Keywords: *Staphylococcus aureus,* osteomyelitis, genome, Hospital

(854) **THE TRANSCRIPTIONAL REGULATOR FasR IS REQUIRED FOR THE SURVIVAL AND REPLICATION OF *Mycobacterium tuberculosis*.**

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Mycobacterium tuberculosis (Mtb), the causative agent of tuberculosis, has the capacity of surviving within infected host and persists for long time. This is due to its distinctive cell wall, which plays a crucial role in the pathogenesis, virulence and survival in macrophages, its intracellular niche. The main component of the cell envelope is the mycolic acids (MA) that together with peptidoglycan form the cell wall skeleton and contributes to the reduced cell wall permeability and acid fastness characteristic of Mtb. The biosynthesis of MA depends on the action of two different types of fatty acid synthases (FAS): FAS-I and FAS-II. The FAS-II complex elongates fatty acyl-CoAs previously synthesized by FAS-I to generate very long precursors of MA. Recently it has been described FasR, a transcriptional activator from Mtb that binds specifically to fas promoter to regulate its expression and the biosynthesis of MA precursors. Evidence shows that FasR is important for bacterial growth. Here, we investigate the role of FasR in the Mtb infection. To address the effect of FasR *in vivo*, we infected BalbC mice with Mtb wild-type (WT), the mutant MtbΔFasR or with the FasR complemented strain (MtbΔFasR::FasR) during 60 days. Our results show that FasR is required for the survival and replication of Mtb in the lung of infected mice. Next we evaluated the impact of FasR on Mtb infection of human macrophages (THP-1). After 120 and 144 h of infection, the total CFU of mycobacteria significantly decreased in cells infected with MtbΔFasR, showing that the intracellular survival of Mtb in macrophages depends on FasR. We also analyse the function of FasR in the phagosome maturation, determined by the acquisition of Lysotracker and LAMP-3 (late endo/lysosomal markers). Interestingly, the absence of FasR increased the phagosome maturation compared to cells infected with WT or complemented strain. We conclude that FasR is an essential regulator for the virulence of Mtb.

Key words: Mycobacterium tuberculosis, FasR, phagosome

(902) **NARINGENIN INTERFERES WITH THE DISPERSAL OF *STAPHYLOCOCCUS AUREUS* BIOFILMS**

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Staphylococcus aureus biofilms are recalcitrant to antibiotic treatment leading to chronic infections. The final benefit to the biofilm development is the potential for seeding dispersal or cellular detachment. The dispersal mechanism requires an active *agr* quorum sensing system and occurs in a protease-dependent manner. Naringenin (N) is a natural flavanone with antioxidant and antimicrobial properties. The aim of this study was to investigate the effect of N on *S. aureus* biofilm. *S. aureus* AR411 (osteomyelitis isolate related to USA300 clone) and SH1000 laboratory strain were used. Citotoxicity using LM2 cell line was determined by MTT assay. Bacteria were treated with N before biofilm formation takes place (prior-to-exposure) and 24 h after biofilms were formed (post-exposure). Biofilms were spectrophotometrically quantified. Inhibition of *agr* system was monitored fluorometrically. Proteolytic activity was assessed using milk agar plates. N (50 μg/ml) did not affect the epithelial cells viability. The ability to form biofilm by AR411 strain (prior-to exposure) significantly diminished in presence of N (AR411: 3.27±0.11 *vs* AR411+N: 1.64±0.08, $p<0.01$, t-test). The percentages of dispersal of AR411 and SH1000 biofilms were 83% and 64%, respectively. However, the addition of N to mature biofilms (post-exposure) induced high biomass in both strains (AR411: 0.57±0.03 vs AR411+N: 1.42±0.08, $p<0.01$; SH1000: 0.45±0.11 vs SH1000+N: 2.03±0.16, $p<0.01$). The activation of *agr* system was inhibited by N (F/DO: 521; C+: 3777 and C-: 572). Supernatants of AR411 mature biofilms treated with N did not show proteolytic activity. Altogether the results indicate that N reduced the ability to form biofilm by *S. aureus* USA300-like strain. The dispersal mechanism was negatively affected by N due to the inhibition of *agr* system. Therefore, mature biofilms increased in presence of N as a result of absence of proteases.

(994) **DETECTION OF *KLEBSIELLA PNEUMONIAE* STRAINS IN WATERS USED FOR IRRIGATION OF VEGETABLE AND RECREATIONAL PURPOSES**

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Klebsiella pneumoniae in an opportunistic pathogen that can cause diseases such as sepsis, p neumonía and infection in the urinary tract and soft tissues, constituting one of the eights more important hospital pathogens. In nature, it is found in the water, soil, as well as in some of the mammalian mucosa. Detection in vegetables and fresh fruits has allowed to hypothesize that these bacterias find microsites in the surfaces of the leaves of the vegetables, whose conditions favour their growth and survival. Previous studies made by our work group detected the presence of several bacterial species of the group Enterobacteriaceae in several points of San Juan River, considering the area of the Partiador San Emiliano as a reference or “pristine” site. In this work, and as part of a larger study, with the antecedent that only *E.coli* was isolated, It was investigated this species and *Klebsiella pneumoniae* both formers of biofilms

in the waters of that site, which are used for recreational purposes and later for irrigation in the cultivation of vegetables. Samples were taken under sterile conditions and in the laboratory were incubated in devices suitable for the development of biofilms for 5 days, which was removed and dispersed in buffered peptone water, incubated 24 hours at 28 ° C.With the suspension was seeded in EMB and SS medium. For their identification, the following biochemical tests were done: Gram stain, INVIC, TSI, LIA. 10% of the randomly selected developed cfu were isolated, with the following results: *E. coli* 5%; *Klebsiella pneumoniae* 15%. Given the risk of contagion through the skin, respiratory tract, etc. and that can reach foods that are consumed without cooking, we consider this result an alert for public health, and since in *Klebsiella spp*. the resistance to antibiotics is very frequent, will be deepened in this subject in future investigations.

Keywords: Enterobacteriaceae, water, *Klebsiella*

(875) **ENTEROPARASITES AND COMMENSALS IN THE PROVINCE OF CORRIENTES, ARGENTINA**

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Intestinal parasitic infections continue to represent a major public health problem and are associated with a lack of adequate sanitation and education.

The objective of this study was to determine the occurrence of enteroparasites and commensals in patients from districts located in the province of Corrientes, Argentina, between 2013-2016.

Stool samples and perianal mucus of 675 patients (range: 10 months-90 years), were processed through the Hoffmann, Pons & Janer, Graham, Baermann and Harada-Mori methods.

Of the total number of people examined, 70% were women.

Parasites were found in 284 (42,0%) patients, corresponding to 52,0% of males and 38,0% of females.

There were 221 cases (78.0%) of monoparasitism, 58 (20.0%) of biparasitism and 5 (2.0%) of polyparasitism.

The prevalence of intestinal helminths was lower (50.3%) than that of protozoa (72.2%).

For pathogenic protozoa, the detection rates were: *Blastocystis hominis,* the most prevalent, 66.5%, followed by *Giardia lamblia*, 5.3% and *Cystoisospora belli* in 0.3%. Non-pathogenic commensal protozoa were: *Entamoeba coli*, 20.1%; *Endolimax nana,* 10.6% and *Chilomastix mesnilii,* 0.3%.

Helminths detection rates were: *Strongyloides stercoralis* 24.6%, *Enterobius vermicularis* 10.6%, *Necator americanus*, 6.7%; *Ascaris lumbricoides* and *Tænia saginata*, 2,5% and *Hymenolepis nana* 1.1%.

Other intestinal parasites were also found, *Trichostrongylus* sp (1.4%) and *Hæmonchus* sp 0.3%, both ruminant nematodes.

The youngest subject was a girl of ten month old with *E. vermicularis* and the eldest was a man of ninety years old with *S. stercoralis* infection.

Our results show that 90% of infections occurred in people over 20 years of age.

The percentages of positive findings of intestinal parasites and/or commensals detected in this study reflected the high exposure of this community to contaminated soil and precarious hygiene habits.

Keywords: enteroparasites, commensals, Corrientes

(940) **EVOLUTION OF BODY WEIGHT AND MUSCULAR LARVAL LOAD (CPR) DURING THE INFECTION WITH *TRICHINELLA SPIRALIS* (*TS*) IN CBI-IGE MICE WITH DIFFERENT SUSCEPTIBILITY TO THE PARASITE**

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T. spiralis infection characterizes by the durable survival of encysted larvae in skeletal muscle. CPr, the number of encysted larvae, depends mainly on the defense mechanisms generated by the host, yet the immune mechanisms behind susceptibility to chronic helminth infections are poorly understood. Previous studies have shown that CBi/L mice infected with the parasite are resistant and have a very low muscle larval count on day 30 post-infection (p-i). The CBi+ line from the same colony is susceptible, exhibiting the highest count. During infection, both lines showed a Th1/Th2 mixed response as assessed by serum cytokine levels (IL-2, IL-4, IL-10, IFN-γ). Nevertheless, CBi+ had IFN-γ values higher than those of IL-4 and CBi/L showed a shift to a Th2 profile from day 6 p-i. This research aimed to evaluate the influence of the host genotype on the evolution of body weight and muscle parasite load in lines of mice with extreme response phenotypes. CBi/L and CBi+ mice of both sexes were infected with *Ts* and sacrificed on days 30, 40, 50, 60, 70 and 110 p-i (n=6 by sex and date) at which time CPr (L1 larvae/g tissue) was estimated in the tongue. Mice were weighed before infection and every 15 days until sacrifice. Body weight did not change significantly during the period studied. CBi+ mice showed a significant decrease in CPr between days 50 and 60 p-i ($P<0.001$) (mean±SEM; ♂:1489±458.0 *vs*. 395±76.4; ♀: 2374±569.5 *vs*. 442±54.4) and remained low up to 110 days p-i whereas in CBi/L no significant changes were observed in that period. The lack of changes in body weight during the experiment could be due to the resilience of the species as well as to the low infective dose used. The results suggest that the host response in the chronic phase is partially regulated by the intestinal phase of the parasite and though helminths are powerful modulators of the host immune response, their ability to ameliorate protective type2 response leading to chronicity is limited by the host.

KEYWORDS: *Trichinella spiralis*; chronic stage; resistance/susceptibility; murine model

(997) **NATURAL TREATMENTS: LACTOFERRIN EXERTS ANTIGIARDICIDAL EFFECTS THROUGH STRESS AND CRITICAL MORPHOLOGICAL DEFECTS**

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Understanding how parasites respond to mechanisms of cellular stress is necessary to identify essential processes on their life. *Giardia lamblia* is a protozoa parasite that is the most common cause of non-viral non-bacterial diarrheal disease world-wide. Despite the effectiveness of metronidazole, a major antigiardial drug, its strong side effects has been matter of concern. Consequently, the search for natural treatments is important for the control of the illness. Glycoprotein bovine lactoferrin (bLF) and its peptide lactoferricin (bLFcin), have exhibited antimicrobial activity in *Giardia*. However, there is little evidence of the underlying mechanisms for their effect. Thus, our goal was to understand how these molecules are internalized and how their microbicide mechanism beyond cell death takes place. To address this point, we performed uptake experiments and different fluorescence and electron microscopy techniques. Our results showed that both molecules are internalized by receptor-mediated endocytosis and are localized in the endo-lysosomal compartments. By transmission and scanning electron microscopy, we observed that both bLF and bLFcin produce morphological changes correlated with the trigger of ER stress. Moreover, trophozoite treatment leads to a partial encystation process probably as a consequence of the general stress induced in the cell. Our findings reinforce the knowledge of a safety use of lactoferrin as treatment for giardiasis.

(1238) **DEVELOPMENT AND FUNCTIONAL CHARACTERIZATION OF A VIRULENT STRAIN OF *MYCOBACTERIUM BOVIS* DELETED IN THE *PHOP* GENE**

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PhoP is a transcriptional regulator that belongs to the two-component system PhoPR and is linked to regulation of the synthesis of numerous lipids, physiological processes and secretion of virulence factors in mycobacteria. In previous studies we observed that a *phoP* mutant strain of *M. bovis* NTC10772 regulates genes related to transport and lipid synthesis as well as genes related to the response to oxidative stress. Some of the genes identified have been described as regulated by *phoPR* in *M. tuberculosis* but others appear to be exclusive of the *phoPR* regulon of *M. bovis*. With the aim to confirm our previous results in this study we propose to elucidate the role of *phoP* in the virulent strain of *M. bovis* 04-303 by quantitative RT-PCR (*RT-qPCR*). The *phoP* mutant of *M. bovis* 04-303 was obtained by a gene knockout by allelic exchange which was confirmed by PCR. The *RT-qPCR* results show that some genes have significant differences ($p<0.05$) between the *M. bovis* 303 and *Mb303ΔphoP* mutant strain, similarly to obtain in previous studies with the *M. bovis* NTC10772 strain. Among them, we highlight the MmpL8 lipid transport protein belonging to an operon involved in the synthesis and export of sulpholipids and the AhpC protein involved in oxidative stress processes which would be positively regulated by PhoP in two strains of *M. bovis* of different virulence, and similarly to *M. tuberculosis*. However, this *phoPR* system would not regulate *lipF* transcription, virulence-related lipase, in any of the *M. bovis* strains unlike what happens in *M. tuberculosis*. These results suggest that PhoP would have a differential regulation between the *M. bovis* and *M. tuberculosis* strains.

Key words: *Mycobacterium bovis*, *phoPR*, *RT-qPCR*

(833) **INFLUENCE OF LER OVEREXPRESSION ON THE PATHOGENICITY OF A SHIGA TOXIN-PRODUCING *Escherichia coli* O157:H7 (EHEC) STRAIN**

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Although the production of Shiga toxin (Stx) by enterohemorragic *Escherichia coli* (EHEC) determines Hemolytic Uremic Syndrome onset, factors that modulate intestinal colonization are key components in pathogenesis and host mucosal immune response. Type III secretion system (T3S) is essential for colonization and is encoded on the locus of enterocyte effacement island (LEE). The first operon encodes its own regulator Ler, which controls the transcription of the other four operons (LEE2-5). The aim of this work was to study whether *Ler* overexpression, with the subsequent overexpression of LEE 2-5, determines an increase on EHEC pathogenesis. To do this we transformed a human-isolated EHEC strain (125/99) with a plasmid containing the *ler* sequence under the control of an IPTG-inducible promotor (125pLer) and also with the empty plasmid (125pW) as a control. We tested the expression of T3S proteins (EspB/D) by SDS-PAGE and Western blot (WB), and the adhesion to intestinal epithelial cells (HCT-8 and Caco-2). We also analyzed Stx2 production by ELISA, as T3S proteins are crossregulated by the Stx prophage. We observed an increased expression of EspB/D on 125pLer by SDS-PAGE and WB. Besides, 125pLer strain showed an increased adhesion to HCT-8 and Caco-2 cells (% adherence HCT-8= 125pLer: 66.7±28.9, 125pW: 15.7±3.8; Caco-2= 125pLer: 33.7±3.2, 125pW: 12.3±4.0; ANOVA $p<0.05$). Stx2 production was dependent on the time of pLer induction, i.e. the more time of pLer induction, the more Stx2 production ((mean±SD, μg/ml) no induction= 125pLer: 0.108±0.000, pW: 0.132±0.022; 3.0 h= 125pLer: 0.171±0.013, 125pW: 0.123±0.004; t-test $p<0.05$; 3.5 h= 125pLer: 0.807±0.127, 125pW: 0.349±0.001, t-test $p<0.05$). We conclude that *Ler* overexpression determines the increased production of T3S proteins (EspB/D) together with an increased adherence to intestinal epithelial cells and Stx2 production. All these parameters could determine an increased pathogenicity *in vivo*.

EHEC-HUS-T3S-pathogenicity-Stx2

BIOPHISICS 10

(781) **EFFECTS OF NITRIC OXIDE *PER SE* ON MITOCHONDRIAL COMPLEX III. FUNCTIONAL CONSEQUENCES**

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At submicromolar concentrations, NO regulates mitochondrial respiration under physiological conditions through two main effects on respiratory chain: the competitive inhibition of complex IV with O_2 and the inhibition of electron transfer at complex III. Previous results from our laboratory showed that NO (~1.2 μM), released from S-nitrosoglutathione or spermine-NONOate, inhibited complex II-III activity (50%) without changes in complex II activity, both measured in submitochondrial particles (SMP) from bovine heart. This inhibition lead to the accumulation of cytochrome b^{2+} (71%), independently on [O_2], and it was not affected by HbO_2 addition. NO produced the increase of [UQH•]ss and, consequently, of $O_2^{\bullet-}$ and H_2O_2 production rates (58%). In this work, experiments using NO solution, obtained by bubbling of NO gas under N_2 atmosphere, on complex III enriched mitochondrial fraction, obtained from bovine heart, were performed. Decylubiquinol:cytochrome *c* reductase activity was inhibited (50%) by 1 μM NO, confirming the results obtained with NOdonors. The absorbance spectra of complex III enriched fraction exposed to NO showed the characteristic peak of cytochrome b^{2+} at 562 nm. In coupled mitochondria, 1 μM NO inhibited state 3 O_2 consumption sustained by succinate (~50%). While 90% of the O_2 consumption was recovered by the addition of HbO_2, the remaining 10% was insensitive to NOscavenger addition, suggesting the blockage of complex III by NO. Moreover, mitochondria exposed to NO showed a less change in $\Delta\psi$ (30%) in the transition from state 4 to state 3 respiration, indicating a decrease in ATP synthesis capacity. Furthermore, H_2O_2 production rate was augmented (55%), according to the electron transfer inhibition and the [UQH•]ss enhancement detected by EPR. Altogether, these results suggest that the interaction of NO with cytochrome *b* inhibits electron transfer at bc_1 complex and stabilizes the UQH•, increasing $O_2^{\bullet-}$ and, as consequence, H_2O_2 productions.

Key words: nitric oxide, cytochrome bc_1 complex, reduced cytochrome *b*, ubisemiquinone, hydrogen peroxide

(234) **A COMPREHENSIVE ANALYSIS OF THE COMPUTED TAUTOMER FRACTIONS OF THE IMIDAZOLE RING OF HISTIDINES IN *LOLIGO VULGARIS***

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A recently introduced electrostatic-based method to determine the pKa values of ionizable residues and fractions of ionized and tautomeric forms of histidine (His) and acid residues in proteins, at a given fixed pH, is applied here to the analysis of a His-rich protein, namely *Loligo vulgaris* (pdb id 1E1A), a 314-residue all-β protein. The average tautomeric fractions for the imidazole ring of each of the six histidines in the sequence were computed by using an approach that includes, but is not limited to, molecular dynamic simulations coupled with calculations of the ionization states for all 94 ionizable residues of protein 1E1A in water at pH 6.5 and 300 K. The electrostatic-calculated tautomeric fractions of the imidazole ring of His were compared with predictions obtained from an existent NMR-based methodology. Our results indicate that: (*i*) the averaged electrostatic-based tautomeric predictions for the imidazole ring of *all*

histidines of *Loligo vulgaris* are dominated by the $N^{\varepsilon 2}$-H rather than the $N^{\delta 1}$-H form, although such preferences from the NMR-based methodology are not so well defined; (*ii*) the computed average absolute difference between the electrostatic- and the NMR-based tautomeric predictions among *all* six histidines vary among 0% to 17%; and (*iii*) the tautomeric predictions for the imidazole ring of His computed with the NMR-based methodology are stable within a certain, well-defined, range of variations of a tautomer-related parameter.

(1574) **BIOPHYSICAL AND MOLECULAR BASIS OF *Listeria monocytogenes* RESISTANCE TOWARDS ENTEROCIN CRL35**

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Enterocin CRL35 is a pediocin-like bacteriocin active against the foodborne pathogen *Listeria monocytogenes*. Resistance to these peptides can be associated with either downregulation of the receptor Man-PTS, a glucose transporter, or changes in the bacterial cell envelope. The aim of this work was to analyze resistant strains of *L. monocytogenes* INS7 by means of biochemical and biophysical studies of the cells and their cytoplasmic membranes. Two resistant clones to enterocin CRL35, *L. monocytogenes* INS7 R2 and R3, which were characterized by 16S RNA gene sequencing and rep-PCR, had been isolated in our laboratory. Bacterial growth was studied in different culture media. Plasma membranes of sensitive and resistant bacteria were obtained and characterized by infrared spectroscopy and Langmuir monolayer techniques. The results indicate that the growth rate of resistant strains in TSB medium is slower than sensitive ones. On the other hand, resistant cells were not able to grow in minimal defined medium with glucose as the sole energy source, suggesting that *Listeria* resistant isolates have an alteration in the glucose transporter Man-PTS complex, which is the receptor of the IIa peptides in *L. monocytogenes.* The assessment of the bacterial cells membranes demonstrated difference in the membrane lipids composition. Moreover, the interaction of enterocin CRL35 with the three *Listeria* lipid samples displayed that this peptide could not get inserted into the hydrophobic core of resistant bacteria. These results indicate that both bacteriocin receptor downregulation and membrane structural modifications as well are involved in enterocin CRL35-resistance of *L. monocytogenes* strains.

Keywords: Bacteriocins, Enterocin CRL35, Listeria, Resistant Listeria cells, lipids

(1420) **CYTOTOXIC EFFECTS OF THE KILLER TOXIN KTCf20 AGAINST *Candida spp.***

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Killer yeasts are able to produce proteins or glycoproteins with antimicrobial activity known as killer toxins, and they can be used as biocontrol agents against pathogens yeasts. In previous studies, we demonstrated the glucanase activity of the killer toxin KTCf20, produced by *Wickerhamomyces anomalus* Cf20. In this study, we evaluated the cytotoxic effects of KTCf20, present in cell-free supernatant (CFS), over two clinical isolates of pathogenic strains of *Candida* spp.

Growth inhibition of *C. albicans* and *C. tropicalis* in *W. anomalus* Cf20 CFS (2×10^4 aU/ml), was studied at different temperatures (20 and 30 ºC) and NaCl concentrations (0 and 1%). OD600 and cell viability were measured to calculate inhibition. Fluorescence microscopy (FM) of pathogenic strains was performed after incubation for 2 h at 20 ºC in CFS+NaCl 1% and live/dead staining using SYTO9 and PI as fluorescent probes. Transmission electron microscopy (TEM) was also performed to evaluate the cytotoxic effects of CFS on *Candida* cells. In every experiment, heat-inactivated CFS (100 ºC, 15 min) was used as control.

CFS produced a growth inhibition of 74 and 80%, and 1-2 log cfu/ml on *C. albicans* and *C. tropicalis*, respectively, at 20 ºC and NaCl 1%. Its inhibitory activity decreased at 30 ºC. Fluorescence microscopy, TEM and viability results showed that CFS has a fungistatic and fungicidal effect at a 2-h treatment on C. albicans 78 and C. tropicalis FBUNT3, respectively.

CFS was able to inhibit the growth of *C. albicans* and *C. tropicalis* with fungistatic and fungicidal effects, respectively. These effects could be explained by the different cell wall thickness of both strains.

Keywords: killer toxin, *Candida*, biocontrol

(1296) **EFFECT OF EXTRACELLULAR CALCIUM ON CILIARY LENGTH IN LLC-PK1 RENAL EPITHELIAL CELLS**

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Polycystin-2 (PC2, TRPP2) is a Ca^{2+}-permeable, nonselective cation channel whose dysfunction generates autosomal dominant polycystic kidney disease (ADPKD). PC2 is present in different cellular locations, including the primary cilium. The accepted hypothesis is that ADPKD is generated by changes in ciliary length, which is controlled by several proteins, chiefly among which is PC2. Previously we determined a functional PC2 in the primary cilium of LLC-PK1 cells, where it may contribute to ciliary Ca^{2+} transport. PC2 activation is also associated with a rise in cell Ca^{2+}, instead associated with functional plasma membrane-located PC2 channels. Little is known, however, as to how renal epithelial cells control ciliary length, and in particular its regulation by extracellular Ca^{2+}. Our most recent studies determined the presence of a feedback mechanism of PC2 function by the Calcium-Sensing Receptor, which is activated by extracellular Ca^{2+}. Here, we explored the effect of external Ca^{2+} on ciliary length of confluent monolayers of LLC-PK1 renal epithelial cells. LLC-PK1 cells (2-3 wk old) were cultured, fixed and immunochemically labeled with a specific antibody against a-acetylated tubulin to identify primary cilia, and DAPI to locate cellular nuclei. Images were collected after exposure of cells for 18 hrs to solutions containing either zero nominal Ca^{2+} (1 mM EGTA), normal Ca^{2+} (1.2 mM), or high external Ca^{2+} (6.2 mM) and primary cilia were measured with IPLab software. Exposure of cells to low Ca^{2+} (n = 212) was associated with a 22 ± 1% decrease in ciliary length respect to control conditions in normal Ca^{2+} (n = 159, $p<0.001$). However, no statistical differences were observed between normal and high Ca^{2+} conditions ($p = 0.49$). The data provide the first experimental evidence that external Ca^{2+} controls ciliary length in renal epithelial cells. This signaling mechanism may be essential in the events that trigger cyst formation in the kidney, and the onset of ADPKD.

Keywords: calcium, primary cilium, length

(132) **EFFECT OF FE SUPPEMENTATION DURING THE DEVELOPMENT OF THE ANTARCTIC DIATOM *FRAGILARIA SP***

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The aim of this work was to evaluate the effect of moderate Fe concentrations on the nitrosative metabolism in cultures of the diatom *Fragilaria sp* (from natural populations of the Antarctica) grown in f-2 media supplemented with either 0.3 (control) or 50 μMFe-EDTA (1:1). The growth was evaluated, over 15-20 days at 4ºC under 12 h light-dark cycle, by spectrophotometry and by cellular counting, the total intracellular Fe content by spectrophotometry, labile iron pool (LIP) by spectrofluorometry, nitrite-dependent nitric oxide (NO) generation by Electro Paramagnetic Resonance, and nitration of total protein by Western Blot. The supplementation with 50 μM Fe increased the total Fe content by 22-fold ($p\leq0.01$) and the LIP by 2.3-fold ($p\leq0.1$), as compared to values in control cultures in exponential phase of development (Exp). However, Fe supplementation from 100 to 500 μM Fe increased linearly the LIP content (m= 0,0155% $R^2 = 0.835$) in Exp. The growth rate of the cultures supple-

mented with 50 μM Fe in Exp was increased by 3-fold, as compared to growth in control cultures ($p \leq 0.05$). The total cellular protein nitration in cultures exposed to 50 μM Fe in lag phase of development (Lag) was slightly increased (1.3-fold) respect to values in control cultures ($p \leq 0.1$). Supplementation with 50 μM Fe produced a significant reduction (7.9-fold) in the nitrite-dependent NO generation rate in Lag, as compared to the values in control cultures ($p \leq 0.01$). These results suggested that increases in Fe in the culture, as those seen in the natural habitat, could be appropriately control by endogenous mechanisms avoiding drastic increases in LIP. However, presumable due to the Fe capacity of NO chelation, associated to Fe-dependent ability of catalyzing reactive species generation, the exposure to an Fe excess in the medium lead to alterations in the nitrosative metabolism in the cell in the lag phase of development when antioxidant pathways are still not fully operative.

(1063) **EFFECT OF LOW FREQUENCY MAGNETIC FIELDS ON THE VIABILITY OF MELANOMA CELLS (B16)**

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The relationship between weak low frequency ($B < 1$ mT, $f < 300$ Hz) magnetic fields (MFs) and cancer has been the subject of discussion for decades, not only in the scientific ambit, but also among the general public. Despite the controversies, and the interest in clarifying whether these fields constitute a health hazard, they are also of scientific interest for the opposite reason: the possibility of therapeutic uses in general and, in particular, for the treatment of cancer. Our objective in this work was to evaluate the effect of a combination of static (DC) and alternated (AC) MFs on the proliferation of the B16 melanoma cell line. The DC field was the one present inside our incubator (i.e., the geomagnetic field, distorted by the incubator metallic parts, $80 < B_{DC} < 140$ μT). The AC MF ($3 < B_{DC} < 20$ μT) was generated with a novel system of coils with a special geometry, and time-modulated with the "Thomas" pattern. Two 96-well plates, one control (only DC) and one treated (AC + DC), were seeded completely with 2,000 B16 cells/well. A 24 h-waiting period (for adherence of the cells) was followed by two daily exposures of 6 h, after which viability (for each separate well) was assessed by the MTT essay. Five such experiments were performed and analyzed by ANOVA and the t-test. Unlike what we expected, we did not find a significant difference due to the AC MF. Instead, we did find a significant difference ($p < 0.05$) of the viability (up to 21 %) for wells located in different regions inside the incubator. Careful measurements of the incubator's background MFs strongly suggested that viability was greater where MFs were stronger. Complementary experiments will be performed to confirm and expand these preliminary findings.

Keywords: Low frequency magnetic fields, magnetic fields and cancer, melanoma

(876) **PHYSICOCHEMICAL CHARACTERIZATION OF CHRONIC VENOUS ULCER EXUDATES BY FT-IR SPECTROSCOPY**

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Chronic venous leg ulcers (CVLU) is a complication of chronic venous insufficiency. According to clinical state, ulcer exudate presents differences in its relative composition (plasma ultrafiltrate, bacteria, inflammatory molecules and cells). To get a better diagnostic and treatment, it's crucial the identification of parameters involved in the clinical state as bacterial infection, inflammation, biofilm formation, protein and proteases, pH, etc. This work aims to present FT-IR spectroscopy as a novel diagnostic method for detect these components. For this, it was obtained FT-IR spectra from human CVLU: exudates, isolated planktonic and biofilm bacteria and different serum and plasma controls. In mid infrared region there are several frequency ranges that correspond to specific biomolecules bonds like lipids, proteins, nucleic acids and polysaccharides. After spectral pre-processing, percentage of the mentioned biomolecules and its ratios were calculated from respective spectral area. Also peaks from spectra 2nd derivative at the same frequency regions were identified. This clinical protocol was performed in patients from dermatology service from Avellaneda Hospital and approved by regional bioethical committee. Exudates showed repetitive and characteristic spectral profiles whose areas (dimensionless units) of lipids were (13.94±5.63), proteins (33.90±6.60), nucleic acids (2.61±1.16) and polysaccharides (19.91±11.01). Exudates from acute patients have higher lipid areas than exudates from chronic patients ($p<0.05$). When patients are infected with biofilm forming bacteria, an increase in the polysaccharides area was observed (proportional to bioburden). In addition, significant differences between controls and exudates areas were observed. There were numerous characteristic peaks of each window and each sample. Preliminary studies indicate that study of exudates by FTIR spectroscopy may have prognostic significance in CVLU.

Keywords: FTIR, Chronic ulcers, exudate.

(272) **PrfA*-EXPRESSING *Listeria monocytogenes* CELLS ARE MORE SENSITIVE TO PEDIOCIN-LIKE PEPTIDES DESPITE IMPAIRED EXPRESSION OF THE BACTERIOCIN RECEPTOR**

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Class IIa bacteriocins are antimicrobial peptides produced by Lactic Acid Bacteria. The accepted mechanism of action involves the permeabilization of plasma membrane, where the mannose phosphotransferase complex (Man-PTS) act as the bacteriocin receptor. As a matter of fact, the lack of expression of this complex renders cells highly resistant to these peptides. The *in vitro* studies on class IIa bacteriocins conducted so far dealt with bacteria in saprophytic state. Therefore, the main objective of this study was to characterize the sensitivity of *L. monocytogenes* cells expressing a virulent phenotype. For that purpose, we obtained cells that constitutively express an active form of the master regulator of *Listeria* virulence (PrfA*) by means of the pNF1002 vector. As expected, the qPCR gene expression assay of the Man-PTS subunits IIC and IID showed that those genes were downregulated in PrfA* cells. We complemented these results with a proteomic analysis of the plasma membranes. However, to our surprise, we found that these cells were more sensitive to pediocin-like peptides.
Biophysical assays showed that membranes from PrfA*-expressing cells seemed to be stiffer and less compressible than the wild type cell ones. Furthermore, we performed a lipidomic analysis to determine the lipid composition of the membranes from the strains under study. This work represents the first report on the susceptibility of virulent *L. monocytogenes* to pediocin-like bacteriocins. In addition, it

emphasizes that the current model for the interaction of bacteriocins and the receptor is incomplete and needs further development.
Keywords: Bacteriocin; *Listeria*; PrfA*; Cell envelope

(134) **THE OXIDATIVE BALANCE IN THE HYDROPHILIC MEDIUM DURING DEVELOPMEMENT OF THE MICROALGA *Phaeodactylum tricornutum***
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The oxidative status in the hydrophilic medium during the culture under laboratory conditions of the template diatom *Phaeodactylum tricornutum* was studied. The curve of growth of the microalgae was characterized. The classic three phases of development, LAG, exponential (EXP) and stationary (ST), were identified by cell counting (under optical microscope) and optical density (spectrophotometrically) at room temperature (18ºC). For each phase, ascorbyl radical (A•, measured by electronic paramagnetic resonance, EPR) and ascorbate (AH^-, measured by HPLC) content were measured. The A• content was significantly increased by 2.7-fold, as compared to values in LAG phase, in both EXP and ST phases. The AH^- content in the EST phase, as compared either to LAG phase (0.108 ± 0.003 nmol/10^6 cell) or to the EXP phase (0.11 ± 0.01 nmol/10^6 cell), was significantly increased by 70%. The A•/AH^- index reflects the oxidative condition of the hydrophilic medium in the cells. The obtained values for this index were significantly different among the three phases ($p<0.05$, ANOVA), been 0.059 ± 0.007, 0.145 ± 0.008 and 0.09 ± 0.01 for the LAG, EXP and ST phases, respectively. Our results indicated that even though the content of AH^- increased in the EST phase of the culture, this increment did not reverted the significant increase produced in the content of A• observed in the EXP phase of development. Thus, the active metabolic activity in EXP phase resulted in an alteration on the oxidative balance in the hydrophilic medium of the microalgae. Future studies will be focused on further characterization of these photosynthetic cells in terms of the effects of growth on the oxidative cellular redox balance, studying the physiologic changes associated to the hydrophilic medium.
Keywords: Diatoms, Electronic Paramagnetic Resonance (EPR), Hydrophilic medium, Oxidative stress ratio

MOLECULAR MODELING 2

(1612) **BIOINFORMATIC PIPELINE FOR PROTEIN-CARBOHYDRATE COMPLEXES STRUCTURE PREDICTION.**
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Proteins that bind carbohydrates are responsible for numerous important biological functions, such as signal transduction, cell adhesion, among many others. Despite the number of reported structures of protein-carbohydrates complexes (PCCs) is constantly increasing, they depends mainly on wet experiments, which are expensive and can be very difficult to perform successfully. Achieving accurate predictions of the sugar binding mode by computational methods remains one of the biggest challenges in computational Glycobiology. This is due mainly because the residues that form the Carbohydrate Binding Site (CBS) can differ from its ideal conformation in protein Homology Models HM, which can thereafter significantly affect Docking algorithms performance. In addition, while generally available docking programs work reasonably well for most drug-like compounds, carbohydrates and carbohydrate-like molecules are often problematic, because Force-Fields (FF) and Scoring Functions are typically designed to reproduce structures of protein-drug complexes.

In this work, we present an integrated approach that combines conformational-space sampling of receptor structures builded from a wide range sequence identity templates with MODELLER software, boosted by Molecular Dynamics simulations with AMBER16 package and scored with a biased docking method, the Water-Site Biased Docking Method (WSBDM), an Autodock4 docking protocol with a key FF modification development in our lab. In order to obtain the most plausible PCCs conformations, clustering over final complexes structures predicted by docking and Energetic/Probabilistic analysis was applied. With WSBDM, we was able to reproduce a 93% of PCCs training set builded from Protein Data Bank against a 67% of the conventional docking method. The results show that this emerges as a promising tool to build reliable 3D-models, which can then be used for rational design or optimization of glycomimetic drugs.
Keywords: Docking, Homology Model, Molecular Dynamics, Water Site.

(673) **DEVELOPING A PLATFORM THAT COMBINES AGENT-BASED AND DIFFERENTIAL EQUATIONS APPROACHES TO MODEL INTRACELLULAR TRANSPORT**
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Abstract: Intracellular transport is a key process in eukaryotic cells. Substances need to be transported in membrane-bound organelles along the endocytic and secretory pathways. Transport inside the cell is required for secretion of hormones and neurotransmitters, for antigen presentation and microbe defense and many other fundamental cell functions. At present, hundreds of factors that are necessary for this process have been identified. However, the way intracellular transport is accomplished is far from being deciphered. We have developed a combined agent (ABM) - and differential equation (ODE) -based model of intracellular transport. The resulting model has the flexibility to accommodate the dynamic nature of intracellular structures that move, interact, fuse and divide, and the associated biochemical reactions. At present the model include five organelles identified by five different Rab domains, that correspond to early, sorting, recycling and late endosomes, plus a secretory compartment corresponding to the trans Golgi network. The behavior of the organelles is specified in ABM, whereas Rabs concentration is controlled by ODEs programmed in COPASI. Antigen digestion and cross presentation is also modelled in COPASI. In the simulation, the organelles are stable for more than 50.000 steps, corresponding to times of about 20 minutes. Internalized soluble proteins are correctly directed to degradative organelles and membrane bound proteins are able to recycle to the cell surface. The expectation is that the model will be a useful tool to understand the dynamic nature of the intracellular organelles that work as platforms to control many key functions of the cell biology.
Keywords: intracellular transport, agent-based model, Repast, Copasi

(603) **MULTIPLE TEMPLATE BASED HOMOLOGY MODEL OF ACTIVE STATE D2 DOPAMINE RECEPTOR**
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Dopamine is an essential neurotransmitter in the central nervous system and exerts its effects through the activation of five subtypes of G protein coupled receptors (D1 to D5). Among subtypes, D2 has high therapeutic relevance for treatment of Parkinson's disease, schizophrenia and other disorders in the central nervous system. While D2 structure has not been solved yet, an homology model (HM) of D2 based on the known structure of the closely related D3 was reported. In the solved structure D3 has been captured in its inactive state, therefore the resulting models also would be in the inactive state.

In this work we constructed an HM of D2 in the activated, G protein coupled state. To build the model two templates were considered:

D3 (PDB-ID: 3PBL) due to its high degree of sequence identity to D2 (78% in the TM helices) and the fully activated b_2 adrenergic receptor (PDB-ID: 3SN6) to model mostly the intracellular region of the receptor in the activated, G protein coupled state.

Sequence of D2 in its long form (NP_000786.1) and of Gi protein inhibitory alpha subunit (PDB-ID: 1GP2) were aligned with the template sequences. The alignment was then used to construct several 3-D models of D2 with the program Modeller 9.15. The model with the lowest value of the Modeller objective function was subjected to quality assessment with PROCHECK. The model was then inserted into an heterogeneous biological membrane using CHARMM-GUI server and subjected to MD simulations with Amber14 for further refinement, in order to get a 3-D modelcloser to the native form of the protein.

HM based on a single model requires the choice of a crystallized structure highly similar to the receptor under study, while the strategy used in this work, of alignment with multiple models, involves the excision of the receptor in several domains and the subsequent selection of the most appropriate template for each of these domains, this strategy being very useful to increase the accuracy of the models.

Keywords: Dopamine Receptor, Homology modeling, G Protein.

(1311) **MULTISCALE APPROACH TO THE ACTIVATION AND PHOSPHOTRANSFER MECHANISM OF CPXA HISTIDINE KINASE REVEALS A TIGHT COUPLING BETWEEN CONFORMATIONAL AND CHEMICAL STEPS**

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Sensor histidine kinases (SHKs) are an integral component of the molecular machinery that permits bacteria to adapt to widely changing environmental conditions. CpxA, an extensively studied SHK, is a multidomain homodimeric protein with each subunit consisting of a periplasmic sensor domain, a transmembrane domain, a signal-transducing HAMP domain, a dimerization and histidine phospho-acceptor sub-domain (DHp) and a catalytic and ATP-binding subdomain (CA). The key activation event involves the rearrangement of the HAMP-DHp helical core and translation of the CA towards the acceptor histidine, which presumably results in a autokinase competent complex.

In the present work we integrate coarse-grain, all-atom, and hybrid QM-MM computer simulations to probe the large-scale conformational reorganization that takes place from the inactive to the autokinase competent state (conformational step), and evaluate its relation to the autokinase reaction itself (chemical step). Our results highlight a tight coupling between conformational and chemical steps, underscoring the advantage of the CA walking along the DHp core to favor a reactive tautomeric state of the phospho-acceptor histidine. The results represent not only an example of multiscale modelling, but also show how protein dynamics can promote catalysis.

Keywords: Coarse Grain; QM/MM; Histidine Kinase; CpxA; two component system.

(566) **STUDY OF THE MICRO RNA PRECURSOR PROCESSING MECHANISM BY QM/MM COMPUTATIONAL SIMULATIONS**

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Rnase III is the main protein in the processing of microRNA precursors (pri-miRNA) in bacteria. It is composed by two domains: dsRBD, which recognizes and binds the target precursors, and RIID, which processes the pri-miRNA. The processing by RIIID is performed through a hydrolysis reaction, in which there is a nucleophilic attack on the phosphate group of the RNA backbone by an activated H_2O molecule or a OH^- ion previously coordinated to a Mg^{2+} ion of the active site. The proposed mechanism on this reaction is based on structural studies that are not able to capture the thermodynamics of the process, nor the protonation states of the residues and solvent molecules involved in the mechanism with atomistic detail. In this regard, computational simulation techniques can provide useful and detailed information about the reaction mechanism.

To study the hydrolysis mechanism through hybrid quantum mechanics/molecular mechanics (QM/MM) methods, we performed 50 ns of classical molecular dynamics (MD) simulation on the initial system. Next, we carried out 30 QM/MM steered molecular dynamics (SMD) simulations from different structures taken from the previous MD, in which we forced the nucleophile to attack the phosphate group. The irreversible work obtained in each SMD was used to calculate the free energy profile of the reaction, through the Jarzynksi equality.

Our results show that the the nucleophile is an OH^- ion and not a H_2O molecule, as the former's energy barrier is considerably lower. Additionally, the network of hydrogen bridges around the active site is important for its regeneration after the reaction. On the other hand, by mutating key residues on the active site which bind the Mg^{2+} ion, we observed that its role is not only activating the nucleophile, but arranging and stabilizing the active site by neutralizing the negative charge of the phosphate, nucleophile, and protein residues.

Keywords: microRNA, QM/MM, hydrolysis mechanism.

(1166) **TARGETING THE TRPV1 CHANNEL TO FIND NOVEL ANTIEPILEPTIC AGENTS. FROM MOLECULAR MODELING AND DOCKING SIMULATIONS.**

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Transient receptor potential cation channel subfamily V member 1 (TRPV1) is a nonselective cation channel modulated by both endogenous and exogenous ligands, pH, temperature and voltage. Many efforts have been made from industry and academia to find novel drugs that modulate the channel activity without undesirable side effects. Besides most of the research is focused on inhibiting the channel to get an antinociceptive effect, in the last few years it has been proposed as a promising target to treat some forms of epilepsy.

Since the 3D structure of the hTRPV1 is not currently available, we have constructed a homology model of the channel. The model was later refined in a membrane specific framework implemented in Rosetta, to get a high resolution structure suitable for docking simulations. This procedure was repeated for three different templates representing biologically relevant states of the channel (APO, Open-Agonist bound and Closed-Antagonist bound). Also three different scoring functions were evaluated for both docking and rescoring the complexes prior minimization with flexible side chains. The docking power of every combination was evaluated by re-docking and cross-docking experiments on the template structures. The scoring power and screening power were assessed with a balanced validation set and two simulated databases, respectively, by means of several enrichment metrics. The best docking model involves docking and then re-scoring the complexes with flexible side chains minimization, using two differents scoring functions. The model shows good scoring power (AUC-ROC=0.894) and also very good screening power ($EF^{1\%}$ = 56, $EF^{10\%}$ = 9.2, BEDROC=0.039). Moreover, it's also really fast which makes it suitable for High-Throughput Virtual Screening of large chemical databases.

As a result, a docking model was developed and extensively validated to be applied in a VS campaign to find novel

TRPV1 antagonists with potential antiepileptic activity.
Keywords: Epilepsy; TRPV1; Molecular modeling; Docking

(1343) **THEORETICAL STUDY OF THE ANTITUMORAL LIPID OHMLINE: SELF INTERACTION AND INCORPORATION INTO MEMBRANES**

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The dysregulation of ion channel activity/expression emerged as a common feature of cancer cells and the modulation of overexpressed ion channels opens new perspectives in cancer therapy. Specifically, the SK3 channel is overexpressed in metastatic cells and Ohmline (1-O-hexadecyl-2-O-methyl-sn-glycero-3-lactose, Ohm) is an ether lipid that has been shown to reduce its activity and the migration capacity of cancer cells after incorporation into the plasma membrane. In this work, molecular dynamics simulations were used to study the behavior of Ohm in aqueous solution and its incorporation into models of the plasma membrane and a membrane nanodomain. The simulated system were: i) *The Ohmline system,* it consist on several Ohm molecules in solution, ii) *The plasma membrane system,* similar to a cell membrane in composition, formed by POPC in one monolayer and POPE and POPS in the other monolayer and iii) *The nanodomain system*, similar to a membrane RAFT in composition, formed by a mixed of DSPC, sphingomyelin and cholesterol (it is supposed that SK3 channel is located into these nanodomains). Specifically, system ii) and iii) were studied in contact with dilute solutions of Ohm molecules.

The results have shown the ability of the Ohm molecule to form a kind of micellar or fibrillar structures in solution, a fact that was also confirmed by electron microscopy. The analysis of the incorporation of the Ohm molecule into systems ii) and iii), demonstrate the ability of Ohm to be absorbed independently of the membrane composition, with similar mechanism. Some structural properties does not change upon incorporation, nevertheless, dynamical properties such as the lateral diffusion is increased only in the raft system. These results allow us to infer that Ohm could induce fluidity in rigid membranes such as the rigid nanodomains, affecting the packing of the lipids and in turn, reducing the activity of the SK3 ion channel.

Keywords: Molecular Dynamics, Antitumoral lipids