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

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

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

VII REUNIÓN ANUAL DE LA SOCIEDAD ARGENTINA DE NANOMEDICINA (NANOMEDAR)

V CONGRESO NACIONAL DE LA ASOCIACIÓN ARGENTINA DE CIENCIA Y TECNOLOGÍA DE ANIMALES DE LABORATORIO (AACYTAL)

15-19 de noviembre de 2016
Hotel 13 de Julio – Mar del Plata

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ARGENTINE SOCIETY FOR CLINICAL INVESTIGATION
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(SAFE)

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(NANOMEDAR)

V NATIONAL CONGRESS
ARGENTINE ASSOCIATION FOR SCIENCE AND TECHNOLOGY OF LABORATORY ANIMALS
(AACYTAL)

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13 de Julio Hotel – Mar del Plata

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LA TAPA

Susana Claret. **Esteros**, 1989

Óleo sobre tela. 80 × 120 cm. Cortesía de la Comisión Nacional de Energía Atómica, Predio TANDAR, Centro Atómico Constituyentes. Comisión Organizadora de la Exposición Permanente: Dr. A.J.G.Maroto, Dr. H. Ceva.

Susana Claret nació en Buenos Aires. Profesora Superior de Pintura. Estudió con Batlle Planas y M. Dávila. Realizó numerosas exposiciones individuales (Wildenstein, Art and Arch. y Erko, en Ámsterdam, entre otras) y muestras colectivas. Obtuvo numerosas menciones y premios, entre ellos: Gran Premio Museo de Bellas Artes de Luján, 1ra Mención LXXI Salón Nacional, Gran Premio Salón de Otoño, etc. Poseen obras suyas los museos de Entre Ríos, Quilmes, Luján, Bernal, Uruguay, Río Negro y colecciones particulares en el país y en el exterior[1].

[1]Comisión Nacional de Energía Atómica. Artistas Plásticos con la CIENCIA, 102 Centro Atómico Constituyentes, Predio TANDAR, Buenos Aires, 1999; p 22. En: *www.medicinabuenosaires.com*, *link: http://www2.cnea.gov.ar/xxi/artistas/artistasplasticos.htm*

the presence of *T. cruzi* DNA in the three tissue types analyzed in both patients (5/8 sclera, 4/4 corneas, and 2/2 samples eye muscles). To analyze parasite populations, and rule out possible contaminations, a comparison of patterns of restriction fragments hypervariable sequence kDNA (PCR-RFLP, double digestion MspI-RsaI) was carried out and analized on polyacrylamide electrophoresis, obtaining specific profiles with high similarities within each patient. These results confirm the presence of *T. cruzi* in the analyzed tissues and, particularly positivity corneas, confirming the risk of being used in transplantation.

Studies have proper authorization of the Ethics Committee INCUCAI, approval by Saint Lucia Eye Hospital, and the Central CIS GCABA registered under No. 156/16.

PRESENTACIÓN DE POSTERS SAI II / SAI POSTER PRESENTATION II

ENFERMEDADES INFECCIOSAS II/ INFECTIOUS DISEASES II

299 (55) **BRUCELLA ABORTUS INFECTION ELICITS HEPATIC STELLATE CELLS (HSC) FIBROSIS THROUGH INFLAMMASOME-DEPENDENT IL-1â PRODUCTION.**

Paula Constanza Arriola Benitez[1], Ayelén Ivana Pesce Viglietti[1], Diego Comerci[2], Guillermo Hernán Giambartolomei[1], Maria Victoria Delpino[1].

[1]Instituto de Inmunología, Genética y Metabolismo (INIGEM-CONICET/UBA). [2]Instituto de Investigaciones Biotecnológicas Dr. Rodolfo A. Ugalde (IIB-INTECH-UNSAM-CONICET).

The liver is affected in human brucellosis. *B. abortus* (*Ba*) triggers on HSC a profibrotic response characterized by inhibition of MMP-9 with concomitant collagen deposition and TGF-β1 secretion in a way that involved a functional T4SS. Taking into account that it has been reported that inflammasome is necessary to induce a fibrotic phenotype in HSC, we hypothesized that *Brucella* infection might create a microenvironment that would promote inflammasome activation and a concomitant profibrogenic phenotype in HSC. Our results indicate that *Ba* infection induces IL-1β secretion (ELISA) by LX-2 cells by a mechanism dependent on a functional T4SS ($p<0.001$). When infection experiments were performed in the presence of glyburide, a compound that inhibits NLRP3 inflammasome, the secretion of IL-1β was significantly inhibited respect to uninfected controls ($p<0.001$). The same effect was observed when infection was performed in the presence of specific caspase-1 inhibitor Ac-YVAD-cmk ($p<0.001$). These results indicate that caspase-1 and NLRP3 are involved in IL-1â secretion by *Ba*-infected LX-2 cells. Then experiments were conducted to determine whether expression of inflammasome components could be upregulated during *Ba* infection. We determine the expression of caspase-1, NLRP3 and ASC by qRT-PCR. Our results indicated that *Ba* infection induces an increase in caspase-1 and NLRP3 mRNA expression ($p<0.01$) but was unable to modified ASC expression. We proposed to determine the role of inflammasome in the induction of a fibrogenic phenotype in LX-2 cells during *Ba* infection. To this end the levels of MMP-9 (zymography), TGF-β (ELISA) and collagen (Sirius red staining) were determined in LX-2 cells that were infected with *Ba* in presence of Ac-YVAD-cmk and glyburide. Both inhibitors were able to reverse the effect of *Ba* infection on LX-2 cells. Taken together this results indicate that *Ba* induce inflammasome activation in HSC with concomitant induction of a fibrotic phenotype.

300 (183) **INTERACTION OF POLYMORPHONUCLEAR AND BACTERIA ISOLATED FROM CHILDREN WITH BRONCHIOLITIS OBLITERANS POST ADENOVIRUS.**

Silvia Orosco[1], Juan Carlos Valdéz[2], Nadia Gobbato[2], Nilda Arias[3], Clara Silva[4], Mirta Rachid[2], Gabriela Castillo[2], María Díaz Zamora[2].

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Introduction: Constrictive Bronchiolitis Obliterans (BO) is characterized by inflammation and fibrosis of the bronchioles wall leading to obstruction and sometimes occlusion of the airways. Elevated numbers of neutrophils within the airways are a hallmark of BO. After a viral infection of the lower respiratory tract, polymorphonuclear leukocyte (PMN) is involved in inflammation and tissue damage. This BO form is common in South America. Here, we studied the functionality of PMN isolated from blood of BO patients when are challenged with bacteria frequently isolated from sputa of these patients to determine any alteration related with the pulmonary affectation. Methods: Expectorated sputum and blood were collected from 10 children attending to our hospital diagnosed with medium and severe BO post adenovirus pneumonia. The study protocol was approved by the Ethics Committee of Hospital, and informed consent was obtained from the parents of children. Neutrophils isolated from blood were challenged with *Staphylococcus aureus*, SARM, *Pseudomonas aeruginosa, and Klebsiella pneumoniae* isolated from sputa. Microbicidal activity and spectrofluorometric determination of NETs formation by Sitox Green (DNA extracellular detection) and NO intracellular production by DAF-FM DA were performed. Serum metalloproteinasas were measured by gelatin zymography. Results and Conclusions: In the group of 6 patients with severe BO, 3 patients had stable lung function, neither recurrent infections nor mucus production, and high values of netosis, intracellular production of NO and microbicidal activity. The remaining 3 patients behave like the 4 patients with medium bronchiolitis showing impaired lung function, recurrent infections, low nets formation, NO production and microbicidal activity. The greater reactivity of PMN when challenged with bacteria is related to severe bronchiolitis, without pulmonary infections and more stable lung function than medium bronchiolitis.

301 (224) **B. ABORTUS RNA: A NOVEL VITA-PAMP INVOLVED IN THE DOWN-MODULATION OF MHC-I EXPRESSION ON HUMAN MONOCYTES.**

Maria Ayelen Milillo[1], Lis Noelia Velasquez[1], Aldana Trotta[1], Maria Victoria Delpino[2], Luciana Balboa[1], Guillermo Hernan Giambartolomei[2], Paula Barrionuevo[1].

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Brucella abortus elicits a strong Th1 immune response which activates cytotoxic T lymphocytes. However, this pathogen is able to survive inside macrophages and generate a chronic infection. Previously we reported that infection of human monocytes/macrophages with B. abortus inhibits the IFN-□-induced MHC-I cell surface expression. More importantly, we have recently demonstrated that B. abortus RNA, described as a viability-associated (vita)-PAMP, is the bacterial component involved in this phenomenon. Thus, the aim of this study was to further characterize the component, signalling pathways and mechanisms implicated in MHC-I down-modulation. For this, RNases-treated B. abortus RNA was employed to stimulate human monocytic THP-1 cells in the presence of IFN-□ for 48 h. Then, the expression of MHC-I molecules was evaluated by flow cytometry. Surprisingly, completely degraded RNA was still able to inhibit MHC-I expression ($p<0.05$) and it also induced the intracellular retention of these molecules within the Golgi apparatus into the same extent as intact RNA. On the contrary, DNase- and Proteinase K-treated RNA as well as eukaryotic RNA controls elicited no effect. Furthermore, B. abortus RNA also inhibited MCH-I expression on human primary monocytes and murine bone-marrow derived macrophages ($p<0.05$). TLR3 is one of the best known RNA immune receptors, therefore we evaluated whether it could be involved in this phenomenon. Yet, in the presence of a TLR3 inhibitor, B. abortus RNA down-

regulated MHC-I expression. On the other hand, neutralization of the EGFR resulted in partial recovery ($p<0.05$) of RNA-mediated MHC-I inhibition. Overall, these results indicate that the vita-PAMP RNA as well as its degradation products constitute a novel virulence factor whereby B. abortus, by a TLR3 independent mechanism and through the EGFR pathway, inhibit MHC-I expression. Thus, bacteria can hide within infected cells and avoid the immunological surveillance of cytotoxic CD8+ T cells.

302 (225) **CROSSTALK BETWEEN PLATELETS, B. ABORTUS AND IMMUNE CELLS.**

Aldana Trotta[1], María Ayelén Milillo[1], María Victoria Delpino[2], Guillermo Hernán Giambartolomei[2], Roberto Gabriel Pozner[1], Lis Noelia Velásquez[1], Paula Barrionuevo[1].
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Brucellosis is an infectious disease elicited by bacteria of the genus *Brucella*. Platelets have been extensively described as mediators of hemostasis and responsible for maintaining vascular integrity. Nevertheless, they have recently got involved in the modulation of innate and adaptive immune responses. We have already demonstrated a crosstalk between *B. abortus* and monocytes. However, the role of platelets during monocyte/macrophage infection by these bacteria remains unknown. The aim of this study was to investigate whether platelets are involved in the development of *Brucella*-mediated infection. To start evaluating this, THP-1 cells (pro-monocytic human cell line) were infected with *B. abortus*-GFP (100:1) in the presence or absence of platelets for 4 h and the effect of platelets on the infectious capacity of *Brucella* was analyzed by confocal microscopy. Our results showed that the presence of platelets stimulated the invasion of monocytes by *B. abortus*. Moreover, we observed that platelets formed complexes solely with infected monocytes. Afterwards, we evaluated the ability of platelets to modulate functional aspects of monocytes during the infection. First, we studied the secretion of immunomodulatory mediators. To address this, THP-1 cells were infected with *B. abortus* in the presence or absence of platelets for 4 or 24 h. The supernatants from infected cells were collected and quantified by ELISA. Next, we studied the expression of adhesion and co-stimulatory molecules on the monocyte surface by flow cytometry. The presence of platelets during monocytes/macrophages infection stimulated IL-1β, IL-8 and MCP-1 secretion ($p<0.01$) while it inhibited the secretion of TNF-α ($p<0.01$). At the same time, platelets stimulated the expression of ICAM-1 (CD54) and CD40 ($p<0.01$). Overall, our results indicate that platelets can modulate the *B. abortus*-mediated infection of monocytes increasing their pro-inflammatory capacity, which could promote the resolution of the infection.

303 (458) **BORDETELLA PERTUSSIS MGTC PLAYS A ROLE IN INTRACELLULAR SURVIVAL BY FACILITATING THE ADAPTATION TO MAGNESIUM LIMITING CONDITIONS AND ACIDIC PH.**

Juan Hilario Cafiero[1], Yanina Lamberti[1], Hugo Valdez[1], María Eugenia Rodriguez[1].
[1]CINDEFI (UNLP - CONICET), Facultad de Ciencias Exactas, Universidad Nacional de La Plata, La Plata, Argentina.

Bordetella pertussis (*Bp*), the causative agent of whooping cough, survives inside host cells, a process that requires the adaptation of the pathogen to this harsh environment. *Bp* genome contains a homolog of mgtC, a virulence factor of several pathogens that is involved in growth under mildly acidic pH and Mg^{+2} limiting conditions and that is crucial for intracellular survival. The aim of this study was to analyse the role of mgtC in *Bp* intracellular survival. A *Bp*ΔmgtC mutant strain and a complemented strain were constructed and used in parallel with the wild type strain (wt*Bp*) in an infection assay of PMA-differentiated THP-1 cells. Immunofluorescence microscopy showed that there were no differences between the strains in the uptake of bacteria by THP-1 cells. The intracellular survival of these strains inside THP-1 cells was analyzed in a polymyxin B protection assay by CFU counts at 3, 24 and 48 hours post-infection (pi). *Bp*ΔmgtC showed a decrease in intracellular survival at 24 and 48 hours pi ($p<0.05$) as compared with both wt*Bp* and the complemented strain. Accordingly, confocal microscopy studies showed a higher traffic of *Bp*ÄmgtC to lysosomes as showed by a higher colocalization with the dye Lysotracker than wt*Bp* at 24 and 48h pi ($p<0.05$). To characterize the defect in the intracellular survival of *Bp*ÄmgtC, we tested the *in vitro* growth of this strain in different conditions. There were no differences in the growth yield in liquid medium between wt*Bp* and *Bp*ÄmgtC in Mg^{+2} replete conditions. However, *Bp*ÄmgtC showed lower biomass yield under Mg^{+2} limited conditions ($p<0.01$). We further found that mgtC is upregulated in Mg^{+2} starvation ($p<0.001$), as determined by RT-qPCR. *Bp*ÄmgtC showed a lower resistance to mild low pH than wt*Bp* strain ($p<0.01$), suggesting that this gene is also involved in acidic tolerance. Altogether, this data suggest that mgtC is involved in *Bp* adaptation to the endosomal environment and plays a key role in bacterial intracellular survival.

304 (529) **BOVINE – ECHINOCOCCUS GRANULOSUS CELL LINE (EGPE) AS ANTIGENIC SUPPORT FOR HYDATIDOSIS DIAGNOSIS AND FOLLOW UP.**

Andrea Florencia Maglioco[1,2], Melisa S Barbery Venturi[1,2], Jorge Gentile[3], Susana Hernández[3], Oscar Jensen[4], María Laura Gertiser[4], Alicia G Fuchs[1].
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Introduction: Echinococcus granulosus is the causative agent of hydatid disease (HD), a widely distributed zoonosis in the world. The usual source of antigens used for immunodiagnosis is the hydatid cyst fluid. Differences were observed in the specificity and sensitivity between different studies, perhaps due to the use of a source of non-standardized antigens. In our laboratory, a cell line from bovine protoscolices (EGPE cells) has been established (patent first instance approved INPI P-090102320). In these cells, the antigen B was detected by immunohistochemistry and DCO1 was detected by PCR (Echeverria et al 2010). Aim: to validate the use of antigens from EGPE cells, as a standardized source of antigens, to diagnose HD and monitoring its follow up. Study design: Presence of relevant antigens to HD were evaluated in EGPE, by western blotting. Three different protein mixtures were assayed: proteic extract from short or long culture of EGPE and supernatant. Serum from 13 patients with HD or non HD patients from an endemic zone (Tandil) were used in a case-control study. Six of these HD patients were evaluated 4 years later. Bands detected exclusively by HD patients were taking into account as positive band. Results: The combination of different antigens from the 3 sources allow the diagnosis of 13/13 patients. The three sources have different protein composition. The bands detected by HD patients in short culture antigens were (KDa): 76-78, 70-73, 64-65, 58-60, 52-55, 44-47 and 40-41; in long culture antigens detected were (KDa): 93-94, 78-81, 73-77, 69-72, 63-66, 56-57, 51-55, 46-47 and 40-42; and in supernatant antigens detected were (KDa): 86-90, 79-80, 75-76 and 58-60. These bands were lost in 5/6 HD patients 4 years later ($p<0.05$, Chi-square test). Conclusion: More than one antigen is necessary to HD diagnosis and EGPE proteins would be a very useful source of antigens for HD diagnosis and follow up. This study was approved by UAI ethics committee.

305 (545) **ANTI-PROLIFERATIVE EFFECT OF NON STEROIDAL GLUCOCORTICOID RECEPTOR AGONIST COMPOUND A IN CELLS STIMULATED WITH SEG.**

María Julieta Fernández Lynch[1], Mariángeles Díaz[1], Sofía Noli Truant[1], María Belén Sarratea[1], María Belén Antonoglou[1], Mauricio de Marzi[2], Emilio Malchiodi[1], Marisa Mariel Fernández[1].
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