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IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES

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P. BRASILIENSIS AND MANNAN USE MR, CR3 AND TLR4 TO INTERACT WITH MACROPHAGES BUT INDUCE OPPOSITE ACTIVATION PROFILES IN MACROPHAGES OF RESISTANT AND SUSCEPTIBLE MICE

FERIOTTI CLAUDIA (1); CALICH VERA LUCIA GARCIA (1)

(1) University of São Paulo

Introduction: In addition to alpha1,3 glucan, mannan and mannan-linked proteins are expressed in the outer layer of *Paracoccidioides brasiliensis* yeasts. Mannosyl residues are recognized by several membrane pathogen recognition receptors (PRRs), such as the mannose receptor (MR), complement receptor 3 (CR3) and toll-like receptor 4 (TLR4) on macrophage membranes. The aim of this study was to clarify the role of these receptors in the interaction between *P. brasiliensis* and macrophages from resistant (A/J) and susceptible (B10.A) mice. **Methods and Results:** Therefore, the phagocytic, fungicidal and secretory abilities of macrophages were evaluated in the presence of mannan and antibodies against MR, CR3 and TLR4. In addition, the mRNA expression of SOCS3, a protein associated with M1 macrophages; was also evaluated. We verified that mannan and anti-MR antibody exert antagonistic effects, the former increasing and the latter decreasing the killing ability and nitric oxide production of macrophages. In addition, the specific blockade of MR, CR3 and TLR4 impaired fungal recognition and modulated the production of cytokines. Interestingly, both mannan and *P. brasiliensis* induced the production of IL-12 by B10.A macrophages, whereas TNF-α, IL-6 and TGF-β were produced by A/J cells. Normal B10.A and A/J macrophages expressed different levels of phagocytic and non-phagocytic PRRs. Mannan and *P. brasiliensis* infection led to decreased expression of MR and TLR2 on A/J macrophages, whereas CR3, TLR4 and TLR2 were reduced on B10.A cells. Accordingly the expression of SOCS3 mRNA was significantly higher in B10.A macrophages indicating a preferential M1 profile. **Conclusion:** macrophages appear to use MR, CR3 and TLR4 to interact with *P. brasiliensis*; however, the resulting divergent activation

profiles appear to be dependent on the different expression and engagement of PRRs by genetically distinct cells.

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TISSUE FACTOR PROMOTER POLYMORPHISM AND CD142 EXPRESSION IN CD 14+ CELLS FROM DENGUE VIRUS-INFECTED PATIENTS

ANA PAULA SARRETA TERRA (PG)[1]; TAYSSIA BEATRIZ DOS SANTOS (IC)[1]; JULIANA ALINE DE SOUZA COSTA (IC)[1] ANA CAROLINA SANTANA DE OLIVEIRA (PG)[1]; ANA CRISTINA LOPES TOSTA (PG)[1]; DENISE MACIAL CARVALHO (PG) [1]; DAVID NASCIMENTO SILVA TEIXEIRA[1];

1.Laboratory of Immunobiology and Cellular Activation (LIPAC), Department of Medical Clinic - Federal University of Triangulo Mineiro, Uberaba-MG

Introduction: Dengue fever is an infection caused by a flavivirus that remains as an important epidemiological problem in tropical countries as Brazil. It can cause a wild variety of symptoms since a self-limiting febrile disease to a significant plasma leakage leading to severe hemorrhagic symptoms. Several immunological and hematological factors are different expressed in those patients. Tissue Factor (TF) is a component of coagulation cascade that converts prothrombin to thrombin. The present work aims to analyze TF expression (CD142) in monocytes correlating to TF polymorphism in dengue infection patients compared to non-infected ones.
Methods and Results: Blood samples were collected from volunteers in EDTA tubes. Clinical exams, Cell Blood Counts and IgM serology were done to confirm Dengue virus infection. TF expression was analyzed by flow cytometry (CD142 expression on CD14+ cells) and a polymorphism on tissue factor gene at promoter region was investigated by polymerase chain reaction (PCR). PCR products were visualized on 1% agarose gel, showing the following bands: Homozygotes for the insert with a 205bp band (II); homozygous for deletion showing an 187bp band (DD) and heterozygous presenting 187bp and 205bp bands (ID). Our results showed an increased CD142 expression on CD14+ cells from dengue patients (mean fluorescence intensity, MFI = 76,99) compared to individuals without dengue (MFI = 39,85). PCR data showed no correlation between clinical forms and TF genotypes. **Discussion:** Increased expression of CD142 suggests an important role for tissue factor during the acute phase of dengue virus infection. However, more studies are needed and a greater number of patients should be investigated to rule out an association between TF gene polymorphisms and development of DHF. Our work may contribute to understand mechanisms involved in immune response and DHF development.

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INVOLVEMENT OF TNF RECEPTORS IN APOPTOSIS OF ALVEOLAR MACROPHAGES AFTER MYCOBACTERIA INFECTION

MICHELE FERNANDES RODRIGUES[(1)]; BÁRBARA BRUNA MUNIZ FIGUEIREDO[(1)]; ALICE BELLEIGOLI REZENDE[(1)]; CAIO CÉSAR DE SOUZA ALVES[(1)]; ANA PAULA FERREIRA[(1)]; HENRIQUE COUTO TEIXEIRA[(1)].

[1] Laboratório de Imunologia, Departamento de Parasitologia, Microbiologia e Imunologia, Instituto de Ciências Biológicas, Universidade Federal de Juiz de Fora, Juiz de Fora, MG, Brasil

Introduction: Apoptosis of macrophages has been reported as an effective host strategy to control the growth of intracellular pathogens, including pathogenic mycobacteria. TNF-α plays an important role in the modulation of apoptosis of infected macrophages. TNF-α exerts its biological activities via two distinct cell surface receptors, the 55-kDa receptor (TNF-R1) and the 75-kDa receptor (TNF-R2) whose extracellular domain can be released by proteolysis forming soluble TNF receptors (sTNFR-1 and sTNFR-2). The signaling through TNF-R1 initiates the majority of the biological functions of TNF-α, leading to either cell death or survival whereas TNF-R2 mediates primarily survival signals. Here, the expression of TNF-a receptors and the apoptosis of alveolar macrophages were investigated during infection with virulent and avirulent mycobacteria in mice. **Methods and Results**: C57BL/6 mice were intratracheally infected with avirulent (BCG Moreau) and virulent (ATCC19274) strains of *M. bovis*. TNFR1$^{-/-}$ mice were infected by BCG only. Mice were killed 3 and 7 days after infection and lung cells obtained by bronchoalveolar lavage (BAL). Alveolar macrophages were differentiated according to their CD11b/CD11c profiles and apoptosis was assessed by flow cytometry using annexin V-FITC/PI labeling. Cell surface TNFR1 and TNFR2 were studied using FITC anti-mouse CD120a (TNFR1) and PE anti-mouse CD120b (TNFR2). Levels of soluble TNF receptors (sTNF-R1 and sTNFR2) in BAL were measured by ELISA. A significant increase of apoptosis and high expression of TNFR1 were observed in alveolar macrophages at 3 and 7 days after infection with *M. bovis* BCG but only on day 7 in C57Bl/6 mice infected with the virulent *M. bovis*. Low surface expression of TNFR1 and increased levels of sTNFR1 on day 3 after infection by the virulent strain were associated with reduced rates of apoptotic macrophages. In addition, a significant reduction in apoptosis of

alveolar macrophages was observed in TNFR1$^{-/-}$ mice at day 3 after BCG infection. Surface expression of TNFR2 in C57Bl/6 mice was lower on days 3 and 7, which was accompanied by increased levels of sTNFR2 in mice infected with both strains of *M. bovis*. **Conclusions:** These results suggest a potential role for TNFR1 in mycobacteria induced alveolar macrophage apoptosis in vivo. In this scenario, shedding of TNFR1 seems to contribute to the modulation of macrophage apoptosis in a strain dependent manner.

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Effects of bioactive *Cryptococcus neoformans* mimotopes on vascular endothelial growth fator, nitric oxide and chemokine production *in vitro* by immune cells.

SILVA, P.R. (PG)[1,2]; BOMBANA, H.S. (IC)[2]; ARAÚJO, A.S. (PG)[2]; NOGUEIRA, M.R. (PG)[2]; GARCIA, F.G. (PG)[2]; OLIVEIRA, A.C.S. (PG)[2]; SILVA-TEIXEIRA, D.N.[2]

[1]Centro de Formação Especial/CEFORES-UFTM, [2]Laboratório de Imunobiologia e Pesquisa em Ativação Celular (LIPAC), Instituto de Ciências da Saúde (ICS), Universidade Federal do Triângulo Mineiro (UFTM), Uberaba, MG.

e-mail: silvateixeira@mednet.com.br

Key words: *Cryptococcus neoformans*, "phage display", mimotopes, VEGF, nitric oxide (NO).

Introduction and objectives: *Cryptococcus neoformans* infected patients show an augmented permeability of blood-brain barrier, which contributes to increase morbidity and mortality in cryptococcosis. *C. neoformans* access to central nervous system (CNS) is facilitated by vasoactive mediators produced by immune cells, such as nitric oxide (NO), vascular endothelial growth factor (VEGF), some cytokines and chemokines. In the present work we investigated the effects of *C. neoformans* mimetic peptides (mimotopes) obtained by *"phage display"* on *in vitro* production of vasoactive mediators (VEGF, NO, IL-1b, CCL-2 and CXCL-8) by human mononuclear cells (PBMC) and ***polymorphonuclear leukocytes*** (PMN).

Methods: PBMC and PMN from healthy volunteers (n=12) were isolated by a double density ficoll-hypaque gradient. Mimotopes were selected by *"phage display"* with a monoclonal antibody (18b7 Mab) against *C. neoformans* capsular glucoronoxylomanan (GXM). After a chemical synthesis, peptides were validated by ELISA. A linear sequence peptide (PL), a conformational peptide containing one disulfide bridge (PC) and a double conformational peptide (PD), made of two PC peptides linked by a GGGS spacer sequence, were used to stimulate cells *in vitro*. VEGF, IL-1b, CCL-2 and CXCL-8

production were evaluated by ELISA and NO synthesis by Griess reaction. This research was approved by UFTM ethical research committee.

Resultados e conclusões: *Phage display* results demonstrated a common motif for *C. neoformans* mimotopes. PC and PD mimotopes significantly ($p < 0,05$) increased mean CXCL-8 (PC=3072,00 and PD=4571,00 pg/ml) and IL-1b (PC=925,00 and PD=1274,00 pg/ml) production by PBMC when compared to not stimulated cells (874,0 pg/ml of CXCL-8 and 501,00 pg/ml of IL-1b). PL mimotope was not able to stimulate cells *in vitro*. PD also stimulates NO production by PMN, but VEGF production was not induced by any of these mimotopes. The higher effect of PD compared to PC and PL also suggests that double conformational peptides (linked by spacer sequences) may potentiate CXCL-8 chemokine production. Identification of bioactive *C. neoformans* mimotopes may lead to development of better diagnostic methods and new therapeutic strategies for cryptococcosis.

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DCs expressing Indoleamine 2,3-dioxygenase (IDO) plays an important role in controls the fungal loads, activation co-stimulatory molecules and T cells proliferation in pulmonary paracoccidiodomycosis (PCM) developed by susceptible and resistant mice to the fungus.

ARAUJO, ELISEU FRANK DE (1); CALICH, VERA LÚCIA GARCIA (1)

(1) USP - University of São Paulo, Dept. Immunology, Institute of Biomedical Sciences, São Paulo, Brazil

INTRODUCTION: *Paracoccidioides brasiliensis* is a pathogenic fungus restricted to Latin America and its natural route of infection is the inhalation of fungal particles. In paracoccidioidomycosis (PCM), the regulatory mechanisms mediated by innate and cellular immunity are still unclear. Indoleamine 2,3-dioxygenase (IDO) is an IFN-gamma induced enzyme which catalyzes the tryptophan metabolism along the kynurenine pathway. It is known that IDO can control host-pathogen interaction by inhibiting the proliferation of intracellular microorganisms due to tryptophan starvation and by its immunosuppressive effect on T cell immunity. The aim of our work was to investigate the influence of IDO on the behavior of dendritic cells (DCs) in the course of the infection of susceptible (B10.A) and resistant (A/J) mice clarifying some important aspects on the immunosuppression associated with the severe forms of PCM. **METHODS and RESULTS:** We worked with B10.A and A/J mice using control and 1-methyl-DL-tryptophan (1MT, an IDO inhibitor)-treated mice. Control and 1MT-treated B10.A and A/J mice were infected i.t. with one million yeasts and analyzed at 2 weeks post infection. Dendritic cells of the lung were purified through magnetic beads and the parameters of influence of treatment with 1MT in IDO were analyzed such as mRNA expression; CFU counts; NO production; levels of kynurenine and cytokines; characterization of subpopulations of DCs present in the lungs; lymphocyte proliferation assay. In 2 weeks post infection of B10.A and A/J mice, 1MT was shown to decrease the frequency of DC cells expressing IDO and also the activation of co-stimulatory molecules CD40, MHC II and CD86. In both mouse strains IDO mRNA expression was augmented, driving tryptophan catabolism, kynurenine production and decreased fungal loads. On the other hand, 1MT restore proliferation of $TCD4^+$ and $TCD8^+$ cells in B10.A mice and $TCD8^+$ in A/J mice, showing a known effect on cells expressing IDO. In total lung cells (before magnetic beads) was observed the same effect of decreased co-stimulatory molecules**,** CD40, MHC II and CD86 in 1MT-treated mice. **CONCLUSION:** DCs cells of resistant and susceptible mice use the

enzyme IDO to control mechanisms of immunosuppression in infected mice, diminishing fungal burdens but also suppressing T cell proliferation.

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DETECTION OF GITR ON PATIENT CERVIX INFECTED BY HUMAN PAPILLOMAVIRUS (HPV)

CACILDA TEZELLI JUNQUEIRA PADOVANI[(1)]; CAMILA MARETI BONIN[(1)]; INÊS APARECIDA TOZETTI[(1)]; ALDA MARIA TEIXEIRA FERREIRA[(1)]; CARLOS EURICO DOS SANTOS FERNANDES[(1)]; THIAGO THEODORO MARTINS PRATA[(1)]; SIMONE DOMINGUES SALES[(1)]; ANA PAULA MACHADO[(1)]; FLÁVIA GATTO DE ALMEIDA[(1)]; IZAIAS PEREIRA DA COSTA[(1)].

[(1)] Universidade Federal de Mato Grosso do Sul.

INTRODUCTION: The Human papillomavirus (HPV), in the anogenital tract, infects basal and parabasal cells of squamous epithelium. The progression of the infection has also been associated with the participation of cells with regulatory properties. Evidence shows that GITR (glucocorticoid-induced tumor necrosis factor receptor) is the second most important surface molecule for the characterization of these cells. This marker, TNFRS superfamily member, is predominantly expressed on CD25 + CD4 + Treg cells and has a strong engagement with these phenotypic and functional cells. So, playing an important role on regulating the immune response. Thus, this study aimed to detect the presence of GITR and CD25 in the patient cervix stroma with and without pathological changes, HPV infected or not. Therefore, to associate the outcomes found with histological findings and viral load, to a better understanding of the immune response in infected microenvironment.

METHODS AND RESULTS: Were used to this study forty-nine cervical samples, included in paraffin, obtained from patients that were submitted to HPV DNA detection by Hybrid Capture (Digene ^{TM}), to the histopathology and to immunohistochemistry analyzes (IHC) of simple marking with antigenic recovery by moist heat and LSAB+Sys HRP (DAKO®) detection system for the presence of CD4. These samples were analyzed by immunohistochemistry for the detection of GITR (R & D systems ®, goat IgG / cod. AF689) and CD25 (Invitrogen ®, clone 143-13/cod.AHS2512) in lymphocytes. This study was approved by CEP/UFMS (nº 975/2007). It was observed that the GITR expression raised with the viral load (p = 0.024) and that the frequency of this marker, in large quantities, in the HPV-positive samples (76.9%) was higher than three times as compared to the negative samples (23.1%). There was statistical significance between the presence of GITR and histopathological findings (p = 0.034), with a GITR predominance, in large quantities, in high-grade lesion samples and carcinoma (53.95%). The results showed a statistical

trend between CD4 and GITR ($p = 0.076$) and CD25 was present in great quantity in all samples.

CONCLUSION: Our results suggest that the detection of GITR may reflect the presence of cells with regulatory profile, indicating a suitable microenvironment for the progression of cervical neoplasia.

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DETECTION OF GITR AND IL-10 ON CERVIX OF HUMAN PAPILLOMAVIRUS (HPV) INFECTED PATIENTS

CAMILA MARETI BONIN[(1)]; CACILDA TEZELLI JUNQUEIRA PADOVANI[(1)]; INÊS APARECIDA TOZETTI[(1)]; ALDA MARIA TEIXEIRA FERREIRA[(1)]; CARLOS EURICO DOS SANTOS FERNANDES[(1)]; LEANDRO SOBRINHO ÁVILA[(1)]; ANA PAULA MACHADO[(1)]; FLÁVIA GATTO DE ALMEIDA[(1)]; IZAIAS PEREIRA DA COSTA[(1)].

[(1)] Universidade Federal de Mato Grosso do Sul.

INTRODUCTION: The progression of Human papillomavirus infection (HPV) has been associated with various factors and recently the involvement of cells with immunoregulatory properties, in particular T regulatory cells (Treg), has been enhanced. Evidence suggests that the Treg constitutively express high levels of GITR (glucocorticoid-induced tumor necrosis factor receptor) and IL-10 (interleukin 10) and these molecules have strong phenotypic and functional engagement with such cells, playing an important role in the negative regulation of immune response. Thus, this study aimed to detect markers of cells with regulatory profile in the cervical stroma of patients with and without pathological changes, HPV-infected or not and therefore to associate the phenotypic characteristics found with histological findings and the viral load to better understand the immune response in the infected microenvironment.
METHODS AND RESULTS: Were used samples of the uterine cervix, included in paraffin, obtained from patients submitted to the detection of HPV DNA by Hybrid Capture (DigeneTM) and to histopathological analysis. Simple marking immunohistochemical reaction was performed with antigen recovery by moist heat and LSAB + LSAB+Sys HRP (DAKO®) detection system. Ninety one samples were used for the detection of IL-10 (anti-IL10 Invitrogen, clone 945A2A5/cod. AHC9102) and 99 for GITR (anti-GITR R&Dsystems®, goat IgG/cod. AF689). This study was approved by CEP/UFMS (n°975/2007). A higher frequency of IL-10 in moderate to large quantity in HPV-positive samples (70.4%) and high viral load (67.9%) was observed. The expression in great quantity of this cytokine predominated in high-grade lesions samples and carcinoma (70.5%). Regarding the detection of GITR, a higher frequency of this marker in moderate to large quantity was also observed in the HPV-positive samples (75.3%) and high viral load (70.5%). The expression of this marker in large quantity predominated in high-grade lesions samples and carcinoma (56.3%).
CONCLUSION: Our results suggest that the detection of GITR and IL-10 in the stroma of the samples may reflect the presence of the cells with regulatory profile, allowing the formation of a suitable microenvironment for the development of cervical neoplasia, without the proper activation of the immune response to the elimination of the antigen.

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VACCINATION WITH SM10.3 ANTIGEN, A MEMBER OF THE MICRO-EXON GENE FAMILY-4 (MEG-4) FROM *SCHISTOSOMA MANSONI*, PROTECTED MICE AGAINST PARASITE CHALLENGING

VICENTE DE PAULO MARTINS[1]; SUELLEN BATISTONI DE MORAIS[1]; NATAN RAIMUNDO GONÇALVES DE ASSIS[1]; BÁRBARA DE CASTRO PIMENTEL FIGUEIREDO[1]; CARINA DA SILVA PINHEIRO[2]; NATASHA DELAQUA RICCI[1]; SÉRGIO COSTA OLIVEIRA[1].

[1] Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Brazil.
[2] Instituto de Ciências da Saúde, Universidade Federal da Bahia, Brazil.

Introduction: The flatworm *Schistosoma mansoni* is a blood fluke parasite that causes schistosomiasis, a debilitating disease that occurs throughout the developing world. Current schistosomiasis control strategies are mainly based on chemotherapy, but many researchers believe that the best long-term strategy to control schistosomiasis is through immunization with an anti-schistosomiasis vaccine combined with drug treatment. The Sm10.3 is a 28 kDa protein expressed in the digestive tract of cercariae, male and female adult worms. Its location is restricted to the esophageal gland and its function may be related to coagulation and digestion of the host red blood cells, processes critical to the survival of the parasite.

Methods and Results: Here, we describe the cloning and expression of the Sm10.3 gene from *S. mansoni* and its potential as a recombinant vaccine. Quantitative real time PCR (qPCR) analysis revealed that Sm10.3 was highly expressed in the schistosomulum stage. Immunization of mice with rSm10.3 induced a mixed Th1/Th2 type of immune response with production of IFN-γ and TNF-α, and low levels of IL-5 into the supernatant of splenocyte cultures. The protection engendered by this vaccination protocol was confirmed by 32% reduction in worm burden, 43% reduction in eggs per gram of hepatic tissue, 24% reduction in the number of granulomas per area and 45% reduction in the granuloma fibrosis.

Conclusion: Taken together, the data herein support the potential of surface exposed antigens from the *S. mansoni* digestive tract as vaccine candidates.

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PHENOTYPIC AND FUNCTIONAL ANALYSIS OF $CD4^+FOXP3^+$ REGULATORY T CELLS (T_{REG}) IN THE EARLY PHASE OF MURINE INFECTION WITH *TRYPANOSOMA CRUZI*.

BEATRIZ VILLAS BÔAS[1]; ROGÉRIO SILVA DO NASCIMENTO[1]; JOSÉ M ÁLVAREZ[1]

[1]Departamento de Imunologia, Instituto de Ciências Biomédicas, Universidade de São Paulo, Brazil.

Introduction: Recently, studying T_{REG} cells in the acute phase of infection with *T. cruzi* (Y strain), we observed their numbers just marginally increase. Besides, acute phase parasitemias and heart pathology in mice treated with monoclonal anti-CD25 (PC61) were not different from that in animals treated with irrelevant monoclonal antibody. These results, although not conclusive, suggest that T cells might not be involved in the regulation of the early immune response to *T. cruzi*. Using $FoxP3^+GFP^+$ mice, we have studied the phenotypic changes in the spleen T_{REG} population along the first weeks of infection with Sylvio X10/4 *T. cruzi* parasites. **Methods and Results**: Spleens from $FoxP3^+GFP^+$ infected and non-infected mice were analyzed for weight, total cell count, number and frequency of $CD4^+FoxP3^-$ and $CD4^+FoxP3^+$ cells, as well as expression of markers representative of the T_{REG} molecular profile. Peak weight and cellularity, as well as peak $CD4^+$ cell numbers, were attained on the 11^{th} day post-infection (d.p.i.). Along the analysis period (4^{th} to 18^{th} d.p.i.) there were no major changes in T_{REG} numbers, but their frequency among $CD4^+$ cells decreased, the percentage of $CD4^+$ cells among total splenocytes being minimally altered. Increased frequencies of large cells (blasts) among $CD4^+FoxP3^+$ and, more notably, among $CD4^+FoxP3^-$ cells were observed. Regarding the expression (MFI) of different markers in the T_{REG} population, we observed a slight increase in FoxP3 levels, a strong progressive increase in CD25 expression that peaked at day 14 p.i., the appearance of a small CTLA-4^{HIGH} population, and a late increase in GITR expression. Besides, we observed increases in ICOS in the last days analyzed and an increased expression of Fas (at day 11 p.i) and FasL (at days 11 to 14 p.i.). In addition, CD69 suffered a slight persistent augment, and no changes were observed in CD127 and OX40 expression. Regarding the expression of these markers on the $CD4^+FoxP3^-$ population, the most relevant changes after infection were a brief small increase in CD25, a persisting increase in Fas with presence of a small Fas^{HIGH} subset and no changes in FasL expression, a persisting increase in ICOS, an increase

of OX40 from 7 to 11 d.p.i., CTLA-4 increases on the 7 and 14 d.p.i. and a late augment of GITR. **Conclusion**: The expression changes observed in spleen T_{REG} cells in the early infection by *T. cruzi* do not indirectly support a functional loss in their suppressive capacity.

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THE MURINE INFECTION BY *TRYPANOSOMA CRUZI* CLONE SYLVIO X10/4: IMMUNE SYSTEM INVOLVEMENT IN PARASITEMIA CONTROL

GIOVANA GIACOMINI[1]; ROGÉRIO SILVA DO NASCIMENTO[1]; RAFAEL MOYSÉS SALGADO[1]; DENISE VILARINHO TAMBOURGI[2]; JOSÉ MARIA ÁLVAREZ MOSIG[1]

[1]Departamento de Imunologia, Instituto de Ciências Biomédicas, Universidade de São Paulo, Brazil; [2]Laboratório de Imunohistoquímica, Instituto Butantan/SP, Brazil.

Introduction: *Trypanosoma cruzi*, the parasite responsible for Chagas´ disease, displays wide heterogeneity and includes virulent isolates and low-virulence ones which only yield patent parasitemias in immunodeficient mice. Few studies analyzed the *in vivo* infection with low virulence *T. cruzi* parasites in spite these may account for a large portion of human cases. Here we explored the involvement of the immune system in the low level parasitemia of mice infected with low-virulence Sylvio X10/4 parasites. **Methods and Results**: C57BL/6 (WT) mice inoculated intravenously (iv) with tissue culture Sylvio X10/4 parasites do not show patent parasitemias after 24 hours, the blood clearance curves of these parasites being similar to those of tissue culture Y strain parasites (a high-virulence isolate). Meanwhile, if the inoculated trypomastigotes were isolated from immunodeficient infected mice (SCID or RAG2KO) we observed that Y parasites leave the bloodstream more slowly than Sylvio X10/4 parasites do. Analysis of parasite loads at different organs 48 hours after iv parasite inoculation revealed that, in spite of the known myotropism of Sylvio x10/4, most injected parasites go to the liver and spleen, suggesting an active removal process. The involvement of natural antibodies in Sylvio X10/4 removal was discarded by comparing the blood clearance curves in wild-type or SCID mice (which lack antibodies). A small participation of the complement system was suggested by analyzing Sylvio X10/4 blood removal in WT mice depleted of C3 by cobra venom factor (CVF) treatment. Our observation that Sylvio X10/4-infected IFNg-KO mice showed intermittent patent parasitemias in the first weeks of infection can not be attributed to deficiency in the production of acute phase proteins, in as much as these mice were found to secrete high levels of serum amyloid A and serum amyloid P. As infection by Sylvio X10/4 parasites progressed, parasite-specific antibodies became crucial for parasite control, given that infected B-KO mice succumbed to infection in 2-3 months. In addition, a protective role was suggested for parasite-specific IgM since CD28-KO mice, which do not produce specific IgG, are more resistant than B-KO to infection by Sylvio X10/4. **Conclusion**: We suggest that the rapid

clearance that follows Sylvio X10/4 iv inoculation is an active removal process. At the long run, not only IgG, but also IgM parasite-specific antibodies play an important role in protection.

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KILLER CELL IMMUNOGLOBULIN-LIKE RECEPTOR GENES IN PATIENTS WITH CHAGAS DISEASE

CHRISTIANE MARIA AYO[1]; PÂMELA GUIMARÃES REIS[1]; CAMILA DE FREITAS OLIVEIRA[1]; EMÍLIA ÂNGELA SIPPERT[1]; MÁRCIA MACHADO DE OLIVEIRA DALÁLIO[1]; JEANE ELIETE LAGUILA VISENTAINER[1]; ANA MARIA SELL[1]

[1] Universidade Estadual de Maringá

Introduction: Chagas disease, caused by *Trypanosoma cruzi,* occurs throughout Latin America and is one of most serious parasitic diseases affecting millions of people. The disease is classified in acute and chronic phase but the clinical manifestations vary from one endemic area to another and this variation can be attributable to genetic factors. KIR genes (killer cell immunoglobulin-like receptor) encode molecules activating and inhibitory function of NK cells (natural killer) and have as ligands HLA (human leukocyte antigen) class I. Both KIR and HLA molecules are highly polymorphic and the specific KIR-HLA allelic combinations may regulate NKcell-mediated immunity against infectious pathogens. The aim of this study was to investigate the association of *KIR* genes with their ligands HLA in chronic chagasic patients and controls in a population from North/Northwest of Parana State, South of Brazil. **Methods and Results:** A total of 50 patients and 65 controls were evaluated and all they were typed for 16 *KIR* genes and *HLA* class I alleles by rSSO technique (One Lambda Inc., USA).The observed gene frequencies were determined by direct counting and statistical analysis was performed using the Fisher exact test and Chi Squares with Yates correction. Individual analysis of these KIR genes did not show significant correlation. However when analyzed the interation between KIR-HLA ligands, KIR3DL2 - HLA-A3/11(p: 0.004; OR: 0.21; CI: 0.05-0.66) was significantly more frequent in the controls. A significant increased of the ligands HLA-A3/11 also was observed in the controls group (p: 0.007; OR: 0.25; CI: 0.07-0.73), when these ligands were analyzed separately. **Conclusions:** We did not observe the influence of *KIR* genes and their HLA ligands (-A,-B and-C) in the susceptibility to Chagas disease. As KIR3DL2 is a framework gene, present virtually all genotypes, significance in the control group may have been because of HLA-A03/11 frequencies only. More investigations will be done in order to understand the role of *KIR* genes in Chagas disease.

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EhMSP-1 - AN ENTAMOEBA HISTOLYTICA SURFACE METALLOPROTEINASE AS A PROMISING CANDIDATE FOR VACCINE DEVELOPMENT

EDUARDO CROSARA RONCOLATO (PG)(1); JOSÉ EDUARDO TEIXEIRA (PhD)(3); GILVAN PESSOA FURTADO (PG)(1); RICHARD JOHN WARD (PHD)(2); JOSÉ ELPIDIO BARBOSA (MD, PhD)(1); CHRISTOPHER D. HUSTON (MD)(3)

(1). Department of Biochemistry and Immunology, School of Medicine of Ribeirão Preto, USP, Brazil; (2). School of Philosophy, Sciences and Letters of Ribeirão Preto, USP, Brazil; (3). Department of Medicine, University of Vermont, USA

Introduction: Amebiasis, regarded by the WHO as one of the major health problems in developing countries, is the disease caused by the enteric protozoan parasite *Entamoeba histolytica*. Although this disease causes approximately 100,000 deaths annually, the mechanism that triggers the switch to a pathogenic phenotype, leading to the extra intestinal disease form, is still unknown. The invasive infection requires penetration of the intestinal wall, a protease-dependent process. Huston's group recently described a new metalloproteinase expressed on the *E. histolytica* surface, EhMSP-1. This protease was shown to be immunogenic and possibly involved in migration through the intestinal mucosa. EhMSP-1 silencing increases *E. histolytica* adherence to host cells, decreases motility and enhances phagocytic ability. The goal of this work is to investigate whether immunization of mice with a recombinant form of EhMSP-1 would protect the animals in a further challenge with the parasite. **Methods and results:** The whole EhMSP-1 encoding gene was cloned within the PPIC9 plasmid, a specific vector for the expression of the metalloproteinase on *Picchia pastoris*. After DNA incorporation by *P. pastoris*, the expressed recombinant protein was recovered from the supernatant after 72h of culture. This system allows the expression of large amounts of protein and is LPS free. Five 17kDa fragments from the metalloproteinase were also cloned and expressed in *E. coli*. The fragment containing the metalloproteinase motif was used as bait for selection of scFvs by Phage Display technology. The next steps will comprise immunization of the animals with the whole protein and further challenge with *E. histolytica*. The response profile after contact with the parasite will be analyzed. Splenocytes will also be stimulated with each fragment to evaluate the immunodominance of the protein. **Conclusion:** The cloning steps were accomplished and the protein was expressed in the culture supernatant. Selection of specific scFvs was also successful. Functional assays will be performed in order to evaluate the functionality of the recombinant protein.

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IDENTIFICATION AND CHARACTERIZATION OF IMUNOGENIC PROTEINS FROM *Fonsecaea pedrosoi* EXOANTIGENS.

POLLYANNA CHRISTINA DA SILVA MARTINS[(1)],, GILBERTO HIDEO KAIHAMI[(2)], GRASIELLE PEREIRA JANNUZZI[(1)], SANDRO ROGÉRIO DE ALMEIDA[(1)]
[(1)]Department of Clinical and Toxicological Analysis, Faculty of Pharmaceutical Sciences, University of São Paulo
[(2)] Department of Biochemistry, Faculty of Pharmaceutical Sciences, University of São Paulo

Introduction and Objects:

The chromoblastomycosis is a subcutaneous mycosis characterized by the appearance of polymorphous post-traumatic lesions, where indeed the fungus was inoculated. These injuries range from erythematous to nodule form and the gravity may involve deeper tissues like muscle and bone. It is endemic in countries with tropical and subtropical climate, generally affects individuals involved to the manipulation of the soil. Knowledge is scarce about the host-parasite relationship in this mycosis. Then is necessary to study the antigens released during infection. For this purpose, mice were infected with the main agent *Fonsecaea pedrosoi*, and serum was used to identify immunologically reactive proteins.

Methods and Results:

BALB / c mice were immunized with *Fonsecaea pedrosoi* and after the period of 15 and 30 days, the serum was collected and used in Western blot with 2D SDS-PAGE with proteins from fungus exoantigen.

Two major proteins of *Fonsecaea pedrosoi* exoantigen at approximately 25 kDa and another at approximately 40KDa were identified.

Conclusion:

We suggest that 40KDa and 25KDa are the major proteins involves in the pathogenic process of experimental chromoblastomycosis. These proteins may be prepared for sequencing that is the next stage of this work.

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THERAPY WITH SCFV TRANSFECTED-DENDRITIC CELLS INDUCE A DECREASE LEVELS OF SPECIFIC IGG UPON *PARACOCCIDIOIDES BRASILIESNSIS* INFECTION

GRASIELLE PEREIRA JANNUZZI[(2)], JOSÉ ROBERTO FOGAÇA DE ALMEIDA[(2)], SANDRO ROGÉRIO DE ALMEIDA[(2)] and KAREN SPADARI FERREIRA[(1)]
[(1)] Institute of Environmental Sciences, Chemical and Pharmaceutical UNIFESP, Diadema/SP; [(2)]Department of Clinical and Toxicological Analysis, Faculty of Pharmaceutical Sciences, University of São Paulo.

Introduction and Objects: Paracoccidioidomycosis (PCM) is a systemic mycosis endemic in Latin America with a high prevalence in Brazil. The etiologic agent of disease is the *Paracoccidioides brasiliensis (Pb)*. A gp43 is the major antigen of *Pb*. It has been shown that mice immunized with anti-gp43 monoclonal antibodies (MAbs) (Ab1), induce the idiotypic cascade in the gp43 system, which produced both, anti-Id antibodies (Ab2) and anti-anti-Id antibodies (Ab3). Ab2 named 7B12 inhibited (>95%) the binding of gp43 to Ab1, suggesting that this anti-Id MAb bind to the idiotope, thus fulfilling the internal image criteria. To better characterization of 7B12 MAbs we developed antiidiotypic antibody single chain variable fragments (scFv) and we showed that dendritic cells (DCs) transfected with scFv from Mab 7B12 mimicking original antigen gp43 induces protection against experimental PCM. Accumulating evidence, however, suggests that immune priming is initiated by professional APC rather than by these myocytes. Since professional APC are not typically found in normal muscle tissue, the objective of this study was analyze the capacity of DCs in migrate to the site of DNA inoculation in response to inflammatory signals and analyze if infiltrating APC may then either take up the plasmid DNA directly or cross-present expressed antigen to initiate immune responses. **Methods and Results:** Mice were immunized subcutaneously with pMAC/PS-scFv and after 7 days we assess the types of cells recruited into regional lymph nodes by flow cytometry (5 animals/group). Signifcance test was carried out by Anova and Turkey. We observed that the population of DCs was increased into regional lympho nodes when compared with control. These DCs expressed high levels of CD40, CD8, DEC205 and MHCII suggesting the capacity of presenting antigen and a decreased of regulatory T cells also was observed. Besides, therapy with pMAC/PS-scFv transfected-DCs induced decrease levels of specific IgG was upon Pb infection. **Conclusion:** These studies describe that pMAC/PS-scFV plasmid can be utilized to recruit DCs into regional lymph nodes and initiate the immune responses of T lymphocytes, besides to induce an efficiently immune response with a decrease of regulatory T cells and antibody production.

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13173 - COMPARISON OF ANTI-LEISHMANIAL IGG ANTIBODIES PROFILE IN SERA FROM BRAZILIAN PATIENTS WITH VISCERAL LEISHMANIASIS AND INDIVIDUALS PRESENTING ASYMPTOMATIC INFECTIONS

LUIZ GUSTAVO ARAUJO GARDINASSI[1]; GUSTAVO ROCHA GARCIA[1]; SANDRA REGINA MARUYAMA[1]; FERNANDO OLIVEIRA DA SILVA[2]; CARLOS HENRIQUE NERY COSTA[2]; ROQUE PACHECO DE ALMEIDA[3]; AMÉLIA MARIA RIBEIRO DE JESUS[3]; ISABEL KINNEY FERREIRA DE MIRANDA SANTOS[1]

[1]Faculdade de Medicina de Ribeirão Preto - USP, Departamento de Bioquímica e Imunologia; [2]Instituto de Doenças Tropicais Natan Portella - UFPI; [3]Centro de Ciências Biológicas e da Saúde – UFS, Depto. de Medicina Interna e Patologia

Introduction: It is intriguing that the majority of infections with the etiological agent of visceral leishmaniasis (VL), *Leishmania infantum*, do not result in disease. The scientific consensus is that antibodies do not have a role in this outcome and, in fact, participate in susceptibility to VL; however recent studies challenge this view. We aim to test the hypothesis that individuals presenting with asymptomatic infections with *L. infantum* remain so because they produce antibodies with functional properties that differ from those produced by individuals who develop VL.

Methods and Results: With immunoblots and ELISAs we will study the correlation of different outcomes of infections with L. infantum (VL or asymptomatic infection) with antigen reactivity profiles and titers of IgG1-4 antibodies in serum from humans of different ages. Description of patterns of IgG antibody responses will be refined by probing sera with a protein microarray constructed with approximately 2300 secreted or plasma membrane proteins of *L. infantum*. Sera were collected from 208 kala-azar patients, 197 from individuals with asymptomatic infection and 138 from healthy donors, all living in endemic areas. Western blots were performed between soluble antigens (SLA) of *L. infantum* and sera from individuals presenting with VL or infected asymptomatically. Indeed, sera from VL patients recognize a set of antigens that is distinct from those recognized by sera from infected, asymptomatic individuals, and the pattern of recognition differs both by specificity and intensity. ELISAs were performed to screen IgG antibodies against SLA

between the groups. The results show that VL patients present higher titers (~10 fold) of IgG against SLA than asymptomatic carriers. Despite this data, a statistically significant difference between asymptomatic individuals and healthy controls was obtained (P<0,001), which indicates that specific IgG propertires (such as their subclasses and specificities) should be evaluated

Conclusion: SLA preparation may not be suitable to evaluate antibodies with the correct functional properties, participating in favorable outcomes of *L. infantum* infections. Thus, secreted and plasma membrane proteins of were extracted and will be used on immunoblots and ELISAs, which may represent the most appropriate antigens to verify efficient immune response mediated by antibodies against *L. infantum*.

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IMMUNE RESPONSE TO *LUTZOMYIA INTERMEDIA* SALIVA IN HOUSEHOLDS CONTACTS OF CUTANEOUS LEISHMANIASIS PATIENTS

AUGUSTO MARCELINO PEDREIRA DE CARVALHO[(1)]; JUQUELINE R. CRISTAL [(1)]; JOSÉ CARLOS MIRANDA[(1)]; EDNALDO LAGO[(2)]; ALDINA BARRAL[(1)]; EDGAR M CARVALHO[(2)]; CAMILA I DE OLIVEIRA[(1)]

[(1)]Centro de Pesquisas Gonçalo Muniz, FIOCRUZ, Salvador, Bahia, Brasil; [(2)]Serviço de Imunologia, Complexo Universitário Professor Edgard Santos, Universidade Federal da Bahia, Salvador, Bahia, Brasil.

Introduction: Cutaneous leishmaniasis (CL) is a disease caused by protozoa of genus *Leishmania* and transmitted through the bites of infected sand flies. Previous studies showed that pre-exposure to *Lutzomyia intermedia* saliva, the main vector of *Leishmania braziliensis* in Brazil, resulted in a large cellular recruitment, modulation of the inflammatory response induced by parasite and enhancement of *Leishmania* infection, in mice. In humans, evidence of humoral immunological response to salivary gland sonicate (SGS) was higher in CL patients than individuals with asymptomatic *L. braziliensis* infection. We hypothesized exposure to sand flies saliva influences the outcome of *L. braziliensis* infection, in residents of an endemic area for CL. **Methods and Results**: Individuals (N=264) living in the endemic area of Corte de Pedra, Bahia, were evaluated regarding serology and cellular immune response to SGS. Anti-SGS antibodies were found in 150 (56.8%) individuals and higher antibody titers were found in younger (<30 years old) individuals. To evaluate the cellular immune response, mononuclear cells from individuals with anti-SGS positive serology were stimulated with SGS. We observed an increased production of IFN-gamma. Anti-SGS serology was also higher in individuals without evidence of immune response to *Leishmania* [OD 0.05 (0 – 0.4)] when compared to subjects with positive *Leishmania* skin test or IFN-gamma production [OD 0.03 (0 – 0.3)], in response to *Leishmania* antigen. **Conclusion**: Evidence of humoral and cellular immune response to SGS was found in residents of an endemic area for CL. Moreover, individuals with cellular immune response to *Leishmania* have lower antibody titers to SGS, suggesting that exposure to sand flies modulates the immune response to infection with *L. braziliensis*.

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TRYPANOSOMA CRUZI PARASITE INDUCES RELEASE OF NEUTROPHIL EXTRACELLULAR TRAPS (NETs).

Daniel Sousa-Rocha[1]; Larissa Figueiredo Alves Diniz[1]; Mariana Thomaz-Tobias[1]; Priscila Silva Sampaio Souza[1];Bruna Castilho Soto Campanha[1]; Phileno Pinge-Filho[2]; Karina Alves Toledo[1]

[1]Faculdade de Ciências e Letras de Assis, UNESP - Univ Estadual Paulista;

[2] Universidade Estadual de Londrina.

Introduction: Diseases triggered by protozoa consist of serious public health problem, mainly due to high relative mortality associated with them. The Chagas Disease, described for the first time by Carlos Chagas in 1909, which the aetiological agent is the *Trypanosoma cruzi*, can develop fatal events as inflammation of the myocardium, the meninges and brain regions. Currently, control of replication of the parasite by drugs involves intense immune response included innate immunity cells such as monocytes, eosinophils and neutrophils. NETs (Neutrophil Extracellular Traps) have been described as a new mechanism of the microbicide neutrophils to

quickly capture and kill many pathogens, including bacteria, fungi and parasites as *Toxoplasma gondii* and *Leishmania sp*. Here, our aim was to investigate if *T.cruzi* parasite and its soluble molecules are able to stimulate to form and release NETs from human neutrophils. **Methods:** In order to answer our questions, human neutrophils were isolated from blood by using sedimentation gelatin method and co-cultivated with different number of parasites or different doses of soluble extract from *T.cruzi* parasite. The end of incubation the cells were (i) fixated and incubated with DAPI for analysis by fluorescence microscopy or (ii) incubated with MTT salt.

Results: Images acquired and analyzed has been demonstrated that *T.cruzi* and its soluble molecules induced NET release by human neutrophils in a parasite number- and dose-dependent manner. This release was similar to positive control (fMLP 10^{-6}M) and opposite to negative control (RPMI only). Interestingly, when the neutrophils were incubated with the parasite, we could see small bright blue dots on the NETs. This effect did not observed when neutrophils were incubated with soluble molecules from parasite. During co-incubation, cell viability was not affected since the MTT salt was equally metabolized in all tested conditions. **Conclusion:** Together, our preliminary results suggest that *T.cruzi* and its soluble molecules are able to induce the

release of NETs from human neutrophils without affecting the viability of these cells. In addition, the NETs could capture extracellular parasites. Extend this study will assist in developing new strategies to control the inflammatory response during infection by *T.cruzi* and to control the pathogenesis associated with it.

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ROLE OF PROSTAGLANDIN E_2 IN MICE LUNG INFECTION INDUCED BY *Achromobacter xylosoxidans.*

MORGANA KELLY BORGES PRADO [2]; PRISCILLA APARECIDA TARTARI PEREIRA [2]; ANA LUCIA DA COSTA DARINI [1]; JOSEANE CRISTINA FERREIRA [1]; LUCIA HELENA FACCIOLI [1].

[1]. Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Departamento de Análises clínicas, Toxicológicas e Bromatológicas - USP.

[2]. Programa de Pós-Graduação em Imunologia Básica e Aplicada, Departamento de Bioquímica e Imunologia - FMRP- USP.

Introduction: *Achromobacter xylosoxidans* is an aerobic bacillus, gram negative, glucose non-fermenting, oxidase positive and mobile, which normally colonizes the digestive tract and the environment. Recently, this bacillus has been found in severe opportunistic infections in immunosuppressed individuals. Virulence factors, escape mechanisms and antibiotic multi-resistance make the *A. xylosoxidans* infections difficult to be controlled. Cancer and cystic fibrosis (CF) seem to facilitate the development of infection. These diseases have as characteristic the production of prostaglandin (PG) E_2. PGE_2 in bacterial pulmonary infections has potent immunosuppressive properties, since this mediator inhibits phagocytosis and killing of the pathogen by alveolar macrophages. However in the literature there are no studies demonstrating the role of PGE_2 in the infection by *A. xylosoxidans*.
Aim: In this context, we hypothesized that the increased PGE_2 production may favor infection and persistence of the microorganism in alveolar macrophages. In the light of this, our purpose is to investigate the role of PGE_2 in *A. xylosoxidans* infections.
Methods: To address this question, C57Bl/6 mice will be infected with *A. xylosoxidans* and treated or not with COX-2 inhibitor (celecoxib) or with PGE_2 synthesis inhibitor (CAY10526). The mortality rate, kinetics of cell recruitment to bronchoalveolar space and cell phenotype, cytokine profile and pulmonary histopathology will be evaluated. Furthermore, alveolar macrophages will be infected *in vitro* to *A. xylosoxidans* and treated or not with celecoxib, EP1/EP2 receptor antagonist (AH6809) and soluble PGE_2 for evaluation of phagocytosis, microbicidal activity and cytokine profile.

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ROLE OF CD18 IN ACTIVATION OF M1 MACROPHAGES INFECTED WITH *LEISHMANIA AMAZONENSIS*

ANA CAROLINA PAGLIARONE(PG)(1,2); KARINA FURLANI ZOCCAL (PG)(1); CARLOS ARTÉRIO SORGI(1); LUCIA HELENA FACCIOLI(1,2)

(1) Departamento de Análises Clínicas, Toxicológicas e Bromatológicas – Faculdade de Ciências Farmacêuticas-USP Ribeirão Preto-SP

(2) Pós-graduação em Imunologia Básica e Aplicada – Faculdade de Medicina - USP Ribeirão Preto-SP

Introduction: Macrophages are pivotal in the primary response against pathogens. Depending on the cytokines that activate these cells, they can be classified as M1 or M2 macrophages, in which activation occurs through IFN-γ and IL-4, respectively. M1 macrophages are essential against infections, by producing high concentration of effector molecules such as nitric oxide (NO) and inflammatory cytokines, while the M2 subtype controls the inflammatory response and inhibit respiratory burst through arginase metabolism (Nat. Rev. Immunol. 8: 958-969, 2008). *Leishmania sp* survive and multiplicate in macrophages due to mechanisms that inhibit cellular microbicidal responses. The parasites interact with macrophages through molecules expressed in cell surface such as CD18, a component of β2 integrins (Trends Parasitol. 28: 335-344; 2012). The aim of this study is to investigate the role of CD18 molecule in activation of M1 macrophages during *L. amazonensis* (*L.a.*) infection *in vitro*. **Methods and results:** bone marrow-derived macrophages (BMDMs) obtained from C57BL/6 (WT) or $CD18^{low}$ mice were cultured in DMEM-medium (supplemented with L-929 supernatant and horse serum) for 10 days in 37°C and 5%CO_2. The adherent cells were washed and cultured with IFN-γ for 18h. After this time, the cells were infected or not with *L.a.* promastigotes at parasite/macrophage ratio 5:1 for 24h, in the presence or absence of LPS. Cellular viability was evaluated by rezasurin reduction method. Supernatants were collected for cytokines quantification by ELISA assay and for NO production by Greiss reaction. More than 80% of cells were viable in all experimental conditions. NO and TNF-α were increased in $CD18^{low}$ M1 macrophages comparing to WT cells after *L.a.* infection, in the absence of LPS. IL-10 production was also significantly increased in $CD18^{low}$ cells comparing to WT macrophages in all conditions. However, IL-10 was produced in higher concentrations than TNF-α by $CD18^{low}$ macrophages during infection. **Conclusion:** these preliminary results show that although leishmania infection induced NO in both WT and $CD18^{low}$ M1 macrophages, low expression of CD18 lead to higher IL-10 production comparing to TNF-α, which could favor the parasite intracellular survival.

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IDENTIFICATION, PURIFICATION AND CHARACTERIZATION OF CHITINASE ENZYME PRESENT IN TOTAL SOLUBLE ANTIGEN OF *TOXOPLASMA GONDII* TACHYZOITES

FERNANDA MARIA SANTIAGO[1]; LAURA MARQUES KNYCHALA[1]; VANESSA DOS SANTOS MIRANDA[1]; MYLLA SPIRANDELLI DA COSTA[1]; AMANDA DE OLIVEIRA CUNHA[1]; JOSÉ ROBERTO MINEO[1]

(1) LABORATORY OF IMMUNOPARASITOLOGY DR. MÁRIO ENDSFELDZ CAMARGO; INSTITUTE OF BIOMEDICAL SCIENCES; FEDERAL UNIVERSITY OF UBERLÂNDIA, MG-BRAZIL.

Introduction: *Toxoplasma gondii* is an obligatory intracellular parasite that has the cat as definitive host and a great varied of other mammals and birds as intermediate hosts. There are three major life stages of *T. gondii:* the tachyzoite, which is involved in acute infection and dissemination of the parasite in its host; the bradyzoite, which is found in tissue cysts and latent infection, and the oocysts. The present study aimed to isolate and characterize chitinase enzyme from *T. gondii* tachyzoites (Ch-Tg). **Methods and results:** After obtaining the total antigen of *T. gondii* (STAG), it was subjected to electrophoresis according to REISFELD for proteins with basic characteristics. Then, Ch-Tg chitinase was withdrawn from the gel, pooled, and added to Ambic buffer, pH 7.4, centrifuged at 14.000*g* and lyophilized. The enzyme characterization was performed by using the synthetic substrate of *p*-nitrophenyl-*N*-acetyl-beta-D-glucosamine at pHs in the range of 4.0 to 7.4 and temperatures ranging from 25ºC to 60 ºC. Balb/c mice were immunized with Ch-Tg and serum samples were collected to be tested for seroconversion. Later on, the animals were challenged with cystogenic strain of *T.gondii* (Me-49). The results showed that the isolated enzyme Ch-Tg presented molecular weight of 115 kDa and had a high stability at an optimal temperature of 37°C in pH 7.4. In terms of immunolocalization of the chitinase enzyme, a delineated fluorescent reactivity was seen at the external membrane of the parasite, whereas the apical pole showed an undefined but specific staining. Concerning the challenge with Me-49 strain, it was observed a decrease of brain cyst numbers of the group of the animals immunized with the Ch-Tg compared to the control group (188 ± 31 and 1757 ± 100, respectively). **Conclusion:** The animals immunized with the enzyme present a protective immune response against *T. gondii* infection, as the challenge with the strain Me-49 results in an amount of 10 times lower number of cysts than the unimmunized animals.

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THE INVOLVEMENT OF HEME OXYGENASE-1 (HO-1) IN THE CONTROL OF *Toxoplasma gondii* REPLICATION IN C57BL/6 MICE

ESTER CRISTINA BORGES ARAUJO[1]; BELLISA DE FREITAS BARBOSA[2]; PAULO VICTOR CZARNEWSKI BARENCO[1]; LUCIANA ALVES DE SOUSA[1]; LOYANE BERTAGNOLLI COUTINHO[1]; ELOISA AMÁLIA VIEIRA FERRO[2]; DEISE APARECIDA OLIVEIRA SILVA[3]; CRISTIANE MARIA MILANEZI[4]; GIULIANO BONFÁ[4]; JOÃO SANTANA SILVA[4]; JAIR PEREIRA DA CUNHA-JÚNIOR[3]; NEIDE MARIA SILVA[1]

[1]Laboratory of Immunopathology, Institute of Biomedical Sciences, Federal University of Uberlândia,

[2]Laboratory of Histology and Embryology, Institute of Biomedical Sciences, Federal University of Uberlândia,

[3]Laboratory of Immunoparasitology, Institute of Biomedical Sciences, Federal University of Uberlândia,
[4]Department of Biochemistry and Immunology, School of Medicine of Ribeirão Preto, University of São Paulo.

Introduction: *Toxoplasma gondii* is an apicomplexan parasite characterized to induce type 1 immune response in infected hosts and the disease occurs mainly in immunocompromised individuals. Heme oxygenase-1 (HO-1) is the enzyme that catabolizes free heme that induces an intense inflammatory response. Furthermore, the expression of HO-1 is induced by different stimuli, triggering an anti-inflammatory response during biological stress. Additionally, it was previously verified that HO-1 are able to induce the indoleamine 2,3-dioxygenase (IDO), an enzyme known to be involved in the parasite control.

Methods and Results: To verify the role of HO-1 during *in vivo* infection, BALB/c and C57BL/6 mice were infected with ME49 strain of *T. gondii* and treated during 12 days with zinc protoporphyrin IX (ZnPPIX) or hemin, which inhibit or induce HO-1 expression, respectively. The results showed that C57BL6 and BALB/c mice treated with ZnPPIX showed higher tissue parasitism in the lung (BALB/c: 3.71 ± 2.36; C57BL/6: 35.00 ± 19.8), whereas treatment with hemin decreased the replication of the parasite in this organ (BALB/c: 0.35 ± 0.43; C57BL/6: 1.82 ± 2.37) and in the small intestine of C57BL/6 infected mice (1.25 ± 0.93). It was observed that *T. gondii* infection induced high levels of HO-1 mRNA in the lung of infected mice. Furthermore, C57BL/6 mice treated

with hemin showed higher levels of IDO mRNA (10.34 ± 2.46) and protein expression (2.71 ± 1.17) than non-infected mice (1.33 ± 1.27; 1.02 ± 0.93) in the lung.

Conclusion: In conclusion, our data suggest that the control of the parasite in the lung of infected mice is at least in part dependent of HO-1 through IDO induction, mainly in C57BL/6 mice. In the small intestine elevated HO-1 expression contributes to the control of *T. gondii* replication, but in an IDO independent manner.

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Characterization of fractions of *Leishmania amazonensis* extract with immunomodulatory activity

Cintia Figueiredo de Araújo[1]; Virgínia Maria Góes da Silva[2]; Lain Carlos Pontes de Carvalho[1]

[1] Centro de Pesquisa Gonçalo Moniz (IGM-FIOCRUZ);[2] Universidade Estadual do Sudoeste da Bahia (UESB)

INTRODUCTION: Parasites of the genus *Leishmania* secrete molecules that modulate the host immune system and thus contribute to the establishment of the infection, changing the microbicidal activity of macrophages (Exp. Parasitol.127: 46-51, 2011). Identification and functional characterization of parasite molecules may clarify the pathogenesis of leishmaniasis and contribute to the development of prophylactic and therapeutic methods. The aim of this study was to purify and characterize molecules with immunomodulatory activity in extracts of *Leishmania amazonensis* amastigotes.

METHODS AND RESULTS: *L. amazonensis* extract (*La*E) was prepared (Infect. Immun. 79: 1236-1243, 2011) and subjected to non-denaturing electrophoresis on 12% polyacrylamide gel. Fractions with molecules with apparent weights of 68 kDa, 45 kDa, 36 kDa and 28 kDa were chosen, based on previous results and processed according to a previously described protocol (PloS One 4: e5820, 2009). To analyze the immunomodulatory effect of the fractions, BALB/c mice (n=8) were injected intradermally with saline or 5 µg in 50 µL of *LaE* or of the fractions. After one week they were inoculated in the right hind footpad with 10^7 promastigotes of *L. braziliensis* in stationary phase. The evolution of the lesions was monitored weekly by measuring with a digital caliper, until six weeks after infection. The fractions of 68 kDa and 28 kDa increased significantly the lesion from the second to the sixth week ($p<0.05$). Parasite loads in the footpads were estimated by limiting dilution, and the fractions of 68 kDa, 36 kDa and 28 kDa produced results significantly ($p <0.05$) different from saline. Supernatants from cultures of anti-CD3 - stimulated draining lymph nodes cells were collected and tested for the presence of IL-4 and IL-10 by ELISA, following the protocol of the kit manufacturer (eBioscience, San Diego, CA). No statistically signicant differences in these cytokine concentrations were observed.

CONCLUSION: The fractions of 68 kDa and 28 kDa of *La*E enhances *Leishmania* infection in BALB/c mice when injected intradermally, however IL-4 and IL-10 do not seem to be involved in this effect.

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SEPTIC ARTHRITIS TRIGGERED BY *S. aureus* ATTC 19095 SEC$^+$ IS ASSOCIATED WITH HIGH IL-17 LEVELS

PRISCILA MARIA COLAVITE (PG)[(1)], LARISSA LUMI WATANABE ISHIKAWA(PG)[(1)], THAÍS GRAZIELA DONEGÁ FRANÇA(PG)[(1)],SOFIA FERNANDA GONÇALVES ZORZELLA-PEZAVENTO(PG)[(1)], LARISSA CAMARGO DA ROSA (PG)[(1)], LAÍS CALISSI BRISOLLA TAVARES (IC)[(1)], FERNANDA CHIUSO-MINICUCCI [(1)], GUSTAVO POMPERMAIER GARLET [(2)], MARIA DE LOURDES RIBEIRO DE SOUZA DA CUNHA[(1)], ALEXANDRINA SARTORI [(1)].

(1) Department of Microbiology and Immunology, Biosciences Institute, Univ. Estadual Paulista (UNESP), Botucatu, São Paulo, Brazil.
(2)Department of Biological Sciences, School of Dentistry of Bauru, São Paulo University-FOB/USP, Bauru, São Paulo, Brazil.

Introduction: *Staphylococcus aureus* is the most common causative agent of septic arthritis that is a severe, rapidly progressing and erosive disease. Superantigens produced by *S. aureus* are considered major arthritogenic factors. IL-17 is able to promote cartilage destruction and bone erosion in experimental arthritis. The objective of this study was to evaluate the level of IL-17 production during the development of septic arthritis triggered by *S. aureus* ATCC 19095 SEC$^+$.
Methods and Results: Male mice were intravenously infected with the *S. aureus* strain ATTCC 19095 SEC$^+$. Clinical parameters as body weight, clinical score (arthritic index scale varying from 0 to 3) and disease incidence were daily evaluated. The production of IL-17 by spleen cells stimulated with a particulate *S. aureus* Cowan I antigen (SAC) and ConA were evaluated 7 and 14 days after the infection. Infected animals (5-8 mice/group) presented a significant weight loss (around 15%) that remained until the end of the experiment (14 days after infection). The average clinical score varied according to the bacterial inoculum. The group infected with 1.10^6 CFU, that was sacrificed 7 days after infection, reached the score 1.5 whereas the group infected with 2.10^7 CFU, sacrificed 14 days after, reached the score 2.3. The arthritis incidence was similar after 7 (75%) and 14 days (80%). A significant production of IL-17 stimulated with SAC was detected during both periods of evaluation, however the levels were significantly higher at the 7th (462 ± 370pg/mL) then at the 14th day (76 ± 45pg/ml). When stimulated with ConA the production IL-17 was also higher at the 7th day.
Conclusion: The *S. aureus* strain ATTCC 19095 SEC$^+$ triggered a high incidence of septic arthritis in C57BL/6 mice that was concomitant with elevated levels of IL-17 production.

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PATTERN RECOGNIZING RECEPTORS (PRRs) INVOLVED IN EICOSANOID PRODUCTION BY HUMAN NEUTROPHILS IN RESPONSE TO *Paracoccidioides brasiliensis*

HELANDERSON DE ALMEIDA BALDERRAMAS (PG)[1]; DANIELA RAMOS RODRIGUES (PG)[1]; TATIANA FERNANDA BACHIEGA (PG)[1]; REGINALDO KELLER FERNANDES (PG)[1]; LUCIANE ALARCÃO DIAS-MELICIO[2]; MARCIMARA PENITENTI[3]; MAURA ROSANE VALÉRIO IKOMA[3]; SILVIO LUIS DE OLIVEIRA[1]; ÂNGELA MARIA VICTORIANO DE CAMPOS SOARES[1]

[1]Microbiology and Immunology Department, Bioscience Institute, UNESP – Univ. Estadual Paulista, Botucatu-SP, Brazil; [2]Department of Pathology, Botucatu Medical School, UNESP – Univ. Estadual Paulista, Brazil; [3]Amaral Carvalho Foundation - Flow cytometry Laboratory, Jaú-SP, Brazil.

Introduction and Objective: *Paracoccidioides brasiliensis* is the agent of paracoccidioidomycosis, the most prevalent deep mycosis in Latin America. Since the etiological agent is considered an intracellular pathogen, studies of its interaction with phagocytes are essential for the understanding of the host-parasite relationship. The interaction between PAMPS (pathogen-associated molecular patterns) and PRRs leads to the activation of phagocytic cells with subsequent production of mediators which modulate antimicrobial activities of these cells. Among these mediators the eicosanoids PGE_2 and LTB_4 have received attention in recent years. Studies on our laboratory have shown that human monocytes in response to *P.brasiliensis* produce PGE_2 and LTB_4 that respectively inhibits and increases antifungal activities of these cells. In this context, we aimed to evaluate whether human neutrophils produce LTB_4 and PGE_2 in response to high (Pb18) and low virulent strain (Pb265) of *P.brasiliensis* and which PRRs are involved. **Methods and Results**: Neutrophils were treated with monoclonal antibodies anti-TLR2, anti-mannose or anti-dectin-1 for 2h followed by challenge with Pb18 or Pb265 for 4 and 18h. After these periods, supernatants were removed and evaluated for LTB_4 and PGE_2 levels, by Elisa. We detected that control cells after 4h produce low amounts of PGE_2 that was significantly increased after challenge with the fungus, mainly with Pb18. At 18h, PGE_2 levels induced by both strains were proportionally increased in comparison to previous period. Blocking assays at 4h revealed that dectin-1 and mannose receptor (MR) were involved in this production. However, at 18h, the role of these receptors was not so clear. In relation to LTB_4, the results showed that Pb18 and Pb265 induce similar levels of this eicosanoid. In addition, this production was similar after 4 and 18h. Of note, differently from PGE_2 results, blocking assays revealed that none of the tested receptors was involved in LTB_4 production. **Conclusions**: *P.brasiliensis* by binding to dectin-1 and MR induces PGE_2 production by human neutrophils,

being higher levels induced by a virulent strain. This difference could represent high capacity of this strain to escape from antifungal mechanisms of neutrophils, since PGE_2 could inhibit some functions of these cells. This fungus also induces LTB_4 production by neutrophils, however by a mechanism independent from dectin-1, TLR2 or MR.

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EXPRESSION OF MMP-2 AND 9 IN SKIN OF DOGS WITH VISCERAL LEISHMANIASIS

ANA PAULA PRUDENTE JACINTHO[1]; MAYARA CAROLINE ROSOLEM[1]; MARCIO DE BARROS BANDARRA[1]; PAMELA RODRIGUES REINA MOREIRA[1]; GISELE FABRINO MACHADO[2]; GUILHERME DIAS DE MELO[2], DANISIO PRADO MUNARI[3]; ROSEMERI DE OLIVEIRA VASCONCELOS[3*].

(1). Programa de Pós-graduação em Medicina Veterinária, FCAV/UNESP, Jaboticabal, SP; (2).FMVA/UNESP, Araçatuba, SP; (3). FCAV/UNESP, Faculdade de Ciências Agrárias e Veterinárias, Jaboticabal, SP; *rosevasc@fcav.unesp.br

Introduction: Dogs infected by *Leishmania (L.) chagasi* may have clinical signs for this zoonosis and they are the main source of infection for man. The skin is the first organ infected by the parasite. The aim of this study was to evaluate the expression of MMP-2 and MMP-9 by immuno-histochemistry and zymography in the skin of dogs naturally affected by protozoa and compare with immunodetection of TNF-α, TGF-β and *Leishmania* sp. and with the alterations of dermal extracellular matrix.
Methods and Results: This study included 36 dogs, without preference for age, breed or sex, from the Zoonosis Control Center of Araçatuba (SP), where they were euthanized due to the positive diagnosis of visceral leishmaniasis (VL). One control group was formed with dogs from non-endemic area for VL (CEUA nº. 020373/09). In immunohistochemical analysis we used the antibodies MMP 2 and 9 (Abcam), TNF-α and TGF-β (Santa Cruz Biotecnology) and for the parasite load was used canine hyperimmune serum (J. Immunol. Meth. 292:17-23, 2004). The gelatinolytic activity was assessed by zymography (Vet. Immunol. Immunopathol. 136:340-345, 2010).The Picrosirius red staining was used to differentiate collagen types I and III, in dermis of the muzzle, ear and abdomen, in asymptomatic (GA), oligosymptomatic (GO) and symptomatic dogs (GS). The parasite load (P=0.0001) and intensity of inflammation were higher in regions of the ear (P=0.1709) and muzzle (P=0.0235) mainly in dogs GS. In the dogs GA and GO the skin inflammation was discrete or absent. The active form of MMP-9 predominated in the GS group and same skin areas. The macrophages were significantly different from lymphocytes in the cutaneous inflammatory infiltrate (P =0.01) in ear and muzzle of GS dogs. In this case there were granulomas associated with degradation of mature collagen (type I) and discrete deposition of the young collagen (type III), mainly in dogs with high

parasite load skin. The TGF-β was detected in all groups of infected dogs (P>0,05). The TNF-α was not detected in infected animals and the control group.

Conclusion: We conclude that the higher parasite load and severity of inflammation in the skin results in increased degradation of mature collagen, production of active MMP 9 and TGF-β. This profile of the host response, possibly favoring systemic dissemination of the parasite**.**

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Key words: gelatinases A and B, Immunohistochemistry, *Leishmania chagasi*, skin, dog, zymography.

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EFFECT OF AUTHENTIC PGE_2 PRODUCED BY *Paracoccidioides brasiliensis* ON EFFECTOR FUNCTIONS OF HUMAN POLYMORPHONUCLEAR AND MONONUCLEAR CELLS

ANA PAULA BORDON-GRACIANI (1), GUILHERME AUGUSTO BIONDO (PG) (1), LUCIANE ALARCÃO DIAS-MELICIO (2), TATIANA FERNANDA BACHIEGA (PG) (1), MARJORIE DE ASSIS GOLIM (3), JOSÉ ROBERTO MARQUES SILVA (4), ÂNGELA MARIA VICTORIANO DE CAMPOS SOARES (1).

(1) Departamento de Microbiologia e Imunologia, Instituto de Biociências de Botucatu, UNESP – Univ Estadual Paulista, Brasil; (2) Departamento de Patologia, Faculdade de Medicina de Botucatu, UNESP – Univ Estadual Paulista, Brasil; (3) Hemocentro, Faculdade de Medicina de Botucatu, UNESP – Univ Estadual Paulista, Brasil; (4) Núcleo de Pesquisa Avançada em Matologia, Faculdade de Ciências Agronômicas de Botucatu, UNESP – Univ Estadual Paulista, Brasil.

Introduction: Prostaglandins (PGs) are eicosanoids synthesized from arachidonic acid oxidation that regulate key aspects of the immune response in different infections. In paracoccidioidomycosis, human monocytes produce PGs that inhibit the fungicidal activity of these cells *in vitro,* by reducing H_2O_2 and TNF-α production. In addition to mammalian cells, another potential source of PGE_2 is the fungal pathogen itself. We demonstrated that *P. brasiliensis* is able to produce authentic PGE_2, which is directly involved in the survival of this fungus. However, the function of PGE_2 produced by this fungus on host cell activities has not yet been evaluated. Thus, the purpose of this work was to assess the effect of PGE_2 produced by *P. brasiliensis* on the effectors functions of human polymorphonuclear and mononuclear cells. **Methods and Results:** Peripheral blood neutrophils (PMN), monocytes (MO) and PBMC obtained from 10 healthy donors were treated with PGE_2 purified from *P. brasiliensis* yeast cells cultures (PGE_2f) or commercial PGE_2 (PGE_2c). After 1, 2, 4, 8 and 12 hours of incubation, PMN and MO cultures were evaluated by phagocytosis assay, surface receptors expression (TLR-2, TLR4, dectin-1, MR and HLA-DR), H_2O_2, cytokines (TNF-α, IL-6, IL-8, IL-10, IL-12p70, IL-15, IL-17, IL-23, IL-27) and chemokines (MCP-1 and MIP-1α) production. PBMC cultures were evaluated after 24, 48 and 72 hours of incubation with PGE_2f and PGE_2c by cellular proliferation and cytokines production (TNF-α, IFN-γ, IL-2, IL-4, IL-10, IL-12 and IL-17). The treatment with PGE_2c or PGE_2f inhibited the phagocytosis of zymozan and H_2O_2 production by PMN and MO cultures stimulated with PMA. The treatment also inhibited TLR-2 on PMN surface and HLA-DR expression on MO, while MR expression was increased. There were a decrease on TNF-α and MCP-1 production, and an increase on IL-6 production by PMNs. MO showed a decrease on TNF-α, IL-12, IL-15 and MCP-1 levels and an increase on IL-6, IL-10, IL-17 and IL-23 production. In relation to PBMC

cultures, both treatments decreased cellular proliferation and TNF-α, IFN-γ, IL-2 and IL-12 levels, but increased IL-4, IL-10 and IL-17 production. **Conclusion:** Our results show that PGE_2 purified from *P. brasiliensis* cultures has the same immunomodulatory effects on mononuclear and polymorphonuclear human cells when compared with PGE2c.

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CAFETERIA DIET CONTAINING 16% FAT, CAUSES LITTLE VARIATION IN PARASITEMIA AND MORTALITY OF FEMALES C57BL / 6 MICE WITH CEREBRAL MALARIA

CHRISTIANE LIMA MACHADO(IC)([1]), ANA CRISTINA M. GUALBERTO(IC)([1]), GABRIELA C. M. EVANGELISTA (IC)([1]), POLLYANNA AMARAL SALVADOR(MS)([1]), RENAN V. H. DE CARVALHO(IC)([1]), SARA MALAGUTI (IC), JACY GAMEIRO([1])

[1] Laboratório de Imunologia, Departamento de Parasitologia, Microbiologia e Imunologia, Instituto de Ciências Biológicas, Universidade Federal de Juiz de Fora, Juiz de Fora, MG, Brasil.

Introduction: The obesity in humans is increasing every year due to lifestyle changes. Obesity is associated with insulin resistance, dyslipidemia, steatosis hepatitis and cardiovascular diseases. Several obesity models have been used to research the relationship between obesity and diseases. One model is used to diet-induced obesity (DIO), which is developed after feeding mice with high-fat diet (HF diet). It is very similar to human obesity that is result of food over-consumption and sedentary lifestyle. The relation among obesity and diabetes or cardiac diseases is well established, but, the role of obesity in the course of infectious diseases is still poorly understood. Malaria is a tropical disease that causes lots of deaths worldwide. Thus, the aim of this work was to establish a model of DIO and to evaluate whether the influences of DIO in parasitemia and mortality of female mice during the course of cerebral malaria infection in obese mice.

Methodos and Results: C57BL/6 female mice, 4-6 weeks age, were given *ad libitum* water and standard chow high-fat diet (HF) that contained 16% of fat. Food consumption and body weight were monitored every week. Obesity was proved by Lee Index and measurement of perigonadal fat pad weight. After 16 weeks, control C57BL/6 female mice, and HF group were infected with *Plasmodium berghei*-ANKA (5 x 10^6iRBCs/mL). Parasitemia was evaluated daily after infection in all of groups by collecting a small fraction of mice's tail blood and spreading it in a slide and stained with *Giemsa* solution. The percentage of infected red blood cells (iRBCs) was evaluated in comparison to total RBCs. Mortality curve was elaborated according to the number of mice dying from infection in each day after the inoculation of Pb-ANKA. Female C57BL/6 DIO mice showed the significant fat accumulation. The parasitemia and the mortality was not significant between obese and control, but we

observed a tendency to lower parasitemia in obese and higher mortality rate when compared to control ones.

Conclusions: We conclude that this DIO model is able to induce obesity in females C57BL / 6, but slightly alters the course of infection.Experiments using diet with higher amounts of lipids are being evaluated to see if the course of infection is changed significantly

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IN PREGNANT MICE, FOXP3+ CELLS ARE INVOLVED IN THE PROTECTION AGAINST EMBRYOS RESORPTION IN CONGENITAL TOXOPLASMOSIS

RÔMULO OLIVEIRA SOUSA[1]; LETÍCIA DE SOUZA CASTRO-FILICE[2]; LOYANE BERTAGNOLLI COUTINHO[1]; LAYANE ALENCAR COSTA NASCIMENTO[1]; ELOISA AMÁLIA VIEIRA FERRO[2]; TIAGO WILSON PATRIARCA MINEO[3]; NEIDE MARIA SILVA[1]

1. Laboratório de Imunopatologia, 2. Laboratório de Imunofisiologia da Reprodução, 3. Laboratório de Imunoparasitologia, Universidade Federal de Uberlândia, roolsou@hotmail.com

Introdution: Congenital toxoplasmosis is associated with adverse pregnancy outcome. Despite the type 1 immune response, C57BL/6 is more susceptible than BALB/c mice to *Toxoplasma gondii* infection. Additionally, the successful pregnancy appears to be correlated with Th2- type maternal immunity and also Treg cells.
Methods and Results: In order to investigate mechanisms of susceptibility/resistance of mice with different genetic background in congenital *T. gondii* infection, groups of C57BL/6 and BALB/c females were orally infected with *T. gondii* ME-49 on day 1 of pregnancy and were sacrificed on day 8 post-infection. The uterus and placenta were evaluated for resorption rate, parasitism, histological analysis and phenotype of cell infiltrates. C57BL/6 presented inflammatory foci in the decidua in a higher frequency compared to BALB/c mice on day 8 of pregnancy and infection, and great number of pregnant C57BL/6 mice presented necrotic implantation sites. The C57BL/6 mice presented higher resorption rate observed on day 19 of pregnancy and infection. Pregnant and infected C57BL/6 mice showed higher CD4, CD8 and Mac1 positive cells in the uterus compared to BALB/c mice. Additionally, it was verified that BALB/c mice presented statistically higher FoxP3 positive cells and a tendency of higher IL-17 positive cells in the uterus compared to C57BL/6 mice at the same time of pregnancy and infection.
Conclusion: Our data suggest that the CD4, CD8 and Mac1 positive cells infiltrated in the uterus are contributing to the higher resorption rate in C57BL/6 mice and FoxP3$^+$ cells that are associated with a regulatory profile in addition to IL-17 positive cells, are contributing to smaller resorption rate in BALB/c mice.

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SERUM COMPONENTS AND MONONUCLEAR CELLS COOPERATE TO RAPIDLY CONTROL AN AVIRULENT PROTOZOAN PARASITE

NICOLI DE BONA HECK[1]; STEFANNY V. MORALES[1]; ÁLVARO MENIN[1]; PATRÍCIA STOCO[2]; DÉBORA LÜCKEMEYER[2]; MÁRIO STEINDEL[2]; EDMUNDO C. GRISARD[2]; ANDRÉ BAFICA[1].

[1]Laboratory of Immunobiology, MIP-UFSC; [2]Laboratory of Protozoology, MIP-UFSC.

Introduction: *Trypanosoma rangeli* has been documented to be a non-virulent protozoan parasite in mammalian hosts. However, information regarding *T. rangeli*-host interactions during infection remains scarce.

Methods and Results: In this study, parasitemia as well as immune responses of C57BL/6 mice i.p. injected with culture-derived *T. rangeli* trypomastigotes (Choachí strain) were assessed. *T. rangeli* trypomastigotes were found in the blood of infected mice as early as 1 day p.i., reaching a peak of parasitemia at 2 days p.i. Parasite clearance occurred by day 9 p.i. Surprisingly, histological analysis of the liver revealed that this parasite elicited a robust inflammatory infiltrate prominent in mononuclear cells that persisted up to 60 days p.i. In addition, flow cytometry analysis indicated that *T. rangeli* infection induces an early increase in $CD11b^{+}Gr1^{int}$ cells, suggesting that myeloid cell populations such as inflammatory monocytes may play a role in controlling infection as well as the observed persistent inflammation. Consistently, when *T. rangeli* trypomastigotes were incubated with murine macrophages, the majority of parasites were observed outside the cells and no sign of division was detected for intracellular forms by means of light-microscopy analysis. However, *T. rangeli*-exposed macrophages were found to produce high levels of TNF and IL-6, suggesting that although macrophage sense *T. rangeli,* controlling of parasite growth was not completely explained by mononuclear cell recruitment and activation. We next evaluated the possible role of serum components in *T. rangeli* killing. Trypomastigotes treated with normal, but not heat-inactivated, mouse serum displayed profound changes on parasites morphology as well as increased propidium iodide staining, indicating serum components participate of parasite killing. Importantly, pre-treatment of serum with mannose, but not galactose, blocked the observed *T. rangeli* killing pointing to a previously

unappreciated involvement for MBL in controlling *T. rangeli* infection. We thus infer that uncharacterized serum components may cooperate to enhance parasite killing by macrophages.

Conclusions: These results suggest that *T. rangeli* is recognized by macrophages eliciting persistent inflammatory responses even after parasite clearance. Furthermore, serum components, perhaps the complement-activating lectin pathway, are involved in controlling *T. rangeli* infection.

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VIABILITY OF *LEISHMANIA (L.) CHAGASI* TO REACTIVE OXYGEN SPECIES

SUELI SILVA DE CARVALHO[1], PRISCILA LIMA DOS SANTOS[1], JUCIENE DE MATO BRAZ[1], JÉSSICA ALMEIDA RODRIGUES[1], KAELLYNE FIGUEIREDO MATOS[1], MURILO BUENO AIRES MONTEIRO[1], TALITA REBECA DE SOUZA LEITE[1], MICHELI LUIZE BARBOSA SANTOS[1,] AMÉLIA RIBEIRO DE JESUS[1], TATIANA RODRIGUES DE MOURA[1], ROQUE PACHECO ALMEIDA[1], PAULO DE TARSO GONÇALVES LEOPOLDO[1].

(1) Universidade Federal de Sergipe – Aracaju, Brazil

Introduction. Infection of macrophages by *Leishmania* leads to activation of these cells, triggering elimination or inhibition of pathogen growth, through production of toxic molecules, such as reactive oxygen species (ROS) and nitrogen (RNI). I*n vitro* exposure to ROS of promastigotes and amastigotes of *Leishmania* results in loss of their viability. This work aimed to evaluate *in vitro* leishmanicidal activity of menadione (a vitamin K derivative and donor of superoxide anion and H_2O_2) on *L. (L.) chagasi* and infectivity of these parasites in a line of J774 murine macrophages**. Methods and Results**: Ten *L. (L.) chagasi* promastigotes (in growth phase), isolated from visceral leishmaniasis patients from Sergipe, were exposed to menadione (0-750 µM), and their viabilities were determined by counting mobile forms on optical microscopy. To evaluate infectivity, two menadione susceptible and one resistant *L. (L.) chagasi* (in stationary phase of growth) were incubated with J774 cells for 2, 24, 48 and 72 hours on 8 wells plates. The plates were stained with Panotipo and parasitic indexes were determined by the product of percentage of infected macrophages multiplied by the number of amastigotes/100 macrophages. Exposure of *L. (L.) chagasi* promastigotes to 750µM menadione resulted in loss of viability of 70% of them. Half of isolates tested presented loss of 50% of viability at 500 µM menadione. In order to test resistance and susceptibility of amastigotes forms of these isolates to superoxide anion produced by macrophages, three isolates of *L. (L.) chagasi* (LVHSE 23, 49 and 60) were tested. LVHSE 23, menadione resistant, presented constant parasitc indexes until 72 hours after infection, while LVHSE 49 and LVHSE 60 (both of them menadione susceptible) decreases their index at 48 and 72 hour after infection. **Conclusion** These data suggest that *L. (L.) chagasi* can resist leishmanicidal action of superoxide anion in vitro as well as intracellular killing in murine macrophages.

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EVALUATION OF THE INTERFERENCE OF SEROPOSITIVES FOR HUMAN PATHOGENS LINKED IMMUNOSORBENT ASSAY FOR TESTING OF ANTI-HIV

Introduction: Sexually transmitted diseases are an important public health problem worldwide. The acquired immunodeficiency syndrome (AIDS) represents one of the major concerns. AIDS is a progressive disease associated with Human Immunodeficiency Virus (HIV) that can lead to destruction of the immune system and consequent death caused by opportunistic infections. Early diagnosis of HIV infection becomes a crucial point in the fight against viral transmission and disease control. The detection of antibodies against HIV is among the mandatory tests for serological screening of blood banks in Brazil, where the format used is ELISA (Enzyme Linked Immunosorbent Assay). In 2009, the Laboratory of Immunodiagnostics Research (LIR) was created in connection with the “triple helix” concept, joining expertise of FK Biotechnology, Faculty of Pharmacy (PUCRS) and Lifemed with governmental financial support (FINEP), aiming the development of immunoassays with fully national technology in all steps of production. Here we report possible interference in samples positive for different serological parameters, such as, anti-HCV, anti-HBs, anti-HBc and HBsAg.
Methods and Results: For the assay were used 90 samples of anti-HCV, 90 samples of anti-HBs, 100 samples of anti-HBc total and 57 samples of HBsAg. The test for anti-HIV standardized by LIR, with sensibility of 99.5% and specificity of 98.4%, showed no interference of the parameters tested.
Conclusion: The performance reported here is comparable to systems available at market and currently in use for blood screening in clinical laboratories and blood banks, with the advantage of being produced with national technology.

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LEISHMANIA BRAZILIENSIS OVEREXPRESSING THE SPLICED LEADER RNA MODULATES MACROPHAGE CELL FUNCTIONS

CAMILA FIGUEIREDO PINZAN (1); JULIANO SIMÕES DE TOLEDO (1); ANGELA KAYSEL CRUZ (1)

1-Departament of Molecular and Cell Biology of Pathogens, University of São Paulo, Ribeirão Preto, Brazil

Introduction: Leishmaniasis is a spectral disease caused by *Leishmania* spp and affects millions of people worldwide. The parasite has unusual mechanisms for the control of gene expression, such as polycistronic transcription and trans-splicing. The major player in the mRNA processing is the spliced leader (SL) RNA, which is spliced at the 5'-end of mRNAs and defines the polyandenylation site of the upstream mRNA. We have previously reported an attenuation of virulence of *Leishmania major* and *Leishmania braziliensis* carrying extra-copies of the spliced leader RNA gene in animal models, but the role of host immune system on this phenomenon is still unclear. Thus, our goal is to understand whether macrophage microbicidal activities are induced, avoided, or actively impaired during infection with *L.braziliensis* transfectants overexpressing the SL gene (Lb [cLHYG ME]). **Methods and Results:** Our approach was to evaluate the kinetics of cytokines, reactive oxygen species production (ROS) and the co-stimulatory molecules expression by infected bone-marrow-derived macrophages (BMDMs). Our results revealed that infections with the Lb [cLHYG ME] have induced high IL-12p40, IL-6, TNF-α and IL-1β production by the BMDMs, whereas significantly reduced cytokines production was verified by BMDM infected with control strains (Lb [cLHYG]). Compared to control, BMDMs infected with Lb [cLHYG ME] parasites showed increased expression of co-stimulatory molecules (CD80, CD86, CD40 and MHCII) and enhanced production of ROS. BMDMs infected with Lb [cLHYG] were unable to restrict parasite multiplication; by 48 h postinfection, most of the cells were infected with 10 amastigotes, in average. In contrast, intracellular multiplication of Lb [cLHYG ME] parasites was impaired; fewer cells bear, in average, 5 amastigotes. **Conclusion:** In conclusion, overexpression of the SL RNA into *L. braziliensis* led to a molecular stress in the parasite that affected the host parasite interaction; the macrophage is then able to control more adequately the parasite replication.

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IMMUNODETECTION OF THE TGF- β IN LIVER OF DOGS WITH VISCERAL LEISHMANIASIS

PAMELA RODRIGUES REINA MOREIRA[1]; MARCIO DE BARROS BANDARRA[1]; DANÍSIO PRADO MUNARI[2]; ROSEMERI DE OLIVEIRA VASCONCELOS[3]

(1). Doutorandos do Programa Pós-graduação em Medicina Veterinária. (2). Depto. de Ciências Exatas; (3). Patologia Veterinária, FCAV/UNESP, Jaboticabal-SP. rosevasc@fcav.unesp.br

Introduction:: In canine visceral leishmaniasis (CVL) reported the involvement of various organs by *Leishmania (Leishmania) chagasi* and consider liver an organ resistant multiplying it. The objective of this study was to analyze liver injury in dogs with CVL and associate them with the immunodetection of TGF-β and the parasite burden by means of immunohistochemical technique in different clinical stages of disease, such as symptomatic (S), asymptomatic (A) and oligosymptomatic (O).
Methods and Results: This study included 35 dogs, without preference for age, breed or sex, from the Zoonosis Control Center of Araçatuba (SP), where they were euthanized due to the positive diagnosis of visceral leishmaniasis (VL). One control group (C) was formed with dogs from non-endemic area for CVL (CEUA nº. 024686/10). In immunohistochemical analysis we used the antibodies TGF-β (Santa Cruz Biotecnology) and for the parasite burden was used canine hyperimmune serum (J. Immunol. Meth. 292:17-23, 2004). The antibodies were used at dilutions of 1:1300 and 1:1000 respectively. The mean cells immunostained for *Leishmania* sp. and TGF-β were analyzed by Kruskal-Wallis and the Dunn test to compare the groups, considering $P < 0.05$. The correlation between parasite burden and the presence of TGF-β was performed by Spearman correlation coefficient within the group. The statistical program used was Graphpad Prism (version 4.0). In histological analysis, the inflammatory reaction ranged from mild to moderate, located in the centrilobular regions, peri-portal and intralobular associated with granulomas composed of macrophages, lymphocytes and plasma cells. The parasite load was significantly different between groups S and A ($P = 0.0476$). The detection of TGF-β differ between groups S and C ($P = 0.0088$), but did not differ between groups of infected dogs. In group S there was a higher average (7.04 + 6.34). The correlation between parasite burden and the presence of TGF-β was not significant.
Conclusion: In group S, the parasite load and the production of TGF-β were higher, suggesting that this cytokine creates a favorable environment for multiplication of the parasite *Leishmania* sp. However, a larger number of

animals in groups are required to assess whether the increase in parasite density in the liver is associated with increased production of TGF-β.

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Key words: Immunohistochemistry, *Leishmania chagasi*, liver, cytokines, dog.

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DIFFERENT LEVELS OF NKT AND NK CELLS IN PATIENTS WITH LEPROSY

CAMILA FERNANDES (PG) [(1)]; FABIOLA FERNANDES HEREDIA (GRAD)[(2)]; PAULA BRITO E CABRAL[(1)]; MÁRCIA BRASIL[(1)]; LILIA MARIA CARNEIRO CÂMARA[(1)].

[(1)] Universidade Federal do Ceará [(2)] Universidade Estadual do Ceará

Introduction: Leprosy is caused by *Mycobacterium leprae* and is characterized by skin lesions with abnormal sensitivity and neuritis, representing an important an important public health problem worldwide. There is a spectrum variable manifestations depending on the pattern of immune response, with paucibacillary (PB) form characterized by Th1 and multibacillary (MB) form by the Th2 response (Lancet Infect Dis. 11:464-70, 2011). NK cells are important in protection against *M. leprae*, being responsible for the initial response as well as the production of Th1 and Th2 cytokines (Clin Exp Immunol. 135:105-113, 2004). Natural killer T lymphocytes (NKT) have effector and regulatory capacity and can recognize glycolipid antigens presented via CD1d, which can be expressed in Schwann cells, providing a link between innate and adaptive immunity in *M. leprae* infection (Clin Immunol. 127 : 214:224, 2008). Despite this evidence, the role of these cells in leprosy is not clearly elucidated. This study we investigated the frequency of NKT cells and NK cells in patients with leprosy without treatment. **Methods and results**: We studied 6 patients (2 PB and 4MB) aged 6 to 16 years (median = 11.5), and an adult patient (MB) and a healthy adult. 2.5 $x10^5$ PBMC were labeled with anti-CD3-APC (BioLegend), anti-CD16FITC (e-Biosciences), anti-CD56PE (e-Biosciences) and anti-CD8PerCp (e-Biosciences). The reading was held on FACSCalibur, purchased 350 thousand events in the lymphocyte gate. In assessing the positivity within the gated CD8 lymphocytes, all patients had more $CD8^+$ (mean = 35.74%, var. 24.32 to 46.89%) than the control (23.8%), correlating positively with age (Spearman = 0.7857, p = 0.0480). The average percentage (0.54%; var. 0.12 to 1.02) of $CD3^+CD16^+CD56^+$ (NKT) was lower than the control (1.26%). 71.4% of patients showed mean percentage of $CD3^-CD16^+CD56^+$ (NK) (8.21%; var. 2.98 to 12.68) greater than control (1.98%). The adult patient, MB form, was the only individual whose percentage of $CD3^+CD8^+CD16^+CD56^-$ was greater than $CD3^+CD16^+$ $CD56^+CD8^+$. **Conclusion**: We observed a lower number of NKT and an increase of NK cells in patients, which may suggest an important role of these cells in leprosy, despite the limited size of the sample. A larger sample of patients and controls should be considered in future work.

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ANALYSIS OF CELL-FREE ANTIGEN OF THE FIRST CULTIVATED STRAINS OF *Lacazia loboi*

PATRÍCIA FAGUNDES DA COSTA[1,2]; CLAUDIO GUEDES SALGADO[1,3]

[1]Laboratório de Dermato-Imunologia UEPA/UFPA/Marcello Candia, Marituba, Pará; [2]Centro Universitário do Estado do Pará, Brasil; [3]Instituto de Ciências Biológicas, Universidade Federal do Pará, Belém, Pará.

Introduction: Lobomycosis is a chronic dermal mycosis, presenting with nodules, which can be localized or diffuse on the skin. Previously regarded as an infection restricted to humans living at the Amazon Region, it has been recently diagnosed in Africa, and it is rapidly becoming a widespread disease of dolphins, as more animals are diagnosed with lobomycosis in different parts of the world. We have recently cultivated *in vitro* the etiological agent *Lacazia loboi*, and the first immunological analysis of Cell-Free Antigens (CFA) are presented. **Methods and Results:** Eight *L. loboi* strains kept on Fava-Netto media for 7 or 17 days at 37ºC/5% CO2 were transferred to eppendorfs, vortex for 2 min and centrifuged at 10.000 rpm for 10 min. The CFA supernatant was removed and kept on -20ºC freezer until use. The samples were applied to 100W electrophoresis SDS-PAGE gel for 90 min. After running, protein bands were transferred to nitrocellulose membranes, and kept on blocking Tris 5% dry milk buffer for 1 h. Then, the membrane was Tris washed 3x and incubated for 1 h with lobomycosis patients plasma diluted 1:50. Goat IgG-HRP anti-human IgG were diluted 1:1000 and incubated for 1 h before DAB revelation. We observed the presence of a strong band of about 200 KDa, recognized by the plasma sample of a lobomycosis patient, besides other protein fractions varying from 50 to 70 KDa. Although gp43 was not observed on these first western blot samples, we have seen 43 KDa bands on silver stains, and new blots with different strains and new plasma samples from other patients are being prepared for analysis. **Conclusion:** CFA immunoblotting of different *L. loboi* samples present a strong ≈200KDa band, and other 50-70 KDa bands. Gp43 has not been observed at this first immunoblotting analysis, but other strains samples, and new plasma obtained from patients with different clinical forms will be tested to confirm these results.

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"ADENOSINE AND ADENOSINE MONOPHOSPHATE FROM *PHLEBOTOMUS PAPATASI* SALIVARY GLAND EXACERBATE LEISHMANIASIS BY INDUCING TREGS DEPENDENT OF TOLEROGENIC DENDRITIC CELLS"

[1]VANESSA CARREGARO, [1]DJALMA LIMA-JÚNIOR, [1]DIEGO L. COSTA, [3]Carlo J. F. Oliveira, [1]CRISTIANE M. MILANEZI, [4,5]JESUS G. VALENZUELA, [4,5]JOSÉ M. C. RIBEIRO, [1,2]FERNANDO Q. CUNHA AND [1]JOÃO S. SILVA

[1]Department of Biochemistry and Immunology, and [2]Department of Pharmacology, School of Medicine of Ribeirão Preto, University of São Paulo, Brazil; Institute of Biological and [3]Natural Sciences, Federal University of Triângulo Mineiro, Minas Gerais, Brazil, [4]Section of Vector Biology, and [5]Vector Molecular Biology Unit, Laboratory of Malaria and Vector Research, NIAID/NIH, USA

INTRODUCTION: Phlebotomines saliva plays a crucial role in the establishment of *Leishmania* infection. Among several potent pharmacologic substances, we recently purified and identified adenosine-(ADO) and adenosine-monophosphate-(AMP) as saliva's active pharmacologic compounds presents on *P. papatasii* that inhibits dendritic cells-(DC) functions through PGE_2/IL-10-dependent mechanism. ***AIM****:* We evaluate whether ADO and 5'AMP are compounds present into *Phlebotomus papatasii* saliva responsible for leishmania establishment into vertebrate host and such immunomodulatory mechanism. ***METHODS.*** C57BL/6WT or C57BL/6IL-$10^{-/-}$ mice were coinoculated with *L. amazonensis* promastigotes forms ($1x10^5$parasites/ear-i.d.route) in the presence of ADO+AMP or vehicle (PBS). The ear lesion size, parasites burden, cytokines production and inflammatory infiltrated were analyzed at 12nd week post infection. ***RESULTS:*** ADO+AMP mimicked the exacerbative effect of saliva in leishmaniasis, increasing parasites numbers and ear lesion. Enzymatic catabolism of salivary nucleosides reversed the SGE-induced immunosuppressive effect. Such effect was associated with pro-inflammatory cytokines reduction (IFN-γ,TNF-α) and IL-10 enhancement. Moreover, ADO+AMP failed to enhance ear lesion and parasite burden in IL-$10^{-/-}$ infected mice. Enzymatic catabolism of salivary nucleosides reversed the SGE-induced immunosuppressive effect. Interestingly, nucleosides increased Tregs makers (GITR; CTLA-4; CD39; CD73 expression) on $CD4^+CD25^-$ population, suggesting the induction of Tregs on T effector cells. Treg induction was associated with nucleosides-induced tolerogenic dendritic cells (tDC) expressing higher levels of COX_2 and IL-10. Furthermore, nucleosides-induced tDC displayed a semi-mature phenotype and produced lower levels of proinflammatory cytokines *in vitro* and *in vivo*. ***CONCLUSION:*** We demonstrated that ADO and 5'AMP are constituents present in *P.papatasi*

saliva that exacerbated leishmania infection. The exacerbative effect is associated with Tregs enrichment generated by nucleosides-induced tDC in the inflammatory foci.

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IL-33/ST2 INHIBITS *COXSACKIEVIRUS* B5-INDUCED EXPERIMENTAL PANCREATITIS

RENATA SESTI-COSTA[1], GRACE KELLY DA SILVA[1], JOSÉ LUIZ PROENÇA-MÓDENA[1], DANIELA CARLOS [1], MARIA LÚCIA SILVA[1], JOSÉ CARLOS ALVES-FILHO[1], FOO YEW LIEW[2], EURICO ARRUDA-NETO[1], JOÃO SANTANA DA SILVA[1].

[1]Medical School of Ribeirão Preto. FMRP/USP, Ribeirão Preto, Brazil, [2]Division of Immunology, Infection and Inflammation, University of Glasgow, Glasgow, United Kingdom.

Introduction: *Coxsackieviruses* are single-stranded and positive-polarity RNA viruses. They have a pancreatic tropism, which often leads to fulminant pancreatitis with subsequent pancreatic insufficiency. Apart of virus proliferation, the Th1 immune response generated to the virus is also responsible to tissue lesion. So, modulation strategies that balance host immune response in eliminating the virus while minimizing injury to the host tissue is the key to treating coxsackievirus-associated pancreatitis. With this purpose, we investigate the effect of IL-33, a type Th2 cytokine, on CVB5-induced pancreatitis. **Methods and Results:** Balb/c and ST2-deficient mice were i.p infected with 10^7 $TCID_{50}$ of *coxsackievirus* B5 (CVB5) and the course of infection and pancreas pathology were determined. $ST2^{-/-}$ mice infected with CVB5 developed significantly more severe pancreatitis, greater weight loss and higher viral titer compared to wild-type (WT) BALB/c mice. Conversely, WT mice treated with recombinant IL-33 developed significantly lower viral titer and attenuated pancreatitis. Infected $ST2^{-/-}$ mice also showed reduced levels of IL-4, mast cells, alternatively-activated macrophages (M2) and $CD4^+Foxp3^+$ regulatory T (Treg) cells in the pancreas. Adoptively transferred mast cells or M2 macrophages reversed the heightened pancreatitis in the $ST2^{-/-}$ mice. In contrast anti-GITR antibody, which inhibits Treg cells, exacerbated the disease in WT mice. Furthermore, IL-4 and Stat6-deficient mice showed similar phenotype as the $ST2^{-/-}$ mice during CVB5 infection, suggesting a role of IL-4/Stat6 pathway in this process. **Conclusion:** Together, these results reveal an unrecognized IL-33/ST2 pathway, which stimulates mast cells and the production of IL-4 that activates Stat6. This pathway enhances the differentiation of M2 macrophages and Treg cells, leading to the attenuation of inflammatory pancreatitis during CVB5 infection. Our finding also suggests that IL-33 may be a potential therapeutic agent against CVB infection.

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BACTERIAL FILAMENTATION: SUPER-RESISTANT OR SUPER-SENSIBLE CELLS?

JOB ALVES DE SOUZA FILHO
Department of Biochemistry and Immunology, Institute of Biological Sciences, Federal University of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil

Introduction*:* Morphological changes induced by different antibiotics in Gram positive and Gram negative, cocci and bacilli, aerobic and anaerobic bacterial cells are widely reported in the literature. However, there is a debate concerning the relation between the bacterial filamentation and the resistance to antimicrobials and other stress, inclusive the immune response. Many authors suggest that filamentation is a mechanism of resistance to various stresses, while others suggest that filamented cells are an intermediary process in cell death. The aim of this study was to provide a methodology to clarifying this question. **Methods and Results:** Flow cytometry was used to measure the relationship between the morphology and the death rate of bacteria after 1h in oxidative stress (21% O_2) and in exposure to subinibitory concentrations (CSI) of antimicrobials. The resident anaerobic of humans *Fusobacterium nucleatum*, the predominant species in clinical samples worldwide, was using as a model. From the strain *F. nucleatum* ATCC 25586 (FnWT), seven strains were obtained selected after ten successive cultivations in CSI (1/2MIC) of ampicillin (FnAMP), ampicillin/sulbactam (FnAMS), clindamycin (FnCLI), chloramphenicol (FnCLO), levofloxacin (FnLEV), metronidazole (FnMET) and piperacillin/tazobactam (FnPTZ). Propidium iodide was used as a marker of cell death. Were analyzed 10,000 cells for each sample. The strains obtained by cultivation in CSI of β-lactams (FnAMP, FnAMS and FnPTZ) have undergone the more pronounced changes in cell morphology and complexity with 38.8%, 75.2% and 85.0%, respectively, of altered cells. The strains FnCLO, FnCLI, FnLEV and FnMET exhibited little morphological and complexity change, ranging from 5.8% to 8.5% of abnormal cells. In all analyzed strains, the exposition to oxygen and CSI of all antimicrobials induced a higher death rate in morphologically altered cells when compared to cells with normal morphology. **Conclusion:** These data suggest that the *F. nucleatum* morphologically altered cells, cultured in the presence of CSI antimicrobials, are more sensitive to the respective antimicrobial and to the oxidative stress, in comparison to cells with normal morphology. The methodology used in this experiment may be useful to evaluate the response of other bacterial species with altered morphology in response to other sources of stress, and thereby elucidate the relationship between filamentation and bacterial resistance.

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THE TNFRP55 MODULATE THE INFLAMMATORY RESPONSE IN LEISHMANIA AMAZONENSIS INFECTION

LEONARDO GOMES VAZ.[1*]; MATEUS EUSTÁQUIO DE MOURA LOPES[1]; MATHEUS BATISTA HEITOR CARNEIRO; LILIANE MARTINS DOS SANTOS; LEDA QUERCIA VIEIRA

[1] Departamento de Bioquímica e Imunologia, ICB, Universidade Federal de Minas Gerais, Minas Gerais, Brasil.

Introduction: The cytokine tumor necrosis factor (TNF) is required for resistance to several pathogens, such as Listeria monocytogenes, Candida albicans, Trypanosoma cruzi and Leishmania major. One protective function of this cytokine is the ability to synergize with IFN-y to induce the expression of iNOS by macrophages, leading to NO production and the killing of parasites. Two cognate receptors for TNF have been described: the TNFR1 (TNFRp55) and the TNFR2 (TNFRp75). TNFR1 promotes cell survival and inflammation or, alternatively, can induce apoptosis. Although many studies had demonstrated that TNF plays a central role in the outcome of many infection models, the role of this cytokine in L. amazonensis infection remains to be completely understood. The objective of this study was to evaluate the role of TNFRp55 in infection by L. amazonensis.
Methods and Results: Our data did not show differences in parasite load, lesion size and production of TNF-α, IFN-γ and IL-10 by lymph node cells stimulated in vitro with the parasite antigen, between C57BL/6 wild-type and TNFRp55$^{-/-}$ mice, 8 weeks post infection. After 16 weeks, an increase in lesion size was seen in the TNFRp55$^{-/-}$ mice, but the parasite load was not different between the groups. At this time of infection, the production of TNF-α, IFN-γ and IL-10 were the same for both groups, but knockout mice showed a higher arginase activity in the footpad, which can reflect a higher inflammatory infiltration. Interestingly, at the beginnig of infection, larger lesions were seen in wild type mice.
Conclusion: These data suggest that TNFRp55 plays an immunomodulatory effect in this infection model that may be is important for the resolution of the inflammatory process, be by mediating may apoptosis, but TNFRp55 was not essential for the control of the parasite replication.
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IMMUNOLOGICAL PARAMETERS ASSOCIATED TO GRANULOMATOUS PATHOLOGY AFTER *SCHISTOSOMA MANSONI* INFECTION AND REINFECTION IN TWO MURINE STRAINS.

GARDENIA BRAZ FIGUEIREDO DE CARVALHO[1]; ANGÉLICA SAMMER LALLO DIAS[1]; CLARICE CARVALHO ALVES[1]; CRISTINA TOSCANO FONSECA[1,2].

[1]Centro de Pesquisas René Rachou, Fiocruz-MG, Belo Horizonte, Minas Gerais, Brazil; [2]Instituto Nacional de Ciências e Tecnologia em Doenças Tropicais (INCT-DT), Brazil.

Introduction: In schistosomiasis, host immune system plays an important role in both parasite development and elimination. Also difference in immune response has been associated to resistance or susceptibility for the disease and to the different clinical forms observed in infected individuals. Granulomatous reaction around eggs is the major pathology associated with schistosome infection, and once again the host immune system plays an important role in granuloma development and modulation. To understand this complex host-parasite interaction we analyzed parasitological, pathological and immunological parameters associated with infection/reinfection in two murine strains: C57BL/6 and Balb-c. **Methods and Results:** Thirty C57BL/6 mice and Balb-c mice were infected with 30 *S. mansoni* cercariae, 45 days after infection, 15 animals were perfused to determine worm burden. The other animals were treated with praziquantel (400mg/Kg) and thirty days after treatment mice were reinfected with 30 cercariae. Forty five days after reinfection, worm burden recovered were determined. Any difference in worm burden was observed between strains after infection or reinfection. Sixty days after infection/reinfection granuloma area was determined in 50 granulomas from each group with a single well-defined egg and at exudative-productive stage. Significant reduction in granuloma area was observed in C57BL/6 infected mice in comparison to Balb infected mice (31%). Also a significant reduction in granuloma area was observed in both strains after reinfection (52% in Balb and 36% in C57BL/6). Sixty days after infection/reinfection spleen cells from individual animals were culture in the presence of soluble eggs antigens and cytokines were measured in culture supernatant. Significant IL-10 production was detected in C57BL/6 mice ($p=0,03$), in contrast significant IL-13 production was detected in Balb-c infected mice ($p=0,04$). Any significant difference in cytokine production was between infected and reinfected mice. **Conclusion:** Our results indicate that although the difference in the genetic background did not influence parasite survival, it leads to differences in pathology that might be related to the different cytokine profile observed between strains. Additional studies are necessary to clarify the mechanisms involved in granuloma modulation after reinfection.

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SMTEG IMMUNIZATION WITHOUT AJUVANTS INDUCES A MODULATORY IMMUNE RESPONSE IN MICE.

CLARICE CARVALHO ALVES[1]; JULIANO MICHEL ARAUJO[1]; TATIANE TEIXEIRA DE MELO[1]; ISABELA CAMPOS DE SENA[1]; NEUSA ARAUJO[1]; FERNANDA DO VALLE DURÃES[2]; DEBORAH LARANJEIRA FERREIRA PIMENTA[1]; SERGIO COSTA OLIVEIRA[2]; CRISTINA TOSCANO FONSECA[1,3].

[1]Centro de Pesquisas René Rachou, Fiocruz-MG, Belo Horizonte, Minas Gerais, Brazil; [2]Departamento de Bioquímica e imunologia, Instituto de Ciências Biológicas, Univesidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil; [3]Instituto Nacional de Ciências e Tecnologia em Doenças Tropicais (INCT-DT), Brazil.

Introduction: Vaccine development is essential to control schistosomiasis since chemotherapy does not prevent reinfections. The *S. mansoni* schistosomula tegument (Smteg) is an interesting parasite structure to be used in a vaccine formulation against schistosomiasis, since it is a dynamic host-interactive layer. We have recently demonstrated that Smteg is able to activate innate immune response and to induce protective immunity reducing parasite burden, egg elimination and disease morbidity in a vaccine formulation with Freunds adjuvant. In this work, we evaluated the immunological response trigged by Smteg immunization in the absence of adjuvant and its ability to elicit protection against *S. mansoni* challenge infection. **Methods and Results:** Female C57BL/6 mice were immunized subcutaneously with three doses of Smteg (25µg) or saline (control group) in a 15 day-interval regimen. Thirty days after the last boost, mice were challenged through percutaneous exposure of abdominal skin. Fifty days after challenge, adult worms were perfused from the portal system and the protection level was calculated. Unexpectedly, immunization failed to induce protection in mice. To evaluate humoral immune response, blood samples were collected from retro orbital sinus of each mouse with an interval of 15 days beginning 15 days after the first immunization for measurement of specific anti-Smteg antibodies. Immunization with Smteg lead to production of specific IgG antibodies indicating that immunization was able to activate immune response. Cytokines production (IL-4, IL-10, TNF-a, IFN-g) was assessed by splenocytes culture and intracellular staining ten days after the last immunization. An increased percentage of CD4+IFN-g+ ($p<0,02$) and CD4+IL-10+ ($p<0,05$) cells in spleen and increased production of IFN-g ($p<0,05$) and IL-10 ($p<0,05$) by spleen cells were observed in Smteg immunological group. To evaluate modulatory profile in the innate immune response induced by Smteg, bone marrow dendritic cells (BMDC) were cultured in the presence of Smteg and IL-10 production was measured in the culture

supernatant. BMDC stimulation with Smteg resulted in significant IL-10 production ($p<0,001$). **Conclusion:** Our results demonstrate that Smteg immunization in the absence of adjuvant failed to reduce worm burden and induce a modulatory immune response.

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EXPERIMENTAL ALLERGIC ASTHMA IS MODULATED BY SCHISTOSOMA MANSONI SCHISTOSOMULA TEGUMENT (SMTEG) WITH DOWN REGULATION OF EOSINOPHILS AND SPECIFIC ANTI-OVA IGE.

SARA CAMILA DE SOUZA(IC)[1]; FABIO A. V. MARINHO(PG)[2]; CRISTINA T. FONSECA(PQ)[1]; SERGIO C. OLIVEIRA(PQ)[2]; LUCILA G. G. PACIFICO(PQ)[1].

[1].Centro de Pesquisa René Rachou (CPqRR) - FIOCRUZ/MG; [2].Universidade Federal de Minas Gerais (UFMG).

Introduction: Allergic inflammations are directed by Th2 cells activation, high levels of IgE and eosinophilia. *Schistosoma mansoni* infection has a negative association with allergic disease in endemic areas, feature that is supported by experimental models. The schistosomulum is the first pathogen stage to keep contact with host immune system. Its tegument (Smteg) represents an important interface host-pathogen, activating antigen presenting cells. Our previous results showed that Smteg stimulated interleukin (IL)-10 production and downregulated lung pathology and CCL11 levels. Our goal is to investigate other inflammatory parameters (IgE and protein extravasation) as well as the cell population involved in IL-10 production to better characterize the mechanism underlying this modulation. **Methods and Results:** Smteg was prepared using cercariae as described by Durães F.V. et al., 2009. Balb/C mice were divided into three groups (n=6): PBS, Asthma and Smteg/Asthma. At days 0 and 14 all groups were immunized with 10µg of ovalbumin chicken egg (OVA) plus alum and at 7^{th} day Smteg/Asthma group received 25µg of Smteg, intraperitoneally. During 21^{th} to 25^{th} days the Asthma and the Smteg/Asthma groups were challenged by OVA aerosol. At 26^{th} day mice were euthanized. Blood samples were collected for IgE measurement in sera, broncho-alveolar lavage was performed to counting of eosinophils numbers and protein analysis. The lungs were collected to flow cytometetry analysis. All parameters were higher in Asthma group compared to PBS group. Importantly, the injection of Smteg (Smteg/Asthma group) reduced the number of eosinophils ($p<0.001$), protein extravasation ($p<0.01$) and specific anti-OVA IgE ($p<0.05$). Analysis of cell population in lungs showed higher percentage of $CD4^{+}IL\text{-}10^{+}$ cells in group Smteg/Asthma compared to asthmatic group. **Conclusion:** The Smteg inoculation modulates allergic asthma reducing inflammatory characteristics

such as number of eosinophils, protein extravasation and specific IgE. This modulation involves $CD4^+$ cells producing IL-10.

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PRODUCTION OF MONOCLONAL ANTIBODIES FOR HUMAN IMMUNOGLOBULIN G CONJUGATED TO PEROXIDASE FOR USE IN DIAGNOSTIC TESTS.

VANESSA SILVA MORAES[1,2]; PAULO MARCOS ZECH COELHO[2]; EDUARDO RIBEIRO DE OLIVEIRA[2]; RAFAELLA FORTINI QUEIROZ GRENFELL[2], JAQUELINE MARIA FERREIRA SIQUEIRA[1]

[1]Federal São João Del Rei University; [2]Research Center René Rachou/ FIOCRUZ

Introduction: The monoclonal antibody (mAbs) has high specificity directed to recognize a single epitope, having a better performance to polyclonal antibodies. Currently, their efficiency in diagnostic kits has gaining attention for allowing the use of low concentrations of specific material. In Brazil the laboratories still lacks the autonomy to execute their own production routine, becoming dependents of the importation theses reagents. This work aims to produce, for *in vitro* methodology, murine mAbs anti-human IgG conjugated to peroxidase, aiming at the widest use in diagnostics studies and in basic and applied research. **Methods and results:** Serum samples collected at intervals of 15 days for 45 days at 2 Balb/C (n1 and n2) immunized with human immunoglobulin G were screened by ELISA test. The n1 mice showed positive for production of anti-human IgG with levels of absorbance of 1,767 (±0,04) and was selected for splenectomy. The B cells obtained were fused with myeloma cells and then expanded into 96-well plate. The supernatant was collected and submitted to a new ELISA and then the polyclonal hybridoma anti-human IgG was selected, coded P2A1 and presenting absorbance of 0,792. This was then again expanded and cloned. After a further step ELISA was selected clone P2A1b6, which showed satisfactory absorbance of 0,560 and was called the producer of monoclonal anti-human IgG. The clone P2A1b6 was cultivated, the supernatant was collected and the antibodies were purified by ammonium sulfate and chromatography column G protein. One portion of the previous purification was subjected to ELISA subtypes, showing the increased production of IgG1 by clone. For the next steps we will performance the protein dosage and SDS-PAGE to confirm the purification. The specificity will be tested by immunoaglutination test with beads coated with G protein. The mAbs selected will be then conjugated to peroxidase and the kit produced will be validated by ELISA utilizing human samples serum of patients from endemic areas for Schistosomiasis mansoni. **Conclusion:** The final mAbs conjugate may be used for a wide range of methods for the identification of numerous diseases, including parasitic, viral and bacterial infections. Therefore, the production of mAbs anti-human IgG implies a good alternative for the routine laboratory and research and national independence in the acquisition of these reagents.
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EVALUATION OF THE PRODUCTION OF TNF-a, IL-10, IFN-g AND IL- 4 BY SPLEEN CELLS FROM CBA MICE IN RESPONSE TO DIFFERENT ANTIGENIC FRACTIONS OF *C. pseudotuberculosis*.

MARCOS DA COSTA SILVA [1, 2], VERA VALE[1,2], ANDRÉIA PACHECO[1], ANA PAULA GOMES[1], LUIZ MIGUEL BARRETO[1], CAMILA AZEVEDO[1], DANIELLE LIMA[1], GABRIELE RODRIGUES[1], MIRIAM REBOUÇAS[1], REJANE SOUZA[1], INARA OLIVEIRA[1], SORAYA TRINDADE[1], ROBERTO MEYER[1].

1 – Universidade Federal da Bahia , 2 – Universidade do Estado da Bahia

Introduction: Of all the diseases that affect small ruminants, caseous lymphadenitis stands out because limitations it imposed on livestock farming. This disease is caused by *Corynebacterium pseudotuberculosis*, a facultative intracellular bacterium, and for its control, both a cellular and humoral immune responses are necessary. It is therefore important to study the effect in the immune system of fractions from the somatic antigen of C. pseudotuberculosis, and also to evaluate the production of cytokines. For this work we studied the citokyne TNF-a, a pro-inflammatory cytokine that help to organize the granulome, which is characteristic of infection by *C. pseudotuberculosis* and also to control an excessive response to IFN-γ. IL-10 is a cytokine that controls an exaggerated inflammatory and anti-inflammatory immune response. IFN-g is an inflammatory cytokine produced by T lymphocytes that controls the immune system. This cytokine also help the organization of the granuloma. However IFN-g excessive production can prevent the formation of this structure. IL-4 is an anti-inflammatory cytokine that promotes alternative activation of macrophages by inducing them to produce collagen.

Methods and results: 35 CBA mice were divided in 3 groups, the first with 15 animals infected with 10^2 CFU T1 line (attenuated) and the second group with VD57 line (wild) of C. Pseudotuberculosis with the same dose of infection and a control group with 5 animals. At 30, 60 and 120 days, five animals from each infected group were sacrificed for analysis, using the ELISA test, the *in vitro* production of the cytokines mentioned. Above from cultures of splenocytes stimulated with fractionated somatic antigens. The molecular of the fractions were weight above 100, between 100 and 50, between 50 and 10 and below 10 kDa. Only mice infected with VD57 showed granulomas, which may explain the

higher production of TNF-α, IL-10 and IL-4. In the study the fractions below 10 and 10-50 kDa did not induce *in vitro* production of IFN-g and IL-4. The fraction between 50-100 kDa stimulated splenocytes to produce TNF-α and the fraction above 100 kDa mainly stimulated the production of IL-10.

Conclusion: It is probable that the virulent strain of this microorganism induces the production of IL-4 as an immune escape mechanism.

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Keywords: *Corynebacterium pseudotuberculosis*, caseous lymphadenitis, somatic antigen fractions.

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Critical motifs of the *Anaplasma marginale* MSP1a functional epitope protect mice by balancing humoral and cellular immune response

PAULA DE SOUZA SANTOS (1); ANGELA APARECIDA SERVINO DE SENA (1); RAFAEL NASCIMENTO (1); THAISE GONÇALVES ARAÚJO (1); MIRIAN MACHADO MENDES (1); TIAGO WILSON PATRIARCA MINEO (1); LUIZ RICARDO GOULART (1)

(1). Federal University of Uberlândia

Introduction: Bovine anaplasmosis is a hemoparasitic disease that causes considerable economic loss to the dairy and beef industries. Cattle immunized with the *Anaplasma marginale* MSP1 outer membrane protein complex present a protective humoral immune response; however, the efficacy of these vaccines is variable. The immunodominance of epitopes seems to be a key-limiting factor for the adaptive immunity. We have successfully demonstrated that a critical motif of the MSP1a functional epitope is essential for antibody recognition of infected animal sera (PLoS One 7(3):e33045, 2012) , but its potential to induce protective immunity is yet to be tested.
Methods and Results: We have evaluated two synthetic vaccine formulations (Am1 and Am2) against *A. marginale*, using epitope-based approach. The experiments were carried out in murine model, which were immunized with peptides conjugated to bovine serum albumin in three 15-day interval intraperitoneal injections before challenge with live bacteria. Blood samples were analyzed for the presence of specific IgG antibodies, along with IgG2a and IgG1 antibodies, as well as for the rickettsemia analysis. Additionally, a panel containing the transcription of innate and adaptive immune responses' cytokines was carried out through qPCR. After challenge with *A. marginale*, it was observed that mice immunized with synthetic peptides presented stable body weight and no mortality, along with reduced number of infected erythrocytes (75%). In addition, the immunization process induced significantly higher production of specific IgG2a than IgG1 antibodies, along with an increased in situ expression of pro-inflammatory cytokines.
Conclusion: we have demonstrated that the two synthetic peptides obtained from critical motifs within the MSP1a functional epitope were able to induce an effective immune response in mice, associated with the upregulation of effector functions of humoral and cellular immune responses, reinforcing the use of synthetic epitopes as vaccine targets against bovine anaplasmosis, aiming bacterial clearance and diminished pathogenesis.

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IDENTIFICATION OF M. LEPRAE DNA IN HOUSEHOLD CONTACTS RESIDENT IN GOVERNADOR VALADARES – MG, BRAZIL

RAFAEL SILVA GAMA(1); THALISSON ARTUR RIBEIRO GOMIDES(1); EUZENIR NUNES SARNO(2); MILTON OZÓRIO MORAES(2); GULNARA PATRICIA BORJA CABRERA(1); LUCIA ALVES DE OLIVEIRA FRAGA(1).

(1). Núcleo de Pesquisa em Imunologia – Faculdade de Ciências da Saúde - Universidade Vale do Rio Doce – UNIVALE – Gov Valadares, MG; (2). Fundação Oswaldo Cruz –FIOCRUZ/RJ.

Introduction: Classical bacteriological methods for identification of pathogenic bacteria can not be applied to the diagnosis of leprosy, especially the inability of in vitro cultivation of *M. leprae*. The histopathology and smear microscopy has been used as auxiliary methods for the clinical classification of cases. The advent of molecular biology techniques with good specificity and high sensitivity has been evaluated as tools for early diagnosis of leprosy. **Objective**: The objective of this study was to evaluate the qPCR as a tool to identify *M. leprae*, and to compare the levels of bacterial DNA in samples of dermal scrapings and blood of patients with leprosy and their household contacts. **Methodology and Results**: The qPCR was performed to amplify 16S rRNA fragments, specific for the *M. leprae*. A total of 156 individuals participated in this study, 43 index cases and 113 household contacts. PCR was positive with 16S rRNA primer in 21 (48.84%) of 43 patients diagnosed with leprosy while the baciloscopic method was positive in only 13 (30.23%) patients. In relation to household contacts 27 (23.89%) of 113 subjects had bacterial DNA. The levels of bacterial DNA contacts were similar to those of DNA of PB. We conclude that qPCR was able to detect bacterial DNA in biological samples in which the baciloscopic method was negative. Furthermore, a positive qPCR smear was higher than in index cases under 5 lesions. We found that 23.89% of the contacts showed *M. leprae* DNA in qPCR and that the level of bacterial DNA in these subjects was similar to the group level CPB, DM 1 and DM 2. Rather, the level of bacterial DNA was significantly lower than in group MB. **Conclusion:** We suggest the incorporation of this technique in the health system, as well as monitoring and prophylactic treatment of contacts positive qPCR as strategies for early diagnosis and control of leprosy.

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SCHISTOSOMA SPP ANTIGENS IMPAIR THE CELLULAR IMMUNE RESPONSE IN HTLV-1-INFECTED INDIVIDUALS

LUCIANE MOTA LIMA(1); SILVANE BRAGA SANTOS(1); ALINE BÁFICA(1); LUCIANA SANTOS CARDOSO(1); SERGIO COSTA OLIVEIRA(2); ALFREDO MIRANDA GÓES(2); ALEX LOUKAS(3); EDGAR M. DE CARVALHO(1); MARIA ILMA ARAÚJO(1).

(1)Serviço de Imunologia, Hospital Universitário Professor Edgard Santos, Universidade Federal da Bahia; (2)Instituto de Ciências Biológicas, Departamento de Bioquímica e Imunologia, Universidade Federal de Minas Gerais; (3)Division of Infectious Diseases, Queensland Institute of Medical Research, Brisbane, Queensland, Australia.

ABSTRACT

Introduction-The HTLV-1 is the causal agent of Adult T cell Leukemia/lymphoma (ATLL) and HTLV-1-associated Myelopathy/Tropical Spastic Paraparesis (HAM/TSP). While the immune response to HTLV-1 infection is polarized to the Th1-type, in the last has been shown that helminthes infections, that had a deviation to Th2-immune response in chronic schistosomiasis, modulate the Th1 response involved in some auto-immune diseases. Our hypothesis is that the *Schistosoma spp.* antigens modulate the *in vitro* cytokine response in HTLV-1-infection. **Methods-** The *Schistosoma* antigens Sm29, ShTSP2 e PIII was added to the cultures of PBMC of HTLV-1- infected individuals and the levels of cytokines in the supernatants were measure using ELISA sandwich method. **Results-** Compared to the levels of cytokine in non stimulated PBMC cultures, the levels of IFN-γ ($p<0.05$) and TNF-α were reduced by the presence of Sm29 in 50 and 53% of patients (mean reduction 59 and 43% $p<0.05$ respectively), in 59 and 29% of them by the ShTsp2 presence (47 and 45%. $p<0.05$ respectively) and in 50 and 41% after PIII addition (35 and 45%, $p<0.05$ respectively). Meanwhile, the levels of IL-10 increased by the addiction of this antigens in 74, 22 and 44% of individuals, mean reduction 327, 573 and 35%, respectively $p<0.05$. The down-regulation of IFN-γ and TNF-α production by the *Schistosoma* antigens was observed mainly in subjects who had lower basal levels of this cytokine. **Conclusion-** We conclude that *Schistosoma spp.* antigens are able to down modulate the inflammatory

cytokine response, mainly in subjects who had lower basal levels of these cytokines.

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DOWN-REGULATION OF IFN-g RECEPTOR ALPHA CHAIN EXPRESSION AND CXCL-10 PRODUCTION BY *CYSTOISOSPORA BELLI* ANTIGENS

FERNANDA GONÇALVES GARCIA[(1)]; MÁRCIA BENEDITA DE OLIVEIRA SILVA[(1)]; ANA CRISTINA LOPES TOSTA[(1)]; DALMO CORREIA FILHO[(1)]; DAVID NASCIMENTO SILVA TEIXEIRA[(1)].

[(1)] Universidade Federal do Triângulo Mineiro, UFTM, Uberaba, MG, Brasil.

Introduction: Extraintestinal cystoisosporosis has already been reported in HIV/AIDS patients, generally involving preferential invasion of mesenteric and tracheobronchial lymph nodes, liver and spleen by unizoic cysts of this parasite, which may infect macrophages. It has been proposed that macrophages may act as *C. belli* host cells in extraintestinal sites. However, the absence of an *in vivo* experimental model has hampered the knowledge of parasite's biology. Moreover, until present, there is no information about chemokine and cytokine profile secreted by PBMCs in response to *C. belli* antigens.
Methods and Results: PBMCs were isolated from healthy volunteers and stimulated with *C. belli* oocysts/sporozoites antigens. Cytokines in culture supernatants were quantifyed by ELISA. The cell surface expression of IFN-g receptor α-chain (CD119) was assessed by staining PBMCs with anti-CD119-phycoerythrin (PE) monoclonal antibody and analyzed with a FACSCalibur flow cytometer using CellQuest software (BD Biosciences). This study was approved by UFTM Research Ethical Committee. We demonstrate that *C. belli* antigens markedly inhibited CXCL-10 production (403,7 pg/ml) by PBMCs when compared to non stimulated cells (1030,0 pg/ml) ($p=0,0093$) without affecting IL-12p40 and IFN-g secretion ($p>0,05$). Since, CXCL-10 is an IFN-g inducible chemokine; we also analyzed the IFN-g receptor α-chain expression on CD14+ positive PBMCs. Results obtained showed that PBMCs stimulation with *C. belli* antigens decreased CD119 expression.
Conclusion: Since IFN-g activates effector mechanisms against intracellular pathogens, the impaired IFN-g response (reduction of CXCL-10 production and CD119 expression) in cells stimulated by *C. belli* antigens may contributes to survival of this pathogen inside host cells. Investigation of mechanisms involved on IFN-g signaling inhibition by *C. belli* antigens may contribute for increase the knowledge about *C. belli* evasion strategies. The results obtained on the present work may improve the understanding of immunological events involved on *C. belli*/hosts cells interactions.

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DENDRITIC CELLS PROFILE INDUCED BY *SCHISTOSOMA MANSONI* ANTIGEN IN CUTANEOUS LEISHMANIASIS PATIENTS

DIEGO MOTA LOPES[(1)]; LUCIANA SANTOS CARDOSO[(1,2,3)]; JAMILLE SOUZA FERNANDES[(1)]; EDGAR M. CARVALHO[(1,2,4)]; MARIA ILMA ARAUJO[(1,2,4)].

[(1)]Immunology Service, HUPES,UFBA, Salvador; [(2)]National Institute of Science and Technology in Tropical Diseases (INCT-DT)-CNPQ/MCT; [(3)]Department of Life Sciences, UNEB, Salvador, Bahia; [(4)]Bahiana School of Medicine and Public Health, Salvador, Bahia, Brazil.

Introduction: The Th1-immune response is associated with tissue injury in human cutaneous leishmaniasis (CL). On the other hand, the immune response induced by some *Schistosoma mansoni* antigens is able to down-regulate the inflammatory response in immune-mediated diseases. The aim of this study was to evaluate the potential of the *S. mansoni* antigen Sm29 in down-modulate the response of monocyte-derived dendritic cells (MoDCs) from individuals with CL stimulated *in vitro* with *L. braziliensis* soluble antigen (SLA).
Methods and Results: Twenty patients with cutaneous leishmaniasis were enrolled in this study so far PBMC were obtained using a Ficoll-Hypaque gradient. Monocytes were cultured in the presence of GM-CSF and IL-4 for differentiation into dendritic cells (DCs), and then stimulated with the soluble leishmania antigen (SLA) in the presence or absence of the *S.mansoni* antigen Sm29. The expression of surface molecules HLA-DR, CD80 and CD86 on MoDCs and the expression of cytokines such as IL-12 and TNF-α and IL-10 receptor (IL-10R) by these cells have been evaluated by flow cytometry. The study was approved by the Ethical Committee of the State University of Bahia (UNEB). We observed that the addition of Sm29 to the MoDCs cultures stimulated with SLA resulted in higher mean fluorescence intensity (MIF) of CD80 (716±721) compared to cells stimulated with SLA alone (281±100; $p<0.05$). Moreover, the expression of IL-12 was lower in the presence of Sm29 (1.0±0.9%) compared to the cultures stimulated with SLA without Sm29 (6.7±8.9%; $p<0.05$). There was no significant difference in the frequency of MODCs expressing HLA-DR, CD86 and TNF-α and neither in the MIF of this molecules between cultures stimulated with SLA and SLA in the presence of Sm29 (data not shown). On the other hand, we observed a higher expression of IL-10 receptor in the presence of Sm29 (14.5 ± 8.5%) compared to the cultures stimulated with SLA alone (2.8± 2.3%; $p<0.05$).
Conclusion: Our results indicate that the antigen Sm29 is able to decrease the *in vitro* expression of inflammatory mediators on MoDCs of cutaneous leishmaniasis patients and that this down-modulation may involves the regulatory cytokine IL-10.

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MONOCYTES SUBSETS IN SCHISTOSOMIASIS PATIENTS WITH PERIPORTAL FIBROSIS

JAMILLE SOUZA FERNANDES[(1)]; MARIA ILMA ARAUJO[(1,2,3)]; DIEGO MOTA LOPES[(1)]; EDGAR CARVALHO[(1,2,3)]; LUCIANA SANTOS CARDOSO[(1,2,4)]

[(1)]Immunology Service, HUPES,UFBA, Salvador; [(2)]National Institute of Science and Technology in Tropical Diseases (INCT-DT)-CNPQ/MCT; [(3)]Bahiana School of Medicine and Public Health, Salvador; [(4)]Department of Life Sciences, UNEB, Salvador, Bahia; Brazil.

Introduction: Periportal fibrosis occurs in about 5-10% of individuals infected with Schistosoma mansoni. This pathology is predominantly caused by the host immune response to parasite egg antigens, however the mechanisms is not well understood. The aim of this study was to characterize the profile of the three monocytes subsets in schistosomiasis patients with different degrees of periportal fibrosis.

Methods and Results: Twenty-seven patients have been enrolled in the study to date. Periportal fibrosis was classified using abdominal USG according to the WHO criteria. Monocytes were obtained from PBMC and classified into classical ($CD14^{++}CD16^{-}$), intermediate ($CD14^{++}CD16^{+}$) and non-classical ($CD14^{+}CD16^{++}$). Cytokine expression was evaluated using flow cytometry. The study was approved by the Ethical Committee of the Maternidade Climério de Oliveira – UFBA. In general, the frequency of classical monocytes was higher than the frequency of the other subsets. The expression of TNF was higher in intermediate (mean±SD of MIF = 269.3±152.6) and non-classical (77.9±28.8 MIF) monocytes from patients with moderate to severe fibrosis, compared to patients without fibrosis (84.8±38.4 and 61.2 ±33.4 MIF to intermediate and non-classical, respectively). Moreover, there was a higher expression of IL-6 in monocytes (74.3±27.5 MIF in classical, 142.4±63.1 in intermediate and 98.9 ±50.4 in non-classical) of patients with moderate to severe fibrosis in comparison to those without fibrosis (15.1±9.4, 21.6±15.1 and 11.9±9.0 MIF, in classical, intermediate and non-classical, respectively). There was also a higher expression of TGF-beta in all subsets of monocytes in patients with periportal fibrosis in relation to patients without fibrosis. Unexpectedly, the expression of IL-10 was higher in classical (119.2±15.8 MIF) and intermediate (170.5±27.4

MIF) monocytes of patients with moderate to severe fibrosis, compared to patients without fibrosis (65.4±46.9 and 100±72.2 MIF in classical and intermediate monocytes, respectively).

Conclusion: In schistosomiasis patients with moderate to severe periportal fibrosis, different subsets of monocytes are characterized by a high expression of proinflammatory and profibrotic cytokines such as TNF, IL-6 and TGF-beta, despite the elevated expression of the regulatory molecule IL-10 in these monocytes.

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THE IN VITRO ANALYSIS OF iNOS-KO MOUSE MACROPHAGES INFECTED WITH PURPUREOCILLIUM LILACINUM FROM DISTINCT CLINICAL FORMS OF HYALOHYPHOMYCOSIS

CARLOS G. G. PONTE[1,2]; JESSICA R. DE LIMA[1]; MARIANA SIQUEIRA[1]; PAULA M. DE LUCA[3]; CÍNTIA M. BORBA[2] & PAULO R. Z. ANTAS[1].

[1]Laboratório de Imunologia Clínica, [2]Laboratório de Taxonomia, Bioquímica e Bioprospecção de Fungos and [3]Laboratório de Imunoparasitologia, Oswaldo Cruz Institute, FIOCRUZ, Rio de Janeiro.

Introduction: *Purpureocillium lilacinum* is currently recognized as an emerging opportunistic fungus, causing the hyalohyphomycosis infection in adults and children, mostly in immunosuppressed ones. Virtually no data is available regarding the immune mechanisms related to host-pathogen interaction for hyalohyphomycosis caused by this fungus. We aim to explore better this initial contact using a genetic deficient model in order to uncover early critical immunological mechanisms that may play an important role during the infection. **Methods and Results:** In this study, we have used 3 distinct isolates of *P. lilacinum* obtained from human clinical cases (skin, tibial area, sinus), and thus in vitro challenged knock-out mouse macrophages, which are believed to be the first host cells to interact with this pathogen. Conidia of *P. lilacinum* were purified and peritoneal macrophages from iNOS-KO mouse were infected at different time-points and at a ratio of 2:1, respectively. After infection, cells were stained both with "Wright-Giemsa" for light optical microscopy, and with surface markers for flow cytometry. In addition, supernatants were harvested for further assays such as IFN-a ELISA and MMP-9 gelatin zymography. Following 12 hours of incubation, germ tubes were promptly produced, suggesting active metabolism by the fungus, as well as development of branched septate hyphae inside macrophages. Ultimately at 24 hours, the macrophages were completely destroyed. Dissimilar data were found among the *P. lilacinum* strains used through. High levels of CD18/CD38-double expression were observed, but the MMP-9 (except for the skin isolate) and IFN-a levels (peaked at half-hour of interaction) were equally produced, disregard the clinical isolate used. **Conclusion:** Besides the mouse model here, highly destructive capability of *P. lilacinum* has been also observed in vitro in wild-type mouse. Additional studies with other immunological parameters are to come in order to correlate these

data with future findings intended to improve our understanding of this neglected fungal infection.

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CCR7 IS CRITICAL FOR RESISTANCE AGAINST EXPERIMENTAL *TRYPANOSOMA CRUZI* INFECTION

ESTER ROFFÊ(1); ANTONIO GIGLIOTTI ROTHFUCHS(2); FLAVIA LIMA RIBEIRO-GOMES(3); ANA PAULA MAIA PEIXOTO MARINO(1); MICHAEL ECKHAUS(4); MICHAIL S. LIONAKIS(1); HELTON DA COSTA SANTIAGO(5); PHILIP M. MURPHY(1)

(1)Molecular Signaling Section, LMI, NIAID/NIH, Bethesda, MD, USA

(2)Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, Stockholm, Sweden

(3)Intracellular Parasite Biology Section, LPD, NIAID/NIH, Bethesda, MD, USA

(4)Division of Veterinary Resources, ORS, OD, NIH, Bethesda, MD, USA

(5)Departamento de Bioquímica e Imunologia, UFMG, Belo Horizonte, MG, Brasil

Introduction: The protozoan parasite *Trypanosoma cruzi* is the etiological agent of Chagas' Disease, which affects ~12 million people in Latin America despite coordinated international infection control efforts. Cardiomyopathy due to chronic inflammation and fibrosis is the most common and serious manifestation of Chagas' Disease, but its pathogenesis is poorly understood. Several inflammatory chemokines and chemokine receptors have been implicated in *T. cruzi* immunopathogenesis in mouse models of the infection; however, the role of homeostatic chemokines has not been previously defined.

Methods and Results: In the present study, we found that mRNA for both the homeostatic chemokine Ccl19 and its receptor Ccr7 was induced in the heart during the acute phase after infection of wild-type C57BL/6 mice. Consistent with an indispensable role of Ccr7 in trypanosomiasis, infection was uniformly fatal during the acute phase in infected *Ccr7*$^{-/-}$ mice, whereas mortality was only 10% in infected WT control mice. Death in infected *Ccr7*$^{-/-}$ mice was associated with uncontrolled parasitemia and increased parasite burden in both heart and liver, whereas infected WT mice controlled parasite burden in all 3 compartments. Leukocyte infiltration was greater in the hearts of infected *Ccr7*$^{-}$

$^{/-}$ mice compared to infected WT controls, and was characterized by a lower frequency of $CD8^+$ T cells and a greater frequency of $Gr\text{-}1^{hi}CD11b^+I\text{-}A^-$ cells (neutrophils). Also, lower expression of IFNg was detected in the hearts of *Ccr7* KO mice. Adoptive transfer of both $CD4^+$ and $CD8^+$ T cells from WT mice was necessary to confer protection to *T. cruzi*-infected TCRa KO mice, whereas transfer of $CD4^+$ and/or $CD8^+$ T cells from Ccr7-deficient mice was not sufficient to confer protection to these mice.

Conclusion: Ccr7 is a critical factor for survival and control of *T. cruzi* infection in C57BL/6 mice. We suggest a role for Ccr7 in the activation and migration of IFNg-producing $CD8^+$ T cells to *T. cruzi*-infected hearts, which may control the parasite and restrict neutrophil-mediated immunopathology. The exact mechanisms of resistance conferred by Ccr7 are currently under investigation.

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EVALUATION OF THE IMMUNE RESPONSE AGAINST *BRUCELLA ABORTUS* IN SPLENECTOMIZED MICE

ALICE BELLEIGOLI REZENDE[(1)]; NÁIRA NEVES NETO[(1)] ; CAROLINA DE SOUZA MIRANDA[(1)], MARCELA MOTA[(1)]; POLLYANNA AMARAL SALVADOR[(1)]; GILSON COSTA MACEDO[(1)]; HENRIQUE COUTO TEIXEIRA[(1)]

[(1)] Laboratório de Imunologia, Departamento de Parasitologia, Microbiologia e Imunologia, Instituto de Ciências Biológicas, Universidade Federal de Juiz de Fora, Juiz de Fora – MG, Brasil

Introduction: The spleen plays an important role in immune function. However, the influence of the spleen in control and progression of intracellular bacterial infections remains unclear. Brucellosis is a zoonosis caused by the facultative intracellular bacteria of the genus *Brucella*. According to World Health Organization, thousands of new cases of human brucellosis are diagnosed each year worldwide. In Brazil, bovine brucellosis is endemic throughout the country, compromising the quality of cattle and causing a large annual loss for the Brazilian economy. *Brucella abortus* has a tropism for the spleen and induces an immune response mediated by cytokines such as IFN-γ and TNF-α. The objective of this work was to investigate the immune response against *Brucella abortus* in splenectomized mice.

Methods and Results: C57Bl/6 mice were divided into a sham-operated control group (SH) and splenectomized group (SP). Thirty days after surgery, animals were intraperitoneally infected with 10^6 bacteria/mouse of *Brucella abortus* (S2308). Twenty one days after infection, *B. abortus* colony-forming units (CFU) were evaluated in macerated liver. Cytokine production (IFN-γ, IL-12, TNF-α, IL-6 and IL-10) was analyzed in liver by ELISA. A higher number of *B. abortus* CFU was observed in liver of splenectomized mice (Ln = 3.8 ± 0.1) in comparison to the sham-operated group (Ln = 3.4 ± 0.1). IFN-γ and IL-12 production was lower in liver of SP mice (600.3 ± 75.9 pg/ml and 1303 ± 99.3 pg/ml, respectively). In addition, SH animals presented a greater production of TNF-α, IL-6 and IL-10 in liver in comparison to the splenectomized group ($p<0.05$).

Conclusion: Splenectomy reduces the capacity of C57Bl/6 mice to control *B.abortus* infection which correlates with a lower cytokine production in splenectomized mice.

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MONOCYTE PRODUCTION OF MATRIX METALLOPROTEINASE 9 IN PATIENTS WITH CUTANEOUS LEISHMANIASIS

TAÍS MENEZES CERQUEIRA CAMPOS[1], RÚBIA COSTA[1], SARA PASSOS[1], ADRIANO QUEIROZ[1], DAVID MOSSER[2], PHILLIP SCOTT[3], EDGAR M. CARVALHO[1, 4,] and LUCAS P. CARVALHO[1,4, 5].

(1). Serviço de Imunologia, Universidade Federal da Bahia; (2). University of Maryland; (3). Department of Pathobiology, University of Pennsylvania; (4) Instituto de Ciência e Tecnologia – Doenças Tropicais (INCT-DT); (5). Instituto de Ciências da Saúde (ICS), Universidade Federal da Bahia.

Introduction: Cutaneous leishmaniasis (CL) caused by *L. braziliensis* is an inflammatory disease characterized by the presence of ulcerated lesion with mononuclear cell infiltrate and few parasites. In *Leishmania* antigen-stimulated peripheral blood mononuclear cells (PBMC) cultures and in lesion of these patients, high levels of pro-inflammatory cytokines (TNF and IFN-gama) are detected. TNF can mediate immunopathology through various mechanisms, including induction of necrosis, cytotoxicity and metalloproteinases (MMPs) secretion. MMPs are a family of enzymes that degrade extracellular matrix proteins produced by many cell types, particularly activated macrophages. Factors contributing to basal membrane degradation that precedes the ulcer development are not known. However, in other pathologies MMP-9 contribute to basal membrane degradation. Thus, our goal is to investigate the contribution of subsets of monocytes to MMP-9 production in CL patients.
Methods and Results: PBMC from uninfected controls and CL patients were obtained and *ex-vivo* labeling for CD4, CD8, CD14, CD16 and MMP-9 were performed to determine cells producing MMP-9 by flow cytometry. We found that monocytes are the main source of MMP-9, and the subset of non-classical ones ($CD14^{dim}CD16^{+}$) expressed more MMP-9 than the classical ($CD14^{+}CD16^{-}$) and intermediate ($CD14^{+}CD16^{+}$) monocytes. Monocytes from CL patients expressed more MMP-9 than the ones from healthy individuals. To determine the balance MMP-9/TIMP-1 (MMP-9 inhibitor), cells were stimulated with *Leishmania* antigen for 72 hours and the levels of MMP-9 and TIMP-1 were determined by ELISA. Higher levels of *Leishmania* antigen-induced MMP-9 and lower levels of TIMP-1 were found in supernatants of PBMC cultures from patients when compared to the ones from healthy individuals. Also, MMP-9 was detected in unstimulated cultures of biopsies from CL patients, whereas unstimulated PBMC from the same individuals did not produce MMP-9. To determine the contribution of TNF to MMP-9 production we cultured PBMC in presence or absence recombinant TNF for 24 hours. Presence of TNF induced MMP-9 secretion as determined by ELISA.
Conclusion: Monocytes from CL patients produce more MMP-9 than cells from healthy individuals and non-classical monocytes are the main source of MMP-9. High levels of MMP-9 were also found in lesion from CL patients. Altogether,

our data suggest that monocyte secretion of MMP-9 may contribute to the tissue damage in CL.

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EFFECT OF THALIDOMIDE TREATMENT ON IMMUNE RESPONSE AGAINST *BRUCELLA ABORTUS*

MARINA COELHO DE QUEIROZ [(1)]; CAROLINA DE SOUZA MIRANDA[(1)]; MARCELA MOTA[(1)]; TÁRSILA GUIMARÃES[(1)]; GILSON COSTA MACEDO [(1)].

[(1)] Laboratório de Imunologia, Departamento de Parasitologia, Microbiologia e Imunologia, Instituto de Ciências Biológicas, Universidade Federal de Juiz de Fora, Juiz de Fora – MG, Brasil.

Introduction: Brucellosis is a zoonosis caused by bacteria of the genus *Brucella* that infects humans and a variety of animals, mainly cattle. According to World Health Organization, this illness remains the commonest zoonotic disease worldwide with thousands of new cases reported every year. In addition to great damages to health, Brucellosis affects the quality of animal-derived products resulting in a great economic impact, especially in Brazil. The immune response against *Brucella* involves several mechanisms of innate and acquired immunity, of which the cytokine IFN-γ and $CD8^+$ T cells have a critical role. In this context, many studies have shown that Thalidomide (a glutamic acid derivative) is able to induce high production of IFN-γ, costimulation, proliferation and cytokine production, mainly by $CD8^+$ T lymphocytes. Thus, the goal of this study was evaluate the effect of Thalidomide treatment on immune response against *B. abortus*.

Methods and Results: The C57BL/6 mice were treated with Thalidomide during seven days and infected with *B. abortus* (S2308). The PBS treated mice were used as control. A week after the infection, the bacterial load was determined in the spleen. IFN-γ and Nitric oxide (NO) production were evaluated in supernatants of splenocytes in response to *B. abortus* stimulation. Cytokine (IFN-γ e IL-12) concentration was also evaluated in macerated liver. Finally, the ability of *B. abortus*-primed splenocytes from Thalidomide treated mice, to lyse infected macrophages was assayed.

The results have shown a significant decrease in the number of bacteria in the spleen of the treated animals compared to control group. Furthermore, the treated group showed greater IFN-γ, IL-12 and NO production in response to *Brucella*. Additionally, was detected an enhanced cytotoxic activity in splenocytes derived from treated animals.

Conclusion: The results suggest that Thalidomide is able to potentiate the immune response against *B. abortus*.

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DIVERGENT OUTCOMES OF THE INTERACTION BETWEEN MACROPHAGES AND *Trichophyton rubrum* CONIDIA

FÁBIO SEITI YAMADA YOSHIKAWA (IC) [(1)]; SANDRO ROGÉRIO DE ALMEIDA [(1)]

[(1)] Department of Clinical and Toxicological Analysis, Faculty of Pharmaceutical Sciences, University of São Paulo

Introduction: *Trichophyton rubrum* is the main etiological agent of dermatophytosis - superficial fungal infections of the skin, hair or nails - in humans. *T.rubrum* infections are inflammatory, chronic and refractory to most available treatment schemes. Unrevealing the immunopathological mechanism behind this mycosis may display new pharmacological targets and help to design more efficient and safer therapeutic approaches. The aim of this study was to evaluate the response of different macrophage models to *T.rubrum* conidia.

Methods and Results: Thioglycollate-elicited peritoneal macrophages (PM) and bone marrow-derived macrophages (BMMs) from C57BL/6 mice were incubated with *T.rubrum* conidia (MOI 1) for 4, 6, 8 and 10 hours. BMMs were primed with LPS (1µg/mL) overnight (BMM_{ov}) or 30 minutes (BMM_{30}) before conidia addition. Phagocytosis was observed under optical microscopy. Fungal viability was assessed by colony forming units (CFU) assay. The supernatants were collected for cytokine measurements by ELISA. Statistical significance was determined by Two-Way ANOVA and Bonferroni posttest. All macrophage populations were able to phagocytose *T.rubrum* conidia, but, in BMM_{30} population, conidia could turn into hyphae, growing inside the cells and ending by disrupting them. On the other side, PMs and BMM_{ov} kept the dermatophyte on it conidial form, without hyphae growth, but conidia were viable even after 10 hours as showed by CFU assay (log CFU: 4h 1,91±0,09; 6h 1,89±0,28; 8h 1,74±0,21; 10h 1,61±0,2). In addition, IL-1β, an inflammatory cytokine produced by the inflammasome in response to the activation of cytosolic receptors (Nod-like receptors), was significantly detected only in BMM_{30} (4h: 44,7±14,0 pg/mL) while neither PMs nor BMM_{ov} produced significant amounts of the cytokine in response to the infection.

Conclusion: Results showed that macrophages efficiently phagocytosed but did not eliminate the fungus. Even though activated macrophages could resist to fungal growth, they showed a fungistatical, but not fungicidal, activity. Furthermore, IL-1β seemed to be dependent on hyphae growth, perhaps because NLRs are intracellular receptors which could only come into contact with fungal determinants through hyphae growth in the cytosol.

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Leptospira evades the human complement attack by the secretion of metalloproteases that directly cleave complement proteins

TATIANA RODRIGUES FRAGA[1], DANIELLA DOS SANTOS COURROL[1], SÍLVIO ARRUDA VASCONCELLOS[2], ANGELA SILVA BARBOSA[3], LOURDES ISAAC[1]

[1] Department of Immunology, University of São Paulo; [2] Faculty of Veterinary Medicine, University of São Paulo; [3] Bacteriology Department, Butantan Institute, Brazil

Introduction: Leptospirosis is a neglected infectious disease of public health importance. Complement represents a central immune mechanism in blood circulation, but the high ability of *Leptospira* to spread indicates a low efficacy of complement against this microorganism. Pathogenic *Leptospira* have successfully developed strategies to evade the complement system. They acquire the regulators Factor H (FH) and C4BP, which control complement activation in pathogen´s surface. However, complement evasion may also occur in the fluid phase, by the secretion of bacterial proteases. The aim of this work was to evaluate the *Leptospira* ability to secrete proteases that directly cleave complement molecules and also to identify the proteins responsible for the cleavages.

Methods and Results: The proteolytic cleavages of complement molecules were analyzed by Western blot. Seven different strains of pathogenic *Leptospira* were able to secrete proteases that cleaved C3, C3b, iC3b, C2, C4b, C5 and FB, but not C1q, C4 and IgG. The cleavages of C3, C4, FB and C2 were also observed when normal human sera were used as a complement source. In contrast, non-pathogenic *Leptospira* did not present significant proteolytic activity. The protease activity was inhibited by ortho-phenanthroline, a metalloprotease inhibitor. We cloned, expressed and purified the leptospiral metalloprotease thermolysin NprT and showed that it was able to cleave C3 and that its activity was inhibited by ortho-phenantroline. NprT specifically cleaved C3 in the alfa chain, generating a C3b-*like* molecule, which was susceptible to the cleavage by host factor FI. NprT also cleaved C3b, but only in the presence of FH, indicating a co-factor activity of this molecule. We also performed a purification of the native proteases from the pathogenic leptospiral supernatant by gel filtration. A fraction of 15 to 30 kDa was able to cleave the complement protein C3b. Finally, we showed the alternative pathway activity of normal human serum was reduced by the treatment with pathogenic leptospiral proteases.

Conclusions: We describe a novel immune evasion mechanism in *Leptospira*: the secretion of proteases that cleave complement proteins. We also identified the thermolysin NprT, a metalloprotease that cleaves the complement molecule C3. The leptospiral proteases can be considered as virulence factors, since

they can deactivate immune effector molecules, being potential targets to therapeutic approaches in leptospirosis.

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Title: *VPAC1* GENE POLYMORPHISM IS ASSOCIATED WITH THE INDETERMINATE FORM OF CHAGAS DISEASE

MARIELLE VALERIO CORREA(PG)(1); MANOEL OTÁVIO DA COSTA ROCHA(2); NATALIA SATCHIKO HOJO DE SOUSA(PG)(1); KARINE SILVESTRE FERREIRA(PG)(1); KENNETH JOHN GOLLOB(3,4,6); WALDEREZ ORNELAS DUTRA(1,3,6); CRISTIANE ALVES DA SILVA MENEZES(5,6).

(1).Departamento de Morfologia da Universidade Federal de Minas Gerais; (2).Faculdade de Medicina da Universidade Federal de Minas Gerais; (3). Center for Infectious Disease Research, SRI International, Menlo Park, CA, USA; (4). Instituto de Ensino e Pesquisa Programa de Pos-graduação em Biomedicina e Medicina, Hospital Santa Casa BH; (5). Instituto de Ciências Exatas e Biológicas da Universidade Federal de Ouro Preto; (6) INCT-DT.

Introduction: Human infection with *T. cruzi* leads to Chagas disease (CD), which presents as several different clinical phenotypes ranging from an asymptomatic form to a severe dilated cardiomyopathy. Host's immune system plays a critical role in parasitemia control; however, exacerbated cellular response can cause tissue damage. Interactions between the immune, nervous and endocrine systems play an important role in modulating host susceptibility and resistance to inflammatory and infectious diseases through the homeostasis maintenance of cellular response. Vasoactive intestinal peptide (VIP) is a neuropeptide with a broad distribution in the body and exerts a role as a modulator of the homeostasis of the immune system through its receptors VPAC1 and VPAC2. Data concerning to VIP or its receptors function in the scenario of CD are lacking in literature. Considering this, and the variation in clinical forms of CD, we hypothesized that an aberrant response to VIP could be related to development of different clinical outcomes and associated to VPAC polymorphisms. Thus, the present study aims to analyze if gene polymorphisms of VIP receptors, may be associated with different clinical forms of CD.
Methods and Results: To assess possible associations between single nucleotide polymorphisms of genes encoding VPAC1 and VPAC2 and the development of CD, DNA was obtained from oral swabs of a cohort of 144 individuals (57 patients with dilated cardiomiopathy, 51 patients with indeterminate form and 51 non infected individuals) and the evaluation of polymorphisms are being performed using Real Time PCR. Statistical analysis of data was performed using the chi(2) likelihood ratio test. The study groups were tested for Hardy-Weinberg equilibrium comparing the expected with the observed genotypes frequencies for VPAC1 SNP rs342511 (2234 C/T) (X^2 =0,0079; p=0,9292). Our data showed that patients carrying the T variant for VPAC1 have four times more chance of developing the indeterminate form as compared to the cardiac form (X^2= 5, 179; OR= 4,267; p=0,023; IC=0,336-2,976).

Conclusions: We observed a significant association with VPAC1 and the indeterminate form of CD. The polymorphism observed is localized at the 3´-UTR that may contain regulatory sequences related to the stability of the mRNA, leading to alteration of VPAC1 expression in indeterminate individuals. Studies regarding cellular VPAC1 expression must be performed.

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THE ROLE OF TLR4 IN THE PRODUCTION OF TNF-α, IFN-γ, IL-10, IL-17 AND IN THE EXPRESSION OF FOXP3 IN A *TOXOPLASMA GONDII* EXPERIMENTAL INFECTION – PRELIMINARY RESULTS

MARIANA MIZIARA DE ABREU[1], MARIANA GATTO[1], LAURA DENISE MENDES DA SILVA[1], FRANCILENE CAPEL TAVARES[1], JOÃO PESSOA DE ARAÚJO JUNIOR[2], CARLOS ROBERTO GONÇALVES DE LIMA[1], SUELI APARECIDA CALVI[1]

[1]Departament of Tropical Diseases, Botucatu School of Medicine – UNESP, Brazil; [2]Departament of Microbiology and Immunology, Biosciences Institute – UNESP Botucatu, Brazil.

Introduction: Toxoplasmosis is a worldwide disease, in immunocompetent individuals are asymptomatic and manifest in different clinical forms in pregnant women and immunosuppressed. The protection of the host against infection is performed by mediated immune response effector cell receptors as TLRs and proinflammatory cytokines. In addition, regulatory T cells and anti-inflammatory cytokines act by regulating the immune response. Our objective was to evaluate the involvement of TLR4 in the production of pro-inflammatory and anti-inflamatory cytokines and Foxp3 expression during acute experimental infection by T. gondii. **Methods and results:** 12 Balb/c mice were infected with $2{,}5x10^4$ tachyzoites of BTU-2 strain and euthanized (n=3) on days 1, 3, 5 e 7 post-infection and parasite load observed in peritoneal fluid these days. Spleen of each animal was held on extraction of RNA by the Trizol ® method, cDNA synthesis and expression quantification of TLR4 and Foxp3 by qPCR. Culture of splenocytes was performed and stimulated or not with LPS (TLR4 agonist), and the supernatants collected for the determination of TNF-α, IFN-γ, IL-10 and IL-17 by ELISA. The parasite load on days 5 ($1{,}9x10^7 \pm 0{,}4x10^7$) and 7 ($2{,}6x10^7 \pm 0{,}9x10^7$) was significantly higher compared to days 1 ($2{,}3x10^4 \pm 1x10^4$) e 3 ($6x10^4 \pm 4x10^4$) p.i.. The expression of TLR4 and Foxp3 increased in all days of infection, especially on day 3 p.i. (139.79 ± 29.64) (307.53 ± 136.49) respectively, compared to control. All cytokines production increased in infected animals when stimulated with LPS. The higher levels of IL-10 were detected on day 1 (247.5 ± 3.786) and 3 (210.50 ± 1.0) p.i.. IL-17 and TNF-α increased on day 3 p.i. (329.75 ± 0.5) (387.25 ± 5.188), respectively, but decreased in other days. IFN-γ was significantly lower on all days of infection compared to control (103.75 ± 6.551). **Conclusion:** The infection increased the expression of TLR4, which could be related to increased production of TNF-α, IL-17 and IL-10, with the Foxp3 expression and low IFN-γ production, which suggests that the IFN-γ is the principal protective cytokine during infection, while others are related to the increase in parasitic load. Our results suggest that TLR4 may be acting in infection with T. gondii as a pathogenic mechanism.

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TLR2 AND TLR4 EXPRESSION AND NUTRITIONAL PROFILE IN PATIENTS WITH VISCERAL LEISHMANIASIS. PRELIMINARY RESULTS.

MARIANA GATTO[1]; MARIANA MIZIARA DE ABREU[1]; MARJORIE DE ASSIS GOLIM[2]; LORAINE GOLLINO[1]; JOSÉ CLÁUDIO SIMÃO[3]; KAREN INGRID TASCA[1]; CARLOS MAGNO CASTELO BRANCO FORTALEZA[1]; PAULO CÂMARA MARQUES PEREIRA[1]; SUELI APARECIDA CALVI[1]

[1]Departament of Tropical Diseases, Botucatu School of Medicine - UNESP, São Paulo State University, Brazil; [2]Flow Citometry Laboratory, Hemocenter, Botucatu School of Medicine - UNESP, São Paulo State University, Brazil; [3]Manoel de Abreu Hospital, Bauru, São Paulo, Brazil.

Introduction: Visceral leishmaniasis is an emerging public health problem, with 500,000 new cases per year. In order to have the development of adaptive immune response is necessary that cellular receptors such as TLR2 and TLR4 recognize and play the parasite effector mechanisms. Moreover, the nutritional profile also has impact on immune response. Our objective was to evaluate the TLR2 and TLR4 expression and correlate with the nutritional profile of patients with visceral leishmaniasis before and after treatment. **Methods and results**: Were evaluated 8 patients pre-treatment, 3 post-treatment and 8 healthy subjects. Analysis of the expression of TLR2 and TLR4 on CD3 and CD14 cells was performed by flow citometry and the nutritional profile was performed by bioelectrical impedance and biochemical tests. In CD3 cells, the percentage of TLR2 expression was higher in patients pre-treatment (36,9±14,5) than those post-treatment (5,7±5) and controls (1,42±1,03); percentage of TLR4 expression, although low in all groups, was higher in pre-treatment (2,8±1,4) compared to controls (1±0,3). Percentage of TLR2/TLR4 coexpression in CD3 cells was higher in pre-treatment (2,4±1) compared to controls (1±0,1). Regarding to CD14 cells, percentage of TLR2 expression was lower in pre-treatment (83,7±13,6) than those controls (98,9±0,8); percentage of TLR4 expression and TLR2/TLR4 coexpression did not differ between groups. The percentage of body fat was lower in patients pre-treatment (16,3±10,6) compared to controls (27,4±6,3); phase angle was lower in patients pre-treatment (5.8º±1,3) compared to pos-treatment (7.0°±0,4). HDL and albumin concentrations were lower in pre-treatment (9,8±11,9 e 2,2±0,6) than those post-treatment (41,5±6,3 e 4,2±0,05) and controls (65,8±13,4 e 4,3±0,4) respectively; LDL and total cholesterol levels in pre-treatment (101,4±30,6 e 58±17,9) were lower than in controls (199±22,5 e 113,32±24,3) respectively. **Conclusion**: Our results suggest that patients with active LV expressed TLR2 and TLR4 in monocytes and lymphocytes, suggesting a possible involvement of these receptors in the development of the immune response. Patients also had nutritional profile changes that may be interfering with the immune response against the parasite.

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DEVELOPMENT OF MURINE PLACENTAL MALARIA IS DEPENDENT ON TLR4/MYD88 PATHWAY.

RENATO BARBOZA[1]; ARAMYS SILVA DOS REIS[1]; WESLEY L. FOTORAN[1]; LEANDRO GUSTAVO DA SILVA[1];GERHARD WUNDERLICH[1]; NIELS OLSEN SARAIVA CÂMARA[3]; SILVIA BEATRIZ BOSCARDIN[1]; SABRINA EPIPHANIO[2] & CLAUDIO ROMERO FARIAS MARINHO[1]

[1] Departamento de Parasitologia, ICB, Universidade de São Paulo, São Paulo, Brasil; [2]Departamento de Imunologia, ICB, Universidade de São Paulo, São Paulo, Brasil; [3]Departamento de Ciências Biológicas, Universidade Federal de São Paulo, Diadema, Brasil.

Introduction: Toll like receptors (TLR) are components of innate immune system that recognize pathogens through distinct molecular patterns. Recruitment of MyD88 is a common pathway of TLR signaling, which leads to induction of NF-kB-dependent inflammatory cytokine production. It has been described that TLR4 recognize components of *Plasmodium sp.* and affect the host immune response. Despite the fact that the activation of innate immune system by *Plasmodium sp.* during pregnancy can result in a range of complications affecting both mother and fetus, the aim of this work was to evaluate the effects of TLR4/MyD88 pathway on the development of placental malaria (PM). **Methods & Results:** Initially, to ascertain if TLR4 could recognize *P. berghei* NK65 infected red blood cells (iRBC), COS-7 cells were co-transfected with mouse CD14 and TLR4. For luciferase reporter assay, cells were also co-transfected with ELAM-1-firefly luciferase and beta-actin-*Renilla* luciferase plasmids. Our results showed that iRBC were able to activate TLR4. To verify the effects of TLR activation, we infected pregnant TLR4$^{-/-}$, MyD88$^{-/-}$ and C57BL/6 (WT) mice with *P. berghei* NK65 on the 13th gestational day. Histopathological analysis of the placentas showed a decrease of vascular spaces in WT infected mice when compared with placentas from non-infected mice (39.44±4.75 vs. 48.13±4.57 % of vascular space, respectively). On the other hand, placentas from infected and non-infected of both strains TLR4 and MyD88 did not present differences when compared with the control. As the reduction of vascular space can be a consequence of inflammatory processes, our next step was to analyze the placental expression of IL-6, TNF-alpha and IP-10 mRNA. Our results show that only WT infected mice presented an increase in the expression of these cytokines as compared with TLR4$^{-/-}$ and MyD88$^{-/-}$ mice. **Conclusion:** In summary, this study illustrates the importance of a severe inflammatory response in the development of PM pathogenesis via TLR4/MyD88 signaling and, additionally, indicates the TLR4 and adaptor protein MyD88 as potential targets for therapeutics intervention.

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INDUCTION OF TH17 LYMPHOCYTES CONTROLS MYOCARDITIS DURING *Trypanosoma cruzi* INFECTION BY RESTRAINING A DETRIMENTAL TH1 RESPONSE

TIAGO DA SILVA MEDINA [(1)]; GRACE KELLY SILVA [(1)]; DENISE MORAIS FONSECA [(1)]; RENATA SESTI-COSTA [(1)]; MARIA CLÁUDIA SILVA [(1)]; BERNARD RYFFEL [(2)]; JOÃO SANTANA SILVA [(1)].

[(1)] Laboratório de Imunoparasitologia da Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo - Ribeirão Preto, Brasil (FMRP-USP).
[(2)] Centre National de la Recherche Scientifique (CNRS), Orléans, France.

Introduction: *Trypanosoma cruzi* infection predominantly induces a Th1 response to control the parasite proliferation; nevertheless an exacerbated Th1 response is deleterious to the host. **Objective:** Because the microenvironment plays a crucial role during $CD4^+$ T cell differentiation, we sought to understand how the immune response is regulated in the absence of molecules involved with Th17 lymphocytes differentiation and maintenance. **Results:** After infecting WT, IL-17R$^{-/-}$, IL-23$^{-/-}$ and IL-6$^{-/-}$ mice with 10^3 forms of *T. cruzi* (Y strain), we observed that IL-17R$^{-/-}$, IL-23$^{-/-}$ and IL-6$^{-/-}$ mice rapidly succumb to *T. cruzi* infection compared with WT mice. As *T. cruzi* mainly affects the heart tissue, we analyzed the inflammation in this organ. Inflammation in the heart tissue was very impressive in IL-17R$^{-/-}$, IL-23$^{-/-}$ and IL-6$^{-/-}$ mice, which was important to control the cardiac parasitism compared with WT mice. Heart inflammation somewhat promoted cardiac damage in IL-17R$^{-/-}$, IL-23$^{-/-}$ and IL-6$^{-/-}$ mice, indicated by high CK-MB serum levels. To assess the mechanisms related to the premature death in the absence of Th17 molecules, we verified that macrophages from IL-17R$^{-/-}$, IL-23$^{-/-}$ and IL-6$^{-/-}$ mice eliminate the parasite more efficiently than macrophages from WT mice, by expressing augmented iNOS and NO levels. We also verified that IL-17R$^{-/-}$, IL-23$^{-/-}$ and IL-6$^{-/-}$ mice induced an increased, deleterious Th1 response in the heart tissue when compared with WT mice, indicated by high levels of IFN-γ, IL-12, iNOS and TNF. Corroborating these data, we detected increased levels of CXCL9 and CXCL10 chemokines (associated with Th1 migration), but reduced levels of CCR6 (expressed on Th17 cells), in the heart tissue of IL-17R$^{-/-}$, IL-23$^{-/-}$ and IL-6$^{-/-}$ mice when compared with WT mice. This intense Th1 response was not efficiently regulated by anti-inflammatory pathways, as observed by decreased IL-10 levels in the heart of IL-17R$^{-/-}$, IL-23$^{-/-}$ and IL-6$^{-/-}$ mice compared with WT mice. To confirm the importance of regulatory mechanisms, we observed that WT mice induced high amounts of regulatory T cells 21 days after infection, the period that coincide with the death of IL-17R$^{-/-}$, IL-23$^{-/-}$ and IL-6$^{-/-}$ mice, suggesting that these cells are controlling the local inflammatory response. **Conclusion:** Collectively, Th17 cells control myocarditis during *T. cruzi*

infection by restraining a detrimental Th1 response, which is tightly regulated by regulatory T cells, leading to mice survival.

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Leishmania amazonensis INCREASES ECTONUCLEOTIDASES EXPRESSION IN LYMPH NODE DENDRITIC CELLS AND T REG CELLS POPULATIONS IN LESION SITES

AMANDA BRAGA DE FIGUEIREDO; PRISCILA MARTINS DE SOUZA, TIAGO DONATELLI SERAFIM; LUÍS CARLOS CROCCO AFONSO

Laboratório de Imunoparasitologia, Departamento de Ciências Biológicas, ICEB / NUPEB, Universidade Federal de Ouro Preto. Ouro Preto, Minas Gerais, Brazil.

Introduction: *Leishmania* infections can result in a wide spectrum of clinical manifestations, and the outcome of disease is determined by parasite species and the host immune response. Dendritic cells (DC) play an essential role in the modulation of immune response and T reg cells are important in immunosuppressive response, including during *Leishmania* infection. Extracellular-ATP exhibits pro-inflammatory properties whereas adenosine is an important anti-inflammatory mediator. Our objective was to investigate the DC activation, the ectonucleotidases expression in these cells and the generation of T reg cells after *Leishmania* infection. **Methods and Results:** C57BL/6J mice were inoculated on ears with 1 x 10^5 CFSE-labelled metacyclic promastigotes of *L. amazonensis*, *L. braziliensis* or *L. major*. Draining lymph nodes and ears were isolated after 20 h, 1 and 3 weeks and cells were stained and analyzed by flow cytometry. After 20 h of infection with *L. amazonensis*, *L. braziliensis* or *L. major*, approximately 12% of lymph node cells were found to be $CD11c^+$ and of these $CD11c^+$ cells 10% were infected. The expression of MHCII, CD86 and CD40 was significantly higher in lymph node infected-DC, regardless of the strain of *Leishmania* used. Interestingly, the infection increases ectonucleotidases expression in DC and at least 50% of infected cells co-expressed CD39 and CD73, independent of the parasite species used. *L. amazonensis*, *L. braziliensis* and *L. major* decrease the percentage of T reg cells (CD4+CD25+Foxp3+ cells) in draining lymph nodes after 1 or 3 weeks of infection, but only *L. amazonensis* increases the percentage of these cells in lesion sites after 3 weeks of infection. **Conclusion:** In conclusion, *L. amazonensis* increases ectonucleotidases expression in lymph node DC and T reg cells populations in lesion sites.

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TYPE II GRANULOMA INDUCED BY *SCHISTOSOMA MANSONI* EGGS IN 5-LIPOXYGENASE KNOCKOUT MICE

GABRIEL TOFFOLI DA SILVA (IC) [(1)]; MILENA SOBRAL ESPÍNDOLA (PG) [(1,2)]; CAROLINE FONTANARI (T) [(1)]; ALYNE FÁVERO GALVÃO (T) [(1)]; OLINDA MARA B. TREVILATO (T) [(2)]; ELAINE MEDINA FLORIANO (T) [(2)]; VANDERLEI RODRIGUES [(2)]; SIMONE GUSMÃO RAMOS [(2)]; LÚCIA HELENA FACCIOLI [(1)]; FABIANI GAI FRANTZ [(1)].

(1). Faculdade de Ciências Farmacêuticas de Ribeirão Preto, USP, SP
(2). Faculdade de Medicina de Ribeirão Preto, USP, SP.

Introduction: In the experimental model to study type 2 granulomas, mice are inoculated intraperitoneally and challenged intravenously with eggs of *Schistosoma mansoni*, previously isolated from liver of chronically infected animals. In this model, granulomas are developed in the lungs 4 days after challenge, involving mainly mononuclear cells and little collagen deposition, with an initial induction of TH1-type immune responses (production of IFN-γ, with low levels of IL-4 and IL-13). This response is transient and passes to a TH2 profile, with lesions containing mononuclear cells, eosinophils and fibroblasts, with significant collagen deposition, characteristic of granulomas type II. Lipoxins and leukotrienes are lipid mediators derived from arachidonic acid metabolites via the 5-lipoxygenase (5LO) pathaway. However, until now no one knows how these mediators are involved in the induction of type II granuloma. In this study, we aim to investigate how type II granulomas develop in mice knockout of the 5-lipoxygenase gene (5LO-KO).
Methods and Results: Wild-type or 5LO-KO mice were inoculated intraperitoneally with 3000 eggs of *S. mansoni*, and 14 days later, mice were challenged intravenously with 3000 eggs of the parasite. Four, eight and sixteen days after intravenous administration of the eggs, the animals were killed for the assessment of pulmonary granuloma formation, determining size, cellular composition, and fibrosis formation by histopathology techniques and production of cytokines and chemokines by ELISA. Apparently, compared to WT mice, 5LO-KO animals had fewer granulomas, in despite of its larger size. Regarding the production of cytokines, the absence of 5LO relates to a high rate of TGF-β after 4 days of intravenous inoculation and a lower production of IL-13 compared to WT in the same period.
Conclusion: This high production of TGF-β in 5LO-KO mice in the first days can be related to the control of the granulomas number. Furthermore, the lower production of others cytokines and chemokines in 5LO-KO compared to WT can be related to the uncontrolled growth of granulomas in the lung.

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HLA CLASS I AND SUSCEPTIBILITY TO CHAGAS DISEASE

CHRISTIANE MARIA AYO[1]; CAMILA DE FREITAS OLIVEIRA[1]; PÂMELA GUIMARÃES REIS[1]; EMÍLIA ÂNGELA SIPPERT[1]; FLAVIA CARDOSO ZAGH[1]; MÁRCIA MACHADO DE OLIVEIRA DALÁLIO[1]; JEANE ELIETE LAGUILA VISENTAINER[1]; ANA MARIA SELL[1]

[1] Universidade Estadual de Maringá

Introduction: Chagas' disease, caused by Trypanosoma cruzi, is endemic in many countries in Latin America, affecting millions of people. It is worth noting that uninfected individuals are found in all reported studies of endemic areas and the variation in seropositivity is attributable to genetic factors. HLA genes are extremely polymorphic and play a central role in the immune response making them attractive candidates for influencing the differential outcomes of *T. cruzi*. The aim of this study was to investigate allele and haplotype frequencies of the HLA-A, B and C in seropositive chronic Chagas patients and controls in a population from North/Northwest of Parana State, South Brazil. **Methods and Results:** Peripheral blood was collected from 158 unrelated individuals (control, 95; chagasic, 63) and the DNA was extracted from leukocytes by a salting out procedure. HLA typing was carried out according to the manufacturer's specification for LABType SSO Typing, testing for each locus using Luminex Technology (One Lambda, INC, USA) and the retrieved output was analyzed by HLA Fusion software (One Lambda, INC, USA) for allele identification. The frequencies were determined by Arlequin 3.1. software and the comparison of allele frequencies was analyzed in 2x2 contingency tables using the chi-square test with Yates' correction or Fisher's Test. To estimate the disease risk, odds ratio and 95% confidence interval were calculated. Statistical analyses were performed using the *SISA* statistics software. The Hardy-Weinberg equilibrium was achieved by calculating the expected genotype frequencies and comparing them to the observed values. Statistically significant susceptibility to Chagas' disease was found for *HLA-B*08* allelic group (p: 0.001; OR: 7.2; 95% CI: 2.01-25.94) and for *HLA-A*02-B*14* haplotype (p: 0.001; OR: 23.9; 95% CI: 1.35-123.8). A significant increase of the frequency of the haplotype *HLA-A*02-B*35* was observed in the control group (p: 0.02; OR: 0.05; 95% CI: 0.003-0.9). **Conclusions:** These results suggest that the *HLA-B*08* and *HLA-A*02-B*14* conferred susceptibility and *HLA-A*02-B*35* conferred protection against the development of the Chagas' disease, regardless of the clinical form of the disease.

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The Role of Eosinophils in *Aspergillus fumigatus* Lung Infection

FREDERICO MARIANETTI SORIANI[1]; REMO CASTRO RUSSO[2]; CAMILA RODRIGUES CHAVES NOGUEIRA[4]; LIRLÂNDIA PIRES DE SOUSA[4]; MILENE ALVARENGA RACHID[3]; MAURO MARTINS TEIXEIRA[1]: [1]Bioquímica e Imunologia, [2]Fisiologia, [3]Patologia, ICB; [4]Farmácia, Universidade Federal de Minas Gerais, Belo Horizonte, Brasil.

Introduction: Eosinophils are important in the pathophysiology of allergic diseases and in host immunity. Although microorganisms directly activate inflammatory cells, the mechanisms that trigger eosinophils activation and its functions in immune responses are unknown. Fungi are ubiquitous and may contribute to the development and exacerbation of pulmonary diseases. In this sense, the aim of this study was to unravel the role of eosinophils during *Aspergillus fumigatus* lung infection. **Methods and Results:** Wild type (BALB/c) and *ΔdblGATA* mice were infected intranasally with 10^8 spores of an *A. fumigatus* wild type strain. Lungs and BALFs were collected from animals after 1 and 3 days post infection and used for the analysis. Results show that *ΔdblGATA* mice had increased survival (40% versus 100% in WT mice). PMN influx in the lungs 1dpi was greater in *ΔdblGATA* mice (1,641±76.3), compared to wild type (392.7±139.1), while macrophage influx to the airways was reduced in *ΔdblGATA* mice after 3 days of infection (10.6±2.1x10^6 cells/BALF) compared to wild type (21.1±2.7x10^6 cells/BALF). However, besides cell influx there was less protein leakage 3 dpi in *ΔdblGATA* mice (2,224±172 versus 3,195±275). Levels of eosinophils were very high in lungs and airways of WT mice after *A. fumigatus* infection but not detectable in *ΔdblGATA* mice. Airway levels of CXCL1, TNF-α, CCL11, CCL2 and IL-1β were reduced in *ΔdblGATA* mice (638.5±205; 865±93; 163±13; 265±8.6 and 19.8±3.5, respectively) compared to wild type (1,771±79; 1,858±197; 216±26; 378±8.3 and 79.4±17.6, respectively). Interestingly, histopathological analysis did not show any differences between animals. Fungal burden was higher in *ΔdblGATA* than WT mice after 1(11.5±0.7x10^5 versus 8.6±0.5x10^5UFC) and 3 dpi (0.46±0.15x10^5 versus 0.15±0.05x10^5 UFC). **Conclusion:** In conclusion, the absence of eosinophils leads to decreased production of key cytokines and increased fungal burden after infection with *A. fumigatus*. However, this is associated with enhanced neutrophil influx and survival, suggesting that eosinophils are crucial in setting the appropriate tone of the immune response to infection by *A. fumigatus*.

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INCREASE TLR2 RECEPTOR, ROS AND NO PRODUCTION IN MONONUCLEAR CELLS FROM DOGS AFTER STIMULATION WITH P-MAPA.

LARISSA MARTINS MELO(PG)[1]; LETICIA DA CRUZ SANCHES(PG)[1]; KATHLENN LIEZBETH DE OLIVEIRA DA SILVA(PG)[1]; JULIANA PEROSSO BORGES(PG)[1]; BRUNA BRITTO DE OLIVEIRA(IC)[1]; MARCOS DE ARRUDA SOMENZARI (PG)[1]; VALÉRIA MARÇAL FÉLIX DE LIMA(PD)[1]

(1)Laboratório de Imunologia, Depto. Clínica, Cirurgia e Reprodução Animal – Faculdade de Medicina Veterinária -UNESP - Araçatuba -SP

Introduction: *Leishmania (L.) chagasi* is the etiologic agent of visceral leishmaniasis (VL), which can be transmitted to humans, and the dogs are main domestic reservoirs. VL in Brazil represents a serious public health problem. The infection in dogs suppress cellular immune response and the therapeutic arsenal against CVL is limited. It is therefore important studying new alternatives for the treatment of infected dogs, which may reduce the incidence of the disease in endemic areas. The new immunomodulator P-MAPA improve the immunocompetence when the immune system was impaired.

Methods and Results: This investigation was performed in Araçatuba, the city is located in the São Paulo state. It is a region known to be endemic for canine VL. A total of 15 adult dogs were selected based on positive serological tests for visceral leishmaniasis by ELISA and the presence of at least three clinical signs. A group of 6 healthy dogs, from a non-endemic area were included in the study as negative controls. These animals showed negative serological tests for visceral leishmaniasis by ELISA.The peripheral blood mononuclear cells were isolated from both group and cultured in RPMI-1640 with different concentrations of P-MAPA 20, 100 and 200 µg/ml in a humid environment at 37°C with 5% CO_2 for 24h. Dyed for determination of TLR2, TLR4 using the monoclonal antibodies conjugated to fluorochromes. The ROS production was measured using carboxy-H2DFFDA (Molecular Probe ®) according to manufacturer's instructions. After data acquisition in EasyCyte mini ® (Guava, Hayward, CA), the analysis of the data was held in the Software Express Plus ® Guava. For determination NO were using the culture supernatant of mononuclear cells stimulated with P-MAPA by Griess reaction. The data were analyzed with non parametric test. Results were considered as significant when $p<0.05$. Mononuclear cells from infected dogs with Leishmania spp stimulated with 100 mg and 200 mg of P-MAPA increase significantly TLR2, ROS in

compared to baseline ($p < 0.05$). The NO production was observed only with stimulation of 200 mg of P-MAPA. Mononuclear cells from healthy dogs NO and ROS production were observed with the stimulation of 100mg and 200mg of P-MAPA compared to baseline ($p < 0.05$) and TLR 2 increased after stimulation with 20mg, 100mg and 200mg of P-MAPA

Conclusion: These findings suggest that P-MAPA has potential as a therapeutic drug in canine visceral leishmaniasis.

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SAG2A PROTEIN OF *Toxoplasma gondii* SHOWS AN INTRINSICALLY UNSTRUCTURED PROTEIN DOMAIN

ARLINDO GOMES DE MACÊDO JÚNIOR[1], JAIR P. CUNHA-JUNIOR[1], THYAGO H. S. CARDOSO[2,3], MURILO V. SILVA[1], FERNANDA M. SANTIAGO[1], JOÃO S. SILVA[4], CARLOS P. PIROVANI[2], DEISE A. O. SILVA[1], JOSÉ R. MINEO[1], TIAGO W. P. MINEO

1. Immunoparasitology Laboratory, Institute of Biomedical Sciences, Federal University of Uberlândia (UFU), Uberlândia, Minas Gerais, Brazil. 2. Biotechnology and Genetics Center, Santa Cruz State University/UESC, Bahia, Brazil. 3. Department of Biochemistry, Escola Paulista de Medicina, Universidade Federal de São Paulo, São Paulo, SP, Brazil. 4 School of Medicine of Ribeirão Preto, São Paulo University/USP, São Paulo, Brazil.

Introduction Toxoplasmosis is a zoonosis caused by an intracellular parasite, *Toxoplasma gondii*. Several studies have been performed in order to understand the interactions between proteins of parasite and its host cells. SAG2A is a 22 kDa protein that is mainly found in the surface of tachyzoites. Additionally, It has been previously found to be serologically immunodominant during the acute phase of toxoplasmosis. In the present work, our major aim was to study the structure modeling demonstrated that SAG2A protein. Methods To construct three-dimensional structures of SAG2A, modeling was based on structures solved by X-ray crystallography or RMN and PSI-Blast. Recombinant protein SAG2A full and without C-terminal region and tachyzoites of RH strain of T. gondii were used in experiments with macrophages derived from mouse bone marrow. Concentrations of IL-12 were measured by commercial ELISA kits and nitric oxide production estimated from the concentration of nitrite by the Griess method. Results Structure modeling demonstrated that SAG2A protein possesses an unfolded C-terminal end forming a loop, characteristic of intrinsically unstructured protein (IUP) domains, which also sheltered the known immunodominant epitope. In addition, distinct conformations of the loop were predicted within strain types of *T. gondii*, due to an additional glycine found in type II strains, which induced the formation of a helical structure in the immunodominant epitope region. Modeling of NcSAG2A, orthologue expressed in *Neospora caninum*, confirmed similar overall structure of the orthologues, although it may be noted that SAG2A from T. gondii presents a largely

disordered loop and NcSAG2A presented a loop sequence composed with beta-sheets. Additionally, we observed that overall structural homology was preserved in the N-terminal end of the sequences. The role of such distinctions in the IUP domain was then checked in innate responses against *T. gondii*. In agreement with the experiments with the recombinant proteins, opsonization of live parasites with monoclonal antibodies specifically directed to the epitope region restored the observed downmodulation of pro-inflammatory responses, although that effect presented a stronger phenotype in type II strains. Conclusion Altogether, we demonstrate that the structure modeling demonstrated that SAG2A protein possesses an unfolded C-terminal end forming a loop, characteristic of intrinsically unstructured protein (IUP) domains, which also sheltered the known immunodominant epitope.

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LEISHMANIA CHAGASI RESISTANT TO NO SHOWED HIGHER CAPACITY TO SURVIVE AGAINST MACROPHAGES MICROBICIDAL MECHANISMS

PRISCILA LIMA DOS SANTOS [(1)]; RICARDO VIEIRA COSTA [(1)]; JUCIENE DE MATOS BRAZ [(1)]; MICHELI LUIZE BARBOSA SANTOS [(1)]; ADRIANA CARDOSO BATISTA [(1)]; MARGARETE RANGEL [(1)]; FABRÍCIA ALVISI DE OLIVEIRA [(1)]; PAULO DE TARSO GONÇALVES LEOPOLDO [(1)]; AMÉLIA RIBEIRO DE JESUS [(1,2)]; TATIANA RODRIGUES DE MOURA [(1)]; ROQUE PACHECO ALMEIDA [(1,2)]

(1) Universidade Federal de Sergipe – Aracaju, Brazil; (2) Instituto de Investigação em Imunologia, São Paulo, Brazil

Introduction: Nitric oxide (NO) plays an important role as a leishmanicidal agent in mouse macrophages. NO resistant *Escherichia coli* and *Mycobacterium tuberculosis* have been associated with poor outcomes of their resulting diseases. NO resistant *Leishmania braziliensis* has also been identified and exacerbates the clinical course of human leishmaniasis.

Methods and results: *L. chagasi* were obtained from bone marrow aspirates from both visceral leishmaniasis patients and the canine reservoirs. Promastigotes were exposed to increasing concentrations of $NaNO_2$ (0-16 mM) and viability was determined by the MTT colorimetric method. We found that 12 *L. chagasi* isolated from humans were considered resistant to NO, showing an average of 87% survival at 16mM $NaNO_2$, while 9 isolates were considered susceptible to NO, showing an average of 50% survival at concentrations of 8 mM. 13 *L. chagasi* promastigotes isolated from dogs were also considered resistant to NO, showing an average of 100% survival at 16mM $NaNO_2$, while 8 isolates were considered susceptible to NO, showing an average of 50% survival at concentrations of 2 mM. The profile of NO resistance of both dogs and humans *L. chagasi* was confirmed using another NO donor (SNAP) showing an IC_{50} of 193.2 mM for $NaNO_2$ and of 48,8 mM for SNAP, as compared to the NO susceptible *L. chagasi* with an IC_{50} of 9.3 mM for $NaNO_2$ and of 636.0 mM for SNAP. To evaluate whether the NO resistance phenotype would enhance the infectivity and survival of amastigote in macrophages, we selected 4 resistant (LVHSE 9 and 14) and 4 susceptible (LVHSE 7 and 17) isolates from humans and infected J774murine macrophages. Interestingly, the resistant isolates survived and proliferated better in murine macrophages, showing a significantly higher number of infected macrophages and parasite numbers.

Conclusion: We demonstrate the existence of *L. chagasi* from humans and dogs that are naturally resistant to NO and that these resistant isolates have a higher capacity to survive against murine macrophages microbicidal mechanisms.

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REACTIVE OXYGEN SPECIES PRODUCTION, DEVELOPMENT OF IMMUNE RESPONSE AND MYOCARDITIS DURING *Trypanosoma cruzi* INFECTION: EFFECTS OF ARYL HYDROCARBON RECEPTOR (AhR) ACTIVATION

ANDRÉIA BARROSO (1); LISIA ESPER (1,3); FATIMA BRANT (1,3); RONAN RICARDO SABINO ARAÚJO (1); MATHEUS BATISTA HEITOR CARNEIRO (1); LEDA QUERCIA VIEIRA (1); MILENE ALVARENGA RACHID (2); FABIANA SIMÃO MACHADO (1,3)

(1) Departamento de Bioquímica e Imunologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Minas Gerais, Brasil. (2) Departamento de Patologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Minas Gerais, Brasil. (3) Faculdade de Medicina, Universidade Federal de Minas Gerais, Minas Gerais, Brasil.

Introduction: The balance of immune response is essential to control parasite growth and pathology development during *Trypanosoma cruzi* infection. The 5-lipoxygenase enzyme activity results in production of Lipoxin $(LXA)_4$, an anti inflammatory eicosanoid, that is important in the regulation of inflammatory cytokines production during this infection. The role of AhR, a LXA_4 nuclear receptor, during *T.cruzi* infection is not known.

Methods and Results: Herein, wild type (WT) and $AhR^{-/-}$ mice were infected with *T.cruzi* (Y strain) and the AhR expression, parasitemia and immune response was assessed. The spleens, livers and hearts were harvested at different days post-infection (dpi) for histology or cytokines analyses by RT-PCR and/or flow cytometry. We found that during *T.cruzi* infection the AhR expression is up-modulated in spleen and heart. The resistance to infection is related with the absence of AhR that resulted in increased levels of IL12, IFN-γ production and number of dendritic and T cells at 10dpi.The inhibition of pro-inflammatory cytokine production was observed in WT, but not in $AhR^{-/-}$ mice, 15dpi. The deficiency of AhR also resulted in an increased inflammation in the heart and liver 10dpi. *In vitro*, we investigated which was the mainly factor responsible for the increased efficiency to control the parasite grown in infected $AhR^{-/-}$ mice. We found an increased trypanocidal activity by *T.cruzi*-infected $AhR^{-/-}$ macrophages (MO), associated with increased reactive oxygen species (ROS) production, but not nitric oxide (NO) production, when compared with

WT. By contrast, uninfected AhR$^{-/-}$ MO is hyperresponsive to IFN-γ stimulation, producing higher levels of NO when compared with WT. Moreover, the Arginase I activity was similarly detected in the supernatants harvested from *T.cruzi*-infected- and -uninfected WT and AhR$^{-/-}$ MO cultures.

Conclusion: All together, our data suggests that AhR activity is essential to modulate innate and adaptative immune response and development of myocarditis during *T. cruzi* infection.

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INVOLVEMENT OF SUPPRESSOR OF CYTOKINE SIGNALING 2 (SOCS-2) PROTEIN IN THE EXPERIMENTAL LUNG INFECTION INDUCED BY PATHOGENIC FUNGUS *PARACOCCIDIOIDES BRASILIENSIS*

PATRÍCIA CAMPI SANTOS[1,2]; LUCAS SECCHIM RIBEIRO[1,2]; TALLES PROSPERI DE PAULA[1,2]; SÍLVIA MARIA CORDEIRO WERNECK[1,2]; ROSANA DE CARVALHO CRUZ[1]; FABIANA SIMÃO MACHADO[2]; MAURO MARTINS TEIXEIRA[2]; PATRÍCIA SILVA CISALPINO[1]; DANIELLE G. SOUZA[1,2].

(1) Laboratory of Microorganism-Host Interaction, Department of Microbiology, Institute of Biological Sciences, Federal University of Minas Gerais, Minas Gerais, Brazil; (2) Laboratory of Immunopharmacology/Department of Biochemistry and Immunology, Institute of Biological Sciences, Federal University of Minas Gerais, Minas Gerais, Brazil

Introduction: *Paracoccidioides brasiliensis* is the agent of paracoccidioidomycosis (PCM), the most prevalent deep mycosis in Latin America, endemic in South and Southeast regions of Brazil. The PCM presents a wide range of clinical manifestations and severity of the disease is related to the pattern of host immune response. The suppressor of cytokine signaling (SOCS) family of proteins is a key controller of cytokine responses, which can down-regulate specific cytokine signals and consequently modify the immune response. The study aimed to evaluate the participation of SOCS-2 protein in the course of experimental pulmonary infection induced by the dimorphic fungus *P. brasiliensis* in mice.

Methods and Results: C57BL/6 wild-type mice (WT) and SOCS-2-deficient mice (SOCS-$2^{-/-}$) were used in all experiments. The experimental protocol was approved by the Committee on the Ethics of Animal Experiments of the Federal University of Minas Gerais (CETEA/UFMG - protocol number 163/2012). After anesthesia, mice were infected with 10^6 yeasts of Pb18 strain, by intratracheal injection, while uninfected mice received buffered saline by the same route. Mice were evaluated for survival and at given times, they were sacrificed and the lungs removed for analysis of the cytokines profile by ELISA, histopathology and in assays of myeloperoxidase (MPO), N-acetylglucosaminidase (NAG) and eoperoxidase (EPO) to evaluate the accumulation of neutrophils, macrophages and eosinophils, respectively, at the site of infection. Moreover, the fungal load was determined from bronchoalveolar lavage fluid (BAL) and lungs of infected mice. The results obtained showed that the absence of SOCS-2 resulted in 100% of death after 30 days of infection. This higher susceptibility to infection was associated with increased pulmonary fungal burden, a significant increase

in the synthesis of proinflammatory cytokines such as TNF-a, IL-1b, IL-6 and CXCL-1/KC chemokine, as well as a significant increase in the neutrophils and eosinophils recruitment into the pulmonary tissue. This inflammatory response profile resulted in exacerbated histopathology of the lungs in SOCS-2$^{-/-}$ mice relative to that observed in WT mice.

Conclusion: These results show that the signaling pathway involving SOCS-2 plays an important role in modulating the immune response during infection by the pathogenic fungus *P. brasiliensis*.

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HUMORAL IMUNE RESPONSE OF BALB/C AND C57BL/6 CHRONICALY INFECTED MICE AGAINST HSP70 OF *Toxoplasma gondii*

PAULO VICTOR CZARNEWSKI BARENCO[1]; JAIR PEREIRA CUNHA JÚNIOR[2]; ELAINE VICENTE LOURENÇO[3]; MARIA CRISTINA ROQUE-BARREIRA[3]; DEISE APARECIDA OLIVEIRA SILVA[2]; JOSÉ ROBERTO MINEO[2]; NEIDE MARIA SILVA[1]

[1]Laboratório de Imunopatologia, ICBIM, Universidade Federal de Uberlândia, Uberlândia, MG;
[2]Laboratório de Imunoparasitologia, ICBIM, Universidade Federal de Uberlândia, MG;
[3]Laboratório de Glicobiologia, Faculdade de Medicina de Ribeirão Preto, USP, Ribeirão Preto, SP;

Introduction: *Toxoplasma gondii* is an obligate protozoan parasite widely distributed that may cause severe damages mainly in the central nervous system (CNS) of immunocopromised hosts, among them HIV positive patients and pregnant women. During its life cycle, *T. gondii* converts from tachyzoite to bradyzoite stage and express the *T.g.*HSP70. The objective of this study was to correlate the humoral immune response against *T.g.*HSP70, tissue pathology and parasite load of infected mice.
Methods and Results: BALB/c and C57BL/6 mice were intraperitoneally inoculated with 10 cysts of ME-49 *T. gondii* strain and sacrificed on 7th (group 7doi), 32th (32doi), and 56th days of infection (56doi). Another additional group was infected and treated for 24 days with dexamethasone (DXM) in drinking water from 32th to 56th days of infection and then sacrificed (56doi DXM). During treatment time, the mice were observed daily for the morbidity score. C57BL/6 and BALB/c loses weight and increased the morbidity score when treated with the drug. The sera were analyzed by ELISA for detection of IgG antibodies against *T.g.*HSP70. BALB/c mice produced higher titers of IgG antibodies against *T.g.*HSP70 than C57BL/6 mice, although when both lineages were treated with DXM, low antibodies titers against *T.g.*HSP70 were detected. The C57BL/6 presented more severe tissue damage and parasite load in the course of infection than BALB/c mice. However, DXM-treated and infected mice presented lower tissue damage than untreated mice (56doi group).
Conclusion: These data suggests that the detection of IgG antibodies against *T.g.*HSP70 is an alternative diagnostic tool for toxoplasmosis in resistant mice lineages.

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PRODUCTION AND EVALUATION OF CHICKEN IGY ANTIBODIES AGAINST RECOMBINANT HSP70 OF *Toxoplasma gondii*

PAULO VICTOR CZARNEWSKI BARENCO[1]; MÁRIO CÉZAR DE OLIVEIRA[1]; POLIANA DE CASTRO MELO[1], JAIR PEREIRA CUNHA JÚNIOR[2]; TIAGO WILSON PATRIARCA MINEO[2]; NEIDE MARIA SILVA[1]

[1]Laboratório de Imunopatologia, ICBIM, Universidade Federal de Uberlândia, Uberlândia, MG;
[2]Laboratório de Imunoparasitologia, ICBIM, Universidade Federal de Uberlândia, MG;

Introduction: *Toxoplasma gondii* presents two developmental stages in the intermediate hosts. During stage differentiation from tachyzoite to bradyzoite, the parasite express *T.g.*HSP70. The objective of this study was to produce, purify and test IgY specific antibodies against *T.g.*HSP70 as an alternative diagnostic tool for toxoplasmosis.
Methods and Results: Recombinant *T.g.*HSP70 was expressed as fusion protein with glutathione-S-transferase (GST) in *E. coli* BL21 DE3 vector and purified with glutathione resin followed by thrombin cleavage and LPS removal. Chickens were immunized with 100 µg/animal of *T.g.*HSP70 diluted v/v with Freund's complete adjuvant and boosted after 15 and 30 days with 100 µg/animal of *T.g.*HSP70 diluted v/v with Freund's incomplete adjuvant. Eggs were collected daily and yolk was frozen at -20°C until use. For purification of IgY antibodies, yolk was diluted 10 times in water (pH 5,0) and centrifuged. The supernatant was pelleted with 19% sodium sulphate. The sediment was ressupended and dialyzed against phosphate buffer saline and then frozen at -20°C for late use. The immunofluorescence assay was used to verify the specificity of the purified IgY antibodies. It was observed that the IgY *T. gondii*-specific antibodies were able to detect T. g.HSP70 in the cytoplasm of *T. gondii* RH strain.
Conclusion: These results suggest that the IgY *T.g.*HSP70 could be used as an alternative diagnostic tool for toxoplasmosis.

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EVALUATION OF MACROPHAGE FUNCTION FROM HTLV-1 INFECTED SUBJECTS

CAMILA FARIAS AMORIM[1]; ANSELMO SOUZA[1]; ANGELA GIUDICE[1]; SILVANE SANTOS[1] and EDGAR M CARVALHO[1].

[1]Serviço de Imunologia, Complexo Universitário Professor Edgard Santos, Universidade Federal da Bahia, Salvador, Bahia, Brasil.

Introduction: The human T lymphotropic virus type 1 (HTLV-1) infection increases susceptibility and worsens the course of other infectious diseases, such as strongyloidiasis and tuberculosis. Since that, it is possible that APCs and/or lymphocytes from HTLV-1 infected individuals have defects in their functions leading to inadequate adaptative immune response against other infectious agent. In this viral infection there is an increased expression of proinflammatory cytokines, and is possible that the constant stimulation of macrophages take these cells to exhaustion. Alternatively, this activation may increase microbicidal ability.

Methods and Results: We compared the macrophage function of HTLV-1 carriers (n=13) with soronegative healthy subjects (n=7), evaluating their ability to phagocyte and kill *L. braziliensis*, analyzing the percentage of infected cells and the number of amastigotes per 100 macrophages. Additionally we determined TNF-α, CXCL9 and CXCL10 levels. The percentage of infected cells and the number of amastigotes per 100 cells were quite variable between the two groups studied. There was a trend for lower percentage of infected cells and lower number of amastigotes after 48 and 72 hours infection in cells from HTLV-1 carriers but there was no statistic difference. The TNF-α, CXCL9 and CXCL10 levels were higher ($p<0.05$) in supernatants of macrophages from HTLV-1 infected subjects than in health subjects macrophages. At 72h of culture the median and variation of TNF-α levels by HTLV-1 macrophages were 2.445 pg/mL (427 pg/mL to 2.863 pg/mL) and in controls macrophages were 89 pg/mL (0 pg/mL to 1.762 pg/mL), $p<0.05$.

Conclusion: While it is clear that macrophages from HTLV-1 infected subjects are activated and produces more TNF-α and proinflammatory chemokines than macrophages from healthy subjects, the limited number of cases studied do not

allow a conclusion about the differences in the killing ability of macrophages from these two groups.

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Activation of P2X7 receptor contributes to parasite control and to ameliorate the clinical manifestations of blood-stage Plasmodium chabaudi malaria

[1]Érika Machado de Salles, [1]Maria Nogueira de menezes,[1]Eduardo Pinheiro Amaral, [1]Sheyla Castillo Mendez, [1]Henrique Borges da Silva, [1]José Maria Alvarez, [2]Robson Coutinho-silva, *[1]Maria Regina D'Império Lima*

[1]Instituto de Ciência Biomédicas, Universidade de São Paulo [2]Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro.

Introduction: Malaria is a potentially serious infection disease caused by protozoa parasites of the genus Plasmodium. The blood stage of infection is responsible for all symptoms associated with malaria, which are mostly related to excessive activation of the immune system. Recently, it has been shown that innate immune receptors are able to detect signals released by damaged cells as ATP. P2X7 receptor (P2X7R) detects extracellular ATP and therefore could contribute to activate the immune response to Plasmodium. **Methods and results:** Six-to-eight-week-old C57BL/6 (B6) and $P2X7R^{-/-}$ (B6 background) female mice were infected by intraperitoneal injection with 10^6 parasitized erythrocytes. Serum samples of B6 mice were collected before and after the erythrocyte rupture and the ATP was quantified using ATP Bioluminescence Assay Kit. ATP levels were significantly higher after the erythrocyte rupture. Erythrocytes were removed from blood cell preparations by Percoll gradient separation (70%), to assess whether the ATP released from infected and non-infected erythrocytes is able to permeabilize spleen cells. Supernatants from lysed infected erythrocytes induced an increase in permeabilization of $CD4^+$ T cells and $CD11c^+$ cells. Four days after infection, spleen cells from B6 and $P2X7R^{-/-}$ mice were subjected to different ATP concentrations and then the cell permeability and proliferation were measured. Some cells were submitted to treatment with a P2X7R antagonist – Brilliant Blue G (BBG). We observed that infection by P. chabaudi is capable of up-modulating the ATP-induced permeabilization of $CD4^+$ T cells and $CD11c^+$ cells. Therefore, we then analyzed several clinical manifestations of the acute phase of malaria. $P2X7R^{-/-}$ and BBG-treated B6 mice had difficulty in controlling parasitemia and failed to reestablish body temperature and weight up to the seventh day of infection. **Conclusion**: Our results suggest that the recognition of extracellular ATP by P2X7R

contributes to parasite control and to ameliorate the clinical manifestations of blood-stage Plasmodium chabaudi malaria.

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PI3Kγ CONFERS RESISTENCE AGAINST *Trypanosoma cruzi* INFECTION DUE TO ITS IMPORTANT ROLE IN LYMPHOCYTE PROLIFERATION AND MICROBICIDAL ACTIVITY OF MACROPHAGES

MARIA CLÁUDIA DA SILVA[(1)]; RENATA SESTI[(1)] ; FABRÍCIO C. DIAS[(1)]; GRACE K. SILVA[(1)]; TIAGO DA SILVA MEDINA[(1)]; FERNANDO Q. CUNHA[(1)]; JOÃO SANTANA DA SILVA[(1)]; THIAGO MATTAR CUNHA[(1)]

(1) School of Medicine of Ribeirão Preto, University of São Paulo (USP), Ribeirão Preto, São Paulo - Brazil.

Background and objective: The phosphatidylinositol-3-kinases (PI3Ks) are a family of lipid kinases that plays a crucial role in several cellular processes including survival and proliferation. Among PI3Ks, PI3Kγ is a member activated by G protein coupled receptors, involved in the signaling of chemotactic factors and leukocytes migration and activation. In this study, we evaluated the role of PI3Kγ during experimental infection by *T. cruzi*. **Methods:** C57BL/6 WT and PI3Kγ$^{-/-}$ mice were infected with 10^3 forms of *T. cruzi* Y strain. Ten days after infection, the heart tissue was harvested for quantification of PI3K activation. For evaluation of PI3Kγ role eighteen days after *T. cruzi* infection, heart tissue was harvested for assessment of inflammation, lesion and parasitism, and we observed daily the parasitemia and survival rate of WT and PI3Kγ$^{-/-}$ mice. The involvement of PI3Kγ in the killing of the parasite and NO production was assessed in bone marrow-derived macrophages from WT and PI3Kγ$^{-/-}$ mice. To verify whether PI3K signaling influenced the *in vitro* T cell proliferation, splenocytes stained with CFSE from both groups were stimulated with ConA and α-CD3 during 72 hours. **Results:** *T. cruzi* infection causes an increase in the PI3Kγ activation in the heart tissue. Although there is no difference in the parasitemia between WT and PI3Kγ$^{-/-}$ mice in all times evaluated, all PI3Kγ deficient mice died until day 25 after infection, whereas WT mice remained alive after 35 days. PI3Kγ$^{-/-}$ mice also showed greater inflammation, parasitism and lesion in the heart tissue. Interestingly, in the absence of PI3K, the heart tissue express five times higher levels of iNOS enzyme after infection compared with WT mice, but the expression of arginase I (ARG1), that also consume the amino acid arginine thus impairing the NO production, is twenty times higher compared with WT mice. *In vitro*, macrophages from PI3Kγ$^{-/-}$ mice, when stimulated with IFNγ, fail to produce NO and to kill the parasite. Corroborating these data, the addition of ARG1 inhibitor reverts the fail in NO production by PI3Kγ$^{-/-}$ macrophages. Finally, PI3K signaling seems to be involved in the adaptive response, augmenting T cell proliferation. **Conclusion:** These results indicate that PI3Kγ is critical for the host to control *T. cruzi* parasitism. In the heart tissue of the infected mice, PI3Kγ is involved in the microbicidal mechanisms of macrophage by mediation of NO production.

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Evaluation of the role of reactive oxygen species during *Leishmania infantum* infection

WALDIONÊ DE CASTRO(PG)[1]; LILIANE MARTINS DOS SANTOS(PG)[1]; ANA CLARA M. MONTUORI(IC)[1]; YURI C. KARLIN(IC)[1] LEDA QUERCIAVIEIRA[1].

1.Universidade Federal de Minas Gerais

Leishmania infantum, which causes South American visceral leishmaniasis, must survive antimicrobial responses of host macrophages to establish infection. Macrophage oxidative responses have been shown to diminish in the presence of intracellular leishmania. **Objective:** In the present study we evaluated the susceptibility of Phox$^{-/-}$ and iNOS$^{-/-}$ mice to *L. infantum* infection and investigated a possible association between NO• and ROS in host resistance to the parasite. **Results:** Our *in vitro* studies suggest that both NO and ROS are important to control the parasite proliferation in infected macrophages. Macrophages from iNOS$^{-/-}$ and Phox$^{-/-}$ showed higher percentage of infection and parasite burden at different times after infection when compared withWT macrophages. *In vivo* studies showed that during *L. infantum* infection in mice, amastigotes multiply rapidly for the first four weeks of infection in the liver, but they are cleared spontaneously by week eight both in WT and Phox$^{-/-}$ mice but not in iNOS$^{-/-}$ mice. The parasite load in the spleen was not controlled throughout the weeks following infection by any of the mice strains. Next we determined the nature of the earliest inflammatory cell response at the site of parasite inoculation. Using the myeloperoxidase (MPO) activity assay we verified that MPO levels in the ear were higher in the Phox$^{-/-}$ mice when compared with WT or iNOS$^{-/-}$ mice after 24 hours of infection. Neutrophils (CD45^{+}Ly6C^{+},Ly6G^{high}) and macrophages (CD45^{+}F4/80^{+}) in the ear dermis were analyzed by flow cytometry. There was a rapid and transient increase in the number of cells expressing the neutrophil surface antigen Ly6G, reaching a peak at 24 h in the 3 groups of mice. Phox$^{-/-}$ mice showed higher neutrophil numbers when compared with iNOS$^{-/-}$ and WT mice confirming the MPO results. The numbers of macrophages in the infiltrate increased after 48 hours of parasite exposure in the 3 groups of mice but were lower in Phox$^{-/-}$ than WT and iNOS$^{-/-}$ mice. Furthermore, Phox$^{-/-}$ mice showed increased production of MIP-2 than WT mice in the ear dermis infected by *L. infantum*. No KC production was detected. **Conclusions:** ROS plays a role in the initial neutrophil infiltrate to the skin. In addition, during the chronic phase ROS seems to have importance in the control of visceral infection. Financial support: CAPES, CNPq and FAPEMIG

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MILTEFOSINE EXERTS ITS LEISHMANICIDAL ACTION VIA PAF RECEPTOR

ANA CLARA M. MONTUORI[2]; WALDIONÊ DE CASTRO[1,2]; YURI C. KARLIN[2] ; MATHEUS B. H. CARNEIRO[2]; DANIEL M. ALMEIDA[1]; PAULA S.MELLO[2]; BHASKAR SAHA[3]; LEDA QUERCIA VIEIRA[1,2].

1 Núcleo de Pesquisa em Ciências Biológicas (NUPEB), Universidade Federal de Ouro Preto,Minas Gerais, Brasil
2 Departamento de Bioquímica e Imunologia, ICB, Universidade Federal de Minas Gerais, Minas Gerais, Brasil.
3 National Centre for Cell Science , Ganeshkhind, Pune, India.

Miltefosine (hexadecylphosphocolina - HPC), which was first used as treatment in patients with cancer, has been used as an effective oral drug in visceral leishmaniasis. There is a structural similarity between miltefosine and platelet activating factor (PAF). PAF acts by binding to the PAF receptor (PAFR) present in target cells. The mechanism by which the drug works is not well established.
Objective: The objective of this study is to verify if the mechanism of action of miltefosine is mediated by the PAF receptor (PAFR). **Methodology:**To test this hypothesis, BALB/c and PAFR$^{-/-}$ mice were orally treated from the 14th day after infection (2x10^7 promastigotes inoculated intra-venously into the tail vein) for 7 consecutive days at dose 20mg/kg/day and killed on day 28. Parasites quantification was made by serial dilution, measurement of serum cytokine analysis by ELISA and the activity of Arginase I and NO production from peritoneal macrophages.**Results:** Here, we report that both miltefosine and PAF bind to PAF receptor (PAFR), as suggested by the modeling data and similarities in activating the p38MAPK and ERK-1/2- and *Leishmania* killing in macrophages. Miltefosine-induced p38MAPK activation was inhibited by PAF antagonist and anti-PAFR antibodies. The PAF antagonist reduced the miltefosine-induced TNF-α production and *Leishmania* killing. Initially we found that macrophages from wild type mice and PAFR-KO showed a similar profile of infection when infected with *L. donovani*. The in vitro infection experiments revealed that macrophages from wild type mice treated with miltefosine were more effective in controlling the growth of the parasites than macrophages from PAFR-KO mice. We found that in vivo infection with *L. donovani* showed no differences in susceptibility between wild-type mice and PAFR-KO. We treated wild type mice and PAFR-KO orally from day 14 after infection with *L. donovani* for seven consecutive days with 20mg/kg/day of miltefosine. Our data revealed that treatment of wild type mice led to a reduction in parasite load in the liver and spleen of these animals. Interestingly, PAFR-KO mice showed a higher parasite load in these organs. **Conclusions:** These data define for the first time the mechanistic basis of host cell-dependent anti-leishmanial function of

miltefosine and identifies PAFR as a possible target for immunomodulation. Financial support: FAPEMIG, CAPES and CNPq

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IMMUNOMODULATORY ACTIVITIES OF B-GLUCAN IN A MICE MODEL OF *CANDIDA ALBICANS* VAGINITIS

ALEIDA MARIA DA SILVA LIMA[1*], KARINA MENDES MELCHUNA[1], LUANDA BÁRBARA FERREIRA CANÁRIO DE SOUZA[2], MARIANA ARAÚJO COSTA[2], DANIELLE CARDOSO GERALDO MAIA[4], KEYLA BORGES FERREIRA ROCHA[3], IRACILDA ZEPPONE CARLOS[4], GUILHERME MARANHÃO CHAVES[2], VALÉRIA SORAYA DE FARIAS SALES[2].

Department of Immunology, University of São Paulo[1], Department of Clinical and Toxicological Analysis, Federal University of Rio Grande do Norte[2], Department of Pathology, University of Rio Grande do Norte[3], Department of Clinical Analysis, State University of São Paulo[4].

aleida_ maria85@yahoo.com.br

Introduction: Vulvovaginal candidiasis (VVC) is an inflammatory disease on vaginal tissue, caused mainly by pathogenic yeasts of *Candida albicans*. There are some resistant strains of *C. albicans* to the conventional antifungal, therefore is necessary to search other forms for therapy. The β-glucans are structural polysaccharides of the cell wall of the fungi *Saccharomyces cerevisiae* and there are several reports that demonstrate the immunomodulatory effect of this structure in infections of bacterial, viral, fungal and parasitic. Knowing this, the present study evaluated if β-glucan has immunomodulatory activity in mice with vulvovaginal candidiasis under the influence of estrogen.

Methods and Results: The animals were inoculated with *C. albicans*, intravaginally, and treated with glucan, vaginal and intraperitoneally, and the control animals received only saline. In the eighth and tenth days after inoculation, when compared the control animals (1.48±1.668 and 0.46±0.887) with the animals treated with intraperitoneal (-0.89±0.524 and -0.22±0.983) and vaginal glucan (1.09±1.269 and 0.34±0.900), the treated with glucan on vaginal fluid showed smaller means in the difference of CFU. The results more noticeable were of the intraperitoneal group, it was confirmed with histopathological analysis. Despite has been some difference in colony counts, no case was statistically significant. Relative to the IFN-γ, the highest peak

(416.35 pg /mL) was found on the 6th day after inoculation, in intraperitoneal group, while in the 9th day there was a decline to 3.64 pg/mL and in the 11th an increased to 101.94pg/mL. In vaginal glucan, levels of IFN-γ virtually remained constant in 6th and 9th days (219.73 and 225.48pg/mL, respectively, whereas in 11th, there was a decline to 17.54 pg/mL. While in the control group not treated with glucan, IFN-γ was not detected in any collection day.

Conclusion: The data suggest that glucan may have an important activity in protection against C. albicans in VVC, mainly via intraperitoneal.

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REGULATION OF IMMUNE RESPONSE AGAINST PLASMODIUM BERGHEI ANKA INFECTION IS DEPENDENT OF SUPPRESSOR OF SIGNALING CYTOKINES 2 (SOCS2) PATHWAY

FATIMA BRANT (PG)[(1,3#)], ALINE SILVA MIRANDA (PG)[(1,3#)], LISIA ESPER (PG)[(1,3)], RONAN RICARDO SABINO ARAÚJO (PG)[(1)], ANDREIA BARROSO (IC)[(1)], CYNTHIA HONORATO VAL (PG) [(1)], BRUNO CABRAL OLIVEIRA (PG)[(1)] , FREDERICO MARIANETTI SORIANI (PhD)[(1)], MILENE ALVARENGA RACHID (PhD)[(2)], HERBERT BERNARD TANOWITZ (PhD)[(4)], ANTÔNIO LUCIO TEIXEIRA (PhD)[(1,3)], FABIANA SIMÃO MACHADO (PhD)[(1,3)]

Instituto de Ciências Biológicas, Departamentos de Bioquímica e Imunologia[1] e Patologia[2], Faculdade de Medicina, Departamento de Clínica Médica[3], Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brasil. Albert Einstein College of Medicine, Department of Pathology[4], Bronx, New York, USA. [#]Equal contribution.

Introduction: The cerebral malaria (CM) is the most severe neurological complication and one of the most life-threatening occurring from *Plasmodium falciparum* infection. Mice infected with *P. berghei ANKA* (PbA) faithfully recapitulate many of the characteristics of human CM and it has been an important tool to investigate the disease pathogenesis. Cerebral malaria (CM) is a complex condition whose pathogenesis is still poorly understood, it likely involves deregulation of inflammatory response and alterations in neurotransmitters. The suppressor of cytokine signaling (SOCS) 2 is an intracellular protein induced by eicosanoids and hormones, and is important to modulate the inflammatory response. Recent studies suggest a role for SOCS-2 in neural development, growth, neurogenesis and stem cell differentiation. However, the involvement of SOCS2 in CM is not known.
Method: C57Bl/6(WT) and SOCS2$^{-/-}$ mice were infected with PbA and the parasitemia, survival and body weight were monitored periodically. The production of cytokines (TNF-α, IL-1β, TGF- β, IL-6, IFN-γ, IL-12 and IL-10) in the brain and spleen was assessed by ELISA and flow cytometry. The expression of SOCS1 and SOCS3 was assessed by RT-PCR. Leukocyte recruitment in the brain was evaluated by intravital microscopy. Nitric oxide (NO) was assessed by the Griess method in the brain. Histopathological analysis was performed in brain.
Results: The parasitemia was significantly lower in SOCS2$^{-/-}$ compared with WT mice and no difference in lost weight was detected among the groups. In the brain of PbA-infected SOCS2$^{-/-}$ mice there was a significant increased expression of TGF-β and IL-17 and increased level of NO when compared with infected WT mice. Additionally, there was a decreased expression in the brain but not spleen of IL-1β, TNF-α, IL-10 and IL-12 of infected SOCS2$^{-/-}$ mice when

compared with WT counterparts. Moreover, a significant decreased of leukocyte rolling in the brain microvasculature and increased microvascular obstruction in the brain was found in the PbA-infected SOCS2$^{-/-}$ mice when compared with WT.

Conclusions: These findings indicate, for the first time, a role for SOCS2 in the immunopathogenesis of PbA-associated CM.

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THE ROLE OF REACTIVE OXIGEN SPECIES DURING TRYPANOSOMA CRUZI INFECTION

GRAZIELLE ALVES RIBEIRO[1]; BRENDA SILVA ROSA DA LUZ[1]; LEDA QUERCIA VIEIRA[1]

[1]Departamento de Bioquímica e Imunologia, ICB, Universidade Federal de Minas Gerais, Minas Gerais, Brasil.

Introduction: Chagas' disease, one of the most prevalent infectious diseases in Central and South America, is caused by the intracellular protozoan *Trypanosoma cruzi,* parasite of many mammalian species. *In vitro*, *T. cruzi* is readily phagocytosed by macrophages and triggers respiratory burst. However, little is known about the role of ROS in *T. cruzi* parasite burden and the results found in literature are contradictory. Previous studies have demonstrated that in macrophages with an exhausted respiratory burst, *T. cruzi* growth increases, suggesting that ROS are protective. However, others have not found evidence that macrophage-derived ROS contribute to *T. cruzi* killing. **Objective:** The aim of this study was to investigate the parasite burden in macrophages from C57BL6 wild-type mice (WT) and from mice deficient in NADPH phagocyte oxidase (phox KO) infected with two strains of *T. cruzi* (Y and CL Brener). **Methods and results:** Macrophages isolated from murine peritoneal cavity of WT and phox KO were infected with culture trypomastigotes of Y and CL Brener strains of *T. cruzi* for different times and the parasite burden was analyzed by optical microscopy. Our results demonstrate that both macrophages uptook parasites similarly. Both parasite strains had the same capacity of infecting macrophages. After 48 hours of infection with Y strain, the number of infected macrophages and amastigotes was quantified and the infection index was calculated. In WT macrophages, the infection index was 36.0±13.8, and 0.01±0.01 in phox KO macrophages. The number of trypomastigotes in the supernatant of macrophage cultures was evaluated after 3 days of infection. In WT macrophage cultures we observed an exponential increase in the trypomastigotes until 7 days after infection. On the 5th day after infection we found $53.5x10^4 \pm 7.7x10^4$ parasites in the supernatant of infected WT macrophages and no parasites in the supernatant of phox KO macrophages infected with Y strain. When the macrophages were infected with CL Brener strain we observed the similar results. In NADPH- dependent phagocyte oxidase-deficient macrophages we did not observe exacerbation of the infection. **Conclusions:** This study indicates that ROS contributes to *T. cruzi* growth inside macrophages and increases overall parasitism.

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DECREASED ACTIVATION OF MONOCYTES IN PATIENTS WITH MULTIBACILLARY LEPROSY

GIOVANA BERGHEME FRANCISCON[1]; SARA PASSOS[1]; TAIS CAMPOS[1]; MAYUME SHIBUYA[1]; EDGAR M. CARVALHO[1,2]; PAULO MACHADO[1,2] AND LUCAS P. CARVALHO[1,2,3].

[1]Serviço de Imunologia, Universidade Federal da Bahia; [2]Instituto de Ciência e Tecnologia – Doenças Tropicais (INCT-DT); [3]Instituto de Ciências da Saúde (ICS), Universidade Federal da Bahia.

Introduction: Leprosy is a chronic granulomatous disease caused by *Mycobacterium leprae*, which has tropism for peripheral nerves and skin. While patients with multibacillary (MB) disease do not mount type 1 immune response facilitating the multiplication of *Mycobacterium*, paucibacillary (PB) individuals produce IFN-gamma in response to *M. leprae* antigens, what control bacteremia but causes immunopathology. Downregultion of type 1 immune response observed in MB patients may be due to decreased antigen presentation and /or co-stimulation. In the present study we aimed to evaluate the frequency and degree of activation of subsets of monocyte in peripheral blood of patients with MB and PB forms of leprosy, prior and after treatment.
Methods and Results: Peripheral blood mononuclear cells from MB and PB patients were obtained; frequency and degree of activation of subsets of monocyte were determined *ex-vivo* by flow cytometry, prior and after treatment. There was no difference between frequency of monocyte subsets between MB and PB patients prior to treatment. However, analysis of co-stimulatory molecules expression in monocytes reveiled that the non-classical ($CD14^{dim}CD16^{+}$) population of monocytes from MB patients had a significant decrease in CD86 expression when compared to the ones from PB individuals. CD86 expression in monocytes from MB patients was restored after polychemotherapy. To test whether *Mycobacterium* antigens could downregulate CD86 expression, we treated monocytes from helathy individuals with *M. leprae* antigens and assesed MHC class II and CD86 expression in these cells. We found IL-10-independent decrease in CD86 expression 8h after treatment with *M. leprae* antigens.
Conclusions: These data suggest that high bacteremia observed in MB patients contribute directly to downregulation of CD86 expression in monocytes from these individuals. Future functional analysis shall be performed to test whether decrease in CD86 expression leads to low Th1 cytokines production in MB patients.

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INNATE AND ADAPTIVE IMMUNE REPONSE AGAINST *LEISHMANIA* INFECTION IS DEPENDENT ON NOD-LIKE RECEPTORS ACTIVATION

DJALMA S. LIMA-JUNIOR[1,2], EULÁLIA L. SILVA[2], FERNANDA MARIM[1,2], SILVIA ULIANA[3], JOÃO S. SILVA[2], DARIO S. ZAMBONI[2]

1- Department of Cell Biology, School of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, São Paulo, Brazil
2- Department of Biochemistry and Immunology, School of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, São Paulo, Brazil

3- Department of Parasitology, Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil.

Abstract

Introduction: The intracellular sensors Nod1 and Nod2 have key role in the host responses. During activation, these proteins signals via the adapter molecule Rip2, which is a protein kinase that leads to activation of NF-κB and MAPK favoring the production of cytokines and chemokines. Also, Nod1 and Nod2 participate in the detection/control of several pathogens as they sense PAMPs contained in the cell walls of Gram-negative and Gram-positive bacteria. However, the role of Nod1 and Nod2 during *Leishmania* infection is unknown. ***Aim:*** Here, we investigated the participation of Nod/Rip2 pathway in host response during *L. major* infection. ***Methods:*** Bone marrow macrophage (BMDMs) or –dendritic cells (BMDCs) derived from C57BL/6, Nod1$^{-/-}$, Nod2$^{-/-}$ and Rip2$^{-/-}$ mice were infected with *L. major* promastigotes and cytokines (ELISA/flow cytometry) and NO (Griess assay) production, surface molecules expression (flow cytometry) and killing (Giemsa staining) were analyzed. Additionally, the lesion development and parasite burden were measured in WT-, Nod1$^{-/-}$-, Nod2$^{-/-}$- and Rip2$^{-/-}$-infected mice. Dendritic cells activation and cytokines production were evaluated at 8th week p.i. by flow cytometry. Finally, we analyzed the susceptibility and cytokines production in chimeras generated by irradiating recipient mice. ***Results:*** Nod1$^{-/-}$, Nod2$^{-/-}$ and Rip2$^{-/-}$ BMDMs had an impaired induction of NF-κB–dependent products in response to infection and failed to restrict *L. major* replication. Moreover, IL-12p40 production and surface molecules expression were decreased in parasite infected-Rip2$^{-/-}$ BMDCs. Nod1 activation was crucial for *in vivo* parasite replication control and

resolved cutaneous lesions. Rip2-dependent response was required for dendritic cells activation and induction of effective Th1 response *in vivo*. Additionally, Rip2-dependent signaling in radio-sensitive compartments was required for the control of the infection and induction of Th1 response. ***Conclusion:*** These studies indicate that Nod1/Rip2-dependent responses account for host resistance against *L. major* infection by mechanisms dependent of cytokine and nitric oxide production. Importantly, this study shows that the Nod-Rip2 axis effectively participate of the induction of innate and adaptive immune responses against a *Leishmania* parasite, thus providing a novel function for Nod-like receptors family or proteins in parasite-host interactions.

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KINETIC OF THE SPECIFIC HUMMORAL IMMUNE RESPONSE IN SHEEP IMMUNIZED WITH *Toxoplasma gondii* SURFACE GLYCOCONJUGATES

PATRÍCIA OLIVEIRA MEIRA SANTOS (PG)(1,2); MARIA TEREZA BARRETO GUEDES (PG)(1); DAN LOUREIRO (PG)(1); BRUNO LOPES BASTOS (1); JOSE TADEU RAYNAL (PG)(1); CIRO DE OLIVEIRA LIMA (2); DANIEL FACUNDO DA SILVA (2); ROBERTO MEYER (1); RICARDO WAGNER PORTELA (1)

(1) Laboratory of Immunology and Molecular Biology, Health Sciences Institute, Federal University of Bahia
(2) Medical School, Federal University of Ceará – Cariri

Introduction: Toxoplasmosis is a relevant disease in human and animal medicine; the infection with the parasite can lead to cause abortion and neonatal death, as well as neurologic symptoms at immunosuppressed individuals. With the growth of Brazilian sheep industry, the knowledge of the immune response against this protozoan and the development of diagnostic assays could significantly contribute to the infection control and productivity improvement. This study had the objective to evaluate the specific humoral immune response kinetics in sheep immunized with *Toxoplasma gondii* surface glycoconjugates (GlyC) or challenged with parasite tachyzoites. **Methods and Results:** Sheep (n=16) were injected with saline (G1), GlyC+saponin (G2) or *T. gondii* tachyzoites (G3). An ELISA was developed to evaluate the specific immunoglobulin production; GLyC specific IgG was measured in several moments pos-inoculation (p.i.). The ELISA developed presented repeatability and reproducibility of 94.3% and 96.2%, respectively. Anti-GlyC IgG levels in the G2 and G3 groups began to rise by 10 days p.i., and the highest levels were found at day 12. At G3, higher IgG levels were found at day 30 p.i. There is a significant difference at IgG seric levels in sheep immunized with *T. gondii* derived glycoconjugates and infected with the parasite between the 10th and 60th days p.i. **Conclusion:** The ELISA anti-GlyC seems to be a promising tool in the diagnostic if infected sheep.

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PROFILE OF THE CELLULAR IMMUNE RESPONSE IN SHEEP AFTER IMMUNIZATION WITH *Toxoplasma gondii* DERIVED GLYCOCONJUGATES

PATRÍCIA OLIVEIRA MEIRA SANTOS (PG)(1,2); GERALDO PEDRAL SAMPAIO (1); BRUNO LOPES BASTOS (1); ROANA LACERDA TAVARES LEITE (2); AMANDA SOEIRO FONTELES (2); LUDMILLA SENA (PG)(1); ROBERTO MEYER (1); RICARDO WAGNER PORTELA (1)

(1) Laboratory of Immunology and Molecular Biology, Health Sciences Institute, Federal University of Bahia
(2) Medical School, Federal University of Ceará – Cariri

Introduction: The protozoan *Toxoplasma gondii* infects humans and animals. In sheep, reproductive problems are frequent and adults become chronically infected, being a source of human infection. Glycosylphosphatidylinositol (GPI)-anchored proteins are largely found at *T. gondii* tachyzoite surface. Several studies demonstrated the immunomodulation properties of these molecules. This work aimed to determine the leukocyte profile in sheep after *T. gondii* infection, or immunization with parasite derived glycoconjugates (PGc). **Methods and Results:** Sheep were injected with saline (G1, n=3), PGc+saponin (G2, n=4) or *T. gondii* tachyzoites (G3, n=5). The cellular immune response was evaluated by FACS at 0, 7, 15, 30 and 60 days pos-inoculation (p.i.). The percentage of CD4+, CD8+, CD21+, TCR γδ+, MHC class I and II cells, as well as the expression levels of theses markers were assessed. The CD8+ counts in G2 at 60th day were significantly higher when compared to the control group at 60 days p.i. A similar situation was found on MHC class II+ cell counts in G2 at 60 days p.i., as well as for MHC class I expression in G3 at 7 days p.i. The highest percentages of CD4+, CD8+, TCRγδ e CD21+ cells were found in G2 and/or G3 at 30th and/or 60th day, but with no statistic significance. **Conclusion:** The PGc+saponin inoculation stimulated the ovine immune response. The increased CD8+ T lymphocyte counts indicate an activation of a cytotoxic response, which is very important in protozoan infections.

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PURINERGIC RECEPTOR P2Y12 ACTIVATION IN SCHISTOSOMAL HOST RESPONSE

VALDIRENE S. MUNIZ[1]; RENATA B. REIS[1]; HILTON A. MATA-SANTOS[2]; ALEXANDRE S. PYRRHO[2]; PETER F. WELLER[3]; RODRIGO T. FIGUEIREDO[4] AND JOSIANE S. NEVES[1]

[1]Institute of Biomedical Sciences, [2]Faculty of Pharmacy, Federal University of Rio de Janeiro, Rio de Janeiro, RJ, Brazil, [3]Division of Allergy and Inflammation, Harvard Medical School, Beth Israel Deaconess Medical Center, Boston, MA, USA, [4]Institute of Biomedical Sciences/Unit of Xerem.

Introduction: Identification of new target molecules through which eosinophils activate and secrete their stored proteins may be highly significant for our understanding about the pathophysiology of host immune responses to parasites and allergic inflammation, as well as reveal new therapeutic targets for the control of the eosinophilic disorders. We have recently reported the expression of the purinergic P2Y12 receptor (P2Y12R) in human eosinophils (JACI 125:477-482, 2010), however, their involvement in eosinophilic inflammatory conditions *in vivo*, particularly in host response to parasites is still unknown.
In this work we investigated the role of the purinergic P2Y12R in the eosinophilic inflammatory response induced by *Shistosoma mansoni (S. mansoni)* infection.
Methods and Results: C57Bl/6 mice were infected with 50 cercariae of the BH strain of *S. mansoni* by the cutaneous route (protocol licence CEUA/UFRJ DFBCICB043). The animals were treated with a P2Y12R antagonist, clopidogrel (500µg/mL), via the drinking water three days before and throughout the infection period (55 days). Histopathological and biochemical analyses were performed in the liver to evaluate the areas of inflammatory granulomatous infiltrate and collagen deposition. The P2Y12R blockage reduced the hepatic inflammatory infiltrate around the granuloma – not treated: 5.47 ± 1.8 *versus* treated 4.2 ± 1.6 ($x10^4$ μm^2/granuloma), means ± EPM, N=5 - as well as liver collagen deposition – not treated: 880 ± 7 *versus* treated 690 ± 6 (µg hydroxyproline/g liver), means ± EPM, N=5 - without altering the parasite oviposition. Furthermore, the P2Y12R inhibition promoted blood eosinophilia (2-fold increase, N=5), whereas decreased the eosinophil count in the bone marrow (60% reduction, N=5) after blood smears and cytospin analyses, respectively.
Conclusion: Our results indicate an important role of the P2Y12R in the modulation of the host inflammatory response caused by *S. mansoni* infection. Our studies might enhance understanding of the importance of eosinophil activation and migration to parasite infected sites.

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REAPPRAISAL OF THE IMMUNOPATHOGENESIS OF DISSEMINATED LEISHMANIASIS: IN SITU AND SYSTEMIC IMMUNE RESPONSE

PAULO R. L. MACHADO(1); MARIA ELISA A. ROSA(1); DIEGO COSTA(2); MOEMA MIGNAC(1); JOÃO S. SILVA(2); ALBERT SCHRIEFER(1); MAURO M. TEIXEIRA(3); OLIVIA BACELLAR(1); EDGAR M. CARVALHO(1).

(1) Serviço de Imunologia, Hospital Universitário Prof. Edgard Santos, Universidade Federal da Bahia, Salvador, BA

(2) Departamento de Bioquímica e Imunologia, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP

(3) Departamento de Bioquímica e Imunologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, MG

Introduction:

Disseminated leishmaniasis (DL) is an emerging form of Leishmania braziliensis infection characterized by multiple cutaneous lesions on different parts of the body and a high rate of mucosal involvement. Systemic production of TNFa and IFNg in DL patients is lower than in cutaneous leishmaniasis (CL), which may account for parasite dissemination due to the decreased ability to control parasite growth. In this study, the systemic and *in situ* immune response of DL and CL patients was characterized through evaluation of chemokine and cytokine production.

Methods and Results:

This study included 32 DL patients recruited from the health post of Corte de Pedra, Bahia State, Brazil. The criteria for diagnosis were a clinical picture of DL as described below, in conjunction with parasite isolation by PCR or a positive skin test for Leishmania antigen plus histopathological features characteristic of leishmaniasis. DL was identified according to a case definition of ten or more mixed-type lesions (e.g. acneiform, papu-lar, nodular and/or ulcerated), located on two or more body parts (head, trunk, arms and legs). The cytokine and chemokine production by peripheral blood mononuclear cells after stimulation with Leishmania antigen was measured by ELISA.

Evaluation of cytokine and chemokine expression *in situ* was determined by immunohistochemistry.

In situ evaluation showed similar production of IFNg, TNFa, IL-10, transforming growth factor-beta (TGFb), chemokine (C-C motif) lig-and 2 (CCL2), CCL3, CCL11 and chemokine (C-X-C motif) ligand 10 (CXCL10) in papular and ulcerative lesions from DL as well as in ulcerated lesions from CL. Serum levels of CXCL9, a chemokine that attracts T-cells, was higher in serum from DL than from CL.

Conclusion:

These data indicate that a decrease in the type 1 immune response in peripheral blood of DL patients is due to attraction of Leishmania antigen-activated T-cells to the multiple cutaneous lesions. This may account for the absence or few parasites in the lesions and for the development of ulcers similar to those observed in CL.

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STANDARDIZATION OF LEUKOCYTE ANALYSIS FROM SPUTUM BY FLOW CYTOMETRY TECHNIQUE

PÂMELA CRISTINA GASPAR[1]; RENATA CRISTINA MESSORES RUDOLF-OLIVEIRA[1]; LETÍCIA MURARO WILDNER[1]; ANA CAROLINA RABELLO DE MORAES[1]; VANESSA MENGATTO[1]; MANOELA LIRA REIS[1]; CHANDRA CHIAPPIN CARDOSO[1]; MARIA LUIZA BAZZO[1]; MARIA CLÁUDIA SANTOS-SILVA[1].

[1]Federal University of Santa Catarina, Florianópolis, Brazil.

Introduction: Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis*. Information related to sputum cytology found in pulmonary tuberculosis is scarce, mainly it is unknown if there are differences in pulmonary immune cellular response between patients HIV-positive and HIV-negative and if there is a correlation between cytology and pulmonary response to TB treatment. Thus, the aim of this study was to standardize a technique to analyze leukocytes phenotype from sputum by flow cytometry. **Methods and Results:** To obtain the cells, sputum was fluidized with dithiothreitol (DTT). Approximately 1 g of sputum was incubated with 4 ml of DTT (0.1% - ratio 1:4) for 15 min on a rolling mixer. The same volume of phosphate buffered saline (PBS) (4 ml to 1 g sputum) was added to the **mixture** homogenized and incubated for another 10 min. The suspension was filtered through a 50 mm cell strainer and the filtrate was centrifuged (200 x g, 10 min). After centrifugation, cells were washed with PBS and the pellet was resuspended in 1 ml of PBS. Cell viability was determined using the tryptan blue exclusion method. To prevent aerosol damages caused by sputum contaminated with *M. tuberculosis* the tubes were left to stand for 10 min after each agitation and after sputum fluidization, mycobacterium bacilli were killed by treatment with paraformaldehyde (0.5% v/v for 40 min or 1.0% v/v for 30 min). In order to evaluate DTT and paraformaldehyde influence on surface markers expression, leukocytes from peripheral blood were submitted to the same fluidization and paraformaldehyde protocol. To asses surface marker expression, samples were staining with anti-CD45, -CD3, -CD4, -CD8 according to the manufacturer's instructions. The results showed that surface markers were preserved. After verify that all described procedures did not affect peripheral blood cells surface markers, the sputum cells obtained and treated with paraformaldehyde were also staining with cited monoclonal antibodies. Monocyte/macrophage, lymphocyte and neutrophils cells were observed. In this way, it was verified that surface markers of sputum cells were also preserved after all procedures and could be detect by flow cytometry technique. **Conclusion:** The standardized technique proved to be efficient in evaluation of cellularity from sputum cells by flow cytometry.

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MIP-1α IN RESPONSE TO *M. LEPRAE* RECOMBINANT ANTIGENS ML2629 AND ML82F ARE MARKERS OF DISEASE

Marise do V. Simon (1); Malcolm S. Duthie (2); Jonnia Scherlock (1); Danillo M. dos Santos (1); Karla Caroline Vieira Rollemberg (1); Telma Rodrigues Santos da Paixão (1); Cristiane Santana Ferreira (1); Gabrielle Souza (1); Tatiana R. de Moura (1); Roque P. de Almeida (1); Steeve Reed (2) and Amélia Maria R. de Jesus (1)

(1)Laboratório de Biologia Molecular e Imunologia do Hospital Universitário da Universidade Federal de Sergipe – UFS – Aracaju-SE

(2) Infection Disease Research Immunology – IDRI – Seatle-USA

Introduction: Leprosy affects the skin and peripheral nerves and can cause irreversible chronic disabilities. *Mycobacterium leprae* infection is followed by a variety of immunological response, leading to multibacilary (MB) or paucibacilary (PB) diseases. Identifying specific antigens that are the target of the cellular immune response to *M.leprae* could improve the understanding of leprosy immunopathogenesis and development of vaccines and/or immunotherapy.

Methods and Results: Cellular immune response was evaluated in 20 patients with confirmed diagnosis of leprosy (5 MB, and 15 PB) and 8 controls without disease. Whole blood assay without stimulus or stimulated with *M. leprae* recombinant proteins (ML2028, ML2531, ML2629 and ML82F), PPD and PHA. The plasma were collected and stored at -70°C and tested for cytokines by Luminex technique. Higher levels of IL-10 were observed in MB (365.9±477.7) as compared to PB patients (1.3±1.9) in response to ML2028 antigen. Lower levels of TGF-β were observed in PB (9.0±34.9) and negative in MB as compared with controls (36.5±96.4) in response to ML2531 antigen. MIP-1α in response to ML2629 and ML82F antigens was a marker of disease, with higher levels in leprosy patients ($2.5x10^5$±$9.4x10^5$ and $5.4x10^5$±$1.6x10^6$, respectively), as compared to controls (362.4±459.9 and 375.8±814.4, respectively).

Conclusion: These data suggest that while IL-10 in response to ML2028 antigen is a marker of susceptibility to MB disease, TGF-β in response to ML2531 antigen is modulating disease, because it is higher in controls without disease than in Leprosy patients. Additionally, MIP-1α in response to two of the antigens, ML2629 and ML82F, is a marker of disease.

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COMPARISON OF VIRULENCE AND IMMUNE RESPONSE OF DIFFERENT STRAINS IN EXPERIMENTAL SPOROTRICHOSIS.

JOSÉ ROBERTO FOGAÇA DE ALMEIDA (PG) [(1)]; GRASIELLE PEREIRA JANNUZZI (PG) [(1)]; GILBERTO HIDEO KAIHAMI (PG) [(2)]; SANDRO ROGERIO DE ALMEIDA [(1).]

[(1)] Department of Clinical and Toxicological Analysis, Faculty of Pharmaceutical Sciences, University of São Paulo.

[(2)] Department of Biochemistry, Institute of Chemistry, University of São Paulo.

Introduction and Objects: Sporotrichosis, a disease caused by the dimorphic fungus *Sporothrix schenckii*, is a chronic subcutaneous mycosis. In previous studies the homogenate of organs from mice infected with the strain M-64 produced a mixed Th1/Th2 response, where the serum of infected mice reacted only with a 70 kDa glycoprotein (GP70) identified as adhesin and as important virulence factor. More studies are needed on the analysis of the difference of immune response in others strains of *S. schenckii*, giving the possibility to elucidate the relationship between the virulence of the fungus with the immune response.

Methods and Results: Three groups of 20 BALB/c mice were infected intraperitoneally with $5x10^6$ *S. schenckii* yeast strain 1099-18 (lymphatic sporotrichosis-less virulence) and strain 15383 (disseminated sporotrichosis-more virulence), and sterile PBS as negative control. Each 7 days, the spleen and liver of these animals were taken for evaluation of the infection by CFU and ELISA. The serum was obtained to perform Western blot using two strains exoantigens. Infection performed with strain 1099-18 has a decrease on fungal load in the spleen in the end of the infection time, but in the liver remained unchanged over time of infection. Cytokine production analyze of the strain 15383, showed a typical Th1 response where was detected high levels of IFN-γ. In the mice infected with the strain 1099-18 was detected high level of IL-10 and IL-4 in organs of these animals. In the Western blot accomplished with the exoantigens by strain 15383 was observed the appearance between 50 kDa and 70 kDa band from the 14^{th} day of infection. All Significance test was carried out by two-way ANOVA Bonferroni post test.

Conclusion: Our preliminary results showed that Th1 response produced by strain *S. schenckii* 15383 infection, wasn't enough to cure the mice, and the less virulent strain induce a mixed Th1/Th2 response. The evaluation of spleen fungal burden suggest that strain 15383 has a tendency to become chronic and strain 1099-18 to move towards healing. From the result of Western blot, can be suggested that the protein with approximately 70kDa from strain 15383 exoantigens, is the most immunoreactive protein.

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THE IMPACT OF TUBERCULOSIS IN THE IMMUNE RESPONSE AND CLINICAL CHARACTERISTICS OF HTLV-1 INFECTED SUBJECTS

NATÁLIA BARBOSA CARVALHO(1); MARIA DE LOURDES BASTOS(1,2,3); YURI C. S. NEVES(1); ANSELMO SOUZA(1); SILVANE B. SANTOS(1); EDGAR M. CARVALHO(1,2)

(1) Serviço de Imunologia do Complexo Hospitalar Universitário Professor Edgard Santos, Universidade Federal da Bahia, Salvador-BA, Brazil; (2) Escola Bahiana de Medicina e Saúde Pública, Salvador-BA, Brazil; (3) Hospital Especializado Octávio Mangabeira, Salvador-BA, Brazil

Introduction: The human T cell lymphotropic virus type 1 (HTLV-1) is a retrovirus that infects mainly T cells, leading to cellular proliferation and activation with exacerbated IFN-γ and TNF-α production. The majority of HTLV-1 infected patients are considered as HTLV-1 carriers, but some individuals will develop HTLV-1 associated myelopathy (HAM) or overactive bladder (OAB), that is considered an oligosymptomatic form of HAM. It is widely documented that HTLV-1 is associated with increased susceptibility to *Mycobacterium tuberculosis* infection and severity of tuberculosis. Until now it is unknown if *M. tuberculosis* influences the outcome of HTLV-1 infection. The aim of this study was to determine the prevalence of tuberculosis (TB) in HTLV-1 infected patients and analyze whether TB influences the immune response, proviral load and the outcome of HTLV-1 infection.

Methods and Results: This was a cross-sectional study, in which the prevalence of tuberculosis was analyzed in 136 HTLV-1 infected individuals. Eighteen percent were classified as TB (subjects with positive tuberculin skin test - TST - with previous history of active TB and/or X-ray features of TB), 44% as latent TB (subjects with positive TST without previous history of active TB and X-ray features of TB) and 38% as not TB (subjects without history of TB and with a negative TST). Additionally, we compared the immune response and proviral load of HTLV-1 infected subjects with or without TB. The spontaneous IFN-γ and TNF-α production by PBMCs, analyzed by ELISA, was similar in both groups. There was no difference in the proviral load between the groups, as shown by real time PCR analysis. A contingency analysis demonstrated that there is no difference in the proportion of tuberculosis subjects in asymptomatic, OAB syndrome or HAM groups of HTLV-1 infected subjects.

Conclusion: There is a high prevalence of tuberculosis in a cohort of HTLV-1 infected subjects analyzed in this study, but *M. tuberculosis* infection does not seem to influence the immune response and clinical features of these patients.

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GALACTOMANNAN ANTIGENEMIA SCREENING IN NEUTROPENIC PATIENTS WITH HEMATOLOGICAL DISEASES IN BAHIA STATE, BRAZIL

KELLY CRISTINE MOURA COSTA PEDROZA (1); SÓCRATES BEZERRA DE MATOS (1); MÔNICA BOTURA (2); MARCO AURÉLIO SALVINO (2); TIAGO THALES (3); ROBERTO JOSÉ MEYER (4); FERNANDA W. DE M. LIMA (1)

(1) Serviço de Imunologia de Doenças Infecciosas - SIDI / Universidade Federal da Bahia; (2) Centro de Transplante de Medula Óssea - CTMO / Hospital Universitário Prof. Edgard Santos; (3) Instituto de Ciências da Saúde / UFBA

Introduction: Invasive aspergillosis (IA) is a major cause of morbidity in immunossuppressed patients, in particular those with cancer or hematological diseases and bone marrow／hematopoietic stem cell transplant recipients (BMT／HSCT). Detection of IA represents a diagnostic challenge and relies on
Detection of IA represents a diagnostic challenge and relies on
a combination of clinical, microbiology and histopathology datas. An important feature in the pathogenesis of *Aspergillus* is angioinvasion, a trait that provides opportunities to develop tests that detect characteristic antigenic signatures molecules in biological fluids, as galactomannan (GM), a heat-steable heteropolysaccharide. Serum GM determination is included as microbiological criteria in the diagnosis of IA in consensus by the European Organization for Research and Treatment of Cancer and the National Institute of Allergy and Infectious Diseases Mycology Study Group (EORTC/MSG). The aim of this work is to evaluate the implantation of GM serial screening in neutropenic patients with hematological disease in a university hospital, in Bahia state, Brazil.

Methods and Results: This study was conducted between April 2011 and July 2012. Patients admitted to the Hematology Center of university hospital were eligible if they had absolute neutrophil count < 500 cells/mm^3. Serum samples were obtained twice weekly and GM antigen was detected by direct double-sandwich ELISA (Platelia *Aspergillus*; Bio-Rad, France). A result was considered positive only when two consecutive GM assays presented O.D. index ≥ 0.5. The study was approved by hospital's Ethic Committe. A total of 99 neutropenic episodes (63 patients – 17 to 74 years old, 31 men and 32 women) were investigated. Antifungal prophylaxis was done in 34 episodes, which not positive GM results were obtained. In the group without antifungal prophylaxis was characterized a probable IA case, with serum GM positive. Patient with multiple myeloma, autologous BMT, neutropenic for 9 days. The serum GM index declined after preemptive therapy.

Conclusion: This Hematology Center is the first in Bahia state to use the serum GM detection as tool to IA diagnostic. Preemptive therapy based on GM-

ELISA can potentially reduce the exposure to expensive and potentially toxic drugs and offer effective antifungal control in risk patients.

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NATURAL ISOTYPIC RESPONSE AGAINST POTENTIAL VACCINE CANDIDATE *PLASMODIUM FALCIPARUM* GLUTAMATE-RICH PROTEIN (GLURP) IN INDIVIDUALS LIVING IN BRAZILIAN MALARIA-ENDEMIC AREA.

LILIAN ROSE PRATT RICCIO[1]; JOSUÉ DA COSTA LIMA-JUNIOR[2]; LEONARDO JOSÉ DE MOURA CARVALHO[1]; MICHAEL THEISEN[3]; FÁTIMA SANTOS[4]; JOSELI DE OLIVEIRA-FERREIRA[2]; CLÁUDIO TADEU DANIEL-RIBEIRO[1] & DALMA MARIA BANIC[5]

[1]Laboratório de Pesquisas em Malária, Instituto Oswaldo Cruz, Fiocruz; [2]Laboratório de Imunoparasitologia, IOC, FIOCRUZ; [3]Statens Seruminstitut, Copenhagen, Denmark; [4]Laboratório de Entomologia, LACEN, Porto Velho, Rondônia; [5]Laboratório de Simulídeos e Oncocercose, IOC, FIOCRUZ.

Introduction: The *Plasmodium falciparum* Glutamate-Rich Protein (GLURP) is a malaria vaccine candidate undergoing clinical trials. GLURP is expressed in all developmental stages of the parasite life cycle in human host. Immuno-epidemiological studies have shown a high prevalence of antibodies to GLURP in adults as well as a significant association between high levels of these antibodies and low parasite densities and protection against clinical malaria. Also, a recent study in controlled experimental *P. falciparum* infections shows that antibodies to GLURP are acquired during a single short low density of *P. falciparum* infection in non-immune individuals. The aim of this study was to evaluate the antibody response profile induced by GLURP in naturally exposed individuals from a Brazilian endemic area. **Methods and Results:** The study was carried out in the farming area of Colina (CL) and the riverside fishing community of Ribeirinha (RB), both near the capital of Porto Velho (RO). The population of CL primarily consists of migrants from non-endemic areas, and the population of RB consists of natives from the Amazon Basin. The antibody response against immunodominant regions of the GLURP (R0 and R2) and synthetic peptides (*R0*: P3, P4, P5, P8, P9, P10, P11, S3; *R2*: S4) was evaluated by ELISA. The results showed a high prevalence of individuals with antibodies to R0 and R2 regions in both CL and RB groups. Different data have been reported in an African area of low endemicity, where antibody responses to R2 were observed in only 16 % of donors. Surprisingly, similar results were reported in an African area of high endemicity. The R0-induced antibodies were

predominantly IgG1 and R2-induced antibodies were predominantly of cytophilic subclasses. S4 and P11 epitopes were identified as immunodominant B-cell epitopes. These results are distinguishable from a study conducted in clinically immune Liberian adults, in whom the most frequently recognized B-cell epitopes of GLURP were P3 and P4. However, in RB group, P3 and S3 epitopes induced higher levels of cytophilic antibodies, effective in ADCI, like clinically immune Liberian adults. **Conclusions:** GLURP is immunogenic in natural conditions of exposure and the seropositivity to GLURP increases with exposure. Also, GLURP is able to induce cytophilic antibodies that can participate of protective acquisition immunity. In conclusion, our results highlight the importance of GLURP like a malaria vaccine candidate.

Characterization of costimulatory molecules and inhibitory receptors during *P. vivax* malaria

Pedro Carvalho Costa (PG), Fabiana M. Leoratti (P), Mauro Shugiro Tada (P), Luis Hildebrando Pereira da Silva (P), Ricardo T. Gazzinelli (P), Lis R. V. Antonelli (P)

Introduction: In Brazil, malaria is still a significant public health problem. Although the numbers of malaria cases are decreasing, the incidence was higher than 300,000 cases in the past two years. Of these cases, *Plasmodium vivax* was found to be the causative agent in 90% of these cases (WHO Fact Sheet, 2008; n° 94). The adaptive immune response, along with the mechanisms of innate immunity, has the task of overcoming the strategies imposed by infectious agents, leading to the control of the disease (*Nat Immunol.* 9(7):725-32, 2008). It is know that T cell-mediated responses are essential for parasite control; however the mechanisms behind this response are not clear. The goal of this study is to assess the phenotype of T cells from *P. vivax*-infected patient focusing on the expression of molecules such as T cell immunoglobulin-3 (Tim-3), cytotoxic T lymphocyte attenuator (CTLA-4) and programmed death 1 (PD-1).

Methods and Results: Peripheral blood mononuclear cells from *P. vivax*-infected patients were obtained in Porto Velho; RO. Lymphocytes were analyzed by flow cytometry. Our data show increased levels of inflammatory cytokines and decreased absolute numbers of lymphocytes during acute infection. Interestingly, the expression of costimulatory and inhibitory molecules has been associated with impairment of T cell function during a variety of infectious diseases. Accordingly, our data show that the above-mentioned costimulatory and regulatory molecules are also upregulated on T cells from malaria patients. The increased expression of some of these molecules correlates with liver damage and platelets during acute *P. vivax* infection.

Despite the expression of some inhibitory markers, these cells also express Ki67, a specific marker for cell proliferation.

Conclusion: *P.vivax*-infected patients display a phenotype that suggests an impaired T cell function.

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P2X7 RECEPTOR ACTIVATION IS REQUIRED TO THE OUTCOME OF SEVERE TUBERCULOSIS INDUCED BY HYPERVIRULENT MYCOBACTERIA

Eduardo P. Amaral[1], Marcelle R. M. de Andrade[2], Simone C. M. Ribeiro[2], Verônica R. Lanes[2], Fabrício M. Almeida[2], Érika M. Salles[1], Sheyla Inez Castillo-Mendez[1], Henrique Borges da Silva[1], Karina R. Bortoluci[3], Robson Coutinho-Silva[4,5], José M. Alvarez[1], Elena B. Lasunskaia[2] and Maria Regina D'Império-Lima[1]

[1]Departamento de Imunologia, Instituto de Ciências Biomédicas (ICB), Universidade de São Paulo (USP), Brazil. [2]Laboratório de Biologia do Reconhecer, Universidade Estadual do Norte Fluminense (UENF), Brazil. [3]Centro de Terapia Celular e Molecular, Departmento de Ciências Biológicas, Universidade Federal de São Paulo, Brazil. [4]Programa de Imunobiologia, Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Brazil. [5]Instituto National de Ciência e Tecnologia para Pesquisa Translacional em Saúde e Meio Ambiente da Região Amazônica, Brazil. [6]Departamento de Química e Toxicologia Clínica, Faculdade de Ciências Farmacêuticas, USP, Brazil.

Abstract

Introduction: Primary Progressive Tuberculosis (PPT) is an aggressive form of mycobacterial infection characterized by pneumonia, atelectasis, lung necrosis and bacillus dissemination, mostly affecting children and immunocompromised adults. **Objective and Methods:** To determine the major features of bacillus-host interaction responsible for this spectrum of the disease, we infected C57BL/6 mice via intratracheal injection with ~100 bacillus of two hypervirulent strains of phylogenetic distant mycobacteria (*Mycobacterium bovis* and *Mycobacterium tuberculosis*). **Results:** Infection with both hypervirulent mycobacteria caused rapidly PPT (RPTB) characterized by extensive areas of pulmonary inflammation and necrosis, culminating in bacillus dissemination and mouse death. Extremely high or relatively low production of pro-inflammatory mediators occurred during RPTB. However, the profile of pro-inflammatory response had a minor influence in the progression of necrotic lesions or the fatal outcome of RPTB, but the disease was attenuated in mice lacking the purinergic P2X7 receptor (P2X7R). P2X7R is a sensor of extracellular ATP (adenosine triphosphate), a damage signal released from necrotic cells that induces cell migration, pro-inflammatory cytokine production and cell death. We observed that hypervirulent mycobacteria grew rapidly inside macrophages and then caused their death by a mechanism dependent on P2X7R. Therefore, P2X7R signaling facilitates the spread of mycobacteria to the liver and spleen and also contributes to pneumonia and pulmonary necrosis associated with exacerbated release of ATP. In accordance with this, low production of MCP-1 was observed in the lung of P2X7R$^{-/-}$ mice infected, suggesting that the P2X7R

signaling is required to induction of MCP-1 and consequently induction of intensive cell influx into lung during RPTB. **Conclusion:** This study shows that several pathological manifestations of human PTT can be reproduced in mice infected with hypervirulent mycobacteria and reveals the crucial role of P2X7R in the aggressive forms of tuberculosis.

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CASPASE-1 AND NLRP3 CONTRIBUTE TO THE CONTROL OF *T. cruzi*

VIRGINIA MENDES GONÇALVES (PG)[1]; KELY CATARINE MATTEUCCI (IC)[1]

KARINA RAMALHO BORTOLUCI (PI)[1]

[1]Departamento de Ciências Biológicas e Centro de Terapia Celular e Molecular (CTC-Mol), Universidade Federal de São Paulo, São Paulo, Brasil.

Introduction: *Trypanosoma cruzi* is an intracellular protozoan parasite and etiological agent of Chagas disease, a severe and chronic infectious illness that affects millions of people in the world. Although the role of TLR in controlling infection by *T. cruzi* is well described in the literature, there is no data about the involvement of inflammasomes.

Methods and Results: In this study, we evaluated the participation of NLRP3 and caspase-1 in host response to *T. cruzi* infection and found that NLRP3$^{-/-}$ and caspase1$^{-/-}$ mice are susceptible to infection. To determine the mechanisms involved in susceptibility by NLRP3$^{-/-}$ and caspase-1$^{-/-}$ mice, we evaluated cytokine and nitric oxide (NO) production by spleen cells from infected mice. Inflammatory cytokines IL-6 and IFN-γ were found in spleen cells from NLRP3$^{-/-}$ and caspase1$^{-/-}$ infected mice, but these mice displayed a defect in the production of NO and IL-1β. Finally, the inhibition of caspase-1 with z-YVAD-fmk, but not the neutralization of IL-1R abrogated the NO production by WT cells.

Conclusion: Together, these data show that NLRP3 and caspase-1 are involved in the control of *T. cruzi* infection through the induction of NO production. Moreover, NO production induced by NLRP3 requires active caspase-1 but it is independent of IL-1R.

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INDUCTION OF $CD4^+CD25^+FOXP3^+$ REGULATORY T CELLS DURING HUMAN HOOKWORM INFECTION MODULATES ANTIGEN-MEDIATED LYMPHOCYTE PROLIFERATION

NATASHA DELAQUA RICCI (PHD) (1), JACQUELINE ARAÚJO FIÚZA (MSC) (1,2), LILIAN LACERDA BUENO(PHD) (1), PEDRO HENRIQUE GAZZINELLI GUIMARÃES (MSC) (1), VIRGILLIO GANDRA MARTINS (IC) (1), GUILHERME GROSSI CANÇADO(MSC) ,STEFAN MICHAEL GEIGER (PHD) (1) (1), RODRIGO CAMBRAIA DE MIRANDA (PHD) (1), LEONARDO MATOSO (PHD) (3), ANDRÉA GAZZINELLI (PD) (3) RODRIGO CORREA-OLIVEIRA (PHD) (2), DANIELLA CASTANHEIRA BARTHOLOMEU (PHD) (4), RICARDO FUJIWARA (PHD) (1)

1-Department of Parasitology, Federal University of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil

2- Laboratory of Cellular and Molecular Immunology, Instituto René Rachou, Oswaldo Cruz Foundation, Belo Horizonte, Minas Gerais, Brazil

3- Nursing School, Federal University of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil

4- Department of Biochemistry, Federal University of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil

INTRODUCTION Hookworm infection is considered one of the most important poverty-promoting neglected tropical diseases, infecting 576 to 740 million people worldwide, especially in the tropics and subtropics. These blood-feeding nematodes have a remarkable ability to downmodulate the host immune response, protecting themselves from elimination and minimizing severe host pathology. While several mechanisms may be involved in the immunomodulation by parasitic infection, experimental evidences have pointed toward the possible involvement of regulatory T cells (Tregs) in downregulating effector T-cell responses upon chronic infection. However, the role of Tregs cells in human hookworm infection is still poorly understood and has not been addressed yet. **METHODS AND RESULTS**: In the current study we observed an augmentation of circulating $CD4^+CD25^+FOXP3^+$ regulatory T cells in hookworm-infected individuals compared with healthy non-infected donors. We have also demonstrated that infected individuals present higher levels of

circulating Treg cells expressing CTLA-4, GITR, IL-10, TGF-β and IL-17. Moreover, we showed that hookworm crude antigen stimulation reduces the number of $CD4^+CD25^+FOXP3^+$ T regulatory cells co-expressing IL-17 in infected individuals. Finally, PBMCs from infected individuals pulsed with excreted/secreted products or hookworm crude antigens presented an impaired cellular proliferation, which was partially augmented by the depletion of Treg cells. **CONCLUSION o**ur results suggest that Treg cells may play an important role in hookworm-induced immunosuppression, contributing to the longevity of hookworm survival in infected people.

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AUTHENTIC LTB_4 PRODUCED BY *Paracoccidioides brasiliensis*: EFFECT ON IMMUNOMODULATORY ACTIVITY OF HUMAN POLYMORPHONUCLEAR AND MONONUCLEAR CELLS

Ana Paula Bordon-Graciani (1), Guilherme Augusto Biondo (PG) (1), Luciane Alarcão Dias-Melicio (2), Tatiana Fernanda Bachiega (PG) (1), Marjorie de Assis Golim (3), José Roberto Marques Silva (4), Ângela Maria Victoriano de Campos Soares (1).

(1) Departamento de Microbiologia e Imunologia, Instituto de Biociências de Botucatu, UNESP – Univ Estadual Paulista, Brasil; (2) Departamento de Patologia, Faculdade de Medicina de Botucatu, UNESP – Univ Estadual Paulista, Brasil; (3) Hemocentro, Faculdade de Medicina de Botucatu, UNESP – Univ Estadual Paulista, Brasil; (4) Núcleo de Pesquisa Avançada em Matologia, Faculdade de Ciências Agronômicas de Botucatu, UNESP – Univ Estadual Paulista, Brasil.

Introduction: *Paracoccidioides brasiliensis* is the agent of paracoccidioidomycosis, the most prevalent deep mycosis in Latin America. The production of eicosanoids during fungal infection has been associated with the biology of fungi, and the modulation of host immune response. Leukotrienes (LTs) are proinflammatory mediators produced by various mammalian cells. Studies in our laboratory have shown that, in addition of host cells, another source of LTB_4 production is *P. brasiliensis* itself. The objective of this study was to assess the effect of LTB_4 produced by *P. brasiliensis* on effector functions of human polymorphonuclear and mononuclear cells. **Methods and Results:** Peripheral blood neutrophils (PMN), monocytes (MO) and PBMC obtained from 10 healthy donors were treated with LTB_4 purified from *P. brasiliensis* yeast cells cultures (LTB_4f) or commercial LTB_4c (LTB_4c). After 1, 2, 4, 8 and 12 hours of incubation, PMN and MO cultures were evaluated by phagocytosis assay, surface receptors expression (TLR-2, TLR4, dectin-1, MR and HLA-DR), H_2O_2, cytokines (TNF-α, IL-6, IL-8, IL-10, IL-12p70, IL-15, IL-17, IL-23, IL-27) and chemokines (MCP-1 and MIP-1α) production. PBMC cultures were evaluated after 24, 48 and 72 hours of incubation with PGE_2f and PGE_2c by cellular proliferation and cytokines production (TNF-α, IFN-γ, IL-2, IL-4, IL-10, IL-12 and IL-17). The treatment with either LTB_4c or LTB_4f increased phagocytosis of zymosan and H_2O_2 production after estimulation with PMA, in both PMN and MO cultures.The treatment also increased TLR-2 expression on PMN surface and MR expression on MO. In relation to cytokines and chemokines produced by PMNs there was an increase on TNF-α, IL-6, IL-8 and MCP-1 production. MO showed an increase on TNF-α, IL-6, IL-12, IL-15, IL-17, IL-23 and MCP-1, and a decrease on IL-8 and IL-10 production. Regarding

PBMC, none of the treatments alter cell proliferation,but increased TNF-α, IFN-γ, IL-2 and IL-17 levels and decreased IL-4 and IL-10 production. **Conclusion:** Our results show that LTB_4 purified from *P. brasiliensis* cultures modulate human innate and adaptive response similarly as LTB4c does.

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TUMOR NECROSIS FACTOR MAY CONTRIBUTE TO *TRYPANOSOMA CRUZI* PERSISTENCE IN THE CENTRAL NERVOUS SYSTEM FAVORING ASTROCYTE INFECTION

RAFAEL RODRIGUES SILVA (PG)(1); ANDREA ALICE DA SILVA (1,2); JOSELI LANNES-VIEIRA (1).

(1).Oswaldo Cruz Institute; (2). Federal Fluminense University

Introduction: The existence of a nervous form in the chronic phase of Chagas disease is matter of discussion. *Trypanosoma cruzi* infection of children under two-year old and immunocompromised patients, with cancer, transplanted or malnourished, as well as 75-80% cases of co-infected individuals who develop the acquired immunodeficiency syndrome (AIDS), present immunopathological alterations in the central nervous system (CNS) with intense parasitism. Although immunocompetent individuals control *T. cruzi* dissemination, parasite persists in the CNS. The mechanisms favoring parasite persistence in an apparent silence in the nervous tissue, while myocarditis progresses from the acute to the chronic phase of infection, remain to be understood. Herein, we approached the participation of tumor necrosis factor (TNF) in the process of parasite invasion of astrocyte, the main glial cells, studying the infection rates and the production of nitric oxide (NO). **Methods and Results**: We used primary cultures of C3H/He mice astrocytes untreated or treated with TNF prior to infection with trypomastigote forms of the Colombian strain of *T. cruzi*. Interestingly, the astrocyte cultures treated with TNF prior to infection presented a higher number of intracellular amastigote forms (327.33±3.8 amastigotes/100 cells in TNF-treated cultures *vs* 219.33±0.94 amastigotes/100 cells in untreated cultures). TNF also increased the frequency of parasitized astrocytes (52.31±0.45% in TNF-treated cultures *vs* 31.15±0.22% in untreated cultures). Further, the infection rates were dependent on the time of infection and the multiplicity of infection (MOI). Moreover, independently of previous exposition to TNF, *T. cruzi* infection of astrocytes did not induce NO production. **Conclusion**: Our data suggest that in the CNS an inflammatory milieu containing TNF may facilitate the invasion of astrocytes by *T. cruzi*, which may persist in the nervous tissue in a silent manner.

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CHARACTERIZATION OF MONOCYTE SUBSETS IN PATIENTS WITH CUTANEOUS LEISHMANIASIS

RUBIA SUELY SANTANA COSTA[1]; GIOVANA BERGHEME FRANCISCON[1]; LUIZ HENRIQUE SANTOS GUIMARÃES[1]; SARA TIMÓTEO PASSOS[1]; DAVID MOSSER[2], PHILLIP SCOTT[3], EDGAR MARCELINO DE CARVALHO[1,4] and LUCAS PEDREIRA DE CARVALHO[1,4,5]

(1). Serviço de Imunologia, Universidade Federal da Bahia ; (2) University of Maryland; (3) University of Philadelphia; (4) Instituto de Ciências e Tecnologia – Doenças Tropicais (INCT-DT) (5). Instituto de Ciências da Saúde, Universidade Federal da Bahia (ICS).

Introduction. Cutaneous leishmaniasis (CL) is an inflammatory parasitic disease characterized by the presence of ulcerated lesion on the skin. Patients with CL due to *Leishmania braziliensis* produce high levels of TNF, cytokine that contribute to tissue damage and ulcer development. Mononuclear cell infiltrate are found in lesions of CL patients, with presence of T and B lymphocytes, and mononuclear phagocytes. Most works have focused on T cells immune response and little attention has been given to the contribution of monocytes to the immunopathology observed in CL. Recently, three populations of monocytes have been described based on expression of CD14 and CD16: Classical ($CD14^{+}CD16^{-}$), intermediate ($CD14^{+}CD16^{+}$) and non-classical ($CD14^{dim}CD16^{+}$) monocytes. The objective of this study was to phenotipically and functionally characterize monocyte subsets from CL patients.

Methods and Results. Peripheral blood mononuclear cells were obtained from healthy controls, CL patients in pre-ulcerative phase and CL patients with skin ulcer. Monocyte subsets characterization was performed by flow cytometry. We observed that the frequency of intermediate and non-classical monocyte populations was increased in individuals on pre-ulcerative phase and CL patients with skin ulcer. Expression of MHC class II was increased in intermediate monocytes, suggesting that these cells might better present antigen to T cells. Also, classical and intermediate monocytes produced more TNF than the non-classical ones in response to soluble *Leishmania* antigen and LPS. CCR2 is the ligand for CCL2, a chemokine known to play important role in immune response against *Leishmania*. Interestingly, the frequency of CCR2

expressing monocytes was significantly lower in peripheral blood from CL patients when compared to healthy controls, indicating that CCR2 expressing cells might migrate to the lesion site. Analysis of biopsies from CL patients showed that the predominant population of monocyte found in lesions of these individuals was the non-classical one.

Conclusion. Altogether, our data show differences among monocyte subsets in CL. While classical and intermediate monocytes produce more TNF, non-classical ones are more frequent in lesions of these patients. The identifation of cells that contribute to the immunopatology observed in CL will help to develop new forms of immunotherapy to this disease.

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EFFECT OF OUABAIN IN MACROPHAGES INFECTED BY *LEISHMANIA AMAZONENSIS*

JACOB, PRISCILA LIMA[1,2]; LEITE, JACQUELINE ALVES[1,2]; ALVES,ANNE KALIERY DE ABREU[2]; RODRIGUES, YARA KATIA SANTOS[3]; NÉRIS, PATRÍCIA LIMA DO NASCIMENTO[3]; AMORIM, FRANCIANNE MEDEIROS[3]; **DE OLIVEIRA, MÁRCIA ROSA[1,2,3]; MASCARENHAS, SANDRA RODRIGUES[1,2]**

[1] Universidade Federal da Paraíba, Centro de Ciências da Saúde, Programa de Pós-Graduação em Produtos Naturais e Sintéticos Bioativos.
[2] Universidade Federal da Paraíba, Centro de Biotecnologia, Laboratório de Imunofarmacologia.
[3] Universidade Federal da Paraíba, Centro de Ciências Exatas e da Natureza, Departamento de Biologia Celular e Molecular, Laboratório de Leishmanioses.

Introduction: Ouabain is a cardiotonic steroid identified as an endogenous substance of human plasma and has been proposed to act as an immunomodulator. Leishmaniasis are diseases caused by pathogenic species of protozoans that belong to the genus *Leishmania (Leishmania)* transmitted by the bite of the female phlebotomine sandfly. Despite the anti-inflammatory role of ouabain, little known about its effect on Leishmaniasis. Previous data from our group, demonstrated that ouabain produced a significant reduction in the mouse paw edema and reduced cell migration into the peritoneal cavity induced by *Leishmania amazonensis*. However, the mechanisms involved remain unclear **Objective:** Evaluate cytotoxic potential of ouabain against *Swiss* mice macrophages and influence of ouabain in intracellular Ca^{2+} levels in *Leishmania amazonensis* promastigotes. **Methodology:** Peritoneal exudate macrophages were obtained 5 days after i.p. injection of 1 mL of thioglycolate. Ouabain cytotoxicity to macrophages was tested by MTT and trypan blue after incubation of macrophages with 10, 100 and 100 nM f ouabain for 24 hours. Additionally, promastigotes forms of *Leishmania (Leishmania) amazonensis* were incubated with the fluorescence indicator of intracellular calcium FLUO 3AM (1,5 µM) in the presence or absence of ionomycin ionophore and ouabain and analyzed by flow cytometry FACSCalibur. **Results**: Ouabain did not interfere with the viability of the cells in both tests MTT and Trypan Blue. Furthermore, ouabain was able to increase intracellular Ca^{2+} levels at all concentrations studied, with a more pronounced effect at the concentration of 100nM (37%). **Conclusion:** This work demonstrated that ouabain is capable to increase intracellular Ca^{2+} levels. It is unlikely that the effect observed by us resulted from cell death, as macrophages, *in vitro*, remained as viable in the presence of different ouabain concentrations as cells kept in medium.

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INVOLVEMENT OF NOTCH AND TLR SIGNALING PATHWAYS IN THE IMMUNE RESPONSE OF MACROPHAGES AGAINST *Paracoccidioides brasiliensis*

LAVÍNIA MARIA DAL'MAS ROMERA[(1)]; SANDRO ROGÉRIO DE ALMEIDA[(1)]
[(1)]Department of Clinical and Toxicological Analysis, Faculty of Pharmaceutical Sciences, University of São Paulo

Introduction: Paracoccidioidomycosis is a systemic mycosis of deep nature and granulomatosis, which affects preferentially the lung tissue caused by *Paracoccidioides brasiliensis*, a fungus that exhibits thermal dimorphism. Macrophages are cells that play an important role in the induction and regulation of the immune and/or inflammatory response and important for controlling pathogen growth. The innate immune system is crucial in the antifungal response and macrophages are important for controlling pathogen growth. It was recently demonstrated the importance of Notch receptors in regulating the activity of macrophages and the immune system. Whereas Notch signaling may be involved in modulation of macrophage function, we evaluate the ability of *P. brasiliensis* to modulate the activation of this pathway. **Methods and Results:** J774 macrophages pre-stimulated overnight with LPS or not were incubed with yeast (1:1) for 24 hours (biological triplicate) followed by analysis of Real-Time PCR (mean value of $2^{-\Delta\Delta CT}$) and ELISA (mean standard deviation). Significance test was carried out by Anova and Tukey. The transcriptional analysis of Notch 1 revealed that there is an increased level of transcription in 24 hours, while there is a reduction of its ligand Delta 4 in 24 hours. To verify the importance of Notch signaling, we pre-treated macrophages with a farmacological inhibitor of g-secretase (DAPT) for 24 hours and then they were incubed with yeast (1:1) for 4, 12 and 24 hours. We found that phagocytosis of yeasts by macrophages become more efficient, while there was reduction of IL-6 production and an increased TNF-a. We saw that the fungus has the ability to promote the production of IL-6 via Notch-TLR, making us suppose that this cytokine is important for the establishment of the disease, leading in a fungal benefit in relation to host. **Conclusion:** We suggest that *P. brasiliensis* uses the Notch signaling pathway as an escape mechanism. The interaction between the yeasts with macrophages promotes the activation of this pathway, by Notch 1 receptor, inducing increased production of IL-6 cytokine important for the growth of fungus on host, and a reduction of TNF-a, contributing to a macrophage activity damage caused by the fungus.

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OspC1 AND OspF: VIRULENCE FACTORS INVOLVED IN THE INHIBITION OF INTESTINAL INFLAMMATORY RESPONSE CAUSED BY ENTEROINVASIVE *Escherichia coli*

RENÉE DE NAZARÉ OLIVEIRA DA SILVA[(1)], LUCAS GONÇALVES FERREIRA[(1)], FRANCIELE HINTERHOLZ KNEBEL[(1)] AND MARINA BAQUERIZO MARTINEZ[(1)]

[(1)]Department of Clinical and Toxixological Analysis, Faculty of Pharnaceutical Sciences, University of São Paulo.

Introduction and objective: Enteroinvasive *E. coli* (EIEC) is one etiological agent of bacillary dysentery, which is characterized by the colonic epithelium destruction caused by the inflammatory response induced by bacteria invasion in mucosa Strains of EIEC are biochemical, genetic and pathogenic similar to *Shigella spp*. The pathogenecity of EIEC and *Shigella* depend of the plasmid pInv, which has the genes necessary for bacterial colonization in the intestinal mucosa. Recently, it was demonstrated that the plasmid genes *ospC1*, *ospG* and *ospF* of *S. flexneri* are involved in inhibition of the inflammatory response in intestinal epithelial cells, which is an important factor in the initiation of bacterial colonization and production of disease. As EIEC has showed less severe disease than *Shigella*, we evaluated the transcription of these plasmid genes and inflammatory response modulated by this microorganism in the intestinal epithelial cell Caco-2. **Metodology and Results:** The Caco-2 cells were infected in different times with many serotypes of EIEC and *S. flexneri* M90T strain. The data about invasiveness and survival of bacteria, bacterial genes expression, and chemokine IL-8 were obtained by CFU, RT-PCR, and ELISA, respectively. The statistical significance was evaluated by two-way ANOVA. All EIEC serotypes studied expressed the genes *ospC1*, *ospG* and *ospF*, suggesting the involvement of them in modulating of the immune response induced by these microorganisms. There were no differences in the invasion the enterocytes among EIEC serotypes ($p>0.05$). However, we observed a difference in the transcription of those genes and production of IL-8. The EIEC serotypes O29:H- and O167:H- showed a low transcription of genes *ospC1* and *ospF*, and an significant increase in IL-8 release compared with other serotypes ($p<0.05$). Furthermore, it was shown that the high transcription of *ospF* and *ospC1* by some EIEC serotypes appear to be related to low induction of IL-8. These data suggested that the proteins OspC1 and OspF play a role in the inflammatory response. However, we did not observed any relation between transcription of *ospG* and the production of IL-8. **Conclusion**: These results suggest that the effector proteins OspF and OspC1 are involved in inhibition of the inflammatory response in intestinal epithelial cells contributing with bacteria colonization and infection in the intestinal mucosa.

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LEISHMANIA CHAGASI RESISTANT TO ANTIMONY *IN VIVO* AND *IN VITRO* DOWN MODULATE MACROPHAGE KILLING MECHANISMS

MICHELI LUIZE BARBOSA SANTOS[(1)]; FABRÍCIA ALVISI DE OLIVEIRA[(1)] ;FLÁVIA MARIA MATOS MELO CAMPOS [(1)]; JUCIENE DE MATOS BRAZ[(1)]; PRISCILA LIMA SANTOS [(1)]; PAULO DE TARSO GONÇALVES LEOPOLDO [(1)] AMÉLIA RIBEIRO DE JESUS[(1,2)]; ROQUE PACHECO ALMEIDA[(1,2)]; TATIANA RODRIGUES DE MOURA[(1)]

(1) Universidade Federal de Sergipe – Aracaju, Brazil; (2) Instituto de Investigação em Imunologia, São Paulo, Brazil

Introduction: Pentavalent antimonials (Sb^V) have been used to treatment leishmaniasis for the past 50 years. Although the requirement of a functional cellular immune response for antimony antileishmanial action was previously reported, the cellular and molecular mechanism of involved is still unclear. Sb^V is known to reinforce the killing mechanisms of macrophages. In the present study, we evaluated if *L. chagasi (Lc)* isolated from refractory to antimony treatment patients has cross-resistance to SbIII and NO and also modulate macrophage infection.

Methods and Results: Four *L. chagasi* isolates from antimony treatment refractory patients and two from antimony treatment responsive patients were used in this study. *In vitro* susceptibility nitric oxide (NO) was evaluated by exposing the parasites to different concentrations of $NaNO_2$ (NO donor) and the viability was determined by MTT colorimetric method. The *Lc* isolates from refractory patients were two times more resistant to NO than those from patients responsive at $NaNO_2$ 8nM (86 ± 9 and 46 ± 9, $p>0.005$, respectively). Promastigotes were submitted to increasing concentrations of antimony trivalente (Sb^{III}) and the viability was determined by cell count. The Sb^{III} 50% inhibitory concentration for the isolates from refractory patients was 741 ± 32 and for the sensitive patients was 381 ± 100, $p>0.01$. To evaluate the susceptibility of amastigotes to Sb^V and macrophages killing mechanism, macrophages were infected in the presence of media, LPS/IFN-γ or Sb^V, at 24, 48 and 72 hours. Isolates from refractory patients were more infective than isolates from patients responsive to treatment, independently of these stimuli and in all times post infection. Although isolates from refractory patients produced more nitrite than those from responsive, the rate nitrite/amastigotes was six times lower in the macrophage infected by isolates from refractory patients.

Conclusion: Taken together, these results suggest that in isolates from refractory patients there is cross resistance between Sb and NO and, also, these parasites were able to down modulate the macrophage killing mechanisms.

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CYTOKINE PROFILE IN HUMAN SCHISTOSOMIASIS AND ITS RELATIONSHIP WITH FIBROSIS AND TREATMENT

GABRIELA DA SILVEIRA E NUNES[1,2]; MÔNICA MARIA DE ALMEIDA[2]; AMANDA CARDOSO DE OLIVEIRA SILVEIRA[3]; GIOVANNI GAZZINELLI[4]; LÚCIA ALVES DE OLIVEIRA FRAGA[2]; LUIZ COSME COTTA MALAQUIAS[2,6]; ELAINE SPEZIALI DE FARIA[2]; OLINDO ASSIS MARTINS-FILHO[3]; ANA MARIA CAETANO DE FARIA[1;] RODRIGO CORREA-OLIVEIRA[3]; ANDRÉA GAZZINELLI[5]; ANDRÉA TEIXEIRA CARVALHO[3] E ALDA MARIA SOARES SILVEIRA[2]

[1] Departamento de Bioquímica e Imunologia, ICB, UFMG, Belo Horizonte, MG, Brasil

[2] Universidade Vale do Rio Doce, UNIVALE, Governador Valadares, MG, Brasil

[3] Centro de Pesquisa René Rachou - Fundação Oswaldo Cruz, Belo Horizonte, MG, Brasil

[4] Santa Casa de Misericórdia, Belo Horizonte, MG, Brasil

[5] Escola de Enfermagem, UFMG, Belo Horizonte, MG, Brasil

[6] Universidade Federal de Alfenas, Alfenas, MG, Brasil

Keywords: Schistosomiasis, Periportal fibrosis, Treatment, Cytokines

Introduction: The cytokine response to *S. mansoni* antigens seems to play an important role in pathogenesis of the periportal fibrosis associated with human schistosomiasis. The aim of this study was to investigate whether the cytokine/chemokine pattern produced by peripheral blood mononuclear cells upon *in vitro S. mansoni* antigen stimulation could be used as a biomarker of periportal fibrosis in schistosomiasis patients and evaluate the impact of Praziquantel treatment in this complex cytokine network. **Methods:** Thirty one volunteers living in an endemic area were classified into sub-groups according to the presence or absence of fibrosis before (FIB and non-FIB) and after treatment (FIB_T and non-FIB_T) Cytokine/chemokine pattern (IFN-γ, TNF-α, IL-4, IL-5, IL-13, IL-10, IL-17, TGF-β, CCL3/MIP-1α, CCL2/MCP-1 e RANTES) was measured in PBMC culture supernatants using Cytometric Bead Array (CBA). Cytokine/chemokine signatures were analyzed using the concept of low and high-cytokine producers. **Results:** Our results demonstrate that FIB presented a decreased cytokine production compared to non-FIB individuals. Furthermore,

FIB produced higher proportion of fibrogenic cytokines whereas non-FIB had higher levels of IL-10 and TGF-β, in the non-stimulated cultures. Two years after treatment, a high proportion of fibrogenic cytokines in response to SEA was observed in FIB_T. Those FIB participants who showed a regression of fibrosis after treatment produced high levels of TGF-β e IFN-γ, while non-FIB individuals who remained without fibrosis continued producing high levels of IL-10 e TGF-β. **Conclusion:** These data suggest that the concomitant production of high IL-10 e TGF-β levels is associated with protection against fibrosis, and that specific treatment induces a balanced profile of fibrogenic and regulatory cytokine production in individuals with and without fibrosis**.**

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LACK OF GALECTIN-1 EXPRESSION AFFECTS *Trypanosoma cruzi* REPLICATION AND INFECTION

THALITA BACHELLI RIUL (PG)[(1)]; CAMILLO DEL CISTIA ANDRADE(PR)[(1)]; SEAN R. STOWELL(PR)[(2)]; RICHARD D. CUMMINGS(PR)[(2)]; MARCOS ANTONIO ROSSI[(3)]; ANDERSON SÁ-NUNES (PR)[(4)]; MARCELO DIAS BARUFFI (PR)[(1)]

[(1)]Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, Brasil.[(2)] Emory University School of Medicine, Atlanta, USA. [(3)] Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, Brasil.[(4)]Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, Brasil.

Introduction and objective: Chagas is a neglected tropical disease caused by *Trypanosoma cruzi (T. cruzi)* infection with global prevalence of 8-9 million people worldwide. Some mechanisms of innate immune response against *T. cruzi* are not fully understood. Galectin-1 (Gal-1) is a multifunctional lectin that participates in several biological processes, including modulation of the immune response. Thus, this work aimed to investigate the role of endogenous Gal-1 during the course of experimental *T. cruzi* infection. **Methods and results:** C56BL/6 Gal-$1^{+/+}$ (WT) and Gal-$1^{-/-}$ (KO) mice were infected with *T. cruzi* tripomastigotes and their parasite load and survival during the acute phase of infection were monitored. WT mice exhibited higher parasitemia levels (5,24±1,18x10^5 parasites/mL *X* 2,91±0,44x10^5 parasites/mL from KO mice) as well as higher mortality than those KO mice. Also, we assessed the ability of the peritoneal macrophages (MØ**)** obtained from WT and KO mice to control the replication of *T. cruzi*. We found that WT-MØ released a higher number of tripomastigote forms after infection than those KO-MØ (1,54±0,10x10^4 released parasites/mL *X* 0,92±0,09x10^4 released parasites/mL, respectively). In addition, KO-MØ produce higher levels of nitrite than those from WT-MØ, specially during the early days of infection (46,84±0,99 µM of nitrite *X* 33,12±2,17 µM of nitrite, respectively). Finally, we analized the gene expression of M1/M2 activation markers in both MØ populations by real time PCR after peritoneal thyoglycolate elicitation, and we found that the WT-MØ show higher mRNA expression of M2-markers when compared to those KO-MØ. **Conclusion:** These findings suggest that the expression of endogenous Gal-1 promotes a

MØ phenotype M2, resulting in decreased production of NO and reduced control of infection by *T. cruzi.*

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PIVOTAL ROLE OF TLR9 IN THE CONTROL OF *LEISHMANIA INFANTUM* INFECTION THROUGH A NEUTROPHIL RECRUITMENT DEPENDENT MECHANISM

LAÍS AMORIM SACRAMENTO(PG)[1]; MANUELA SALES LIMA NASCIMENTO(PG)[1]; DJALMA SOUZA LIMA-JÚNIOR(PG)[1];DIEGO LUÍS COSTA(PG)[1]; SILVIA CELLONE TREVELIN(PG)[2]; ROQUE PACHECO DE ALMEIDA[3]; MARCOS ANTÔNIO ROSSI[4]; FERNANDO QUEIROS CUNHA[2]; JOÃO SANTANA DE SILVA[1]; VANESSA CARREGARO[1];

[1]Department of Biochemistry and Immunology, School of Medicine of Ribeirão Preto, University of Sao Paulo; [2]Department of Pharmacology, School of Medicine of Ribeirão Preto, University of Sao Paulo; [3]Center for Human Science and Biological, Federal University of Sergipe; [4] Department of Pathology and Forensic Medicine, School of Medicine of Ribeirão Preto, University of Sao Paulo

Introduction The protozoan *Leishmania infantum chagasi* (*Lic*) is the causative agent of visceral leishmaniasis (VL) in Brazil and South America, causing high morbidity and mortality. The resistance in leishmaniasis is induced by IL-12 secreting-dendritic cells (DC), and their ability to produce relates to the ability to recognize microbial products by Toll-like Receptors (TLRs). Among several TLRs, it has been showed that TLR9 is required for IL-12 production by DC in a model of cutaneous leishmaniasis. In the present study, our aim were to determinate the role of TLR9 in VL infection control.

Methods and Results Our results demonstrate that TLR9 is upregulated *in vitro* and *in vivo* during *Lic* infection. Using genetically resistant C57BL/6 mice deficient in TLR9 ($TLR9^{-/-}$), we show that these mice are more susceptible to infection, displaying higher parasites numbers into the spleen and liver, and less inflammatory cells (liver) at 4^{th} and 6^{th} weeks p.i. Phenotyping the leukocytes by flow cytometry, $TLR9^{-/-}$ failed to recruit neutrophils to inflammatory foci. Likewise, imunohistochemistry analyses showed the reduced $7/4^{+}$ cells (neutrophil marker) staining into the $TLR\text{-}9^{-/-}$ liver. The failure of neutrophils recruitment was associated with reduced CXCL1 and IL-17 (neutrophils chemoattractants) levels into the spleenocytes culture supernatant from $TLR9^{-/-}$. Furthermore, *in vitro* and *in vivo*, Lic failed to activate DC from $TLR9^{-/-}$, showing reduced surface costimulatory molecule expression and proinflamatory cytokines release.

Conclusion Altogether, our results suggest that TLR9 has a critical role for neutrophils recruitment in the protective response against *L.infantum* that could be associated with DC activation stage. However, the mechanism by which DC participates into the neutrophils recruitment through TLR9 pathway remains to be elucidated.

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ROLE OF P2X7 RECEPTOR IN INNATE IMMUNE RESPONSE TO *Plasmodium chabaudi* MALARIA

MARIA NOGUEIRA DE MENEZES; ÉRIKA MACHADO SALLES; ALEXANDRA DOS ANJOS CASSADO; HENRIQUE BORGES SILVA; EDUARDO PINHEIRO AMARAL; JOSÉ MARIA ÁLVAREZ; MARIA REGINA D'IMPÉRIO LIMA

Immunology Department, Biomedical Science Institute, São Paulo University, São Paulo.

Introduction: Malaria is characterized by intense activation of the immune system that seems to contribute to protection against infection and to clinical manifestations related to disease. ATP recognition by P2X7R in immune cells is important for cell activation, death and migration. The main goal of this study is to evaluate the effects of P2X7R-mediated signaling on activation, death and migration of macrophages, monocytes and dendritic cells during acute and chronic infection with *Plasmodium chabaudi* AS. **Methods:** Six- to eight-week-old C57BL/6 and P2X7R$^{-/-}$ male mice received an intraperitoneal inoculation of 10^6 *P. chabaudi* infected red blood cells (iRBC) and pathological parameters were obtained before infection and daily after infection. Kinetics of phagocytic cells in the spleen of these infected animals was determined by flow cytometry, in relation to number of cells and cell death. **Results:** Between days 9 and 17 post-infection, 80% of P2X7R$^{-/-}$ mice died, while the C57BL/6 group presented no death. The parasitemia peak of P2X7R$^{-/-}$ group occurred at day 8 and C57BL/6 mice presented the peak at day 7. Pathological parameters as weight change and body temperature were also delayed in knockout mice in comparison to wild-type (WT) group, and animals showed a late improvement in clinical conditions. Regarding kinetics, P2X7R$^{-/-}$ mice showed a higher number of CD11b^{+} cells (20.4 x 10^6 cells) at day 7 post-infection compared to the WT mice (10.9 x 10^6 cells). In addition, the percentage of leukocytes cell death found in spleen in the same day was lower in knockout group (12.65%) compared to C57BL/6 mice (21.25%). **Conclusion:** Mice lacking the P2X7R seem to be more susceptible to the exacerbated immune response that is responsible for clinical symptoms, thus, presenting more severe pathological parameters for more time. The elevated number of phagocytes in spleen from P2X7R$^{-/-}$ mice when compared to C57BL/6 mice could be due to the reduced rate of cell death found in the same spleen.
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ALTERATION IN THE DISTRIBUTION OF PERITONEAL MACROPHAGE SUBSETS IN A MURINE MODEL OF DIABETES MELLITUS DURING FUNGAL INFECTION.

THAIS FERNANDA DE CAMPOS FRAGA-SILVA(PG)(1,4); CAMILA MARTINS MARCHETTI(PG)(2,4); MARJORIE DE ASSIS GOLIM(PG)(3); JAMES VENTURINI(PG)(2,4); MARIA SUELI PARREIRA DE ARRUDA(4).

(1) Instituto de Biociências, UNESP - Univ Estadual Paulista, Botucatu, Programa de Pós-Graduação em Biologia Geral e Aplicada; (2) Faculdade de Medicina de Botucatu, UNESP - Univ Estadual Paulista, Botucatu, Programa de Pós-graduação em Doenças Tropicais; (3) Faculdade de Medicina de Botucatu, UNESP - Univ Estadual Paulista, Botucatu, Laboratório de Citometria de Fluxo do Hemocentro de Botucatu; (4) Faculdade de Ciências, UNESP - Univ Estadual Paulista, Bauru, Departamento de Ciências Biológicas, Laboratório de Imunopatologia Experimental (LIPE).

Introduction: Macrophages (MØ) display heterogeneous phenotype according to the distribution to different tissue and the cytokine-chemokine networks. Recently, two peritoneal MØ subsets were described under homeostatic condition according to morphological size and CD11b and F4/80 expression. The small peritoneal MØ (SPMs) is identified for exhibit lower expression of CD11b and F4/80 and the large peritoneal MØ (LPMs) for exhibit high levels of the CD11b and F4/80. Considering that the distribution of this cells has not been studied in pathological condition such as fungal infection neither in Diabetes Mellitus, in the present study we investigated the distribution of SPM and LPM in the peritoneal cavity from hypoinsulinemic-hyperglicemic (HH)-mice during dermatophytic infection. **Methods and Results:** HH-mice, induced by alloxan (60mg/kg), were inoculated into the footpad with 10^7 *Trichophyton mentagrophytes* conidia (Group HHTM). Non-infected HH-mice (HH), only infected-mice (TM), non-infected and free-HH mice (CTRL) were used as control groups. The mice were evaluated at 24 and 48 hours and 7 days after the inoculation. Fragments of the footpad, popliteal lymph node, liver, spleen and kidney were submitted to determination of the fungal load. The distribution of peritoneal MØ subsets was performed by flow cytometry. Our results showed that mice from TM group exhibited an increase of LPMs soon after the fungal introduction. Overtime, they exhibited a reduction of fungal load and, the percentage of this subset returned to normal levels. In the HH group, we observed an increase of the LPMs at all times evaluated. In the HHTM group the distribution of LPMs was similar to the TM group, except on day 7, which the mice exhibited a lower percentage of LPMs in comparasion to HH-mice. The percentage of SPMs in HHTM group was always lower than in the HH group; in the TM group, this phenomenon was only observed on day 7. **Conclusion:** Our results demonstrated that both infectious and HH conditions triggered changes in the distribution of SPM and LPM. Thus, factors from the fungus such as

soluble antigens and/or inflammatory mediators released from the infectious process as well as from the HH condition affect the phenotype of peritoneal macrophages.

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BENEFICIAL EFFECTS OF PENTOXIFYLLINE ON CARDIAC AND IMMUNE TRAITS OF EXPERIMENTAL CHRONIC CHAGASIC CARDIOMYOPATHY

ISABELA RESENDE PEREIRA[1], GLAUCIA VILAR-PEREIRA[1], JOSELI LANNES-VIEIRA[1].

[1]*Laboratório de Biologia das Interações*, IOC–Fiocruz, RJ, Brasil;

Introduction: *Trypanosoma cruzi* infection results in chronic Chagas cardiomyopathy (CCC) in about 30-40% of the infected individuals. CCC is characterized by prominent inflammation associated with fibrosis and electrical dysfunction. In the affected cardiac tissue, production of inflammatory mediators, chiefly pro-inflammatory cytokines and chemokines, might drive leukocyte migration contributing to CCC. **Methods and Results**: We tested the effect of pentoxifylline (PTX), a phosphodiesterase inhibitor, that also acts as an immunoregulator, upon immune response and heart dysfunction in C57BL/6 mice chronically infected with the Colombian strain of *T. cruzi.* PTX therapy did not alter the survival and parasite load, however improved cardiac function, reducing fibronectin (FN) deposition (13.5 ± 3.0% of FN positive area in saline-injected *vs* 6.9 ± 1.7% of FN positive area in PTX-treated mice, $p<0.05$) and connexin 43 (Cx-43) loss (129.4 ± 10.9 mm of Cx-43 distance in saline-injected *vs* 103.5 ± 7.2 mm of Cx-43 distance in PTX-treated mice, $p<0.01$) in heart tissue, decreasing CK-MB activity in serum (0.35 ± 0.04 CK-MB activity in saline-injected *vs* 0.27 ± 0.03 CK-MB activity in PTX-treated mice, $p<0.05$), and ameliorating electrical conduction. Further, PTX preserved IFN-gamma status in the blood, spleen and heart tissue, but increased the cytotoxic activity of splenic CD8+ T cells (22.6 ± 1.6 % of specific lysis in saline-injected *vs* 33 ± 3.7 % of specific lysis in PTX-treated mice, $p<0,05$) and decreased the number of perforin+ cells in the heart tissue (29 ± 3.3 in saline-injected *vs* 15.7 ± 7.8 in PTX-treated mice, $p<0,05$). These data suggest a recompartmentalization of effector cells with cytotoxic activity, which may play a pivotal role in *T. cruzi*-induced heart injury. Also, PTX acts on the process of cell migration, reducing the frequency of splenic CD8+CCR5+LFA1+ cells (7.6 ± 1.8 in saline injected *vs* 3.3 ± 0.7 in PTX treated mice, $p<0.05$) and the expression of ICAM-1 in blood vessels of the cardiac tissue (3.4 ± 0.4 % positive area for ICAM-1 in saline-injected *vs* 2.3 ± 0.1 % positive area for ICAM-1 in PTX-treated mice, $p<0.01$). **Conclusion**: Together, our data demonstrate that during *T. cruzi* infection, PTX

prevents the development of severe CCC, acting selectively on CD8+T cell populations, cardiac endothelial cells and cardiomyocytes, supporting that PTX deserves to be explored as therapeutic tool to ameliorate CCC.

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MODULATION OF IMMUNE RESPONSE AND CARDIOMYOCYTE FUNCTION BY ZILEUTON DURING EXPERIMENTAL *TRYPANOSOMA CRUZI* INFECTION

RONAN RICARDO SABINO ARAÚJO(PG)[1]; DANILO ROMAN CAMPOS(PhD)[1]; ANDRÉIA BARROSO(IC)[1]; FÁTIMA BRANT(PG)[1,2]; LÍSIA ESPER(PG)[1,2]; ROSA MARIA ESTEVES ARANTES(PhD)[3]; DANIELE DA GLÓRIA SOUZA(PhD)[4]; JADER DOS SANTOS CRUZ(PhD)[1]; HERBERT BERNARD TANOWITZ(PhD)[5]; MAURO MARTINS TEIXEIRA(PhD)[1,2]; FABIANA SIMÃO MACHADO(PhD)[1,2]

[1]Departamentos de Bioquímica e Imunologia , [3]Patologia e [4]Microbiologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, MG, Brazil. [2]Departamento de Clínica Médica - Medicina Tropical e Infectologia, Faculdade de Medicina da Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil. [5]Departments of Pathology and Medicine, Albert Einstein College of Medicine, Bronx, NY.

Introduction: The Chagas' disease is an illness highly neglected caused by *Trypanosoma cruzi* parasite. The *T. cruzi* infection may lead a mega syndrome and Chagasic cardiomyopathy development. Lipoxin A (LXA) is a modulator of immune response, synthesized for 5-lipoxygenase (5-LO) activities, via aracdonic acid pathway. Zileuton is a 5-LO inhibitor, which can modulate the immune response through manipulation of 5-LO derived production. Here we study the effects of Zileuton treatment on some parameters of immune response and development of myocarditis during *T. cruzi* infection.

Methods: Mice C57BL/6 was infected with *T. cruzi* Y strain and treated with Zileuton (30mg/Kg) via oral at different time points after infection. The parasitemia was observed, and heart, spleen and liver were removed for mRNA expression of cytokines and histological analysis. Cytokine levels were detected in serum and splenic cells by ELISA and flow cytometry, respectively.

Results: We found reduced parasite load in mice treated with Zileuton compared with untreated mice. Zileuton treatment also resulted in decreased production of IL-12 and IFN-g detected in serum during the acute phase of infection. Moreover there was decreased IFN-g mRNA levels in the heart and spleen of treated mice. During the acute phase of infection, T $CD4^+$ and T $CD8^+$ cells harvested from spleen of treated mice produced reduced levels of IFN-g when compared with untreated mice. Additionally, we found reduced IL-6 and IL-10 mRNA levels in spleen of treated mice. Furthermore, the Zileuton

treatment resulted in lower leukocyte infiltration in cardiac tissue and our electrophysiological analyses, using the patch-clamp technique, demonstrated that the treatment also resulted in the protection of cardiomyocytes activities, including decreased in the repolarization time.
Conclusion: Therefore, our results suggest that Zileuton treatment could be a "powerful tool" in the therapeutic field to modulate the development of cardiomyopathy in Chagas' disease.

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FAS/FASL AND TRAIL CAUSE APOPTOSIS OF T CELL CD4 AND CD8 IN CANINE NATURALLY INFECTED VISCERAL LEISHMANIASIS

KATHLENN LIEZBETH OLIVEIRA SILVA (PG)[1]; BRUNA BRITO OLIVEIRA (IC)[1]; JULIANA PEROSSO (PG)[1]; LARISSA MARTINS MELO (PG)[1]; VALÉRIA MARÇAL FÉLIX DE LIMA[1].

1-Laboratory of cellular immunology, Faculty of Veterinary Medicine – FMVA – UNESP - "Júlio de Mesquita Filho" - Araçatuba/SP

Introduction: Visceral leishmaniasis (VL) is an emerging disease caused by *L. chagasi* in Brazil. The dogs are the main reservoirs urban of the disease. In symptomatic dogs the VL is characterized by immunosuppression of cellular immune response with the parasites wide dissemination, mainly in the spleen, liver and bone marrow, and T cell apoptosis was observed. The molecules involved in the apoptosis are the FAS/FASL and TRAIL. However, the role of these receptors in canine visceral leishmaniasis is not yet clear. The objective of this research was to evaluate the rate of apoptosis in CD4 and CD8 T cell from spleen and peripheral blood of dogs infected by *L. chagasi*, investigate the expression of cell surface molecules FAS / FASL and TRAIL, as well as to identify whether apoptosis is dependent cell contact. **Methods and Results:** Mononuclear cells from spleen and peripheral blood of 15 dogs with VL and 5 healthy dogs were used to assess the rate of apoptosis in T lymphocytes CD4 and CD8 and the expression the FAS/FASL and TRAIL. The cell staining for CD4 and CD8 was performed using monoclonal antibodies conjugated to fluorochromes; apoptosis was measured using the kit Guava Nexin ® Assay. The FAS, FAS-L and TRAIL were measured using monoclonal antibodies conjugated to fluorochromes. To check if the apoptosis in T cell is dependent on cell contact, macrophages were infected with $5x10^6$ promastigotes of *L. chagasi* at the bottom of a culture plate and the upper part comprises a transwell was added cell from healthy dogs and apoptosis rate measured. Data were acquired in the cytometer EasyCyteMini and analyzed with the Cytosoft ® software. The results were compared using the Mann-Whitney test, with significance level of 5%. It was observed a increased of apoptosis in CD4+ and CD8+ T cell from spleen and peripheral blood of infected dog compared to healthy. The FAS increase in CD4+ T cell from peripheral blood and also increase CD8 T cell from spleen in infected dog, while FAS-L and TRAIL decrease in CD4 and CD8 T cell from PBMC and spleen from infected dogs compared to healthy ones. The data from transwell plate suggests that the apoptosis in CD4+T cell depends on cell contact. **Conclusions:** The FAS and FASL in T cell from infected dogs are involved in the apoptosis; this knowledge may allow future therapeutic interventions in order to reduce the depletion of lymphocytes, thereby increasing the ability of defense.

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LYMPHOCYTIC APOPTOSIS IN HUMAN *P. FALCIPARUM* AND *P. VIVAX* MALÁRIA: RELATIONSHIP WITH IMUNE RESPONSE TO THE PARASITES

EVELYN KETY PRATT RICCIO[1]; PAULO RENATO RIVAS TOTINO[1]; VÍTOR ENNES VIDAL[2]; IRENE DA SILVA SOARES[3]; MAURÍCIO MARTINS RODRIGUES[4]; JOSÉ MARIA DE SOUZA[5]; LILIAN ROSE PRATT-RICCIO[1]; RENATO PORROZZI DE ALMEIDA[6]; MARIA DA GLÓRIA BONECINI DE ALMEIDA[7]; GRAZIELA MARIA ZANINI[8]; CLÁUDIO TADEU DANIEL RIBEIRO[1]; MARIA DE FÁTIMA FERREIRA DA CRUZ[1].

[1]Laboratory of Malaria Research, Fiocruz, [2] Laboratory of Molecular Biology and Endemic Disease, Fiocruz, [3]Department of Clinical Analyses and Toxicology, University of São Paulo, [4]Department of Microbiology, Immunology and Parasitology, Federal University of São Paulo, [5]Ambulatory and Laboratory of Malaria Clinical Assays, Instituto Evandro Chagas, [6]Laboratory of Leishmaniasis Research, Fiocruz,[7]Immunology Service of Evandro Chagas Clinical Research Institute, Fiocruz and [8]Parasitology Service, Evandro Chagas Clinical Research Institute, Fiocruz.

INTRODUCTION: Infection with *Plasmodium* is characterized by both activation and suppression of the immune system during the course of the disease. As apoptosis is an important mechanism regulating the activation of lymphocytes, we decided to study the role of lymphocytic apoptosis during a non complicated malaria attack. **METHODOLOGY:** Blood samples were collected from 35 individuals living in malaria endemic area with positive blood smears, as well as from 17 individuals without previous reported malaria episodes. Apoptosis in T and B cells were analyzed *ex–vivo* and after 96 hour-culture. Cellular and humoral immune responses after antigenic stimulation, absolute numbers of T and B cells, B cells polyclonal activation and the auto-immune response against components of erythrocytes membrane, cardiolipin, actin and DNA were also investigated. **RESULTS:** In *ex-vivo* analyses low levels of apoptosis and high levels of cellular activation in $CD4^+$ cells were observed in malaria patients, although increased levels of early apoptosis in $CD4^+$ and $CD8^+$ cells had been detected. High levels of IFN-γ and IL-10 and low levels of TNF were also verified in malaria patients. No difference was observed between TNF and IL-10 plasmatic levels between *P. vivax* and *P. falciparum* infections, although high levels of IFN-γ was registered in *P. vivax* malaria patients. No relationship was observed between cytokines levels and activation, viability or PBMC apoptosis. After 96 hour-culture, apoptosis levels in malaria patients were increased in $CD4^+$, $CD8^+$ and B cells, and even in the presence of stimuli the cellular proliferation was lower than in healthy individuals. Antibodies against membrane erythrocyte components, cardiolipin and DNA were similarly detected in malaria patients and healthy individuals, while antibodies against

actin were more frequently detected in malaria patients. **CONCLUSIONS:** The higher levels of apoptosis observed in cells from *P. vivax* and *P. falciparum* malaria patients could contribute to lymphopenia associated to malaria. However, the lack of correlation among apoptosis and parasitemia, number of past malaria attacks and low cellular response, specially against malaria antigens, suggest that apoptosis associated to uncomplicated malaria could be a physiological reaction of the immune system to control polyclonal activation and maintain the balance of these cellular population densities.

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EVALUATION OF THE PROTECTIVE ABILITY OF *LEISHMANIA INFANTUM* HISTONESIN THE INFECTION BY *LEISHMANIA CHAGASI* INFECTION IN HAMSTERS

LAÍS PEREIRA[1]; MELISSA ABBEHUSEN[1]; JUREMA CUNHA[1]; ALDINA BARRAL[1]; MANOEL BARRAL-NETTO[1]; MANOEL SOTO[2]; CLÁUDIA BRODSKYN[1].

1:FIOCruz - Centro de Pesquisas Gonçalo Moniz – LIMI 2:Universidade Autónoma de Madrid,Centro de Biología Molecular Severo Ochoa, Departamento de Biología Molecular

Introduction: Histones proteins are highly conserved antigens produced by *Leishmania*, which are capable to induce a strong immune response. In previous studies, genetic immunization with four histones, protected BALB/c mice after challenge with *L. braziliensis*, controlling inflammation at the site of infection and parasite spreading. We evaluated the immunoprotectiveability of hamsters immunization using DNA or/and recombinant histones proteins employing homologous and heterologous strategy against infection by *L. chagasi*.

Methodology and Results: Two to 4 months male hamsters (15 per group) were immunized with pcDNA3, pcDNA3LiH2A-H3, pcDNA3LiH2B-H4+ and /or HIS(pQE-LiH2A; pQE-LiH2B, pQE-LiH3 e pQE-LiH4) plus CpG, three times, at fifteen days of interval. After last immunization, sera and draining lymph node were collected for serologic tests and quantification of cytokine, respectively. Animals were challenged by intradermal route with 10^5*L. chagasi* promastigotes plus 1/2 pair of *Lutzomya longipalpis* salivary gland. Two, 5 and 7 months after challenge, draining lymph node, spleen and liver were obtained to evaluate parasite load during infection by limiting dilution. Only animals that received heterologous strategy of vaccination showed significantly IgG antibodies anti-His ($p<0,0079$) analyzed by ELISA, when compared to non-immunized hamsters.In cytokine analysis by Real Time PCR, we observed that hamsters immunized with homologous strategy presented significant higher expression of IL-10 ($p<0,0147$) and IFN-γ ($p<0,022$) when compared to heterologous strategy. No difference was observed in the parasite load in the spleen, liver and lymph node between immunized hamsters and controls at all points of evaluation.

Conclusions: There was no protection in immunized animals suggesting that histones are not immunogenic in hamsters and possibly not able to induce a strong Th1 response that would protect in the infection by *L. chagasi* in hamsters.

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NEUTROPHILS AND MACROPHAGES ARE INVOLVED IN THE PATHOGENESIS OF MALARIA ASSOCIATED ACUTE LUNG INJURY/ACUTE RESPIRATORY DISTRESS SYNDROME IN A MURINE MODEL

LUANA DOS SANTOS ORTOLAN[1*]; MICHELLE KLEIN SERCUNDES[2*]; DANIELA DEBONE[2]; ELIZABETH HELEN AITKEN[3]; MOMTCHILO RUSSO[3]; JOSÉ M. ALVAREZ [3]; CLAUDIO ROMERO FARIAS MARINHO[4] AND SABRINA EPIPHANIO[1,2,3]

[1] Departamento de Ciências Biológicas, Universidade Federal de São Paulo, Diadema, Brazil

[2] Instituto de Medicina Tropical de São Paulo, Universidade de São Paulo, São Paulo, Brazil

[3] Departamento de Imunologia, Universidade de São Paulo, São Paulo, Brazil

[4] Departamento de Parasitologia, Universidade de São Paulo, São Paulo, Brazil

[*] These authors contributed equally to this work.

INTRODUCTION: Malaria is a huge burden on global health. In 2010, this parasitic disease was the underlying cause of death for 1,24 million individuals. Severe malaria can manifest in the lungs, an illness known as acute lung injury/acute respiratory distress syndrome (ALI/ARDS). We previously established a murine model that mimics various human ALI/ARDS aspects, such as pulmonary edema, hemorrhages, pleural effusion and hypoxemia. Using this model we have evaluated the cellular immune profile as well as other aspects of the immune response to investigate the possible role of the inflammatory response in the pathogenesis of malaria associated ALI/ARDS. **METHODS AND RESULTS:** DBA/2 mice were infected with *Plasmodium berghei* ANKA. Lungs and bronchoalveolar lavage were collected and analyzed at various time-points during infection. By using the parameters respiratory pattern (enhanced pause and respiratory frequency) and parasitemia from a control group (our gold standard survival group), we established ROC curves and were able to classify experimental animals euthanized at the different time-points as suffering ALI/ARDS or HP (hyperparasitemia). Levels of *18s*

Plasmodium berghei ANKA mRNA in the lungs 7 days post infection were higher in the ALI/ARDS group compared to the HP group (qRTPCR). We observed an increase in neutrophils, measured by *Ncf2* mRNA expression (qRTPCR) and also by Gr-1^+ $CD11b^-$ expression (flow cytometry) in the lungs of the ALI/ARDS group compared to the HP group. An increase of neutrophils was also observed in the bronchoalveolar lavage (light microscopy). We observed larger numbers of alveolar macrophages ($F4/80^+/CD11_C^+$) and fewer interstitial macrophages ($F480^+$) in the ALI/ARDS group compared to the HP group (flow cytometry). We also observed inflammatory monocytes ($F480^+$ Gr-1^+ $CD11b^+$). Besides, we also found increases in mRNA expression of *iCAM-1* and also of *HO-1, IL-10* (anti-inflammatory) and *IFNγ* (pro-inflammatory) genes, however no differences in mRNA expression of *IL-6, TNF-α, IL-12, IL1-β, TGFβ* and *iNOS, VCAM* or *KC*, in lungs of the ALI/ARDS group compared to the HP group (qRTPCR). **CONCLUSION**: Our data suggests that accumulation of *P. berghei* in the lungs results in an increased inflammatory response and that neutrophils and alveolar macrophages play a role in ALI/ARDS development. We hypothesize that the neutrophil recruitment is partly dependent on increased ICAM-1 expression and that the high *IL-10* mRNA expression is an attempt to re-establish homeostasis.

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EXPLORING THE INFLAMMATORY ROLE OF THE MAST CELL/KALLIKREIN-KININ CELL PATHWAY IN THE MODULATION OF T CELL RESPONSES AGAINST *TRYPANOSOMA CRUZI*

BORSOI-COUTO, T.[1]; MARIANNO-COSTA, L.J.[1]; PESQUERO, J.B.[2], SCHARFSTEIN, J.[1]; OLIVEIRA, A.C.[1]

[1] Laboratório de Imunologia Molecular, IBCCF[o], UFRJ, RJ. BRASIL.
[2] Departamento de Biofísica, UNIFESP, SP, BRASIL.

Introduction: We have previously demonstrated that Dm28c trypomastigotes (TCTs) activate the kinin system through activation pathways involving trans-cellular cross-talk between TLR2 and Bradykinin B2 receptors (BK2R) (Monteiro *et al*., 2006). Initiated by tGPI-mucin, the TLR2/CXCR2-driven inflammation promotes the diffusion of plasma borne-kininogens to peripheral sites of infection, allowing for downstream generation of kinins by cruzipain. Once released in extravascular sites of infection, kinins (most likely aided by other immunostimulory peptides) drive dendritic cell maturation via BK2R, converting these APCs into Th1 inducers. Previous data show that $B2R^{-/-}$ mice succumb to acute (systemic) *T. cruzi* infection. Although the susceptible phenotype was linked to primary dysfunction of DCs, $BK2R^{-/-}$ mice gradually lost the ability to generate type 1 CD4 and CD8 T cell effectors (Monteiro *et al*., 2007). **Methods and Results:** Using the subcutaneous route of *T. cruzi* inoculation, here we examined in further detail the relationship between inflammatory edema and T cell effector function. Experiments in $BK2R^{-/-}$ mice showed that CCR5 was not as strongly upregulated in CD4 T cells as in their WT counterparts. Consistent with these findings, frequencies of intracardiac CD4 Th1 cells in infected WT mice were significantly higher as compared to $BK2R^{-/-}$. Unlike the CD8 deficient response observed in the intraperitoneal model of infection, we found similar frequencies of intracardiac CD8 T cells and IFNg producing CD8+ cells in $BK2R^{-/-}$ and WT mice. Moreover, we did not detect differences in CTL activity (TSKB20-splenic targets). Intriguingly, the expression of the pro-apoptotic receptor CD95 (Fas) was not as strongly upregulated in CD8 T cells from $BK2R^{-/-}$ mice as in WT. **Conclusions:** Our results suggest that BK2 receptor signaling is important for the development of a Th1 response but not for CD8 T cell response in our model of *T. cruzi* infection. Experiments with B1K-deficient mice are currently being performed. Preliminar studies from our group suggest that pharmacological intervention on the mast cell compartment (see accompanying abstract by Nascimento *et al.*) may have significant impact on T cell effector function, which will also be evaluated.

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CERVICAL LEVELS OF INTERLEUKIN-1 BETA AND INTERLEUKIN-6 IN RESPONSE TO HUMAN PAPILLOMAVIRUS IN THE PRESENCE OF BACTERIAL VAGINOSIS

LARISSA DODDI MARCOLINO (MSc)[1]; CAMILA MARCONI (PhD)[1]; CAROLINA SANITÁ TAFNER FERREIRA (BSc)[1]; GABRIEL VITOR SILVA PINTO (BSc)[1]; CRISTINA MARIA GARCIA LIMA PARADA (PhD)[2]; MARCIA GUIMARÃES SILVA (PhD)[1]

[1]Department of Pathology; Faculdade de Medicina de Botucatu, Univ. Estadual Paulista - UNESP – Botucatu-SP, Brazil;
[2]Department of Nursing; Faculdade de Medicina de Botucatu, Univ. Estadual Paulista - UNESP – Botucatu-SP, Brazil;

Introduction: Infection by the Human Papillomavirus (HPV) is one of the most commom sexually transmitted infections (STIs). HPV infection is frequently associated with bacterial vaginosis (BV), an abnormal type of vaginal flora characterized by the replacement of lactobacilli by the overgrowth of anaerobic bacteria. Both, HPV and BV are associated with changes in the levels of cytokines produced in the lower genital tract. Therefore, the aim of this study was to compare the cervical levels of Interleukin (IL)-1b and IL-6 in HPV-positive women according to the status of BV. **Methods and Results:** In this cross-sectional study, a total of 161 women attending the gynecology service at one unit of primary medical care in Botucatu-SP were enrolled. Only non-pregnant women with ages between 18 and 50 years were invited to participate. Women positive for endocervical infection by *Chlamydia trachomatis* and *Neisseria gonorrhoeae*, in addition to those with candidosis and trichomoniasis, were excluded. During speculum examination cervical samples were obtained for the detection of HPV using polymerase chain reacion (PCR) and mesurement of IL-1b and IL-6 levels by immunoenzymatic assay (ELISA). Smears from the mid-third lateral vaginal wall were collected and submitted to Gram stain for the microscopic diagnosis of BV, according to Nugent scoring system. The comparison between the cervical levels of cytokines in response to HPV infection in the presence and absence of BV was performed by the non-parametric Mann-Whitney test, and the level of significance adopted was of 5%. A total of 116 cervical samples tested positive for HPV and were assessed for the levels of IL-1b and IL-6. From this total, 61 women presented normal pattern of vaginal flora on microscopic analysis, while 55 tested positive for BV. The measurement of the cervical cytokine levels showed increased IL-1b (median 267.9 pg/mL (range:0.0-998.9) versus 79.5 pg/mL (range: 0.0-848.4); p=0.01) and decreased IL-6 (median 48.4pg/mL (range: 0.0-440.9) versus 118.2 pg/mL (range: 0.0-917.7); p=0.01) in BV when compared with normal vaginal flora. **Conclusion:** Bacterial vaginosis modulates the cervical levels of IL-1b and IL-6 in the presence of HPV infection.

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Title: IDENTIFICATION BY PHAGE DISPLAY OF THE MYCOBACTERIUM TUBERCULOSIS ANTIGEN

SEBASTIÃO MARCOS TAFURI[1,2]; FABIANA DE ALMEIDA ARAÚJO SANTOS[2]; MAYARA INGRID SOUSA LIMA[2]; LÉA DUARTE DA SILVA MORAIS[2]; LUIZ FERNANDO ALMEIDA MACHADO[2]; ISABELA MARIA BERNARDES GOULART [3]; MARCELO SIMÃO FERREIRA[4]; AÉRCIO SEBASTIÃO BORGES[4]; ANDRÉ LUIS DE MORAES CARVALHO[5]; LUIZ RICARDO GOULART[6]; CARLOS UEIRA-VIEIRA[6].

[1]Mestrando do Programa de Ciências da Saúde da Faculdade de Medicina/UFU;
[2]Laboratório de Nanobiotecnologia da Universidade Federal de Uberlândia;
[3]Centro de Referência Nacional em Hanseníase e Dermatologia Sanitária – Hospital de Clínicas/UFU;
[4]Setor de Moléstias Infecciosas do Hospital de Clínicas/UFU;
[5]Programa Nacional de Controle da Tuberculose de Uberlândia-MG;
[6]Instituto de Genética e Bioquímica/UFU.

Introduction: Tuberculosis is an infectious disease very important to public health worldwide, becoming the second most lethal infectious process, approximately 1,7 million deaths per year, second only HIV / AIDS. The big challenge for tuberculosis control is the identification of individuals with the bacillus, but that are showing the disease from those who have TB infection, because the diagnostic methods to identify the pathogen still have a low sensitivity, between 65% and 85%, and 10% of those individuals can develop active process at some stage of life.
Methods and results: The methodology of phage display of biomolecules, developed by Smith (1985), was used in this work. The coupling for purification of IgG from a pool of sera from TB+ (N = 10) and contacts with PPD+ (N = 10), PPD- (N = 10) as positive and negative controls were performed by using magnetic beads conjugated to protein G. In the selection of ligands IgG TB+ individuals, PPD+,PPD-, was used a library of random amino acids in the protein expressed from the bacteriophage pIII. The biopanning was composed by three cycles of selection. The DNA and amino acid sequences of peptides were analyzed and aligned using programs of bioinformatics. Individual phage clones were amplified, purified and validated by ELISA in duplicates. For the alignment of the peptides to each other and with specific proteins of MTB were used a CLUSTALW2 and PEPSURF programs, respectively. The reactivity of 26 selected phage was measured by ELISA, and 11 (24.44%) showed higher reactivity with sera from individuals with active tuberculosis and PPD+, compared with PPD- individuals. The in silico analyzes showed that the sequence of the peptides has a similarity with the MPT64 protein immunodominant *Mycobacterium tuberculosis* complex, and the alignment occurred in a possible antigenic region.

Conclusion: This same clone showed greater sensitivity to recognize antibodies from patients with active tuberculosis and individuals PPD+, and promising for immunological diagnosis of tuberculosis. The phage display methodology was presented as an important tool in the development of a diagnostic platform, allowing the characterization of antigenic mimotopes to differentiate active tuberculosis infection.

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PROFILE OF PRO- AND ANTI-INFLAMMATORY CYTOKINES IN PATIENTS WITH MALARIA ASSISTED AT THE INSTITUTO DE MEDICINA TROPICAL OF COARI-AM

ALLYSON GUIMARÃES DA COSTA (PG)(1,2); JOÃO PAULO DINIZ PIMENTEL (PG)(2,3); NADJA PINTO GARCIA (PG)(2); ANDRÉA MONTEIRO TARRAGÔ (PG)(1,2); LUCYANE BASTOS TAVARES DA SILVA (IC)(2); JÉSSICA PEREIRA MARQUES (IC)(2); WALTER LUIZ LIMA NEVES (PG)(1,2); ADRIANA MALHEIRO (PG)(1,2).

(1). Universidade Federal do Amazonas; (2). Fundação de Hematologia e Hemoterapia do Amazonas; (3). Instituto Leônidas e Maria Deane/Fiocruz Amazônia.

Introduction: Malaria is a major parasitic disease of tropical regions. Three species are associated with this infection in Brazil *P. vivax, P. falciparum* and *P. malariae*. The immune response has many defense mechanisms which result in the inflammation process and the individual resistance to the pathogen. In general, the clinical manifestations are dependent on the concentrations of pro and anti-inflammatory cytokines, which contribute to the immunopathology of the disease. The aim of this study was to quantify the inflammatory and anti-inflammatory cytokines in individuals with malaria treated at the Instituto de Medicina Tropical of Coari-AM. **Methods and Results**: Serum levels of cytokines (IL-2, IL-4, IL-6, IL-10, TNF-α, IFN-γ and IL-17) were measured in the plasma of 50 individuals (25 malaria patients infected by *Plasmodium vivax* and 25 uninfected - control group) using the technique of CBA (Citometry Bead Array) using the kit T_H1, T_H2 and T_H17 (BD Biosciences). The analyzes of serum concentrations were performed with the software *FCAP-Array* (v3.0) and the results were analyzed using the statistical software *GraphPad Prism*® (v5.0), using the nonparametric Mann-Whitney test. The concentrations of IL-2, IL-4 and TNF-α were lower among patients with malaria compared with control subjects ($p=0.1252$, $p=0.4707$ and $p=0.0456$, respectively). While serum levels of IL-6, IL-10, IL-17 and IFN-γ were significantly higher among the infected group compared to uninfected individuals ($p<0.0001$, $p<0.0001$, $p=0.0329$ and $p=0.0403$, respectively). **Conclusion:** Thus, there is an exacerbation in the immune response in malaria caused by infection with *P. vivax*, with simultaneous production of proinflammatory, anti-inflammatory and T_H17 profile cytokines.

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Title: PRO-INFLAMMATORY CYTOKINES PRODUCTION IN PATIENTS INFECTED WITH DIFFERENT CSP MOLECULAR VARIANTS OF *Plasmodium vivax*

Authors: BRUNO DE PAULO RIBEIRO[1]; ANA PAULA SILVA DE AZEVEDO DOS SANTOS[1]; DALILA NUNES CYSNE[1]; GUILHERME TUDE COELHO NETO[1]; ERICKA MIRANDA MESQUITA[2]; GUSTAVO CAPATTI CASSIANO[3]; ELOÍSA DA GRAÇA DO ROSÁRIO GONÇALVES[4]; MARCOS AUGUSTO GRIGOLIN GRISOTTO[2]; RICARDO LUIZ DANTAS MACHADO[3]; FLÁVIA RAQUEL FERNANDES DO NASCIMENTO[1]

[1]Laboratório de Imunofisiologia - Universidade Federal do Maranhão (UFMA) – São Luís, Brazil; [2]Laboratório de Imunologia das Parasitoses - Universidade CEUMA (UniCEUMA) – São Luís, Brazil; [3] Centro de Investigação de Microrganismos - Faculdade de Medicina de São José do Rio Preto (FAMERP) – São José do Rio Preto, Brazil.[4]Centro de Referência em Doenças Infecto-parasitárias - Universidade Federal do Maranhão (UFMA) – São Luís, Brazil.

Introduction: *P.vivax* causes the majority of human malaria cases worldwide, despite this it remains largely neglected. Vivax malaria was thought to be a benign and self-limited disease for a long time; currently, complications associated to this disease in the Amazon region and other places throughout the world have motivated research of the related immunopathological events. One of the many possible causes for complications may be the genetic characteristics of the species.Taking in consideration that pathological changes in malaria, which determine either alleviation or exacerbation of the disease, also depend of a delicate balance in cytokines milieu, we assessed the production of pro-inflammatory cytokines according to the molecular variants of *P. vivax* Circunsporozoit Protein (CSP) -VK210 and VK247.
Methods and Results: Blood samples from untreated vivax malaria patients (n=28) were used to provide the plasma and red blood cells containing parasites. Pro-inflammatory cytokines levels (IFN-g, IL-6 and TNF-a) in the plasma were assessed through Cytometric Bead Array (CBA) and the vivax CSP molecular variants were characterized by RFLP-PCR. The values were described in means ± SEM. There was a tendence of higher IL-6 and IFN-g in VK210 patients (IL-6: VK210 217±152 and VK247 34.9±22; IFN-g: VK210 8.5±6.7 and VK247 0.31±0.31), but there were no statistical differences. TNF-a and *P.vivax*-like variant was not detectable in patients.
Conclusion: CSP vivax variants VK210 and VK247do not influence the expression of pro-inflammatory cytokines in humans.

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IMMUNOMODULATORY MECHANISMS OF SOLUBLE ANTIGEN OF TACHYZOITES (STAg) IN THE INTESTINAL INFLAMMATORY LESIONS INDUCED BY ORAL INFECTION WITH *Toxoplasma gondii*

LUCIANA ALVES DE SOUSA[1]; ALEXSANDRA ALVES BEZERRA MARTINS[1]; ESTER CRISTINA BORGES ARAÚJO[1]; PAULO VICTOR CZARNEWSKI BARENCO[1]; DEISE APARECIDA OLIVEIRA SILVA[2]; JOSÉ ROBERTO MINEO[2]; NEIDE MARIA SILVA[1]

[1]Laboratory of Immunopathology, Institute of Biomedical Sciences, Federal University of Uberlândia
[2]Laboratory of Immunoparasitology, Institute of Biomedical Sciences, Federal University of Uberlândia

Introduction: C57BL/6 mice orally infected with 100 cysts of *Toxoplasma gondii* ME-49 strain are highly susceptible to acute infection, with massive necrosis of the intestinal villi and mucosal cells, as well as intense influx of inflammatory cells in the small intestine. Intraperitoneal treatment with STAg 48 hours prior oral infection with high parasitic load of *T.gondii* is able to reduce the intestinal inflammatory response caused by parasites in the susceptible mice, C57BL/6.
Methods and Results: In order to verify the role of STAg in oral infection with *T.gondii*, resistant, BALB/c, and susceptible, C57BL/6 mice were treated with phosphate buffered saline (PBS) or STAg 48 hours before oral infection with 30 cysts of *T.gondii* ME-49 strain and sacrificed on day 8 after infection. Pretreatment with STAg attenuated the intestinal inflammatory immune response induced by the parasite and increased the Goblet, Paneth and IgA positive cell numbers in the small intestine of susceptible and resistant animals.
Conclusion: The results suggest that the prior injection of STAg is able to promote protective mechanisms in the small intestine of orally *T. gondii* infected animals, which are mediated by goblet and Paneth cells and local secretion of IgA.

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SITE OF *L. MAJOR* INFECTION DETERMINES DOMINANT HOST CELL PHENOTYPE

ERIC HENRIQUE ROMA[1,2], FLÁVIA RIBEIRO-GOMES[1], DAVID SACKS[1], NATHAN PETERS[1].

[1] National Institutes of Allergy and Infectious Diseases, NIH – Bethesda, USA
[2] Institute of Biological Sciences, Universidade Federal de Minas Gerais - Belo Horizonte, Brazil

Introduction: The impact of the inoculation site on the outcome of infection with the intracellular parasite *Leishmania* is poorly understood. We investigated the acute interactions between *L. major* and phagocytic cells at different sites of infection commonly used in studies of Leishmaniasis.
Methods and results: C57BL/6 mice were infected with *L. major* expressing red fluorescent protein (RFP) by the subcutaneous (footpad) and intradermal (ear) routes. At 2 and 48 hours post-infection, ears and footpads showed similar numbers of $CD11b^+$ cells, where as ears had higher numbers on day 9. At all time points tested, the number of infected cells was higher in the ear versus the footpad. Phenotyping of RFP^+ cells revealed that the dominant infected population in the ear were neutrophils at 2h, inflammatory monocytes at 48h, and inflammatory and $Ly6C^{low}$ monocytes at 9 days post infection. In the footpad, infected cells were predominantly macrophages/dendritic cells (MΦ/DC) at 2h, $Ly6C^{low}$ monocytes at 48h, and MΦ/DC at 9 days. Infection in the ear was associated with higher levels of IL-10 and CXCL1 mRNA versus the footpad. **Conclusion:** The acute interaction between *L. major* and phagocytic cells is different at different sites of infection. These observations may help to explain divergent observations in studies of Leishmaniasis that employ different inoculation sites.

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EVALUATION OF THE TH1, TH2 AND TH17 CYTOKINES LEVEL IN HCV$^+$ PATIENTS ATTENDED IN THE FUNDAÇÃO DE MEDICINA TROPICAL OF AMAZONAS - BRAZIL

ADRIANA MALHEIRO(PG)[(1,4)]; ANDRÉA MONTEIRO TARRAGÔ(PG)[(1,4)]; RAPHAEL ALLE MARIE[(2)]; ALLYSON GUIMARÃES DA COSTA(PG)[(1,4)]; JOÃO PAULO DINIZ PIMENTEL(PG)[(3,4)]; ANA RUTH ARAÚJO(PG) [(1,2)]; MARILÚ VICTORIA(PG) [(2)]; FLAMIR VICTORIA(PG) [(2)].

(1). Universidade Federal do Amazonas; (2).Fundação de Medicina Tropical do Amazonas; (3).Fundação Oswaldo Cruz; (4). Fundação de Hematologia e Hemoterapia do Amazonas Universidade Federal do Amazonas.

Introduction: Hepatitis C infection is a major cause of acute and chronic hepatitis. It is estimated that 170 million people worldwide are infected by virus C. Most of infected individuals are not able to clear the virus and may progresses to cirrhosis or hepatocelular carcinoma. The immune responses play an important role in both HCV clearance and persistence of the infection. This study aimed to evaluate the cytokines level of HCV$^+$ patients untreated with ribavirin and interferon alpha and assisted at Fundação de Medicina Tropical of Amazonas. **Methods and Results**: The concentration of IL-2, IL-4, IL-6, IL-10, TNF-α, IFN-γ and IL17A were performed by flow cytometry using the kit TH1, TH2 and TH17 (BD cytometric Bead Array (CBA) Human Cytokine Th1/Th2/Th17 Kit) following the manufacturer's technical guidelines. The results were analyzed using the statistical software GraphPad Prism® and the test applied was Mann Whitney test. The sample consists of 74 HCV $^+$ patients and 47 controls. When we compared the HCV$^+$ patients with the control group the results were statistically significant for IL-2 (p< 0, 0001), IL-6 (p<0,0001), IL-10 (p<0,0001), IFN (p< 0,0001), TNF (p=0,0061) e IL17 (p<0,0001). On the other hand, the IL-4 level (p= 0, 1511) was not statistically significant. **Conclusion:** We conclude that HCV infection induces the Th1 and Th17 profiles simultaneously overlapping the Th2. However, further analysis should be conducted to better understand the interaction of the virus studied and know the immunological profile of HCV$^+$ patients.

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Key word: Cytokines, Hepatitis C, Th1, Th2, Th17.

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ROLE OF PD-1 AND PD-L1 IN THE REGULATION OF T CELL RESPONSES TO BLOOD-STAGE *Plasmodium chabaudi* MALARIA

LETÍCIA SARTURI PEREIRA SEVERI[1]; GENOÍLSON DE BRITO ALVES[1]; ÉRIKA MACHADO DE SALLES[1]; SHEYLA CASTILLO-MÉNDEZ[1]; JOSÉ MARIA ÁLVAREZ[1]; MARIA REGINA D'IMPÉRIO LIMA[1]

[1]Instituto de Ciências Biomédicas IV – Universidade de São Paulo (USP)

Introduction: Malaria, a disease caused by *Plasmodium* species, is a major world health problem with over a million deaths every year. Severe anemia, cerebral malaria and respiratory distress account for severe cases and deaths. Exacerbated immune responses have been implicated in etiology of these syndromes. T cell responses have a pivotal role in both pathogenesis and protection. Then, regulation of these responses is necessary to preserve host homeostasis. As PD-1 and its ligand PD-L1 are regulatory molecules that inhibit T cell receptor signaling, we investigated the role of this molecular pathway during acute malaria.

Methods and Results: Female adult C57BL/6 mice (n≥3) were inoculated intraperitoneally with $1x10^6$ infected red blood cells (iRBC). Expression level (MFI=Median Fluorescence Intensity) of PD-1 and PD-L1 in splenic $CD4^+$ and $CD8^+$ T cells were analyzed on days 4, 6, 7, 8 or 10 post-infection (p.i.). Expression of PD-1 in $CD4^+$ T cells augments on day 7 p.i. (MFI=5.23±0.09) and 8 p.i. (MFI=6.75±0.04), compared to controls (MFI=3.38±0.53). In $CD4^+$ and $CD8^+$ T cells, the expression of PD-L1 increases from day 0 p.i. (MFI=16.94±2.73; 19.05±1.77) to day 4 p.i. (MFI=35.35±17.59; 41.04±20.77), reaching peak values on day 7 p.i. (MFI=53.90±1.11; 78.61±3.38). Also, there is a decrease in PD-L1 expression on days 8 p.i. (MFI=40.58±3.10; 59.59±1.27) and 10 p.i. (MFI=32.14±2.34; 43.31±5.87). Interference of PD-L1 blockade on IFN-g secretion was evaluated, as this cytokine is produced in large quantities during acute malaria and contributes to parasite control. Spleen cells from day 6 p.i. were stimulated with iRBCs for 72 h in presence or absence of anti-PD-L1 monoclonal antibodies. Inhibition of PD-L1 results in significantly higher IFN-g concentration in supernatants (14.18±1.24 ng/mL), in comparison with untreated cultures (4.11±2.05 ng/mL). When spleen cells from day 7 p.i. were cultured for 24 h, we also observed an increase of IFN-g concentration (5.06±0.35 ng/mL), in relation to untreated cultures (0.74±0.09 ng/mL).

Conclusion: PD-1 and PD-L1 are involved in T cell regulation during acute *P. chabaudi* malaria. Kinetics of PD-1 and PD-L1 expression indicate the participation of these molecules during the early T cell response to infection. Furthermore, our data suggest that this molecular pathway inhibits the IFN-g

production, as the blockade of PD-L1 augments IFN-g concentration in culture supernatants.

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RHIZOPUS SP INFECTION TRIGGERS H_2O_2 PRODUCTION BY PERITONEAL PHAGOCYTIC CELLS ONLY IN HYPOINSULINEMIC-HYPERGLICEMIC MICE

DÉBORA DE FÁTIMA ALMEIDA (IC)(1); GABRIELA PICCARO ERAZO(IC)(1); AMANDA RIBEIRO DOS SANTOS(IC)(1); CAMILA MARTINS MARCHETTI(PG)(2); JAMES VENTURINI (PG)(2); MARIA SUELI PARREIRA DE ARRUDA(1).

(1) Faculdade de Ciências, UNESP - Univ Estadual Paulista, Bauru, Departamento de Ciências Biológicas, Laboratório de Imunopatolgia Experimental (LIPE);(2) Faculdade de Medicina, UNESP - Univ Estadual Paulista, Botucatu, Programa de Pós-graduação em Doenças Tropicais.

Introduction: Mucormycosis is the third most common invasive fungal infection among immunocompromised patients and *Rhizopus* sp are the most prevalent etiological agents. It is a progressive disease which is almost always fatal. Furthermore, the Diabetes Mellitus is the main underlying disease associated with this fungal infection, especially during diabetic ketoacidosis episodes. Although macrophages are one of the first cells to recognize and trigger the immune response against the pathogens, there are few studies evaluating the macrophage activity in a murine experimental model of diabetes during mucormycosis. In the present study we evaluate the *in vitro* production of hydrogen peroxide (H_2O_2) and nitric oxide (NO) by peritoneal phagocytic cells from hypoinsulinemic-hyperglicemic (HH) mice infected with *Rhizopus* sp.
Methods and Results: Female Swiss mice were divided into the following groups: Group HH: composed by HH-induced mice by alloxan (60 mg/kg); Group Rhi: composed by *Rhizopus*-infected mice which were intravenously inoculated with 3x104 spores of *Rhizopus* sp; Group Rhi-HH: HH-induced and *Rhizopus*-infected mice; Group CTL, composed by naïve mice inoculated with sterile saline solution. After 24 hours, the mice were killed and sample of spleens, brains, livers, lungs and kidneys were collected and submitted to microbiological evalation fungal load determination. Also we performed an *in vitro* assay to determine the H_2O_2 and NO by peritoneal phagocytic cells. The results showed no differences in the fungal load in the infected groups as well as in the NO production by peritoneal phagocytic cells culture. Moreover, we only observed high production of H_2O_2 in the Rhi-HH mice.
Conclusion: The *Rhizopus* sp infection triggered H_2O_2 production by peritoneal phagocytic cells only when both infectious and HH condition were associated.

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CHARACTERIZATION OF POLYMORPHISM TNF-α -308 G/A IN PATIENTS WITH CHRONIC HEPATITIS C.

SORIANE DE SOUZA CRUZ[1,2] ANDREA MONTEIRO TARRAGÔ[1,2], FLAMIR VICTÓRIA[3], MARILU VICTÓRIA[3], AYA SADAHIRO[1,3], ADRIANA MALHEIRO[1,2]

(1) Universidade Federal do Amazonas (2) Fundação de Hematologia e Hemoterapia do Amazonas (3) Fundação de Medicina Tropical do Amazonas (4) Instituto Nacional de Pesquisas da Amazônia

Introduction: The elimination or persistent infection by Hepatitis C virus (HCV) is associated with viral and host factors, as well as polymorphisms cytokine gene. The TNF-α cytokine is present in the beginning of the immune response against the virus C. The TNF-α gene is located on human chromosome 6p21.3, where several polymorphisms were identified and considered to affect the production of cytokine. The aim of this study was to characterize the polymorphism -308 G/A *TNFα* in patients HCV^+. **Methods and results:** The samples were collected from 22 patients HCV^+ untreated with interferon and ribavirina at the Fundação de Medicina Tropical of Amazonas. The DNA was extracted according to the Brazol protocol followed by PCR amplification of the DNA fragment, subsequently visualized on agarose gel stained with 2.5% DNA Knowing Safe. The data distribution of the -308 G/A *TNFα* demonstrated that 22 patients infected with HCV, 16 (72.2%) had GG genotype, of which 02 (12.5%) were diagnosed with liver cirrhosis, while 06 (27.3 %) were GA genotype and they had no diagnosis for liver cirrhosis. **Conclusions:** The data suggest an association between liver cirrhosis patients and 308 G/G *TNF-α* genotype, because it was more prevalent in this population.

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ROLE OF CD163 IN THE *Mycobacterium leprae* SURVIVAL AND PERSISTENCE IN LEPROMATOUS MACROPHAGES

Roberta Olmo Pinheiro[1], Danielle Fonseca de Moura[1], Katherine Antunes de Mattos[2], Thaís Porto Amadeu[1], Priscila Ribeiro Andrade[1], Jorgenilce Souza Sales[1], Verônica Schmitz[1], José Augusto da Costa Nery[1], Euzenir Nunes Sarno[1].

(1) Oswaldo Cruz Institute, Leprosy Laboratory, Rio de Janeiro, Brazil.

(2) Oswaldo Cruz Institute, Cellular Microbiology Laboratory, Rio de Janeiro, Brazil.

Introduction: Lepromatous (LL) macrophages possess a regulatory phenotype that contributes to the immunosuppression observed in leprosy. CD163, a scavenger receptor that recognizes hemoglobin-haptoglobin complexes, is expressed at higher levels in lepromatous cells although its functional role in leprosy is not yet well-established.

Methods and Results: Lepromatous lesions are microenvironments rich in CD163 - IDO positive cells. Isolated cells from these lesions are $CD68^{+}IDO^{+}CD163^{+}$, while higher levels of sCD163 (6017.0 ± 593.9 in LL *vs.* 1435.0 ± 129.6 in HC; 6017.0 ± 593.9 in LL *vs.* 2150.0 ± 112.1 in BT, $p<0.001$) in lepromatous sera correlate positively with IL-10 levels (36.08 ± 11.80 in LL *versus* 3.88 ± 1.27 in HC, $p<0.01$; 36.08 ± 11.80 in LL *versus* 9.48 ± 4.93 in BT, $p<0.01$) and IDO activity. Different *M. leprae* concentrations in healthy monocytes likewise revealed a positive correlation between increased concentrations of the mycobacteria and IDO, CD209, and CD163 expressions. The regulatory phenotype in *M. leprae*–stimulated monocytes is accompanied by increased TNF (46.91 ± 10.44 in non-stimulated *versus* 206.8 ± 21.78 in *M. leprae*-stimulated, $p<0.01$), IL-10 (154.4 ± 71.34 in non-stimulated *versus* 571.5 ± 199.5 in *M. leprae*-stimulated, $p<0.05$), and TGF-β (71.3 ± 12.9 in non-stimulated *versus* 1093 ± 386.5 in *M. leprae*-stimulated, $p<0.01$) levels whereas the IL-10 blockade reduces *M. leprae* - induced CD163 expression (7.60 ± 1.93 in *M. leprae versus* 1.53 ± 0.60 in *M. leprae* + neutralizing IL-10, $p<0.05$). CD163 blockade also reduces *M. leprae* uptake in human monocytes. *M. leprae* uptake was higher in HEK293 cells transfected with the cDNA for CD163 than in non transfected cells (9807 ± 235 in non transfected cells vs. 22811 ± 1724, $p < 0.001$). At the same time, increased CD163 expression in lepromatous cells

seems to be dependent on *M. leprae* uptake besides contributing to augmented iron storage levels in lepromatous macrophages.

Conclusion: CD163 expression induced by *M. leprae* modulates host cell phenotype to create a favorable environment for mycobacterial entry and survival.

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HYPOINSULINEMIA-HYPERGLICEMIA CONDITION INTERFERES IN THE OUTCOME OF INFLAMMATORY RESPONSE DURING FUNGAL SKIN INFECTION IN A MURINE MODEL OF DIABETES MELLITU

BEATRIZ CESCHIM(IC)(1); LUIZA AYUMI NISHIYAMA MIMURA(IC)(1); JAMES VENTURINI(PG)(1,2);MARIA SUELI PARREIRA DE ARRUDA(1).

(1)Faculdade de Ciências, UNESP - Univ Estadual Paulista, Bauru, Departamento de Ciências Biológicas, Laboratório de Imunopatolgia Experimental (LIPE);(2) Faculdade de Medicina, UNESP - Univ Estadual Paulista, Botucatu, Programa de Pós-graduação em Doenças Tropicais.

Introdution: The high hyperglycemia observed in diabetic patients results in the advanced glycation endproducts (AGEs) which are able to binding in receptors present in the endothelial and immune cells that result in the induction of a variety of cytokines. As a consequence, several disorders have been described in the tissue matrix network such as the development of renal glomerulosclerosis and the difficulties in the wound healing process in skin tissue. Considering that: 1) hypoinsulinemia-hyperglicemia (HH) condition is associated with impaired immune response to fungal infections, 2) infection in the diabetic foot is one of the severe predisposing factors to amputation, 3) the mechanisms involved in skin tissue wound healing during infection in diabetic patients are not fully understood; in the present study, we evaluated the local inflammatory response and the distribution of collagen and reticulin fibers in the footpad from HH-mice during dermatophitic infection.

Matherial and Results: Male Swiss HH-mice, induced by alloxan (60mg/kg), were submitted to subcutaneous inoculation of *Trichophyton ntagrophytes* into the footpad. Non-infected HH-mice, free-HH infected-mice and non-infected and free-HH mice were used as control groups. The mice were evaluated on days 7 and 14 after the fungal inoculation. The footpad were collected and submitted to histological analyses by routine procedures for embedding in paraffinand. The sections were stained with hematoxylin-eosin (HE), periodic acid-Schiff staining (PAS), Masson trichrome and reticulin silver stain. Our results showed that the introduction of the fungus in the footpad in free-HH mice resulted in an acute inflammatory response followed by a granulomatous reaction; the deposition of the collagen fibers and the thickness of reticular fibers were more intense on day

7 with tendency to normalization on day 14. In the infected-HH mice the inflammatory response was more intense and spread, and no typical granulomatous formation was observed; the deposition of the collagen fibers and the thickness of reticular fibers were always more intense than free-HH infected mice.

Conclusion: Our results suggest that the HH condition interferes in the outcome of footpad inflammatory response during dermathophytic infection as well as on the distribution of collagen and reticulin fibers. Furthermore, we suggest that this experimental model is one interesting option to studies about wound healing process in HH condition.

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EXPLORING THE ROLE OF TOLL-LIKE RECEPTOR-2 IN THE HOST RESISTANCE TO TICKS

LAUREN CRISTINA DA SILVA RIBEIRO[1], ELEN ANATRIELLO[3], CARLO JOSÉ FREIRE DE OLIVEIRA[2], ISABEL KINNEY FERREIRA DE MIRANDA SANTOS[1], BEATRIZ ROSSETTI FERREIRA[3].

[1] School of Medicine of Ribeirão Preto, USP, Ribeirão Preto-SP, Brazil
[2] Federal University of Triângulo Mineiro, UFTM, Uberaba-MG, Brazil
[3] School of Nursing of Ribeirão Preto, USP, Ribeirão Preto-SP, Brazil

Introduction: Ticks are blood-feeding arthropods that secrete modulatory molecules through their saliva to antagonize host haemostatic and immune responses as they feed. Our group has shown that tick infestation induces a Th2 response in mice which keep on susceptible to repeated tick infestations. In addition, tick saliva inhibits the expression of co-stimulatory molecules (CD40, CD80 and CD86), decreases the production of IL-12 and IFN-gamma, and increases the production of IL10 by dendritic cells (DCs) after stimulation with LPS (TLR-4 ligand) and LTA (TLR-2 ligand). Interestingly, this effect was independent of change on TLR-4 expression, whereas a significant enhancement of TLR-2 on DC surface was observed. Recently, different groups showed that components from microbes/parasites, via TLR-2 present on DCs, can stimulate Th2 responses (J. Immunol**.** 172:4733-4743, 2004; J. Biol. Chem. 277:48122–48129, 2002). Here, we analyze the role of TLR-2 molecules on mice susceptibility to *Rhipicephalus sanguineus* tick infestation. **Methods and Results:** TLR2 -/- and WT C57Bl/6 mice (n=20 for each group) were submitted to a tick infestation with adult ticks (3 couples/mice) in plastic chambers glued to the animals back's and the biological parameters of the ticks were determined. The biological parameters used were number and weight of engorged females, tick oviposition competence, egg mass weight, reproductive index and hatching rate. Our data showed no statistical differences between experimental groups. **Conclusion:** The results suggest that TLR-2 molecules do not affect tick infestation on mice. Subsequently, we will perform experiments to elucidate the role of the overexpression of TLR-2 in DCs treated with tick saliva *in vitro*. This work can provide new perspectives for the control the *R. sanguineus* ticks.

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CHARACTERIZATION OF THE CONSERVATION AND ANTIGENICITY OF NEW PROTEINS FROM *L. CHAGASI* WITH THE POTENTIAL FOR USE IN THE DIAGNOSIS AND IMMUNITY AGAINST VISCERAL LEISHMANIASIS

NASCIMENTO, MARÍLIA BARBOSA.[1*]; PONTES-DE-CARVALHO, LAIN CARLOS[2]; OLIVEIRA, GERALDO GILENO DE SA.[2]; DHALIA, RAFAEL [1]; MAGALHÃES, FRANKLIN BARBALHO.[3] AND DE MELO NETO, OSVALDO POMPÍLIO[1].

[1]Centro de Pesquisas Aggeu Magalhães– FIOCRUZ, Recife, Brasil. [2]Centro de Pesquisas Gonçalo Muniz– FIOCRUZ, Salvador, Brasil.[3]Associação Caruaruense de Ensino Superior- Faculdade ASCES, Caruaru, Brasil. *E-mail: mariliabarbosa@cpqam.fiocruz.br.

Introduction: Strategies to combat the visceral form of leishmaniasis, the most serious form of this disease, have so far been unsuccessful. In this respect, the identification of novel antigens for diagnosis and vaccine development against visceral leishmaniasis becomes fundamental. Our group has been studying new antigens from *L. chagasi* identified through the screening of genomic and cDNA expression libraries using sera from infected humans and dogs. 13 different clones were isolated encoding proteins. On ELISA pilot tests using sera from infected individuals, and designed to study their use as diagnostic tools, these displayed sensitivity ranging from 34% to 93%. This work focused on some of these proteins, which have the property of being rich in repetitive motifs. The aim was to describe aspects of their localization and conservation in different species of trypanosomatids, as well as to better assess their antigenicity. **Methods and Results**:The selected proteins, called Lci5, Lci9, Lci11, Lci12 and Lci10, were analyzed by immunofluorescence and Western blot using rabbit polyclonal sera. Regarding their subcellular localization, they display different patterns with three localizing to specific regions within the cytoplasm (Lci10, Lci12) or flagellum (Lci5), while two are uniformly dispersed throughout the cytoplasm (Lci9, Lci11). Proteins Lci9, Lci10 and Lci11 are constitutively expressed in growth curves of *L. chagasi* promastigotes and may be restricted to *Leishmania* species. For Lci10, its gene sequence was then redesigned and commercially synthesized so that, after cloning , two different protein constructs (Lci10, complete, and Lci10-nr, non-repetitive region) could be expressed and purified by affinity chromatography. These polypeptides were evaluated by Indirect ELISA using sera from dogs and humans infected with *L. chagasi*. When evaluated with dogs' sera, the results showed that Lci10-nr recognizes 96% of the sera tested and this non-repetitive region is largely responsible for the protein antigenicity. With human samples Lci10-nr recognizes only 40% of the sera tested, with the repetitive motifs contained in the complete protein being responsible for increasing its antigenicity against humans' sera. **Conclusion**:Further studies are ongoing for these proteins which show promise

for both the diagnosis and for vaccine development in order to understand the reasons which make them so immunogenic.

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ASC INFLAMMASOME IS NECESSARY TO ACTIVATE THE HOST INNATE IMMUNE RESPONSE IN *TRYPANOSOMA CRUZI* INFECTION

GRACE KELLY SILVA[1]; TATIANA NUNES SILVEIRA[2]; RENATA SESTI COSTA[1]; BRAULIA COSTA CAETANO[3]; FREDY ROBERTO SALAZAR GUTIERREZ[1]; PAULO MARCOS DA MATTA GUEDES[1]; WARRISON ATHANÁSIO ANDRADE[3]; RICARDO TOSTES GAZZINELLI[3]; DARIO SIMÕES ZAMBONI[2]; JOÃO SANTANA SILVA[1].

(1) Department of Biochemistry and Immunology and (2) Department of Cell Biology from School of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, São Paulo, Brasil; (3) Department of Medicine, Division of Infectious Diseases and Immunology, University of Massachusetts Medical School. Worcester, Massachusetts, United States.

Introduction: An efficient innate immune recognize of the intracellular parasite *Trypanosoma cruzi* is essential for host protection against Chagas disease, a severe and chronic illness that affects millions of people in Latin America. Indeed, mechanisms modulated by TLRs via MyD88 are necessary for resistance to *T. cruzi* infection. However, Myd88$^{-/-}$ infected mice present cytokine production suggesting that other innate pathways are engaged by *T. cruzi*. There are other family of pattern recognition receptors, the Nod-like receptors (NLRs), including NLRP3 that associate to ASC forming a complex with active caspase-1, called inflammasome. This scaffold is cleavage the active form of the IL-1β and IL-18. Here, we aimed to study the role of the inflamassomes in the immune response against this parasite.

Methods and Results: First, we differenced macrophages from bone morrow (BMMs) of C57BL/6(WT) and ASC$^{-/-}$ to analyze the presence of the active caspase-1(p20) by Western blotting and release of IL-1β by ELISA. We found that macrophages from WT presented robust activation of caspase-1 and IL-1β secretion 24 pi, while macrophages from ASC$^{-/-}$ mice were not activated. To verify the mechanisms involved in this signalling, we investigated the influence of the potassium efflux, oxygen radicals reactive (ROS) and lisossomal acidification which are important to the activation of caspase-1 via NLRP3/ASC. The blockade of potassium efflux, oxygen radicals reactive (ROS) and lisossomal acidification resulted in reduce of caspase-1 in WT BMMs. To test the susceptibility of these animals *in vivo*, WT, caspase-1$^{-/-}$ and ASC$^{-/-}$ mice were infected with 10^3 forms of *T. cruzi* Y strain and the mortality, heart inflammation and cytokine production were measured 17 dpi. Strikingly, 100% of ASC$^{-/-}$ and 90% of caspase-1$^{-/-}$ mice succumbed the infection, whereas 40% of the WT were resistant to infection. Moreover, the ASC$^{-/-}$ and caspase-1$^{-/-}$ mice presented high inflammation. We did not observe significant difference in the IFN-γ production however, the IL-1β production, on the heart of infected mice was reduced in ASC$^{-/-}$ and caspase-1$^{-/-}$ as compared with WT infected mice.

Conclusion: Together, these results show that ASC inflamassome is crucial to the resistance against *T. cruzi* infection. Therefore, therapies modulating the caspase-1 via can be used in the future to treatment of Chagas disease.

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CASPASE-1 IS REQUIRED TO CONTROL OF THE INFECTION BY PARACOCCIDIOIDES BRASILIENSIS

NATÁLIA KETELUT CARNEIRO(1); FERNANDA AGOSTINI ROCHA(1); GRACE KELLY SILVA(1); DJALMA DE SOUZA LIMA JÚNIOR(1); DARIO SIMÕES ZAMBONI(1); JOÃO SANTANA SILVA(1).

(1) Department of Biochemistry and Immunology, School of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, SP, Brazil

Introduction: Paracoccidioides brasiliensis (Pb) is a pathogenic dimorphic fungus that causes paracoccidioidomycosis (PCM), a systemic human mycosis highly prevalent in Latin America. An effective innate immune response is essential to control this infection. In this context the NLRs (Nod-like receptors) that activate the caspase-1 pathway are candidate receptors that deserve to be brought into consideration. Caspase-1 is a cysteine protease that cleaves IL-1β into mature forms. In the presence a wide variety of stimuli it is recruited by molecular scaffold called inflammasomes. In this family, the NLRP3 sense damage associated molecular patterns (DAMPs) and recruits ASC (adaptor molecule) to cleave caspase-1. The ASC inflammasome signaling is necessary to eliminate the fungus Candida albicans. Therefore, we hypothesized that NLRs also recognize the fungus Pb and their antigens. Then, the aim of this study is to evaluate the importance of inflammasome in the experimental PCM. Methods and Results: We found that bone marrow-derived macrophages (BMDM) from C57BL/6 (WT) mice stimulated with LPS and Pb (1 yeast: 50 macrophages) presented an increased IL-1β production and caspase-1 activation compared with non-stimulated BMDM or stimulated only with Pb. In vivo, we verified that experimental PCM induced an increase mRNA and protein expression of IL-1β at 30 days post-infection, in lung of infected mice compared with uninfected group. To analyze the role of inflammasome components regarding protection to Pb-infection, WT, ASC-/- and caspase-1-/- mice were intravenously infected with 1x106 viable yeasts from Pb18 virulent strain. At day 7 post-infection, lung lobes were collected, and the recovery of colony forming units (CFU) was evaluated. Interesting, ASC-/- and caspase-1-/- mice were more susceptible to Pb-infection compared with control group. Conclusion: Collectively, these results suggest that Pb-infection triggers IL-1β production by macrophages and that ASC inflammasome are required to control fungus

growth in vivo. We suggest that IL-1β can be important during the inflammatory granumatosis response against the fungus P. brasiliensis and can be used as a possible therapeutic target, however further studies are needed to characterize the mechanisms by which IL-1β is involved in development of this infection.

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The role of CD4$^+$ T lymphocytes during B cell activation induced by *Plasmodium chabaudi* infection

SHEYLA INÉS CASTILLO-MÉNDEZ[1]; SANDRA MARCIA MUXEL[1]; EDUARDO AMARAL[1]; CLAUDIA AUGUSTA ZAGO[1]; ÈRIKA SALLES[1]; LUIZ ROBERTO SARDINHA[2]; JOSÉ MARIA ÁLVAREZ[1] and MARIA REGINA D'IMPÉRIO LIMA[1]

[1]Departament of Immunology, ICB-USP, São Paulo, Brazil

[2]Hospital Israelita Albert Einstein, São Paulo, Brazil

Text: Polyclonal B cell activation is a feature of the early spleen B cell response to *Plasmodium chabaudi* malaria, which results in intense production of parasite-specific low-affinity IgM and IgG and autoantibodies. As the disease progresses, a specific B cell response is generated, guaranteeing complete parasite clearance and protection against subsequent infection. **Methods and Results:** According to our results, in the acute phase of the disease, T-B cell cooperation through the MHC class II molecules is essential for B cell activation and proliferation, and also for antibody production. The CD4$^+$ T cell population involved in the polyclonal B cell activation shows a huge increase of ICOS and OX40 molecules. CD4$^+$ T cells from acute infection also express high levels of CXCR4 but low levels of CXCR5, suggesting that T-B cell interaction occurs in the extra-follicular areas of the spleen. As the disease progresses, we observed an increase in the levels of CXCR5 and PD1 on the CD4$^+$ T cells, a characteristic of follicular T cells. Furthermore, our *in vitro* assays show that T cells from the acute infection are potent helpers for naïve B cells in the presence of iRBC. These interactions result in an increase of CD69 expression, proliferation, and IgM and IgG production. These antibodies contribute not only to the uptake of the infected erythrocytes exposed to phagocytes, but also to the uptake of BCG mycobacterium. The expression of class II MHC molecules on B cells is crucial for their activation in terms of CD69 expression, proliferation and antibody production. Moreover, co-cultures of B cells with or without class II MHC molecules suggest that these molecules are mainly required for T cell activation. In addition, our results show that CD4$^+$ICOS^{HIGH} T cells from the acute infection are responsible for polyclonal B cell activation *in vitro* in the presence of infected red blood cells.

Conclusions: Our results suggest that the cooperation between conventional CD4$^+$ T cells and B cells has a central role in the polyclonal antibody response

to *P. chabaudi.* This study opens the possibility to develop strategies aiming to minimize the exacerbated activation of the immune system in the early phase of the disease, thus improving the humoral immunity against malaria.

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CRITICAL ROLE OF MYD88 EXPRESSION IN TH1 CELLS DURING INFECTION WITH *TRYPANOSOMA CRUZI*.

ANA CAROLINA OLIVEIRA[1], ALESSANDRA GRANATO[2], MAURICIO RODRIGUES[3], ALBERTO NÓBREGA[2], MARIA BELLIO[2]

[1]Laboratório Imunologia Molecular,IBCCFº - UFRJ, Rio de Janeiro, RJ.
[2]Laboratório Integrado de Imunobiologia, Depto. de Imunologia, IMPG - UFRJ, Rio de Janeiro, RJ.
[3] Centro de Terapia Celular e Molecular (CTCMol), UNIFESP, SP.

Introduction: The myeloid differentiation protein 88 (MyD88) is the main adaptor protein of the TLR signaling pathway. It has been implicated in the Th1 immune response associated with the production of IL-12, induced in dendritic cells (DC) by PAMP recognition trough TLRs. Accordingly, MyD88-deficient mice are highly susceptible to infection with different intracellular parasites, including *Trypanosoma cruzi*. On the other hand, we have shown recently that IFN-gamma production and cytotoxicity mediated by CD8 T cells are intact in MyD88-deficient mice infected with *T. cruzi,* suggesting that DC function is not completely abolished in MyD88-deficient mice. As MyD88 is also an essential adaptor for signaling through IL-1R and IL-18R, we aimed to investigate the intrinsic role of MyD88 expression in CD4 and CD8 T cells during infection with this parasite. **Methods and Results**: In order to answer this question, we analyzed the production of IFN-gamma by spleen CD4+ and CD8+ T cells in mixed (WT + MyD88KO→WT) bone marrow chimeric mice infected with the Y strain, by intracellular staining and flow cytometry. In these chimeric mice, at least half of the DCs are of WT phenotype, expressing MyD88. While no important difference was found between the percentage of effector MyD88$^{-/-}$ and WT CD8+ T cells, the percentage of CD4+ MyD88$^{-/-}$ (CD45.2+) IFN-gamma+ T cells is severely reduced compared to the ratio of CD4+ WT (CD45.1+) IFN-gamma+ T cells among total CD4+ T lymphocytes. We also analyzed mixed (WT + IL-1R$^{-/-}$→WT) and mixed (WT + IL-18R$^{-/-}$→WT) bone marrow chimeras and measured CD4+ T cell *in vivo* proliferation by BrDU incorporation. **Conclusion:** Our results show that MyD88 expression in CD4+ T cells, by signaling through IL-18R, is necessary for the expansion of the Th1 subset induced during infection with *T. cruzi*.

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ENCAPSULATED *Cryptococcus neoformans* EVADES INFLAMMASOME ACTIVATION

PEDRO H. V. SAAVEDRA[1]; MANUELLA CAPPARELLI[1]; LARISSA F. MATOS[1]; DARIO S. ZAMBONI[2]; ARTURO CASADEVALL[3]; KELLY G. MAGALHAES[1]; ANAMELIA L. BOCCA[1]

[1]Department of Cell Biology, University of Brasília, Brasília, Brazil; [2]Department of Cell Biology and Microbial Pathogenesis, Medical School of Ribeirão Preto, University of São Paulo, São Paulo, Brazil [3]Department of Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, NY, USA

Introduction: *Cryptococcus neoformans* is an encapsulated yeast widely distributed around the globe, capable of causing pulmonary infection and meningitis in immunocompromised individuals. Its capsule is well known for conferring *C. neoformans* the ability to evade host immune system, leading to its proliferation. Regarding the importance of initial innate immune response to cryptococcal infections, we decided to investigate if *C. neoformans* is capable of activating the inflammasome as well as the role of its capsule in this pathway.

Methods and Results: In order to verify whether *C. neoformans* could trigger inflammasome activation, bone marrow-derived macrophages (BMMs) were infected with wild type *C. neoformans* or its acapsular mutant. Our results showed that acapsular mutant of *C. neoformans*, but not the wild type, induced IL-1β secretion. Flow cytometry and western blot analysis revealed that caspase-1 is activated upon infection with acapsular but not with WT *C. neoformans*. To understand the role of inflammasome proteins in IL-1β secretion induced by acapsular *C. neoformans*, WT, *Asc*$^{-/-}$, *Casp1*$^{-/-}$, *Nlrp3*$^{-/-}$ and *Nlrc4*$^{-/-}$ BMMs were infected. IL-1β secretion was dependent on the NLRP3-ASC-Caspase-1, but independent on the NLCR4 inflammasome. In addition, inflammasome proteins were dispensable for fungal uptake or killing as demonstrated by CFU analysis. The mechanisms underlying IL-1β processing and release by *C. neoformans* infection were dependent on reactive oxygen species (ROS), lysosomal destabilization and Syk tirosine kinase signaling, based on inhibition assays. Interestingly, cells treated with IL-1β in a dose-dependent manner were unable to restrict intracellular yeasts, however, the rate of infection diminished significantly compared to untreated cells.

Conclusion: Taken together, our data show for the first time that acapsular *C. neoformans* activates the NLRP3 inflammasome and IL-1β secretion in a caspase-1 dependent manner. The mechanisms that orchestrate this event rely

on ROS, cathepsin B and Syk kinase signaling, leading to restriction of infection rate. Furthermore, *C. neoformans* capsule plays a major role in avoiding inflammasome activation and promoting host immune evasion.

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Assessment of extracellular matrix proteins in the host skin from tick-resistant and tick-susceptible bovine breeds

Nádia C. S. Gauna[1]; Alessandra M. Franzin[1]; Sandra R. Maruyama[1]; Gustavo R. Garcia[1]; Lauren C. Ribeiro[1]; Isabel K. F de M. Santos[1]; Beatriz R. Ferreira[2]

[1] School of Medicine of Ribeirão Preto, USP, Ribeirão Preto-SP, Brazil

[2] School of Nursing of Ribeirão Preto, USP, Ribeirão Preto-SP, Brazil

Email: nadiagauna@usp.br

Introduction: *Rhipicephalus boophilus microplus* ticks are parasites that feed for several days attached to their bovine hosts, which depends on the inoculation of saliva into the skin feeding lesion. Composition of tick saliva is complex and contains several molecules that modulate the innate and acquired host immune response. The extracellular matrix (ECM) of the skin is a network of components, which contribute to cell differentiation and proliferation, gives tensile strength, as well as, facilitates the connection of chemokines that are essential to recruit cells like basophils and eosinophils. In a previous study of microarray, our group compared the Nelore bovine skin (tick-resistant breed) with Holstein (tick-susceptible breed). We observed that a large profile of genes were differentially expressed between the breeds, including genes coding for ECM proteins, such as tenacyn, keratocan, collagen type I alpha 1, osteoglycin, osteonectin, metalloproteinases and fibronectin. Our present work aimed to evaluate some of these ECM proteins in the skin of tick-resistant and susceptible bovine breeds. **Methods and Results:** We infested Nelore (n=4) and Holstein (n=4) with 10,000 *R. (B) microplus* tick larva/animal and collected skin biopsies containing attached tick larva and nymphs or without ticks. Using qPCR and immunohistochemistry techniques we evaluated the gene expression for keratocan, osteoglycin and collagen type I and their respective proteins. So far, no statistical differences in the gene expression for keratocan, osteoglycin and collagen type I was observed between the bovine breeds tick-infested skin. In standardizing assays we did not detect collagen type I protein in Holstein non-infested skin sections; on the other hand, this same skin was stained for keratocan and strongly labeled for osteoglycin. The comparisons between the skin of Nelore and Holstein breeds by immunohistochemistry are still ongoing. **Conclusion:** The experiments are being repeated. This study will contribute to a better understanding of the mechanisms by which resistant bovines can prevent tick infestation.

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EVALUATION OF A SYNTHETIC PEPTIDE DERIVED FROM A LEISHMANIA CYTOSOLIC FACTOR AS NEW DIAGNOSTIC MARKER TO DETECT *Leishmania* INFECTED DOGS

LEOPOLDO FERREIRA MARQUES MACHADO[(1)] ; Adriana Canavaci Martins[(1)] ; Frederico Crepaldi Nascimento[(1)] ; Mariana Silva dos Santos[(1)] ; MIGUEL A. CHÁVEZ-FUMAGALLI[(2)] ;; ALEXANDRE BARBOSA DOS REIS[(3)] EDUARDO ANTONIO FERRAZ COELHO[(2)]; ANA PAULA FERNANDES[(1)].

[(1)] Departamento de Análises Clínicas e Toxicológicas, Faculdade de Farmácia, Universidade Federal de Minas Gerais; [(2)] Departamento de Patologia Clínica, Colégio Técnico. Universidade Federal de Minas Gerais; [(3)] Laboratório de ImunoPatologia, Departamento de Análises Clínicas, Escola de Farmácia, UFOP.

Introduction: Visceral leishmaniasis (VL) is the more severe form of Leishmaniasis and is usually fatal if untreated. Dogs are the main peridomestic reservoirs and an important step during the parasite life cycle and human infection. Those animals may develop symptoms or remain asymptomatic for long periods although both can transmit infection. Therefore, it is essential to develop effective diagnostic markers to detect symptomatic and asymptomatic dogs. Moreover, the study of new proteins or peptides may contribute to discover the role of in immune responses. The *Leishmania* cytosolic factor gene has been involved to visceralization of parasites. Thus, the aim of this work is to evaluate the sensibility of predicted peptides during antibody detection by ELISA. **Methods and Results:** The protein sequence was submitted to epitope prediction and a synthetic peptide (P35) was obtained. The selected synthetic epytopes were used for antibody detection by ELISA. The assays used sera from known negative control, clinically asymptomatic and symptomatic dogs, PCR positive/serologic negative control, and *T. cruzi*-infected dogs. The ELISA reaction conditions were optimized to determine the concentrations of each antigen and serum dilutions. ELISA plates were previously coated with the synthetic peptide and incubated with sera. The binding of antibodies was detected using anti-canine IgG peroxidase conjugated and the absorbance was read at 492 nm. The cut-off was determined using mean absorbance of negative samples plus two standard deviations, to discriminating positive from negative results. The results of the assays using the synthetic peptide (P35) and the peptide derived from the A2 antigen (A2-P40), which was previously tested for diagnosis of canine visceral leishmaniasis, were comparatively analyzed. The P35 peptide detected as positive 8% of sera in the control group;

80% in symptomatic group; 45% in asymptomatic group; 75% positive in PCR+/sero- group; and 25% positive in *T. cruzi*-infected group. The A2-P40 peptide detected as positive 16% of sera in control group and 86.67% in symptomatic, but none in the other groups. **Conclusion:** The P35 synthetic peptide showed a good performance as a diagnostic marker to detect infected animals by ELISA assay. However, further improvements may lead to better specificity values.

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APOPTOSIS AS A MODULATING FACTOR IN T CD8+ LYMPHOCYTE SUBSETS IN HUMAN CUTANEOUS LEISHMANIASIS

RAQUEL FERRAZ1,3; CLARISSA F. CUNHA1; ADRIANO GOMES-SILVA2; ALDA MARIA DA-CRUZ2; ARMANDO SCHUBACH4; SÉRGIO C.F. MENDONÇA1; ERICA VASCONCELLOS4; CLÁUDIA M. VALETE-ROSALINO4; MARIZA M. SALGUEIRO4; ÁLVARO LUIZ BERTHO1,3.

1– Laboratório de Imunoparasitologia, 2- Laboratório Interdisciplinar em Pesquisas Médicas,

3 Plataforma de Citometria de Fluxo, Instituto Oswaldo Cruz, FIOCRUZ, Rio de Janeiro, RJ;

4- Serviço de Referência em Leishmaniose, Instituto de Pesquisa Clínica Evandro Chagas, FIOCRUZ, Rio de Janeiro, RJ.

Introduction: In human cutaneous leishmaniasis (CL), the interaction between the immune response and the parasite is crucial for the clinical manifestations of this disease. It has been strongly reported the key role of CD8+ T cells in the adaptive immune response leading to the cure of CL. Besides that, apoptosis acts as a modulating factor in immunological events in several parasite diseases. Knowing that the lymphocytes undergoes differentiation under antigenic stimulation, it becomes fundamental an evaluation of naïve, effector and memory lymphocyte subsets. Furthermore, these functionally distinct cell categories may be induced to death by apoptosis in accordance of different different antigenic stimulations. The goal of this research was to evaluate the frequency of effector CD8+ T cells, those in apoptosis and the specificity of response by Leishmania braziliensis antigen during and after the disease. **Methods and Results:** Peripheral blood mononuclear cells were obtained from CL patients, with active disease, during the treatment (DT); of patients cured after the treatment (AT); and healthy controls (HC). These cells were evaluated, ex vivo and 5–day in vitro cultures,stimulated with specific antigens, by an multiparametric flow cytometry protocol including 7-AAD and monoclonal antibodies antiCD8, antiCD27 and antiCD45RA. Ex vivo experiments showed that the DT patients have a higher percentage of effector CD8+ T lymphocytes compared to HC and to AT. However, it is important to note a higher percentage

of cells undergoing apoptosis in DT patients. Moreover, compared to background, after stimulation in vitro L. braziliensis antigens induced an increase in frequency of effector T CD8+ cells in both groups of patients as well as apoptosis of these cells, thus demonstrating an antigenic specificity. **Conclusion:** Our results showed that the occurrence of apoptosis in effector CD8+ T cells may reflect a downregulation of the role of their functional activity and may contribute to the persistence of disease. Thus, despite a lower percentage of effector CD8+ T lymphocytes from AT, the highest percentage of viable and functional cells may be associated with the control of infection and healing of the lesions in these individuals.

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FREQUENCY OF POLYFUNCTIONAL CD8$^+$ T CELLS TO HIV-1 Gag REGIONS IN HIV-EXPOSED UNINFECTED INDIVIDUALS

JOSENILSON FEITOSA DE LIMA[1]; LUANDA MARA DA SILVA OLIVEIRA[1]; GABRIELLE EIMI MITSUNARI[1]; ALBERTO JOSÉ DA SILVA DUARTE[1]; MARIA NOTOMI SATO[1]

[1]Laboratory of Dermatology and Immunodeficiencies (LIM-56)

School of Medicine - USP – São Paulo – Brasil.

Introduction and Objectives: Individuals who are highly exposed to HIV-1 that remain seronegative (exposed uninfected - EU), are important targets to identify the possible factors and mechanisms involved in the resistance to HIV-1. Subjects ESN may display a polyfunctional response of CD8 HIV-specific T cell for secretion of several cytokines/chemokines simultaneous secretion. The aim of this study was to evaluate the profile of polyfunctional CD8$^+$ T cells to the three regions of the Gag peptides in EU individuals and their HIV-1 infected partner.

Methodsand Results: Serodiscordant couples (n=6) were from the Emílio Ribas Institute of Infectology/São Paulo, and healthy control subjects (n=10) LIM-56/FMUSP. All subjects HIV-1 infected were treated with antiretroviral drugs and low viral load (mean plasma viral copies – 466.66). Peripheral blood mononuclear cells (PBMC) were stimulated with three peptides pools of HIV-1 Gag from the NIH (residues 15-mer) which corresponding to p24, p17 or p15 or with Ionomycin + PMA for 6 hours adding brefeldin after 2 hours. CD8$^+$ T cells were evaluated by flow cytometry. Frequency of polyfunctional CD8$^+$ T cells (CD107$^+$ IFN-g$^+$ TNF-a$^+$ MIP-1b$^+$) induced by PMA + Ionomycin were higher in EU than in healthy controls and infected patients with HIV-1. In addition, the p17 Gag peptides induced a significant increased percentage of CD8$^+$ T cells secreting CD107a IFN-g TNF-a MIP-1b compared to healthy controls and infected individuals. In contrast, the simultaneous expression of the four cytokines induced by Gag p15 and p24 peptides were decreased in EU compared to HIV infected group.

Conclusion: The profile of polyfunctional CD8$^+$ T cells to certain regions of the HIV Gag, may contribute to understanding the mechanisms of protection in EU individuals.

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EVALUATION OF THE IMMUNOLOGICAL AND PARASITOLOGICAL EFFECTS OF *CAESALPINIA PYRAMIDALIS* IN GOATS NATURALLY INFECTED BY *HAEMONCHUS CONTORTUS*

GEYANNA DOLORES LOPES NUNES[1]; FAROUK ZACHARIAS[2]; JORGE MAURÍCIO DAVID[3]; JUCENI PEREIRA DAVID[4]; SÍLVIA CAROLINE OLIVEIRA SANTOS[4]; MÁRCIA CRISTINA AQUINO TEIXEIRA[4]; FERNANDA WASHINGTON DE MENDONÇA LIMA[4].

[1]Escola de Medicina Veterinária e Zootecnia-UFBA, Brasil; [2]Empresa Bahiana de Desenvolvimento Agrícola-EBDA, Brasil; [3]Instituto de Química-UFBA, Brasil; [4]Faculdade de Farmácia-UFBA, Brasil.

Introduction: Helminthiasis leads to enormous economic losses in small ruminant production. Development resistance to the most commercially available anthelmintics awakened the interest in phytotherapy as an alternative treatment (Rev. Bras. Pl. Med. 7:97-106, 2005). *Caesalpinia pyramidalis* has its leaves used in traditional medicine to combat fever, diarrhea, gastritis (J. Ethnopharmacol. 131:326–342, 2010). The aim of this study was to evaluate the possible effect immunomodulator and parasiticide of *C. pyramidalis* aqueous extract in the control of *Haemonchus contortus* in naturally infected goats.

Methods and Results: A total of 60 mixed breed goats were equally divided into six groups: the first group (G1) received only water, the second group (G2) was treated with doramectin, an anthelmintic drug, and the remaining groups (G3 to G6) received *C. pyramidalis* extract orally. The goats were naturally infected with helminths, predominantly *Haemonchus contortus* (81.6%). Before initiation of treatments, and at 30, 60 and 90 days, it was verified in each animal the number of eggs per gram of feces (EPG) by the McMaster technique, and the levels of IgG anti-*H. contortus* by indirect ELISA. The mean values of each group were compared by ANOVA followed by Tukey post-test ($P<0.05$). The procedures were approved by the Ethics Committee for the Use of Animals of UFBA (protocol no. 18/2010). The groups G2 (doramectin) and G6 (extract at a dose of 10 mg/kg) were the only ones who showed a reduction of OPG more than 50% after 30 days, where G2 reduced from 560 to 280, and G6 declined from 780 to 315 eggs per gram. However, at 90 days post-treatment, all the groups showed similar levels of reduction, with an average of 170 EPG. The determination of IgG anti-*H. contortus* levels did not show significant differences among treatments groups, with average optical density (O.D.) of 0,676 in the moment zero. A marked decline in the levels of anti-*H.contortus* IgG ($P<0.05$) was observed in all groups at 60 days post-treatment (O.D. 0,361), with the values returning to levels similar to time zero at 90 days (O.D. 0,706).

Conclusion: The *C. pyramidalis* extract demonstrates a promising potential in controlling helminthiasis in goats. However, further research is needed to

identify the active components and mechanism of action of the specific substances present in this extract.

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INTERFERON-GAMMA PRODUCTION BY HUMAN NEUTROPHILS UPON CHALLENGE WITH *PARACOCCIDIOIDES BRASILIENSIS*

DANIELA RAMOS RODRIGUES (PG) (1); REGINALDO KELLER FERNANDES (PG) (1); SUELI APARECIDA CALVI (2); TATIANA FERNANDA BACHIEGA (PG) (1); HELANDERSON DE ALMEIDA BALDERRAMAS (PG) (1); LUCIANE ALARCÃO DIAS-MELICIO (3); ÂNGELA MARIA VICTORIANO DE CAMPOS SOARES (1)

(1) Departamento de Microbiologia e Imunologia, Instituto de Biociências de Botucatu, UNESP - Univ Estadual Paulista, Brasil; (2) Departamento de Doenças Tropicais, Faculdade de Medicina de Botucatu, UNESP - Univ Estadual Paulista, Brasil; (3) Departamento de Patologia, Faculdade de Medicina de Botucatu, UNESP - Univ Estadual Paulista, Brasil.

Introduction: Paracoccidiodomycosis is a systemic mycosis caused by *Paracoccidioides brasiliensis* (Pb), which is endemic in Latin America. The host innate immune response against the fungus has been well characterized and several studies have clearly shown the important role played by phagocytic cells. Our laboratory has studied the relationship between human neutrophils (PMNs) / Pb focusing the effector mechanisms of these cells against the fungus. However, in last years, studies have shown that in addition to their phagocytic and killer functions, PMNs can modulate and instruct the immune response, since these cells have been shown to produce and release several cytokines. Thus, we asked whether PMNs challenged with *P. brasiliensis* could modulate the immune response towards a Th_1 phenotype, by producing and releasing IFN-γ. In addition, we asked if cells activation with the cytokines IL-12, IL-15 and IL-18 would increase this production. **Methods and Results:** Peripheral blood PMNs obtained from 12 healthy donors were non activated or activated with IL-12, IL-15 or IL-18 in different concentrations and challenged with *P. brasiliensis* strain 18 (Pb18) for 2h, 4h, 12h, 24h and 48h. After, cultures were evaluated for IFN-γ production by ELISA. We found that PMNs alone produced low levels of IFN-γ that was significantly increased after Pb18 challenge, during 2h of incubation and maintained at the subsequent periods. Cells activation with IL-18, IL-15 or IL-12 in some concentrations, increased IFN-γ production induced by the fungus. **Conclusion:** PMNs, by producing IFN-γ in response to *P. brasiliensis*, mainly after activation of some cytokines of innate immune response, could be involved in modulation of the adaptative immune response to this fungus.

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EVALUATION OF HUMORAL AND CELULLAR IMUNE RESPONSE OF SHEEP TO ANTIGENS OF *CORYNEBACTERIUM PSEUDOTUBERCULOSIS* TREATED WITH AQUEOUS EXTRACT OF *CAESALPINIA PYRAMIDALIS*

SANTANA, MARTA MARIA OLIVEIRA[1]; MEYER, ROBERTO[5]; LIMA, DANIELLE DANTAS[5]; DAVID, JORGE MAURÍCIO[3]; DAVID, JUCENI PEREIRA[2]; ZACHARIAS, FAROUK[4]; OLIVEIRA, RICARDO RICCIO[2]; LIMA, FERNANDA WASHINGTON DE MENDONÇA[2].

[1]UFBA, Escola de Medicina Veterinária e Zootecnia, Salvador - BA, Brasil.
[2]UFBA, Faculdade de Farmácia, Salvador - BA, Brasil;
[3]UFBA, Instituto de Química, Salvador - BA, Brasil;
[4]Empresa Baiana de Desenvolvimento Agrícola, Salvador - BA, Brasil
[5]UFBA, Instituto de Ciências da Saúde, Salvador - BA, Brasil

Introduction: Many popular plant extracts seem to stimulate cellular and humoral immunity in animals. This study evaluated the effect of an aqueous extract of *Caesalpinia pyramidalis* on the immune response of sheep experimentally stimulated with *Corynebacterium pseudotuberculosis*.
Methods and Results: The study included 20 sheep of both gender that were 90 and 270 days of age and serologically negative for *C. pseudotuberculosis.* The sheep were divided into four experimental groups: G1 was the control, G2 received treatment with extract of *C. pyramidalis*, G3 received an extract of *C. pyramidalis* associated with Antigen MQD and G4 was treated with Antigen MQD. After antigenic stimulation, blood was collected for serology during eight months. The specific cellular and humoral immune responses against *C. pseudotuberculosis* were assessed by specific IgG production and IFN-γ, respectively. G2 presented the highest IgG values 120 (O. D. 0,2498) days after treatment. G3 showed higher values than all other groups after 90 (O. D. 0,9582), 120 (O. D. 0,7638) and 180 (O. D. 0,8383) days and G4 showed its greatest values after 150 (O. D. 0,7685) days. G2 and G3 displayed the greatest values of IFN-γ 120 (O. D. 257,8 and 691,8) days after treatment ($p < 0.05$), while the greatest values in the G4 group were obtained 150 (O. D. 723,0) days after stimulation.
Conclusion: The extract of *C. pyramidalis* seemed to promote the stimulation of the immune responses; however, other studies are needed to investigate its mechanisms of action.

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MODULATION OF AIRWAY ALLERGIC INFLAMMATION BY *TOXOPLASMA GONDII* ANTIGENS

BRUNA BUENO CHAHUD[(1)], MURILO SOLANO-DIAS[(1)], LUCIANA BENEVIDES[(1)], RODRIGO R. RODRIGUES[(1)], MARCELA DAVOLI FERREIRA[(1)], DENISE MORAIS DA FONSECA[(1)], JOÃO SANTANTA DA SILVA[(1)]

[(1)] Department of Biochemistry and Immunology - Ribeirão Preto Medical School - University of São Paulo - Ribeirão Preto, Brazil

Introduction: Antigens from different *Toxoplasma gondii* strains may interact with the immune system leading to distinct effects, such as paralysis of dendritic cells or IFN-γ production by T lymphocytes. These events are associated to the parasite virulence and could modulate Th2 allergic response by different mechanisms. In this study, we evaluated the modulation of allergic airway inflammation using soluble antigens of *T. gondii* tachyzoites (Stag) from different parasite strains. **Methods and Results:** C57BL/6 were sensitized and challenged with ovalbumin (OVA). After induction of allergic response, mice were treated with 3 weekly doses of Stag from RH or ME49 *T. gondii* strains. One week after the treatment, mice received a second challenged with OVA for the analysis of the allergic response. Both treatments, ME49-Stag and RH-Stag, were able to reverse the allergic response leading to a significant reduction in the IL-4 and IL-5 production, in the total cell numbers and eosinophil counts in the bronchoalveolar lavage fluid (BALF) as compared to allergic untreated group. C57BL/6 mice treated with RH or ME49-Stag also exhibited a lower cellular infiltration and a greater preservation of lung parenchyma as compared to allergic untreated animals. The effect of treatment with ME49-Stag was not observed in animals deficient for IFN-γ or MyD88. On the other hand, the effect of RH-Stag treatment was not seen in IL-10 knockout mice. In order to evaluate if the therapeutic effects of Stag were cell-mediated, animals transgenic for green fluorescent protein expression (GFP-mice) were immunized with RH or ME49-Stag and the spleen cells were transferred to allergic mice to evaluate the airway eosinophilia and migration of transferred cells. The transfer of spleen cells from animals immunized with ME49-Stag, but not RH-Stag, reversed BALF eosinophilia compared to the mice that received cells from unimmunized GFP-mice. The transferred $CD4^+$ and $CD8^+$ cells migrated to the lungs and draining lymph nodes from recipient mice and were able to secrete IFN-γ and IL-17 and suppress IL-5 production by recipient lymphocytes. **Conclusion:** Stag antigens obtained from different strains of *T. gondii* are able to reverse the allergic airway inflammation via distinct mechanisms. The therapeutic effect of ME49-Stag is cell-mediated and dependent on the induction of IFN-γ via MyD88 activation, while the effects of RH-Stag depends on IL-10 and is not mediated by lymphocytes.

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Study of infection and signaling activity of cardiomyocytes infected by *Trypanosoma cruzi* strain Sylvio X10/4

Christian Emerson Rosas-Jorquera (PhD) [(1)]; Maria Regina D'Império Lima (PhD) [(1)]; José Maria Álvarez (PhD) [(1)].

[(1)] Departamento de Imunologia – Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, Brasil.

Introduction: Infection with *Trypanosoma cruzi* causes a chronic and progressive cardiac inflammation in 30% of individuals. Host genetic factors as well as the strain of the parasite may lead to differences in the infection process and signaling infected cardiomyocytes. Such differences may contribute to elimination of the parasite or the chronicity of the disease. In this sense, our study seeeks to identify the analogy breaks of cardiomyocytes from neonatal strains C3H/HePAS and C57BL/6 during infection by the parasite strain Sylvio X10/4. C3H/HePAS mice infected develop a strong cardiopathy, whereas C57BL/6 do not show cardiac damage in chronic phase of the infection. Methods and Results: Cardiomyocytes of C3H/HePAS and C57BL/6 strain were isolated from neonatal mice. The cells were maintained in culture with medium alone, or treated with IFNg and infected with tripomastigostas 5:1 cells. After 24 and 48 hours of infection, the cultures were fixed and stained for the analysis of the infection or, RNA was extracted, converted into cDNA and quantifying the expression of genes for cytokines, TNFa, IL6 and TGFb, besides the chemokines, CCL2 , CXCL9 and CXCL10. Our results show that infection by the parasite Sylvio X10/4 is similar in both strains of mice, with or without treatment with IFNg. The infection did not alter gene expression of TGFb and minimally increased expression of chemokines CCL2 and CXCL9. However, the genes for TNFa, IL6 and CXCL10 are widely expressed in both strains. In

addition, significant expression of TNFa in cardiomyocytes of C3H/HePAS and the expression of IL-6 in cardiomyocytes of C57Bl/6. Conclusion: Even though the infection was similar in both strains, it is possible that subtle differences in gene expression of proinflammatory cytokines and chemokines are responsible for maintenance or elimination of the parasite in the cardiac tissue. Financial

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PARTICIPATION OF CYTOKINES AND CHEMOKINES IN HOST SUSCEPTIBILITY TO INFECTION BY *Legionella longbeachae*

GRAZIELE ZENARO MANIN[1]; JULIANA ISSA HORI[1]; LILIANA MOURA MASSIS[1]; DARIO SIMÕES ZAMBONI[1].

(1). Department of Cellular and Molecular Biology, School of Medicine of Ribeirão Preto, University of São Paulo (FMRP/USP).

Introduction: Bacteria from *Legionella* genus are recognized as etiological agents of Legionnaires' disease or legionellosis, a severe and atypical pneumonia that is an important cause of mortality and morbidity worldwide. The main *Legionella* species that causes legionellosis is *L. pneumophila* and the disease caused by it has been extensively studied. However, there is little information regarding the mechanisms by which the immune system eliminates the pulmonary infection caused by *L. longbeachae*, despite its higher importance in some countries. In the present study, we aimed to analyze if IL-17R, IL-23 and CCR5 are related with susceptibility or resistance to *L. longbeachae,* since IL-17R ligand and CCR5 and IL-23 have been described as factors involved in neutrophil recruitment, histological characteristic observed in lungs of infected individuals.
Methods and Results: To verify the importance of IL-17R, IL-23 and CCR5 to infection with *L. longbeachae,* we intranasally infected mice deficient in these molecules (n=10) and analyzed their survival. When challenged with *L. longbeachae* (MOI 10^6/mouse), IL-17R-deficient mice and CCR5- deficient mice (both in C57BL/6 background) had significantly higher survival rates than Wild-type mice (respectively 50% and 75% vs 20%), while IL-23-deficient mice had survival rate similar to Wild-type (10% vs 20%). We also analyzed the bacterial numbers in the lungs of infected mice after 48 hours post-infection and found a strong replication of the bacteria in the lungs of all mice tested: IL-17R ($4x10^7 \pm 2x10^7$ bacteria per lung) and CCR5-deficient mice ($4x10^7 \pm 3x10^7$ bacteria per lung) and Wild-type ($7x10^7 \pm 2x10^7$ bacteria per lung).
Conclusion: These results indicate that IL-17R and CCR5 are molecules associated with susceptibility to *L. longbeachae* in experimental model of Legionnaires' disease. Additional experiments should be done to clarify their specific role in this disease.

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THE ROLE OF K^+ TRANSPORTERS IN THE *Legionella pneumophila* REPLICATION

JULIANA ISSA HORI[1] AND DARIO SIMÕES ZAMBONI[1].

(1). Department of Cellular and Molecular Biology, School of Medicine of Ribeirão Preto, University of São Paulo (FMRP/USP).

Introduction: *Legionella pneumophila* is a facultative intracellular bacteria found ubiquitously in lakes and rivers where parasitizes a broad number of amoebae species. Potassium is an essential nutrient for organisms and certain bacterial species, such as *L. pneumophila*, requires large amounts of potassium to replicate. It is known that bacteria, as well as plants, accumulate potassium by a number of different transport systems that vary in kinetics, energy coupling, and regulation. So, we investigate the role of *L. pneumophila* K+ transporters during bacteria growth in macrophages and in axenic media.
Methods and Results: *In silico* analysis of the *L. pneumophila* genomic revealed that the bacterium presents three genes predicted to encode K+ transporters. These genes showed strong sequence similarity to Kup family K+ transporters that are highly conserved among different organisms. To evaluate the expression of these genes in *L. pneumophila* we performed reverse transcriptase-PCR analyses and found that the bacteria express them all. By measuring the K^+ concentrations in the media we found that KupA is a functional K+ transporter and its absence does not interfere with bacterial growth in axenic medium and bacterial multiplication in *Acanthamoeba castellanii*. In contrast, *kupA* was required to the early multiplication in mouse macrophages and this defect was correlated with a defect in the establishment of the LCV (Legionella Containing Vacuole).
Conclusion: Collectively, our data indicates that *L. pneumophila* expresses three gene products predicted to function as K^+ transporter; KupA is a functional K^+ transporter required for bacterial replication in macrophages.

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STUDYING THE INFLAMMATORY RESPONSES TRIGGERED BY *STREPTOCOCCUS PNEUMONIAE* INFECTION IN A MURINE MODEL – EFFECTS OF A PDE4 INHIBITOR

LUCIANA PÁDUA TAVARES[1,2], CRISTIANA COUTO GARCIA[1], JULIANA PRISCILA VAGO DA SILVA[2], RAQUEL GREGORIO ARRIBADA[2], MAURO MARTINS TEIXEIRA[1], LIRLÂNDIA PIRES SOUSA[2]

[1]Laboratório de Imunofarmacologia, Departamento de Bioquímica e Imunologia, ICB, UFMG

[2]Departamento de Análises Clínicas e Toxicológicas, Faculdade de Farmácia, UFMG

Introduction and Objectives: Pneumonia, caused most frequently by *Streptococcus pneumoniae*, is a major global health problem and is a leading cause of morbidity and mortality worldwide, especially in developing countries. The inflammatory response that follows microbial infections controls dissemination of bacteria but may also cause tissue damage and mortality. Drugs such as phosphodiesterase 4 (PDE4) inhibitors decrease inflammatory responses effectively. Here, we describe the establishment of an animal model of *S. pneumoniae* infection and evaluated whether the anti-inflammatory action of PDE4 inhibitors interferes with the ability of the murine host to deal with infection.**Methods and Results:** Male Balb/C mice were infected intranasally with *S. pneumoniae* serotype 3 (ATCC 6303, 10^3-10^6 CFU). Weight loss and lethality were accompanied from the first day of infection (n=6 per group). There was an inoculum-dependent death rate(10^6 and 10^5 CFU caused 100%, while 10^4 CFU caused 67% and 10^3 CFU 50% of lethality). Mice were infected with 10^5 CFU (100% lethality rate at day 4) and killed at 1, 2 or 3 days after infection. Lung neutrophils (assessed by myeloperoxidase levels) and airway neutrophils (recovered by bronchoalveolar lavage - BAL) peaked at 1 day after infection and declined thereafter. Inflammatory mediators (TNF-α and CXCL1/KC), phagocytosis and number of bacteria in BAL fluid had similar kinetics. Treatment with Rolipram (ROL, 6 mg/kg, 1 h before infection) decreased neutrophil recruitment in lungs, but not in BAL fluid, and decreased levels of cytokines in BAL fluid at 24 h. Number of bacteria in BAL fluid were similar in Rolipram and vehicle-treated animals. The percentage of leukocytes phagocytosing bacteria was slightly greater in drug-treated animals.

Conclusion: In this study, we established a murine model and the kinetics of the inflammatory parameters that follow lethal *S. pneumoniae* infection**.** We show that inflammation was an important determinant of morbidity after infection with *S. pneumoniae* as seen by the large neutrophil influx, protein leakage and cytokine production during infection. Pretreatment of mice with Rolipram partially decreased several parameters of the inflammatory response without interfering with bacterial load suggesting that partial blockade of pulmonary inflammation may be beneficial for the host.

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MATURATION AND ACTIVATION OF MURINE DENDRITIC CELLS stimulated with CELL WALL COMPONENTS OF *Paracoccidioides brasiliensis*

YASMIN SOARES DE LIMA[1]; ANA CAMILA OLIVEIRA SOUZA[1]; RAFFAEL JUNIO ARAÚJO DE CASTRO[1] ALDO HENRIQUE P. TAVARES[2], ANAMÉLIA L. BOCCA[1]

[1]Department of Cell Biology, University of Brasília, Brasília, Brazil; [2]Ceilândia College, University of Brasília, Brasília, Brazil.

Introduction: The cell wall of *Paracoccidioides brasiliensis* contains several components capable to modulate the immune response of the host. The fungi cell wall can be fractionated into two principal cell wall fractions: one composed mainly by beta-1,3-glucan (F1) and another composed basically of alpha-1,3-glucan (F2). Beta-1,3-glucan can recruit inflammatory cells, stimulate cytokine production and granulomatous reaction. Alpha-1,3-glucan is in major related to the virulence of the yeasts cells. The interaction between the immune cells and cell wall fractions can induce different patterns of response. In this study, our objective was to evaluate the influence of the cell wall fractions during *in vitro* dendritic cells maturation. **Methods and Results:** Bone marrow cells of C57/bl6 mice were collected from femur and tibia and differentiated into dendritic cells in the presence of GM-CSF. After 8 days of differentiation, the cells were incubated for 24 hours with F1 and F2 fractions at different concentrations. The expression of MHCII, CD83, DCSIGN and CD11c were evaluated by FACS. The F1 fraction induced an increase in the $CD11c^{-}DCSIGN^{-}$ and $MHCII^{+}CD11c^{-}$ cells, besides a decrease in the $MHCII^{+}CD11c^{+}$ cells. The F2 fraction in low concentrations increases the $MHCII^{+}CD11c^{+}$, $MHCII^{+}CD11c^{-}$, $DCSIGN^{-}CD11^{-}$, $CD83^{+}CD11c^{-}$ and $CD83^{-}CD11c^{-}$ cells. **Conclusion:** By the proportion of $MHCII^{+}CD11c^{+}$ cells, these results indicate that F1 fraction inhibits the maturation of dendritic cells, while F2 fraction probably induces an early maturation and a larger capacity to present antigens.

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Heme activates oxidative mechanisms and induces cell death in human neutrophils infected with *Leishmania chagasi.*

GRAZIELE QUINTELA DE CARVALHO (PG) [(1)(2)]; NÍVEA LUZ (PG) [(1)(2)]; NATÁLIA TAVARES(PG) [(1)(2)];; DANIELE ANDRADE (PG) [(1)(2)]; CLAUDIA BRODSKYN [(1)(2)(3)]; MARCELO TORRES BOZZA[(4)]; VALÉRIA M. BORGES [(1)(2)(3)]

[(1)] Centro de Pesquisa Gonçalo Moniz - CPqGM, FIOCRUZ-BA; [(2)] Universidade Federal da Bahia - UFBA; [(3)] Institute for Investigation in Immunology (iii - INCT); [(2)] Universidade Federal do Rio de Janeiro - UFRJ.

Introduction and Objectives: Human visceral leishmaniasis (VL) is frequently associated with severe hematologic manifestations, including hemorhage and hemolysis. However, the role of heme on human neutrophils in infection *by Leishmania chagasi*, causative agent of VL in Brazil, has not yet been explored. We hypothesized that the presence of increased concentrations of free heme upon VL affects the neutrophils and the inflammatory response. In this study we analyzed the effect of heme on the activity and survival of neutrophils infected with *L. chagasi.*

Methods and Results: Neutrophils were isolated from periperal blood of healthy donors and incubated with *L. chagasi* in the presence or absence of heme (30µM). After 3h of infection, we evaluated the production of reactive oxygen species (ROS) by flow cytometry. Infection of neutrophils with *L. chagasi* in the presence of heme increased the ROS production (MFI: 365.5 ± 144.7) when compared with *L. chagasi*-infected neutrophil (MFI: 250.5 ± 87.63) or non infected controls (MFI: 131.5 ± 53.81). We also observed by staining with Annexin V that treatment with heme in *L. chagasi*-infected neutrophils significantly increased the percentage of death cells (62.85% ± 8.604) when compared to infected neutrophils (2.41% ± 0.724) or neutrophil control (2.66 % ± 0.805). In addition, higher amounts of myeloperoxidase (MPO), was increased in infected neutrophils in the presence of heme (1.292 ± 0.486) when compared with *L. chagasi*-infected neutrophil (0.234 ± 0.042) or non infected control (0.245 ± 0.506).

Conclusion: Taken together, these data suggest that heme induces oxidative stress on *L. chagasi*-infected neutrophils and decreases the survival of these cells. Thus, our study suggests that heme can interfere in the establishment of Leishmania infection in host neutrophils.

Key-words: *Leishmania chagasi*, neutrophils, heme.

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UNDERSTANDING IMMUNE AND INFLAMMATORY RESPONSES DEVELOPED BY BALB/C MICE INFECTED WITH *LEISHMANIA (VIANNIA) BRAZILIENSIS,* A RESISTANT INFECTION MODEL FOR LEISHMANIASIS

MÍRIAM CONCEIÇÃO DE SOUZA TESTASICCA[(1)]; AMANDA BRAGA DE FIGUEIREDO[(2)]; JULIANA PRISCILA VAGO[(1)]; THAÍS ROLLA DE CAUX[(1)]; SIMONE APARECIDA REZENDE[(2)]; LUÍS CARLOS CROCCO AFONSO[(2)]; LIRLÂNDIA PIRES DE SOUSA[(1)]; ANA PAULA FERNANDES[(1)]

[(1)]Faculdade de Farmácia, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil; [(2)]Universidade Federal de Ouro Preto, Ouro Preto, MG, Brasil;

Introduction: Human infection with *L. braziliensis* has been associated with a broad range of clinical manifestations, from simple single lesions to the mucosal form. However, there are fewer studies about infection with this parasite species in mice models in comparison with other species of this genus, which makes difficult the identification of the immune and inflammatory mechanisms associated to infection. Studies characterizing the interaction between these parasites and their hosts can expand the understanding of the pathology associated with infection and potentially identify new biomarkers for resistant and susceptibility profiles of infection that could be applicable to vaccine and chemotherapy studies. Thus, the aim of this work was to characterize the kinetics of the immune and inflammatory responses developed by BALB/c mice after experimental infection with *L. braziliensis*. **Methods and Results:** Our results show that *L. braziliensis* infection of BALB/c mice causes a lesion that is self-limiting, in which parasites are found at initial time of infection (2 weeks) and declined thereafter (analyzed by limiting dilution assay) as expected for a resistant infection profile. Splenocytes of the infected animals respond to the re-stimulation with specific antigen producing IFN-γ. In our model, the mice did not develop T multifunctional cells and memory $CD4^{+}CCR7^{+}CD62L^{+}$ in response to infection. Analysis of signaling pathways involved in inflammatory responses showed an increase of ERK1/2 phosphorylation at initial stages of infection that decreased at the times of resolution of the lesion. Interestingly, it was observed an increase in the anti-inflammatory protein annexin A1 in the initial stages post-infection. **Conclusion:** Together, these results confirm that infection of BALB/c mice by *L. braziliensis* is an infection resistance model and let us formulate strategies for better characterize the inflammatory response. Further investigations of the immune and inflammatory profile caused by *L. braziliensis* compared to those induced by other species may permit a better understanding of the role of inflammatory signaling pathways in leishmaniasis and its possible role in the prognosis of the disease development.

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ROLE OF ADENOSINE AND PROSTAGLANDIN E2 ON TICK INFESTATION

MICHELLE CHRISTIANE RODRIGUES BARBOSA[1]; CARLO JOSÉ FREIRE DE OLIVEIRA[2]; ELEN ANATRIELLO[1]; LAUREN CRISTINA DA SILVA RIBEIRO[1]; ISABEL KINNEY FERREIRA DE MIRANDA SANTOS[1]; BEATRIZ ROSSETTI FERREIRA[3].

[1]School of Medicine of Ribeirão Preto, USP-SP
[2]Federal University of Triângulo Mineiro, UFTM, Uberaba-MG
[3]School of Nursing of Ribeirão Preto, USP-SP

Introduction: Saliva of ticks contains several components with anti-haemostatic, anti-inflammatory and immunomodullatory properties. Our group recently demonstrated that *Rhipicephalus sanguineus* tick saliva contains adenosine (ADO) and prostaglandin E2 (PGE2), two non-protein molecules that have important immunomodullatory properties, including cytokine production. PGE2 acts on the immune response cells via receptor EP1, EP2, EP3, and EP4, as ADO via receptors A1, A3, A2a and A2b.
Objectives: Evaluate the importance of ADO and PGE2 on tick-infested mice in vivo.
Methods and Results: C57/Bl6 mice were submitted to a R. sanguineus tick infestation with adult ticks (3 couples/mice) and in the 1st, 4th and 7th day we collected the skin at the tick-infested site. Subsequently, we evaluated the expression of genes for the receptors of ADO and PGE2, as well as IL-4 and IL-13 using the real time PCR assay. Tick infestation increased in 5 times (p<0.05) the mRNA expression of the EP2 receptor in the skin collected on day 7, while did not affect the expression of ADO receptors. Related to the cytokine analysis, we observed a significant increase in IL-4 and IL-13 (4 and 8 times rise, respectively, p< 0.05) in the tick-infested skin compared to the controls. To further evaluate the role of adenosine in tick-infestation, we treated C57Bl/6 mice (n=10/per group) daily with sulfophenyltheophylline 8-p-(8-pSPT, 20 mg/kg, ip), a non-selective antagonist of adenosine receptors, and evaluated the biological performance of ticks during an infestation on mice. Ticks fed on mice treated with 8- pSPT presented a reduction in the female engorged weight (29% of decrease, p<0.05) compared to the control group. Other biological parameters, such as percentage of engorged female ticks, egg mass weight e reproductive index were not impaired.
Conclusion: These results suggest that a better understanding of the role of non-protein molecules, such as ADO and PGE2, can help the development of products to be used to combat ticks.

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CRITICAL ROLE OF IL-17 IN THE CONTROL OF *Leishmania infantum* INFECTION THROUGH THE MODULATION OF NITRIC OXIDE PRODUCTION

Manuela S. L. Nascimento[1], Vanessa Carregaro[1], Djalma S. L. Júnior[1], Diego L. Costa[1], Laís A. Sacramento[1], Fabrício Dias[1], Dario Zamboni[1], Marcos A. Rossi[1], Amélia de Jesus[2], Roque P. Almeida[2], João S. Silva[1].

[1] – School of Medicine of Ribeirão Preto. São Paulo, Brazil – USP
[2] – Biological and Health Science Center. Aracaju, Sergipe, Brazil – UFS.
E-mail: manuelasales@usp.br

Introduction: Although IL-17 is involved in host defense against several pathogens, its function during *Leishmania infantum* infection, the etiologic agent of visceral leishmaniasis (VL) in Brazil, is unknown. Our aim was to evaluate whether IL-17 producing cells participates in the host immune response against *L. infantum* and such mechanisms involved. **Methods and Results:** Our results showed that *L. infantum* induces an up regulation of *tgf-β* (7 times), *il-1β* (1.7 times), *il-6* (2.3 times), and *il-23p19* (2.5 times) mRNA expression by infected-bone marrow-derived dendritic cells (BMDC). In addition, we detected an up regulation of IL-17 production by lymphocytes co-cultured with infected-BMCDs. In fact, *in vivo,* we observed the IL-17 production in the liver (1300 pg/mL) and spleen (145 pg/mL) of C57BL/6 WT infected mice, being peaked at the 4th and 6th weeks after infection. Furthermore, IL-17R$^{-/-}$ mice fail to kill the parasite and showed lower hepatosplenomegaly than WT mice. In accordance, a lower inflammatory infiltrate was observed in IL-17R$^{-/-}$ animals as a consequence of significant lesser expression of *cxcl10*, *cxcr3*, *ccl5* and *ccr5* in the liver and spleen, when compared to WT mice. Interestingly, an up regulation of *ccl17* and *ccr4* was observed in knockout animals. In agreement, IL-17R$^{-/-}$ mice had an increase on Foxp3 and IL-10 expression in the site of infection. Corroborating these data, IL-17 production was 3 times potentiated in IL-10$^{-/-}$ infected mice, which were more resistant to infection when compared to WT. Besides, in the absence of IL-17R a smaller expression of IFN-γ, Tbet and iNOS was detected in the liver and spleen. Indeed, IL-17R$^{-/-}$ infected-macrophages showed a deficiency in the NO production. The stimulus with only IL-17 in WT infected-macrophage increase the NO amounts, moreover, IL-17 acts synergistically with IFN-γ potentiating the NO production and the macrophage leishmanicidal capacity. The mechanism by which IL-17 production is regulated involves both caspase-1, that potentiates its production, and RIP2, that inhibits. **Conclusion:**

Taken together, our results indicate that *L. infantum* triggers IL-17 producing cells through a caspase-1 dependent mechanism, that promotes the control of parasite replication by inducing NO expression and IL-10 modulation.

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MODULATION OF T AND B CELL TLR EXPRESSION DURING TICK INFESTATION

ELEN ANATRIELLO[1]; LAUREN CRISTINA RIBEIRO[2]; MANUELA SALES LIMA NASCIMENTO[2]; LAIS AMORIM SACRAMENTO[2]; CARLO JOSÉ OLIVEIRA[3]; MICHELLE CHISTIANE RODRIGUES BARBOSA[1]; JOÃO SANTANA DA SILVA[2]; ISABEL KINNEY FERREIRA DE MIRANDA SANTOS[2]; BEATRIZ ROSSETTI FERREIRA[1].

[1] School of Nursing, University of São Paulo, Ribeirão Preto, Brazil

[2] School of Medicine, University of São Paulo, Ribeirão Preto, Brazil.

[3] Institute of Biological and Natural Sciences, Federal University of Triângulo Mineiro –

UFTM, Uberaba, Brazil.[3]

Introdution: The brown dog tick, *Rhipicephalus sanguineus*, is found worldwide, and is one of the most important vectors of diseases to dogs. In order to feed with success, ticks inoculate saliva that modulates both, innate and acquired immune responses of their hosts. Our group has shown that mice infested with *R. sanguineus* ticks develop a Th2 type immune response, and that tick saliva modulates the function of dendritic cells (DCs) stimulated by Toll like receptors (TLRs) ligands. We also observed that tick saliva induces *in vitro* a high expression of TLR2 on the DCs surface. These results suggest that saliva may modulate the host immune response via TLR impairment in diverse cell kinds. **Objectives:** The present work evaluates the role of tick infestation on the expression of TLRs molecules on cells from the acquired immune response.

Methods and Results: We examined the percentage of T and B spleen cells expressing TLR1, 2, 4, 5, 6 and 9 from twice-tick-infested C57Bl/6 mice (n=4/group, 6 ticks/mice) using flow cytometry. The results showed that T and B cells expressing TLR4 were diminished (22 and 28% of reduction, respectively, $p<0.05$ for both), while expressing TLR5 were enhanced (16 e 67% of increase, respectively, $p<0.05$ for both) in the tick-infested group compared to the controls. No significant differences were observed for T and B cells expressing TLR1, 2, 6, and 9 molecules.

Conclusion: Our findings suggest that tick saliva alters the expression of TLR not only on cells from the innate immune response, but also on cells of the acquired immune response. A better understanding of this phenomenon can contribute to new perspectives for the control of the ticks.

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IMMUNOENDOCRINE INTERACTIONS DURING LYMPHOCYTE MIGRATION IN HUMAN CHAGAS DISEASE

LUIZ RICARDO BERBERT[(1)]; ANA ROSA PÉREZ[(2)]; ROMINA MANARIN[(2)]; FLORENCIA GONZALEZ[(2)]; JACKELINE PETRUCCI[(2)]; JUAN BELOSCAR[(2)]; OSCAR BOTTASSO[(2)]; WILSON SAVINO[(1)]

[(1)]Laboratory on Thymus Research - Oswaldo Cruz Institute – Brazil; [(2)]Immunology Institute – Rosario National University – Argentina.

Introduction: Chagas Disease remains a public health issue in Americas. In the pathological processes seen in patients, we found changes in imunoneuroendocrine interactions, which might be related to an imbalance in lymphocyte migration to inflammatory sites. We evaluated T lymphocyte migratory responses from chagasic patients with different forms of cardiopathy, correlating these events to immunoendocrine alterations that occur during chronic disease. We first observed that pro-inflammatory cytokines were more expressed in parallel with the severity of disease. Additionally, we found an imbalance on Hypothalamus-Pituitary-Adrenal axis, where a decreasing of DHEA hormone results in changes of circulating cortisol/DHEA ratio. Also in parallel, we found an enhanced migratory response over fibronectin, CXCL12 and TNF-α, as well as with Cortisol and DHEA pre-treatment. These results suggest that endocrine disturbances, correlated to an inflammatory profile, may contribute to increase migratory potential of T lymphocytes to inflammatory sites and myocarditis.

Methods and results: Chronic chagasic patients were grouped into INDETERMINATE (n=18), MODERATE (n=18) and SEVERE (n=15) cardiopathy degrees, as well as CONTROL donors (n=7). By ELISA assays we observed that pro-inflammatory molecules such as IFN-γ (10-13 pg/ml SEV *vs* 5 pg/ml CT), TNF-α (29-37 pg/ml SEV *vs* 3 pg/ml CT), IL-17 (30-45 pg/ml SEV *vs* n.d. CT) and IL-6 (4-4.5 pg/ml SEV *vs* 2-2.2 pg/ml CT) were higher expressed during chronic disease, and it was directly related to cardiopathy degrees, as well as an imbalance on Hypothalamus-Pituitary-Adrenal axis, where a decreasing of DHEA hormone leads to dirturbances on circulating cortisol/DHEA ratio (3-3.5 AU SEV *vs* 1-1.2 AU CT). We also observed by *in vitro* Transwell migration, an enhance on migratory response over fibronectin ($9.5x10^4$ cells ± 4 SEV *vs* $3.8x10^4$ cells ± 6 CT), CXCL12 ($12.6x10^4$ cells ± 5 SEV *vs* $1.8x10^4$ cells ± 5 CT), fibronectin + CXCL12 ($13x10^4$ cells ± 9 SEV *vs* $2.9x10^4$ mean ± 4 CT) and fibronectin + TNF-α ($25x10^4$ cells ± 7 SEV *vs* $9.3x10^4$ cells ± 4 CT) as well as with Cortisol and DHEA pre- treatment in different concentrations (preliminary results).

Conclusion: These results indicate that endocrine disturbances, correlated to a systemic inflammatory profile, may also contribute to enhance migratory potential of T lymphocytes to inflammatory sites, including the heart tissue, being thus involved in the cardiopathy seen in this disease.

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LYMPHOID ORGANS BEHAVIOR AND DINAMIC OF LYMPHOCYTE POPULATIONS IN EXPERIMENTAL *TRYPANOSOMA CRUZI* ORAL INFECTION

JULIANA BARRETO DE ALBUQUERQUE (PG)1; DÉSIO AURÉLIO FARIAS DE OLIVEIRA1; DANIELLE SILVA DOS SANTOS1; LUIZ RICARDO BERBERT1; CARLA EPONINA DE CARVALHO PINTO2; JOSÉ JURBERG1; VANDA CUNHA1; WILSON SAVINO1; JULIANA DE MEIS1

1Oswaldo Cruz Institute, Oswaldo Cruz Foundation, Brazil;

2Immunobiology Institute, Federal Fluminense University, Brazil

Introduction: *Trypanosoma cruzi* (*T. cruzi*), the causative agent of Chagas´ disease, infects about 10 million of people worldwide (WHO, 2010). The oral transmission by ingestion of contaminated food is causing outbreaks in several Brazilian states and other Latin America countries, and is potentially unrestricted to endemic regions (Int J Parasitol 39:615-623, 2009). Although we have previously described regional immune response in lymphoid organs in intraperitoneal (IP) murine infection (PLoS Negl Trop Dis 3(7):e417, 2009), there is a lack of data in oral infection/intragastric (IG). Here we analyze peripheral lymphoid organs behavior and dinamic of lymphocyte subpopulations in mice infected by IG or IP route. **Methods and Results:** BALB/c mice were infected with 5x104 *T. cruzi* trypomastigotes IP or IG and parasitemia was followed. After 9 (IP:n=8, IG:n=8), 12 (IP:n=3, IG:n=5), 15 (IP:n=1, IG:n=2), 17 (IP:n=1, IG:n=3) and 27 days (IG:n=3), subcutaneous (SCLN) and mesenteric lymph nodes (MLN), spleen (Sp) and Peyer patches (PP) were harvested for cell counting and flow cytometry. Hearts were harvested at 15 dpi (IP:n=3, IG:n=2) and sections stained with Hematoxilin and Eosin. IG infected mice presented lower parasitemia and mortality than IP infected mice. In IP infection, 95% of mice died before 18 dpi, while in IG group, 90% were still alive 27 dpi. SCLN (controls=19,5x106±2,9 cells; IP=57,6x106±9,1 cells; IG =31,8x106±3,2 cells) and Sp (controls=239,1x106±49,7 cells; IP=776,3x106±180,9 cells; IG=307,5x106±80,3 cells) showed increased number of cells in both groups 9 dpi and similar profile in later days; however, hypertrophy of these tissues as well as T and CD19+ cells expansion was less evident IG mice than IP. In mucosal-associated lymphoid tissues, such as PP, after 9 dpi the atrophy was more evident in IP mice than IG (controls=1,5x106±0,4 cells; IP=0,5 x 106±0,2 cells; IG=0,8x106±0,1 cells), due to decrease in CD19+ and CD4+ cells. MLN atrophy was only observed in after 15 dpi (controls= 40,1x106±4,5 cells; IP =11x 106 cells; IG=19,3x106±9,3 cells), due to depletion of CD19+ cells. Heart histological analysis showed similar alterations in 15 dpi (parasite nests, inflammatory infiltrate and tissue damage) in both groups, even with the lower

number of circulating parasites in IG mice. **Conclusion:** Our results indicate that the route of *T. cruzi* infection is a key factor to stimulate the immune response against the parasite in the host.

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Analysis of serial lectin affinity chromatography (SLAC) for diagnostic of Toxoplasmosis.

TAMIRES LOPES SILVA[a], FERNADO DOS REIS CARVALHO[a], ANA CLÁUDIA ARANTES MARQUES PAJUABA[a] , DEISE APARECIDA DE OLIVEIRA SILVA[a], FERNANDA MARIA SANTIAGO[a],JOSÉ ROBERTO MINEO[a].

[a] Laboratory of Immunoparasitology Dr. Mário Endsfedlz Camargo, Institute of Biomedical Sciences, Universidade Federal de Uberlândia, 38400-902 Uberlândia, MG, Brazil

Introduction: Toxoplasmosis is a disease of great importance due to a big number of people in all world infected with *Toxoplasma gondii*. The problem of this disease is when the individual is immunosuppressed or for the fetus casing bad formation or loss of the baby. An efficient diagnostic is necessary considering the different stages of the disease. So we pretend isolate a protein of the parasite that can be used as a marker of infection. **Methods and Results:** For this work, serum samples from patients of Clinical Hospital of Uberlândia, positive for toxoplasmosis were selected by using ELISA. In this study we use Spin columns of Agarose bound to ConA, WGA, or Jacalin for serial lectin affinity chromatography (SLAC). A previous experiment showed that the most purified separation occurred after passing through all columns. The band in western blotting that refers to p30 can be seen in all eluates. The next step is testing the eluate which is more sensible to Western Blotting. **Conclusion:** It is very hard to obtain an appropriate diagnostic in the time of infection. The p30 is a great candidate to identify the infection but the same does not happen for the period of infection.

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CRITICAL ROLE OF IL-6 IN REGULATORY T CELLS DETERMINES THE OUTCOME OF GUT INFLAMMATION DURING *TOXOPLASMA GONDII* INFECTION

MURILO SOLANO-DIAS(1), LUCIANA BENEVIDES(1), MARIA C. SOUZA(1), GIULIANO BONFÁ(1), RODRIGO R. RODRIGUES(1), BRUNA B. CHAHUD(1), TIAGO W. P. MINEO(3), ALEXANDRE S. BASSO(2), DENISE M. FONSECA(1), JOÃO S. SILVA(1)

(1) Department of Biochemistry and Immunology - Ribeirão Preto Medical School - University of São Paulo - Ribeirão Preto, Brazil; (2) Department of Microbiology, Immunology and Parasitology, Federal University of São Paulo-São Paulo – Brazil; (3) Institute of Biomedical Sciences - Federal University of Uberlândia - Uberlândia – Brazil

Introduction: Recent findings point out for the role of regulatory T cells (Treg) in the protection against inflammatory diseases induced by parasite infections. The oral infection with *Toxoplasma gondii* leads to an intense inflammation in the gut of susceptible C57BL/6 mice, which succumb to the infection due the reduction of Treg frequency and suppressive function. Even though the Th1 related cytokines have been associated to this event, it has been reported that pro-inflammatory cytokines, such as IL-6, might induce the conversion of natural Tregs in Th17 cells. Therefore, in this study we evaluated the role of IL-6 in the impairment of Treg during the intestinal inflammation induced by *T. gondii.* **Methods and Results:** Susceptible C57BL/6 and resistant BALB/c mice were orally infected with 40 cists of *T. gondii*. Eight days post infection we detected a significant reduction on CD3+CD4+ Foxp3+ Treg cells in the Lamina Propria, Peyers Patches and spleens of C57BL/6 mice as compared to BALB/c mice. The infection compromised mainly the population of induced Treg cells, since the ratio of induced/natural Tregs (determined by Helios expression) was also reduced in mesenteric Lymph nodes (LNM) and spleens of C57BL/6 mice, which also exhibited an increased IL-6 production compared to BALB/c. The protective role of Tregs were shown by depleting Tregs from resistant mice with anti-CD25 antibody (PC61). The anti-CD25 treated BALB/c mice became susceptible to the infection, presented a higher effector/Treg ratio, and produced a larger amounts of IFN-□ and IL-6 than non-treated mice. Since C57BL/6 infected mice produced higher levels of IL-6 than infected BALB/c mice, we supposed that this cytokine could be involved in the Treg impairment during the infection. To evaluate this, spleen cells were infected *in vitro* with *T. gondii* and treated with recombinant IL- 6. The *in vitro* infection reduced the frequency of Tregs and the addition of IL-6 to the cultures accentuated even more the impairment of Tregs. Indeed we found that IL-6-/- mice were resistant to the infection and presented higher Treg frequency and IFN-□ production in the LNM and spleens. After the anti-CD25 treatment IL-6-/- mice became

susceptible to the infection and produced higher amounts of IFN-□. **Conclusion:** Our data show that the IL-6 production induced during *T. gondii* infection subverts Treg cell population resulting in the susceptibility to the intestinal inflammation induced by the parasite.

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PPAR-α AGONIST GEMFIBROZIL DECREASES SPLEEN TREG CELLS AND AUGMENT MICE SUSCEPTIBILITY TO *Toxoplasma gondii* INFECTION

GIULIANO BONFÁ [(1)]; LUCIANA BENEVIDES [(1)]; MONIQUE THAÍS COSTA FONSECA [(1)]; FRANCIELLE RODRIGUES GUIMARÃES [(2)]; DENISE MORAIS FONSECA [(1)]; NEIDE MARIA SILVA [(3)], JOÃO SANTANA SILVA [(1)]; CRISTINA RIBEIRO DE BARROS CARDOSO [(2)]

(1) Department of Biochemistry and Immunology - School of Medicine of Ribeirao Preto - FMRP/USP - SP - Brazil; (2) Department of Clinical Analyses, Toxicology and Food Sciences - School of Pharmaceutical Sciences of Ribeirao Preto - FCFRP/USP - SP - Brazil; (3) Biomedical Science Institute - Federal University of Uberlândia - ICB/UFU - MG - Brazil. E-mail: giubonfa@usp.br

Introduction: Peroxisome proliferator-activated receptors (PPARs) are nuclear receptors of which PPARα is the most studied. PPARs function as lipid sensors, regulating metabolism and immune response by acting as transcription factor resulting in the inhibition of pro-inflammatory cytokines. *Toxoplasma gondii* infection induces a robust Th1 inflammatory response similar to Inflammatory Bowel Disease (IBD) with excessive IFN-γ, TNF and nitric oxide (NO) production. The role of PPAR in the gut inflammation caused by *T. gondii* in some mice lineages and its possible association with regulation of immune response is still unclear. Then, the aim of this work was to evaluate the role of activated PPAR-α in the susceptibility to *T. gondii* infection.
Methods and Results: C57BL/6 mice were orally infected with cysts of *T. gondii,* ME-49 strain. The transcripts of PPAR-α were evaluated at 0, 4, 6 and 8 days post-infection (p.i.) by real time PCR (qPCR) of ileum and liver on mice inoculated with 40 cysts. One group of animals infected with 5 cysts was treated with PPAR-α agonist (Gemfibrozil, GEM) for mortality assessment, ileum and liver histological evaluation. Spleen cells were phenotyped by flow cytometry. PPAR-α expression was downregulated in ileum and liver at day 8 p.i. when compared to the previous periods evaluated. Interestingly, when mice were treated with GEM at 10mg/Kg/day for 7 days p.i. there was an increase in mortality. Infection increased inflammatory infiltrate in the ileum associated with visible edema at the villi in vehicle and GEM treated mice. However, GEM group presented an intense inflammation with reduced length and larger villi. Liver inflammatory infiltrate was also increased and more diffuse in GEM treated mice. In addition, there was an increased in NK and NKT cells frequency and decreased number of T CD8 and T regulatory cells ($CD4^+CD25^+Foxp3^+$) in the spleen of GEM treated mice.
Conclusion: These data indicate that PPAR-α is modulated by *T. gondii* infection in susceptible mice. Besides its known anti-inflammatory function, activation of this nuclear receptor led to increased susceptibility to parasite induced intestinal damage associated to decreased splenic Treg cells. These

results lead to a better understanding of the susceptibility to this infection and provide basis for future approaches aimed at controlling exacerbated gut inflammation.

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Cutaneous Leishmaniasis in Amazonas, BR: Levels of citokines IL5 and IL12 in patients infected by different species of *Leishmania.*

THAIS TIBERY ESPIR [1,2]; LUANDA DE PAULA FIGUEIRA [1,2]; MARICLEIDE DE FARIAS NAIFF [2]; ALLYSON GUIMARÃES DA COSTA [1,3]; JOÃO PAULO DINIZ PIMENTEL[3]; ADRIANA MALHEIRO[1,3]; ANTONIA MARIA RAMOS FRANCO[2].

(1). Federal University of Amazonas; (2) National Institute of Amazonia Research; (3) Hematology and Hemotherapy Foundation of Amazonas.

Introduction: The northern region has the highest incidence of leishmaniasis in Brazil, estimated at more than 2,000 new cases per year only in the state of Amazonas. Clinical manifestations are related to different species of *Leishmania* and the host immune response. The objective of this preliminary study was to evaluate the immune response of patients infected with *Leishmania* by analysis of cytokines and different species involved in the disease. **Methods and Results:** This study of case series was conducted from June 2009 to February 2012, from five cities in the state of Amazonas. Was analyzed twelve patients aged between 21 and 58 years old, with the duration of the injury about a month in 83% of cases, most activities in the forest area in 100% of cases. Species were characterized as *L. amazonensis*, *L. braziliensis* and *L. guyanensis* by isoenzyme electrophoresis technique. Cytokines were measured in the sera by ELISA (BD Biosciences). The levels of IL-12 in the sera of patients infected with all species, was 68% higher after treatment with pentavalent antimony N-methylglucamine compared with samples before the treatment, the level of IL-5 showed inverse proportion compared to IL-12 and the level of IL-5 had higher elevation before treatment with the same drug. The predominant parasite species was *L. guyanensis* (80%) and there was no significant difference ($p > 0.005$) in cytokine levels before and after treatment to infection by different species. **Conclusion:** The data presented contributes to the understanding of the immune response to infection by species of Leishmania. It is noted that the determination of Th1 and Th2 responses can be influenced differently depending on the species of *Leishmania* and other factors in addition to the genetic and immunological features of the host.

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MYCOBACTERIUM BOVIS-HOST INTERACTIONS DURING NATURAL INFECTION OF BOVINES: STUDIES ON THE TISSUE PATHOLOGY AND GRANULOMATOUS RESPONSE

RENATA FLEITH[1]; ALVARO MENIN[1]; CAROLINA RECK[3]; MARIEL MARLOW[4]; PAULA FERNANDES[1]; CELSO PILATI[3] AND ANDRE BÁFICA[1,2]

[1]Laboratory of Immunobiology, Universidade Federal de Santa Catarina, Brazil;
[2]Howard Hughes Medical Institute Early Career Scientist, HHMI, USA
[3]Laboratory of Histology and Immunohistochemistry, Universidade do Estado de Santa Catarina, Brazil
[4]Laboratory of Protozoology, Universidade Federal de Santa Catarina, Brazil

Introduction: Rational discovery of novel immunodiagnostic or vaccine candidate antigens to control bovine tuberculosis requires knowledge of disease immunopathogenesis. However, there remains a paucity of information on the *Mycobacterium bovis*-host immune interactions during the natural infection.
Methods and Results: Clinical-pathological and mycobacterial analysis in 247 naturally *M. bovis*-infected cattle were performed. Patterns of host-immunopathological response and infection severity were analyzed and correlated with bacterial burden. Statistical analysis was assessed by nonparametrical analysis applying the Spearman rank correlation (r). Data of pathology analysis revealed that 92% (228) of these animals were found to display no clinical signs, but presented severe as well as disseminated bTB-lesions. Moreover, dissemination of bTB-lesions positively correlated with both pathology severity score (Spearman r = 0.48; p <0.0001) and viable tissue bacterial loads (Spearman r = 0.58; p = 0.0001). Additionally, granuloma encapsulation negatively correlated with *M. bovis* growth as well as pathology severity, suggesting that encapsulation is an effective mechanism to control bacterial proliferation during natural infection. Moreover, multinucleated giant cell numbers were found to negatively correlate with bacterial counts (Spearman r = 0.25; p=0.03) in lung granulomas. In contrast, neutrophil numbers in the granuloma were associated with increased *M. bovis* proliferation (Spearman r = 0.27; p=0.021).
Conclusion: Together, our findings suggest that encapsulation and multinucleated giant cells control *M. bovis* viability, whereas neutrophils could serve as a cellular biomarker of bacterial proliferation during the natural infection. These data integrate host granuloma responses with mycobacterial dissemination and could provide useful immunopathological-based biomarkers of disease severity in natural infection with *M. bovis*, an important cattle pathogen.

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CYTOTOXICITY IN IMMUNOPATHOGENESIS OF AMERICAN TEGUMENTARY LEISHMANIASIS – PRELIMINARY RESULTS

CLARISSA FERREIRA CUNHA (PG)[1]; RAQUEL FERRAZ (PG)[1,2]; ALDA MARIA DA-CRUZ[3]; ARMANDO SCHUBACH[4]; MARIA INÊS F. PIMENTEL[4]; MARCELO R. LYRA (PG)[4]; CLÁUDIA M. VALETE-ROSALINO[4]; ÁLVARO L. BERTHO[1, 2]

[1]Laboratório de Imunoparasitologia; [2]Plataforma de Citometria de Fluxo; [3]Laboratório Interdisciplinar em Pesquisas Médicas; Instituto Oswaldo Cruz, FIOCRUZ, Rio de Janeiro, RJ; [4]Serviço de Referência em Leishmaniose, Instituto de Pesquisa Clínica Evandro Chagas, FIOCRUZ, Rio de Janeiro, RJ.

Introduction: The clinical course of American tegumentary leishmaniasis (ATL) depends on the interaction between the parasite and the immune response of patients. Is well known that $CD8^+$ T lymphocytes play a key role in this response due to their cytotoxic potential and cytokine production. Moreover, natural killer cells (NK), natural killer T cells (NKT) and a cytotoxic subpopulation of $CD4^+$ T lymphocytes present similar functions, and therefore few studies point to the roles of these cell populations in the pathogenesis in ATL. The aim of this study is to evaluate the frequency and the cytotoxic effector role of these cell populations, by immunophenotyping and evaluating the expression of CD107a, in blood samples from patients with active disease and at different stages of treatment. **Methods and Results:** Peripheral blood mononuclear cells were obtained from healthy individuals and from patients with active disease, during and after clinical antimonial treatment. These cells were submitted to a flow cytometry analysis to assess the distributions of $CD4^+$ or $CD8^+$ T lymphocytes, NK and NKT cells, and their cytotoxicity profiles by identifying the CD107a expression. Preliminary results have allowed us to observe that the percentage of $CD8^+$ T cells is higher in patients after treatment compared to patients with active disease and, conversely, is noted a decrease in the frequency of $CD4^+$ T lymphocytes, NK and NKT cells in this patients. In addition, it was observed a higher percentage in both $CD8^+$ T and NK cells that express CD107a in patients after treatment under specific in vitro stimulation. **Conclusion:** These preliminary results corroborate the important role of $CD8^+$ T lymphocytes in the resolution of the disease, showing still a most remarkable cytotoxic activity of this cells as well as NK after clinical cure of ATL. More experiments are being performed in our laboratory so such results will be discussed further.

Support: IOC/FIOCRUZ; IPEC/FIOCRUZ; CAPES; CNPq; Beckman Coulter

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AREA: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

TOLL-LIKE RECEPTOR 4 MEDIATE IL-6 AND TNF-α PRODUCTION DURING PLACENTAL MALARIA.

Aramys Silva dos Reis[1]; Renato Barboza[1]; Niels Olsen Saraiva Câmara[2]; Silvia Beatriz Boscardin[1]; Sabrina Epiphanio[3] & Claudio Romero Farias Marinho[1]

[1] Departamento de Parasitologia, ICB, Universidade de São Paulo, São Paulo, Brasil; [2]Departamento de Imunologia, ICB, Universidade de São Paulo, São Paulo, Brasil; [3]Departamento de Ciências Biológicas, Universidade Federal de São Paulo, Diadema, Brasil.

Introduction: Immune system activation induced by *Plasmodium* ssp. during pregnancy leads a production of pro-inflammatory cytokines that may culminate in a range of complications affecting both mother and fetus. It has been described that Toll-like receptor 4 (TLR4) recognize components of *Plasmodium* sp. and affect the host immune response. The objective of this work was to evaluate the effects of TLR4 on development of placental malaria induced by *P. berghei* NK65. **Methods & Results:** To verify the effects of TLR4, we infected pregnant TLR4$^{-/-}$ and C57BL/6 (WT) mice with *P. berghei* NK65 on the 13th gestational day. To obtain the spleen cells, at 19th gestational day the animals were killed and their spleen were removed and processed. Analysis of spleen cell population of infected WT mice by flow cytometry showed an increase of T CD4^{+} cells, B cells (CD19^{+}), granulocytes (Gr1^{+}) and NK1.1^{+} when compared with non-infected mice. However, analysis of TLR4$^{-/-}$ strain not showed difference between infected and non-infected mice. Moreover, TLR4$^{-/-}$ mice cell populations of both groups were similar to the control group (non-infected WT). Next, we evaluated serum cytokine production from infected and non-infected pregnant mice by cytokine beads array (CBA). CBA analysis showed that infected WT and TLR4-/- mice presented an increase of pro-inflammatory cytokines IL-6, TNF-alfa and IFN-gamma when compared with the controls. Comparison of infected mice groups of both strains showed that WT mice presented a huge increase of IL-6 and TNF-alfa, but the IFN-gamma production was not different. To evaluate the effect of inflammatory process on the placenta, we conducted a histopathological analysis. Our results showed that only infected WT mice presented a decrease of vascular space (indicative of placental injure). **Conclusion:** The results presented here suggest that TLR4 is important for the development of placental malaria due an inflammation process mediated by the IL-6 and TNF-alfa.

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ROS production and TLR2 and 4 expression in PBMC from dogs with visceral leishmaniasis.

LARISSA MARTINS MELO(PG)[1]; LETICIA DA CRUZ SANCHES(PG)[1]; KATHLENN LIEZBETH DE OLIVEIRA DA SILVA(PG)[1]; JULIANA PEROSSO BORGES(PG)[1]; BRUNA BRITTO DE OLIVEIRA(IC)[1]; MARCOS DE ARRUDA SOMENZARI (PG)[1]; VALÉRIA MARÇAL FÉLIX DE LIMA(PD)[1]

[(1)]Laboratório de Imunologia, Depto. Clínica, Cirurgia e Reprodução Animal – Faculdade de Medicina Veterinária -UNESP - Araçatuba -SP

Introduction: The canine Visceral Leishmaniasis is a zoonotic disease and serious public health problem, because the infected animals are important reservoirs of the parasite. Leishmaniasis visceral is an endemic disease that has spread over several continents, mainly in tropical and subtropical regions. It is caused by *Leishmania chagasi* and affects millions people worldwide. The parasite is transmitted by sandflies, which infect mammals including man and dog, which is considered the most important urban reservoir of *L.chagasi*. Dogs with visceral leishmaniasis are highly susceptible to infection and Th2 immune response in target organs facilitates the spread of the parasite in the host. Toll-like receptors 2 and 4 were involved in *Leishmania* recognition, but the role of TLRs in dog pathogenesis of canine visceral leishmaniasis and the effect of the interaction with parasite in the antimicrobial activity has not been addressed.
Methods and Results: Thirty dogs presenting clinical symptoms compatible with leishmaniasis and positive detection of antibodies anti-leishmania were used, five healthy dogs were used as control. To examine the expression of TLR 2 and TLR 4 in PBMC of infected dogs with *L. chagasi* and controls, PBMCs were double-stained with specific fluorochrome-conjugated antibodies: FITC conjugated monoclonal antibody anti-human TLR2, and PE conjugated monoclonal antibody anti-human TLR4 or control isotypes. Intracellular ROS levels were measured in PBMCs using H2DCFDA (29,79-dichlorodihydroflurescein diacetate), according to manufacturer's intructions. After the acquisition of data in EasyCyte mini ® (Guava, Hayward, CA), the analysis of the data was performed in the Software Guava Express ® Plus. The results were compared using nonparametric tests. Comparison between the groups showed a decrease of TLR4 on PBMC from infected dogs ($p<0.05$), the TLR2 was equally express on PBMC from infected and control dogs. The ROS production was higher in PBMC from infected dogs compared to control dogs ($p<0.05$).

Conclusion: The data reveals that the receptor TLR4 is involved in immune response in symptomatics dogs chronic infected. The high ROS production observed in infected dogs indicates that ROS not showed antimicrobial effect and suggest that ROS could be useful by the protozoa to chronic the infection. Studies are in progress to further determine the mechanisms involved in this process.

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ROLE OF IL-4 IN THE INTESTINAL INFLAMMATION INDUCED AFTER *Toxoplasma gondii* INFECTION

RODRIGO PEREIRA DE ALMEIDA RODRIGUES1; MURILO SOLANO-DIAS1; LUCIANA BENEVIDES1; BRUNA BUENO CHAHUD1; DENISE MORAIS DA FONSECA1; JOÃO SANTANA DA SILVA1.

1- Department of Biochemistry and Immunology - School of Medicine of Ribeirão Preto - University of São Paulo - Ribeirão Preto, Brazil;

Introduction. The oral infection with *Toxoplasma gondii* induces an intestinal inflammation in susceptible mice which succumb to the infection due to the development of a strong Th1 response. However, BALB/c resistant mice control the disease. Much has been studied about the participation of Th1 lymphocytes in the infection and, recently, the role of regulatory T cells (Tregs) and Th17. However, the participation of Th2 lymphocytes is not completely understood. Here we evaluated the involvement of Th2 response during the intestinal inflammation established after *T gondii* infection. **Methods and Results**: Six-to-8 week old female C57BL/6, BALB/c mice and IL-4-/- mice were orally infected with 40 cysts of *T. gondii* (ME49 strain). Mice mortality was monitored daily for 4 weeks or mice were sacrificed after eight days of infection. Spleens and mesenteric lymph nodes were collected for cell isolation and staining with anti-CD3, CD4, CD25, Foxp3 and ROR□t for flow cytometry analyses. In parallel, cells were stimulated for detection of IFN-□, IL-4 and IL-17. Intestinal homogenate was also done to evaluate the production of IL-5 and IFN-□, in the course of the disease. We observed that C57BL/6 infected mice produced higher concentrations of, not only IFN-□, but also IL-5 and IL-4, as compared to BALB/c infected mice, suggesting that Th2 cytokines could be involved in the susceptibility to the disease. Unexpected, IL-4-/- mice were slightly more susceptible to infection than C57BL/6 wild type (WT) mice. IL-4-/- mice died earlier and presented an increased intestinal inflammation. We also observed higher frequency of Treg cells in IL-4-/- compared to WT mice. Indeed, even with the increase on Tregs, the effector (CD4+CD25+Foxp3-) cells were also higher in IL-4-/- mice and there was no change in the ratio between Treg and effector cells compared to WT mice. In addition, there was no change in IL-17 production, but we also detected higher frequencies of IFN-□, -producing CD4+ lymphocytes in IL-4-/-. **Conclusion**: Taken together, these data show that Th2 lymphocytes participate in

the regulation of pro-inflammatory response and, subsequently, in the susceptibility to *T. gondii* infection. Our data also suggest that in the absence of IL-4 the Th1 cells could be more activated or less suppressed despite the fact that we detected more Tregs. Further experiments are undergoing to evaluate the role of IL-4 in the suppressive activity of Tregs during *T. gondii* infection.

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In vitro CULTURE SYSTEM FOR *Plasmodium vivax*

CAROLINE JUNQUEIRA (1),(2); RICARDO TOSTES GAZZINELLI (2),(3),(4); KASTURI HALDAR (1)

(1) The Center for Rare and Neglected Diseases, University of Notre Dame, IN, USA; (2) Centro de Pesquisas René Rachou, FIOCRUZ, Belo Horizonte, MG, Brazil; (3) Departamento de Bioquímica e Imunologia, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil; (4) Division of Infectious Disease and Immunology, University of Massachusetts Medical School, MA, USA

Introduction: *Plasmodium vivax* is estimated to cause approximately 100 million cases of clinical malaria each year, with many more exposed individuals at risk for asymptomatic parasitemia. Although not as virulent as *P. falciparum*, *P. vivax* nonetheless causes significant illness in Latin America, Asia and some parts of Africa. *P. vivax* causes chronic anemia during infancy, and potentially life threatening morbidities such as severe anemia, respiratory distress, renal failure and splenic rupture. A significant obstacle in developing therapies and eradication strategies against the human malaria parasite *Plasmodium vivax* has been the lack of a culture system to propagate sufficient numbers of blood and liver stage parasites. This is because *P. vivax* infects reticulocytes, which are present in low frequency in peripheral blood (generally <2% of the total erythrocyte population). Our project goal is to establish continuous, *in vitro* culturing of blood-stage *P. vivax.* This will enable production of blood stage parasites as well as gametocytes needed for progression of the parasite through the mosquito vector to yield sporozoites, which in turn can be used to infect hepatocytes and produce liver stage parasites. **Methods and Results**: Our critical milestones are to produce pure 'fully functional' reticulocytes at a maximal capacity of 10^{10} per week. The second milestone is to optimize nutrients to stimulate continuous *in vitro* culturing of parasites. Here we show the preliminary data for reticulocytes purification from cord blood and peripheral blood and *in vitro* culture of *P. vivax*. **Conclusion**: Our results can open a new field to evaluate the cell biology of *P. vivax* and also to evaluate neutralizing antibodies induced by vaccines against *P. vivax*.

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STUDIES ON A SECRETED *MYCOBACTERIUM TUBERCULOSIS* RICIN-LIKE LECTIN (SMTL-13) DURING INITIAL INTERACTIONS WITH MACROPHAGES

STEFANNY LUCÍA VILOCHE MORALES(PG)(1), NICOLE MENEZES DE SOUZA, (PG)(1), LUCAS NOGUEIRA(2), VERÔNICA HOREWICZ(PG)(1), DANIEL MANSUR (1), ANDRÉ BÁFICA(1).

(1) Laboratório de Imunobiologia, Departamento de Microbiologia, Imunologia e Parasitologia, Universidade Federal de Santa Catarina
(2) Núcleo de Estudos e Pesquisa em Saúde, Centro de Ciências da Saúde, Universidade Estadual Vale do Acaraú

Introduction: Infection by *Mycobacterium tuberculosis* (Mtb) can lead to a latent state in which the host is able to control the pathogen growth. While effective cellular immune responses are suggested as critical to control Mtb growth inside macrophages, it has been demonstrated that mycobacteria-associated factors play an important role in the outcome of infection. For that matter, mycobacterial proteins are an incredible source for potential adjuvant molecules. We have previously described a novel secreted 13-kDa lectin in pathogenic Mtb (sMTL-13). Experimental data suggest that this protein is recognized by B and T cells in TB patients and acts as a PAMP inducing APC activation. Thus, sMTL-13 can be considered as a potential adjuvant model to stimulate both cellular and humoral immune responses.
Methods and Results: To confirm the presence of sMTL-13 in Mtb cell wall, bacteria were stained with mAb anti-sMTL-13 and analyzed by flow cytometry. This approach demonstrated that sMTL-13, in addition to being secreted, is anchored within the Mtb cell wall, probably by the signal peptide sequence. To gain insights on the mechanisms by which sMTL-13 modulates cellular infection, murine bone marrow-derived macrophages (BMM) were exposed to mycobacteria pre-incubated with or without D-Gal, an sMTL-13 cognate carbohydrate. BMM infected with bacteria pre-treated with D-Gal, but not L-Gal, were found to present decreased numbers of CFU, suggesting that galactose-binding proteins, perhaps sMTL-13, are involved in macrophage-Mtb interactions. Accordingly, compared to untreated or L-Gal pretreated groups, D-Gal-exposed Mtb-infected macrophages displayed diminished TNF production. We next evaluated whether sMTL-13 is secreted during macrophage infection. Confocal microscopy analysis revealed that following 48h of infection, sMTL-13 was found to colocalize with intracellular mycobacteria in macrophages. In addition, the lectin appeared to be present in the cell cytoplasm.
Conclusions: These findings suggest that anchored sMTL-13 probably functions during the initial interactions of Mtb and macrophages. Moreover, this lectin seems to be actively secreted by the bacterium and gains the cytosolic pathway. To elucidate the pathways involved in sMTL-13-macrophage

interactions, Tandem Affinity Purification strategies are in progress to isolate the native multiprotein complex formed by sMTL-13 interaction with host cells.

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ACTIVATION PROFILE OF HUMAN DOUBLE-NEGATIVE T CELLS STIMULATED IN VITRO BY VACCINIA VIRUS AND APEU VIRUS

FERNANDA NOBRE AMARAL VILLANI(1); THAÍS ELOI DA SILVA(1); ERNA GEESSIEN KROON(2); JAQUELLINE GERMANO DE OLIVEIRA(1); PAULO CESAR PEREGRINO FERREIRA(2); RODRIGO CORRÊA-OLIVEIRA(1).

(1)Centro de Pesquisas René Rachou, Fiocruz, Belo Horizonte - MG; (2)Laboratório de Vírus, Departamento de Microbiologia, ICB, UFMG, Belo Horizonte - MG.

E-mail: fernanda.villani@cpqrr.fiocruz.br

Introduction: In the last 30 years we have seen a marked appearance of emerging viral diseases, with approximately 100 new viruses identified, some being related to major epidemics in humans. Among them the *Apeu virus* (APEUV), a *Orthobunyavirus* of group C, has been described in human infections. In addition to emerging viruses, the reemergence of pathogenic viruses with high dissemination rate as the Poxvirus is a reality. Zoonotic human infections caused by *Vaccinia virus* (VACV), a *Orthopoxvirus*, in Brazil reached worldwide recognition. Most researchers that study antiviral immune response mechanisms target the classical cell populations, such as $CD4^+$ T lymphocytes (T helper), $CD8^+$ T lymphocytes (cytotoxic) and NK cells (natural killer). Although these cells certainly have key functions in the immune response to viral infections, other cell populations can also play an important role. Recently it was shown that monkeys infected with immunodeficiency virus SIV, similar to HIV, do not develop clinical disease (AIDS) and that the $CD4^-CD8^-$ T cell population (double-negative-DN) was responsible for compensating at least partially, the function of $CD4^+$ cell depleted in this disease. This suggests that the DN T cells play an important role in the antiviral immune response. The aim of this work was to evaluate the activation profile of DN T cells expressing alpha-beta (αβ TCR) or gamma-delta T-cell receptors (γδ TCR) from healthy humans.

Methods and Results: The peripheral blood mononuclear cells were isolated from four donors and exposed to UV-inactivated APEUV and VACV (WR isolate). The expression of surface markers (CD4, CD8, αβ TCR and γδ TCR) and the intracellular cytokines (IL-1β. IL-6, IL-10, IL-17A, IFN-α, IFN-β, IFN-γ and TNF-α) were assessed by flow cytometry. We observed a higher frequency of αβ DN T cells expressing IL-10, IFN-α and TNF-α in VACV stimulated cells as compared to non-stimulated cells. IFN-γ expression was higher in VACV-stimulated γδ DN T cells compared to non-stimulated cells. We did not observe differences in the expression of these markers in cells stimulated with APEUV.

Conclusion: These results show that DN T cells from healthy humans are highly activated *in vitro* by VACV and suggests an important role of these cells

in the course of human infection with this virus. Further studies will be conducted to investigate the role of these subpopulations in the immune response against APEUV.

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CRUCIAL ROLE OF REGULATORY T CELLS IN THE IL-17- AND IL22-MEDIATED CONTROL OF INTESTINAL INFLAMMATION

DENISE MORAIS DA FONSECA(1); LUCIANA BENEVIDES(1); MARIA DO CARMO SOUZA(1); GIULIANO BONFÁ(1); MURILO SOLANO-DIAS(1); TIAGO W. P. MINEO(2); BERNHARD RYFFEL(3); ALEXANDRE SALGADO BASSO(4); JOÃO SANTANA DA SILVA(1)

(1)Department of Biochemistry and Immunology - Ribeirão Preto Medical School - University of São Paulo - Ribeirão Preto, Brazil; (2)Institute of Biomedical Sciences - Federal University of Uberlândia - Uberlândia - Brazil; (3)Centre National de la Resarche Scientifique, Immunologie et Embryologie Moléculaire – University of Orléans - Orléans – France; (4)Department of Microbiology, Immunology and Parasitology, Federal University of São Paulo-São Paulo – Brazil

Introduction: Understanding the mechanisms involved in the control of mucosal inflammation in diseases established after the breakdown of intestinal tolerance is one of the biggest challenges for science nowadays. In the gut inflammation induced after *T. gondii* infection, the relative importance of each individual Th1, Th17 and regulatory T (Treg) cells has been partially investigated. However, the main question that remains is whether these cells interact and work together to regulate the intestinal immune-mediated disease. Here we determined how Th17, Th22 and Treg cells contribute to intestinal homeostasis after *T. gondii* infection. **Methods and Results:** We found that C57BL/6-susceptible and BALB/c-resistant mice (orally infected with *T. gondii*) exhibited a progressive increase on the frequencies of Th17 and Th22 cells in Lamina Propria and Peyer´s patches. However, whereas C57BL/6 mice showed higher frequency of Th17, BALB/c mice showed higher counts of Th22 and Treg cells. There was higher frequency of induced-Tregs (Foxp3+Helios-) in BALB/c mice, on contrary to C57BL/6 mice in which these cells were almost absent. The ratio between Tregs/Effector cells was significantly increased in C57BL/6 mice and the infection impaired the effector function of Tregs in susceptible hosts. Tregs obtained from C57BL/6-infected mice, but not from BALB/c, showed reduced expression of GITR, CTLA-4, increased PDL1 and reduced suppressive activity *in vitro.* The transfer of Foxp3+ cells sorted from non-infected mice, but not from infected mice, increased C57BL/6 recipient survival. *In vitro* infected spleen cells experiments showed that Tregs impairment was related to the presence of IFN-□, IL-6 and IL-17, but IL-2, IL-22 and TGF-□ were responsible for Treg maintenance. Because of this, we evaluated the role of IL-17 and IL-22 in the disease and we observed that IL-17R-/- and IL-22-/- mice were more susceptible, presented higher parasite burden and intestinal inflammation; and lower Treg frequency than C57BL/6. The transfer of Th17 cells increased C57BL/6 mice survival and transfer of Treg cells restored IL-17R-/- and IL-22-/- mice resistance. **Conclusion:** Taken together, these

findings suggest that IL-17 and IL-22 production are essential for resistance against *T. gondii* infection, by controlling parasite burden and regulating Th1 inflammation. In addition, the presence of Treg cells are critical to the IL-17- and IL22-mediated control of intestinal inflammation.

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THE ROLE OF IL-6, IL-17 AND IL-23 IN THE EXPERIMENTAL PARACOCCIDIOIDOMYCOSIS

FERNANDA AGOSTINI ROCHA[(1)]; NATÁLIA KETELUT CARNEIRO[(1)]; MARCOS ANTÔNIO ROSSI[(2)]; JOÃO SANTANA SILVA[(1)]

(1) School of Medicine of Ribeirão Preto (FMRP) – Department of Immunology University of São Paulo (USP) Brazil; (2) School of Medicine of Ribeirão Preto (FMRP) – Department of Pathology, University of São Paulo (USP) Brazil;

Introduction: *Paracoccidioides brasiliensis* (Pb), a thermally dimorphic fungus, is the causative agent of paracoccidioidomycosis (PCM), one of the most frequent systemic mycosis that affects the rural population in Latin America. T helper cells producing IL-17 (Th17) are involved in host defense against several infections caused by bacterias, protozoarious and fungus, contributing to eliminate the infectious agent. Considering that is necessary better understand the mechanisms involved in resistance to *P. brasiliensis* infection, in this view, we aimed to evaluate the Th17 pathway and related cytokines in resistance mechanisms against to *P. brasiliensis* infection.
Methods and Results: To verify if Pb-infection induces expression of cytokines related with the Th17 immune response, we measured IL-6, IL-17 and IL-23 mRNA by RT-PCR and protein expression by ELISA method in samples from lung homogenate before and after experimental Pb-infection of C57BL/6 (WT) mice. It was possible observe an increased mRNA expression and protein production of the cytokines in the lung from infected compared with uninfected mice. To determine the role of this cytokines during the Pb-infection we used male WT and IL-6, IL-17 receptor (R) and IL-23 knockout (KO) mice that were intravenously inoculated with $1x10^6$ yeast forms of Pb18, a highly virulent Pb strain. The mice were accompanied about the susceptibility, and the IL-6KO mice showed higher mortality rate compared to WT mice. In addition, we quantified the fungal growth by recovering of colony forming units (CFU). We observed that at 15 and 30 dpi, the CFU from the lung, liver and spleen of all KO mice were increased compared to WT mice. To assess the inflammatory response and granulomas formation, histopathological analysis revealed that IL-6 and IL-17 deficiency abrogated the granuloma formation due to impaired induction of reticulin fibers accompanied of diffuse $CD4^+$ T cell infiltration at lung tissue, favoring an increased fungal load. The deficiency of IL-6, IL-17R or IL-23 also induced lower IFN-γ and IL-10 production in the lung compared with WT mice. Additionally, through flow cytometry, it was verified that the frequency of $CD4^+IL\text{-}17^+$ T cells and IL-17 production were decreased in the mice lacking IL-6 or IL-23.
Conclusion: Taken together, these results demonstrate that IL-6, IL-17 and IL-

23 contribute to immunological control of Pb-infection in the experimental model.

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ROLE OF APOPTOSIS IN IL-17 PRODUCTION DURING *CANDIDA ALBICANS* INFECTION

GUSTAVO FERNANDO DA SILVA QUIRINO (IC) (1); CARINA ZERBETTO SEGATO (PG) (1); TACITO GRAMINHA CAMPOIS (PG) (1); RICARDO SERGIO ALMEIDA (2); IONICE FELIPE(1)

(1) - Lab. de Imunologia Experimental, Depto. de Ciências Patológicas – CCB/UEL; (2)–Lab. de Micologia Médica, Depto. de Microbiologia – CCB/UEL

Introduction: *Citrobacter rodentium* is one pathogen that targets mitochondria and induces apoptosis, and blockade of apoptosis during *C. rodentium* infection impairs the characteristic T_H17 response (J. Leukoc.Biol.89, 1-12, 2011). However, there are no evidences that IL-17 production against *Candida albicans* is triggered by this pathway. Our group previously demonstrated that *C. albican*s CR15 was able to induce apoptosis of macrophages *in vitro* whereas *C. albicans* 577 was not. In order to clarify if apoptosis is involved in IL-17 production, the profiles of cytokines production during *C. albicans* infection were compared.

Methods and Results: Strains 577 and CR15 were cultivated in YPD medium for 24h at 30°C, harvested, diluted to $1x10^7$ ml^{-1} PBS and inoculated intraperitoneally in Swiss mice (n=3 per group). After 0.5, 2, 6, 18 and 24 hours, the peritoneal exsudate of the animals was collected with 2ml RPMI 1640 medium, following centrifugation. The supernatant was stored at -20°C to analyze cytokine levels through ELISA method and the cell pellet was added to coverslips in cell culture plates for 1h at 37°C, 5% CO2, to allow the adherence of phagocytes. Groups infected for 0.5 and 2h had their cells stained with Annexin-V-FITC, followed by 4%-paraformaldehyde fixation and then analyzed under fluorescence Leica microscope, to qualitatively assess the induction of apoptosis of phagocytic cells by *C. albicans*. In addition, all groups had their cells fixed in methanol and stained with Giemsa, to assess the cell population in the exsudate. Our results show that *C. albicans* CR15 was able to induce apoptosis of phagocytic cells 30 minutes postinfection, whereas 577 was not. CR15 induced peak levels of IL-1β (45.06 ± 19.9 pg/mL after 2h), IL-6 (33.6 ± 5.1 ng/mL after 24h), TGF-β (155.6 ± 31.21 pg/mL after 6h) and IL-17 (206.6 ± 61.9 pg/mL after 18h). On the other hand, we show that neither of cytokines that precede IL-17 production were detected in *C. albicans* 577 infection, although it has induced IFN-γ production (252.2 ± 124.4 pg/mL). The neutrophil and macrophage population in the exsudate reaches a peak after 18h of infection with both strains.

Conclusions: The results suggest that apoptosis of macrophages induced by *C. albicans* CR15 could be involved in IL-17 production since this cytokine was

not produced during infection by *C. albicans* 577 that is unable to induce apoptosis.

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ANALYSIS OF CYTOKINE PRODUCTION IN THE INTERACTION OF *Leishmania amazonensis* WITH BALB/c PURIFIED LANGERHANS CELLS CO-CULTURED WITH LYMPH NODE CELLS

SIMONE RODRIGUES CAMPELO (PG)(1); MOISES BATISTA DA SILVA (1,2); FERNANDO TOBIAS SILVEIRA (3); CLAUDIO GUEDES SALGADO (1,2)

(1) Laboratório de Dermato-Imunologia - UFPA/MC, Marituba, Pará
(2) Instituto de Ciências Biológicas - UFPA, Belém, Pará
(3) Departamento de Parasitologia - Instituto Evandro Chagas, Ananindeua, Pará

Introduction: *Leishmania amazonensis* is a major etiological agent in a wide spectrum of clinical forms of cutaneous leishmaniasis, an important neglected disease that has wide geographical distribution in the Americas. Resistance against leishmaniasis may result from development of an efficient immune response, and many studies have demonstrated that specific cytokines or combinations of cytokines could be factors of resistance or susceptibility to infection by *L. amazonensis*. Langerhans cells (LC) are antigen presenting cells that play a crucial role in immune responses of the skin, and their role as regulators of anti-Leishmania immune response is still poorly studied. In this study, we investigated the effects *in vitro* of the protozoan *L. amazonensis* on cytokine production of LC, as well as the ability of these cells to activate the production of IFN-g and IL-4 by cultured lymph node cells. **Methods and Results:** Fresh immature LC, highly purified from BALB/c mice skin were incubated with *L. amazonensis* promastigotes, TNF-a and/or anti-CD40 mAb. After 24 h, LC were co-cultured with lymph nodes cells for additional 72h. Culture supernatants were tested for IL-6, IL-12p70, IFN-g and IL-4 by ELISA. Compared with cells cultured in medium alone, *L. amazonensis*-exposed LC showed significantly increase in IL-6 levels, but had no modulatory effect on IL-12p70 secretion. Treatment with TNF-a did not affect the production of IL-6 or IL-12p70 in the *L. amazonensis*-exposed LC culture, while treatment with anti-CD40 or anti-CD40 plus TNF-a enhanced the production of IL-6 and IL-12p70. In the presence of *L. amazonensis* promastigotes, lymph node cells showed an increased production of IFN-g and IL-4, but the presence of *L. amazonensis*-exposed LC did not change these levels. However, lymph node cells produced more IFN-g and IL-4 in the presence of *L. amazonensis*-exposed LC treated with TNF-a and/or anti-CD40.

Conclusion: The production of cytokines by *L. amazonensis*-exposed LC can be amplified by inflammatory mediators, especially TNF-a and CD40 signaling, which can prime lymph node cells to produce significantly higher levels of IFN-g and IL-4.

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VISCERAL LEISHMANIOSIS IN AN HIV-1-INFECTED CHILD IN SÃO PAULO STATE, IMMUNOLOGICAL ASPECTS

PATRÍCIA RODRIGUES NAUFAL SPIR[1], LOURDES APARECIDA ZAMPIERI D'ANDREA[2], LUIZ EURIBEL PRESTES-CARNEIRO[3]

Departamento de Infecto-Pediatria, Hospital Regional de Presidente Prudente, SP[1]; Laboratório Regional, Instituto Adolfo Lutz , PP, SP[2]; Departamento de Pós Graduação, Unoeste, PP, SP e Departamento de Infectologia, Hospital Ipiranga, SP[3].

INTRODUTION: Brazil was one of the first developing countries to adopt measures against mother-to-child-transmission (MTCT) of HIV, and rates decreased from 20% to 1-2% in some regions. The country contains about 80% of individuals infected with Visceral Leishmaniosis (VL) in Latin America, and the West region of São Paulo State faces an outbreak of the disease. The HIV-infection increases the risk of developing VL by 100 to 2,320 times in endemic areas. **OBJECTIVE:** to analyze immunological aspects of an HIV-infected, 11.4 years old girl, from Paulicéia, São Paulo, with a diagnosis of VL admitted in a reference public hospital in an inner city of São Paulo State. **METHODOLOGY AND RESULTS:** CD4, CD8 and HIV viral loads were determined by FACS and PCR, respectively. VL diagnosis was obtained by Indirect Fluorescent Antibody Test (IFAT), rK39 rapid test and bone marrow aspirate. The patient was admitted with a diagnosis of AIDS, irregular antiretroviral therapy adhesion, severe diarrhea, protein-caloric malnourishment, electrolyte disturbance, oral and perineal candidiasis, precarious teething and depression. CD4/CD8 lymphocytes resulted in 28/801 cells/mL, respectively. HIV-viral load resulted in 45.089 copies/mL, and Neutrophilia, severe thrombocytopenia (18.000/mL) and hypoalbuminemia (1,98 g/mL) was found. At day 17^{o}, she presented with decrease of consciousness and hepatic dysfunction, and was sent to a pediatrics critical care unit with clinical sepsis, and received antibiotics+human IgG immunoglobulin. At day 23^{o}, she presented elevated fever and returned to the pediatric critical care unity with a diagnosis of blood infection, sepsis of fungal origin, cardiac and renal failure. Although a rapid test and IFAT for VL resulted negative, liposomal amphotericin B was administered, and a VL bone marrow aspirate resulted positive. The patient's symptoms improved, although a relapse occurred and new doses of amphothericin were administered. The re-treatment was not sufficient to recover normal levels of platelets ($p>0.05$).

CONCLUSION: It is likely, in this patient, that VL advanced the clinical progression of the HIV disease and the development of AIDS-defining conditions. Results of IFAT and rK39 rapid test in diagnosis of HIV-LV co-infected patients are contradictory and lack specificity. Despite all efforts, MTCT continues to happen, and clinicians should be aware of the possibility of LV in HIV-positive patients, particularly in patients from endemic areas.

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ASSOCIATION POLYMORPHISMS OF THE ACTC1 GENE IN SUSCEPTIBILITY TO EVOLUTION CHAGAS' DISEASE CARDIOMYOPATHY

AMANDA FRADE1,6*; PRISCILA TEIXEIRA1,6*;LUCIANA NOGUEIRA1,6; STHEFANNY ALVES1,6;MONIQUE BARONI1,6; BARBARA IANNI[1]; ABILIO FRAGATA[3]; MARIO HIRATA[3]; MARCELO SAMPAIO[3], BRUNO SABA[3]; HUI TZU LIN-WANG[3]; FABRICIO DIAS 4; EDUARDO DONADI4; VIRMONDES RODRIGUES JR.5; CRISTINA PISSETTI5, ALEXANDRE PEREIRA[1]; JORGE KALIL1,6; CHRISTOPHE CHEVILLARD[2]; EDECIO CUNHA-NETO1,6.

1- Instituto do Coração- InCor/FMUSP

2 -INSERM UMR 906– Marseille, France

3 -Instituto Dante Pazzanese de Cardiologia

4 -FMRP, USP

5 -Universidade Federal do Triangulo Mineiro

6-Institute of Immunological Investigation-iii

*Authors had the same contribution to the abstract.

Introduction: Mechanisms underlying differential progression to Chronic Chagas' Cardiomyopathy (CCC) are still incompletely understood. Familial aggregation of CCC cases has been described and polymorphisms in several immune response genes have been associated to risk for development of CCC. Variations in sarcomeric genes, including the alpha-cardiac actin gene (ACTC1), are known to play a role in dilated and hypertrophic cardiomyopathies. We investigated whether single nucleotide polymorphisms (SNPs) in the ACTC1 gene were associated to risk towards the development of CCC. We also assessed the ACTC1 protein expression levels in myocardial samples from CCC and heart donors.

Methods and Results: 315 CCC and 118 indeterminate form (IF) patients were genotyped with Illumina Golden Gate Assay. Statistical analysis was performed with SPSS (univariate and multivariate analysis). In this work we studied 17 polymorphisms in ACTC1. The SNP rs640249 showed significant results when was compared CCC patients and IF individuals including the gender as covariate (CC+AC vs. AA, p=0.006; OR=1.47; 95%CI: 1.11-1.93).This SNP was associated with increased disease susceptibility. Analysis of ACTC1 expression by Western blots and densitometry showed that median ACTC1

expression in CCC myocardium was 64 % lower than in control myocardium ($p<0.001$).

Conclusion: A SNP in the ACTC1 gene was associated to risk of development of CCC. It is possible that this polymorphism is associated to the reduced levels expression of the ACTC1 protein in the myocardium of CCC patients, which may have deleterious consequences on myocardial contraction.

Keyword: ACTC1; Chagas disease; Genetic polymorphism.

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TREATMENT WITH BONE MARROW CELLS INFLUENCE IN CHRONIC LESION CUTANEOUS OF MICE (TNFRp55$^{-/-}$ BL/6) INFECTED BY *LEISHMANIA MAJOR*

PAULA SEIXAS MELLO[1]; DANIEL MANZONI DE ALMEIDA[1,2;] CAIO COTTA NATALE[1]; MATHEUS HEITOR CARNEIRO[1]; LILIANE MARTINS DOS SANTOS[1]; LUIZA MIRANDA[3];PHILLIP SCOTT[4]; ROSA MARIA ESTEVES ARANTES[3]; LEDA QUERCIA VIEIRA[1,2]

[1]*Laboratório de Gnotobiologia e Imunologia – Depto de Bioquímica e Imunologia– Universidade Federal de Minas Gerais;*
[2] *Núcleo de Pesquisa em Ciências Biológicas (NUPEB)- Universidade Federal de Ouro Preto;*
[3] *Laboratório de Neuroimunopatologia Experimental – Depto de Patologia Geral – Universidade Federal de Minas Gerais.*
[4] *Laboratory Pathobiology - Veterinary Medicine - University of Pennsylvania*

INTRODUCTION Leishmaniasis is caused by parasites of the genus *Leishmania*. The mucocutaneous form is characterized by a low number of parasites at the site of infection and chronic lesions caused by an exacerbated inflammatory response. We showed that TNFR1 KO mice, when infected with *L. major,* control parasite growth at the site of infection, but maintain an intense inflammatory infiltrate and develop chronic lesions when compared to wild-type mice (WT). **OBJECTIVE** The aim of this study was the characterization of inflammatory profile in chronic infection in *L. major*-infected TNFR1 KO mice and analysis of the effect of cell therapy in these chronic lesions. **METHODOLOGY** WT and TNFR1 KO mice were inoculated in the foot-pad with *L. major* and were followed for 15 weeks of infection for analysis of the immunopathology profile. TNFR1 KO mice, in the chronic phase of infection, were treated with of purified mononuclear cells from bone marrow and analyses were performed 1 and 2 months after the treatment. **RESULTS** Our results showed that chronic lesions from *L. major*-infected-TNFR1 KO presented high levels of pro-inflammatory cytokines (IFN-g, TNF-α and IL-17) and chemokines (CCL2 and CCL5) when compared with WT animals. Persistence of these lesions in TNFR1 KO mice is due to intense inflammatory infiltrate with increase percentage of $Ly6G^+$and $TCD8^+$ cells at site of infection compared to WT mice. Treatment with mononuclear cells derived from bone marrow caused lesion healling. After the treatment, donor-cells were located at the site of infection and differentiated into $CD11c^+$ $MHCII^+$ cells 7 days after the transfer; these lesions were significantly smaller showing evidence of healing. We found increased expression of IL-10 and decreased expression of IL-17 and decreased number of polymorphonuclear cells in cell-treated mice when compared to PBS-treated animals. **CONCLUSIONS** These results showed

that mononuclear cells derived from bone-marrow controlled chronic lesions in *L.major*-infected TNFR1 KO mice.

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Pseudomonas aeruginosa 1-Cys Peroxiredoxin LsfA is involved in virulence

GILBERTO HIDEO KAIHAMI (PG)[1]; SUELEN SILVANA DOS SANTOS (PG)[2]; JOSÉ ROBERTO FOGAÇA DE ALMEIDA (PG)[2]; SANDRO ROGÉRIO DE ALMEIDA [2]; REGINA LÚCIA BALDINI [1]

(1) INSTITUTO DE QUÍMICA – USP (2) FACULDADE DE CIÊNCIAS FARMACÊUTICAS – USP

Introduction: Bacteria are recognized by macrophages via Toll-Like Receptors (TLR), leading to a signaling pathway that activates NF-kB and MAPKs and killing in phagossomes in macrophages is achieved by reactive oxygen and nitrogen species (ROS/RNS) generation. *P. aeruginosa* is a common cause of ventilator associated pneumonia and it uses several strategies for virulence and defense, including antioxidant mechanisms. In this work, we show for the first time that the 1-Cys peroxiredoxin, LsfA, is implicated in bacterial virulence.

Methods and Results: Mutant strains with a deletion in *lsfA* or a C45A mutation were constructed and they were more sensitive to H_2O_2 than the wild type strain PA14, as measured in a halo inhibition assay. *In vitro* peroxidasic activity of LsfA was measured by ferric-thiocyanate assay, and while the wild-type protein was active, the mutation in Cys45 abolished its activity. Infection of J774 macrophages with DLsfA or C45A strains resulted in lower cell death, increased bacterial clearance and higher TNF-a in comparison to PA14-infected macrophages, suggesting a higher level of MAPKs and NF-kB activation in macrophages infected with the mutants strains. To verify if LsfA could modify the oxidative state of infected macrophages, they were infected with PA14 and C45A strains and incubated with carboxy-H_2DCFDA. Macrophages infected with mutant strains showed higher oxidative state in comparison to PA14-infected cells, thus confirming that LsfA limits macrophages activation that lead to TNF-a production and cytotoxic activity. MAPKs and NF-kB pathways are required to full production of TNF-a in macrophages infected with C45A, as shown using pharmacological inhibitors for those pathways. When macrophages were infected with C45A in the presence of the antioxidant NAC, there was a reduction in TNF-a production as compared to PA14, as expected. In an acute pneumonia model, all PA14-infected mice died at 48h p.i., while C45A-infected mice survived as long as 60 days after infection. There was also CFU reduction in the lungs, spleen and liver of mice infected with C45A, in comparison to PA14-infected mice.

Conclusion: Altogether, our data show for the first time the role of a bacterial 1-Cys Prx (LsfA) to modulate host immune response *in vitro* and *in vivo*.

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Ehrlichia spp. IN DOGS IN UBERLÂNDIA, BRAZIL: SEROPREVALENCE AND ASSOCIATED FACTORS

SUSANA ELISA RIECK[1]; ROSIANE NASCIMENTO ALVES[2]; MARCELO ARANTES LEVENHAGEN[2]; ROGÉRIO DE MELO COSTA PINTO[3]; MATIAS PABLO SZABÓ[4]; MARCELO EMÍLIO BELETTI[2]

[1] Instituto Federal de Educação, Ciência e Tecnologia do Triângulo Mineiro, Campus Uberlândia. [2] Instituto de Ciências Biomédicas, Universidade Federal de Uberlândia. [3] Faculdade de Matemática, Universidade Federal de Uberlândia. [4] Faculdade de Medicina Veterinária, Universidade Federal de Uberlândia.

Introduction: The canine monocytic ehrlichiosis (CME) is a disease of veterinary importance and are also responsible for emerging life-threatening anthropozoonoses. The primary etiologic agent is a α-proteobacteria that belongs to the order *Rickettsiales* called *Ehrlichia canis,* obligate intracellular phatogen with a tropism for monocytes and macrophages. This organism has a worldwide distribution, concentrated in tropical and subtropical regions due to the geographical distribution of its main biological vector tick, *Rhipicephalus sanguineus*. At this time, however, there have been few epidemiological data collected regarding the prevalence of *Ehrlichia* in the southeast region of Brazil. Thus, the aim of this study was to determine the seroprevalence and factors associated with a positive antibody response to *Ehrlichia* spp. in dogs in Uberlândia, Brazil.
Methods and Results: Blood samples were obtained from a total of 400 dogs, and each animal was examined to determine age, sex, source, localization and exposure to ticks. The samples were analyzed for antibodies to *Ehrlichia* spp. using an indirect immunofluorescence assay. The statistical analysis was performed by the chi-square test to determine the relation between positive and negative results and associated factors. Two hundred and eleven (52.8%) of the 400 dogs were found to be seropositive to *Ehrlichia* spp. The factors sex and exposure to ticks were not significant. Meantime, the following factors associated with seropositive animals were: age (>1 year: 56.3%, $P = 0.002$), source (stray dogs: 68.8%, $P = 0.002$) and localization (district dogs where the economic development is low: 76.7%, $P = 0.0001$).
Conclusion: Results suggest that Uberlândia is endemic to CME. Therefore dogs over one year old, wandering and living in localities with less economic development are predisposed to this disease.

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PARACOCCIDIOIDES BRASILIENSIS INHIBITS HUMAN DENDRITIC CELLS MATURATION AND EICOSANOIDS PRODUCTION

REGINALDO KELLER FERNANDES (PG) (1), LUCIANE ALARCÃO DIAS-MELICIO (2), MARJORIE DE ASSIS GOLIM (3), TATIANA FERNANDA BACHIEGA (1), DANIELA RAMOS RODRIGUES (1), HELANDERSON DE ALMEIDA BALDERRAMAS (1), ÂNGELA MARIA VICTORIANO DE CAMPOS SOARES (1).

(1) Departamento de Microbiologia e Imunologia, Instituto de Biociências de Botucatu, UNESP – Univ Estadual Paulista, Brasil; (2) Departamento de Patologia, Faculdade de Medicina de Botucatu, UNESP – Univ Estadual Paulista, Brasil; (3) Hemocentro, Faculdade de Medicina de Botucatu, UNESP – Univ Estadual Paulista, Brasil.

Introduction: The dimorphic fungus *Paracoccidioides brasiliensis* (Pb) is the etiologic agent of paracoccidioidomycosis, a systemic mycosis that is endemic in Latin America. Dendritic cells (DCs) are believed to be critical in the detection of pathogens and the initiation of the host response to microbial invasion. Many mediators secreted by these cells, by an autocrine manner, can modulate their functions. Among these mediators, the eicosanoids such as prostaglandins and leukotrienes have become the focus of investigation in last years. Interactions of human DCs with *P. brasiliensis* and their consequences in relation to the functions of these cells, such as maturation, migration and cytokine production are poorly understood. In this context, we sought to evaluate the maturation of human of DCs and the production of PGE_2 and LTB_4 in response to high and low virulent strains of the fungus. **Methods and Results:** Human DCs were obtained from differentiation of monocytes cultured in the presence of GM-CSF (80ng/mL) and IL-4 (80ng/mL) for 7 days. Flow cytometry assays identified these cells as $CD14^{-}/CD1a^{high}/CD83^{low}$, confirming the phenotype of immature dendritic cells (iDCs). After, iDCs were incubated with LPS or challenged with high and low virulent strain of the fungus, Pb 18 and Pb 265, respectively, during 1h, 2h, 4h, 8h, 12h, 18h, 24h or 48h, followed by measurement of PGE_2 and LTB_4 levels using competitive ELISA Kits. The results showed that control cells produced substantial concentrations of PGE_2 and LTB_4. Incubation with LPS resulted in PGE_2 increase (mainly at 18, 24 e 48h) but not in LTB_4. Differently, the inhibition of PGE_2 and LTB_4 release was detected after

challenge of iDCs with Pb18 and Pb265, in most periods. In addition, phenotyping assays showed that these cells, during incubation with the fungus, did not change the immature phenotype to mature one. **Conclusion:** *P. brasiliensis* inhibits endogenous PGE_2 and LTB_4 release by DCs. This effect could result in fail of these cells to differentiate in mature DCs in the presence of the fungus.

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CANINE VISCERAL LEISHMANIASIS AND SPLEEN CELLS REDISTRIBUTION: MARGINAL ZONE MACROPHAGES AND PLASMA CELLS

THAÍS CARVALHO SANTOS[1,2]; JOSELLI SANTOS SILVA[2]; DEBORAH BITTENCOURT MOTHÉ FRAGA [2]; MANUELA DA SILVA SOLCÀ[2]; WASHINGTON LUIS CONRADO DOS SANTOS[1,2]

[1] Escola Bahiana de Medicina e Saúde Pública; [2] Laboratório de Patologia e Biointervenção, Centro de Pesquisas Gonçalo Moniz – Fundação Oswaldo Cruz, Salvador, Bahia, Brasil.

Introduction: Visceral leishmaniasis is associated with disruption of splenic lymphoid tissue and redistribution of cell populations involved with the immune defense. In this work, we studied the alterations in distribution of marginal zone macrophages and plasma cells in the spleen of dogs naturally infected with *Leishmania infantum*.
Methods and Results: Spleen samples from 30 dogs grouped into three categories (N=10): noninfected animals with organized white pulp, infected animals with organized white pulp and infected animals with disorganized white pulp, were used in the study. The sections were labeled with IH1 antibody (anti-canine marginal zone macrophages) and anti-dog IgG, IgM and IgA antibodies for plasma cells identification. The number and distribution of marginal zone macrophages and plasma cells were estimated. Additionally, the clinical and laboratory data (biochemistry and hematology) of the animals were reviewed. Plasmacytosis was greater in infected animals with disorganized white pulp (7/10) than in noninfected animals (2/10, Chi-square, $P < 0.04$). The albumin/globulin ratio and the clinical score related to canine visceral leishmaniasis were higher in the animals with disorganized white pulp in comparison with the animals with organized white pulp. No difference was observed by morphometric analysis in the number and distribution of marginal zone macrophages between the animal groups.
Conclusion: The plasmacytosis and disglobulinemia associated with visceral leishmaniasis may result of a disruption of the white pulp of the spleen, affecting B cell differentiation.

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HYPERNOCICEPTION DEVELOPMENT AND IMMUNE CELL INFILTRATION IN DORSAL ROOT GANGLION OF MICE INFECTED WITH HSV-1

JAQUELINE RAYMONDI SILVA[(1)]; ALEXANDRE HASHIMOTO PEREIRA LOPES[(1)]; JHIMMY TALBOT[(1)]; RAFAEL FREITAS DE OLIVEIRA FRANÇA[(1)]; BENEDITO ANTONIO LOPES DA FONSECA[(1)]; THIAGO MATTAR CUNHA[(1)]; FERNANDO DE QUEIRÓZ CUNHA[(1)]

[(1)]. Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo

Introduction: Herpes Zoster (HZ) is a disease caused by reactivation of latent herpesvirus Varicella Zoster (VZV) in the sensory ganglion, characterized by dermal rash and severe pain. VZV infects only humans, and there are no animal models available to study the disease. However, when mice are inoculated with herpes simplex virus type-1 (HSV-1) on the skin of the hind paw, they develop HZ-like skin lesions and show pain-related responses to noxious mechanical stimulation and innocuous tactile stimulus. For this reason, this model has been used to study the pathophysiology of herpes zoster. So far, there are no data available about the immune response in dorsal root ganglion (DRG) of mice infected with HSV-1 in this model. Thus, the aim of this study was to evaluate cells and inflammatory mediators present in DRGs and its relationship with hypernociception during HSV-1 cutaneous infection.

Methods and results: Briefly, mice were depilated with a chemical depilatory and three days later 2 x 10^5 plaque forming unities (PFU) of HSV-1 were inoculated in the skin of the right hind paw after scarification. Mice were observed daily and behavioral tests were performed from 0-21 day post inoculation. The DRGs L1-L6 were collected at 7, 15 and 21days post infection (dpi) and flow citometry analysis and RT-PCR were performed. Viral load was measured by quantitative Real-Time PCR. Mice developed hypernociception from 3 to 21 dpi in the ipsilateral (ips) paws, but not in the contralateral (cl) paws. A higher viral load was detected in DRGs L3, L4 and L5 of infected mice at 7 dpi, when compared to control or naïve mice. We observed an inflammatory infiltrate composed by CD4+, CD8+, CD11b+, Gr1+ cells in DRGs L4, L5 and L6, but not in spinal cord. In infected mice, a higher expression of COX-2 and TNF-α was detected in DRGs L4, L5 and L6 of ips paws at 7 dpi, but not at 15 and 21 dpi. However, GFAP expression was not detected at 7 dpi, but was increased at 15 and 21 dpi, when compared to naïve mice.

Conclusions: Our results show the presence of an intense inflammatory infiltrate, composed by cells from immune system, in DRGs of infected mice, and the early expression of inflammatory mediators in this local that might contribute for the induction of hypernociception in this model.

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IMMUNOMODULATORY EFFECTS OF BIOTHERAPIC IN EOSINOPHILIA DURING SYNDROME OF VISCERAL LARVA MIGRANS MURINE

LUCIANA CAMILLO (1); SANDRA REGINA PEREIRA DE OLIVEIRA (1); JOICE MARGARETH DE ALMEIDA RODOLPHO (1); ROSA DOMINGUES RIBEIRO (2); FERNANDA DE FREITAS ANIBAL (1).

(1). DMP, Laboratory of Parasitology – UFSCar; (2).UNIFRAN – University of Franca

Introduction: The *Toxocara canis* is a parasite that belongs to the nematode phylum and has dogs as their definitive host. The men are accidentally contaminated by ingesting eggs containing infective larvae of the parasite. These larvae, when ingested, pass through the intestinal mucosa, reach the portal circulation and migrate through different tissues of the host. During this process excretory-secretory antigens are released causing an intense inflammatory reaction, which induces a characteristic syndrome, called Visceral Larva Migrans (VLMS). The main features of this chronic disease are the presense of eosinophils in blood and tissue, and high levels of serum IgE. Important disorders such as allergic diseases and parasitic infections may provide the striking accumulation of eosinophils. Thus, it is important to search for therapies which control intense inflammatory conditions with eosinophilia. The use of chemical or biological agents as therapy for various diseases has been used as an alternative to cure or control diseases caused by them. In this context, we use a biotherapic produced from total antigen extract of eggs and larvae of *Toxocara canis* in order to evaluate the recruitment of eosinophils to the blood and bronchoalveolar space of mice infected with T. canis. **Methods**: We used female mice of Swiss strain, divided in three groups: control (no treatment), Infected (*T. canis*), Infected treated (*T. canis* + bio) and immunized and infected (Im + *T. canis*). The infected animals immunized / treated or / not received 500 eggs / animal by gavage. Subsequently, the animals were euthanized and the number of eosinophils was determined. **Results:** Our results demonstrated a reduction in the number of eosinophils in both compartments analyzed in immunized animals, as in the treated compared to the group only infected. However, this reductive activity remained at greater efficiency in treated animals. **Conclusion**: In this regard, we concluded that this type of biotherapy can negatively modulate the recruitment of eosinophils, being the best activity arising in the treatment process.

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IMMUNOPATHOLOGY OF MURINE CHROMOBLASTOMYCOSIS MODELS

RAFFAEL JÚNIO ARAÚJO DE CASTRO[1]; KARINA SMIDT SIMON[1]; ISAQUE MEDEIROS SIQUEIRA[1]; LUIZA CHAVES DE MIRANDA LEONHARDT[1]; ANA CAMILA OLIVEIRA SOUZA[1]; ANAMÉLIA LORENZETTI BOCCA[1].

[1] Department of Cell Biology, University of Brasília - UnB.

Introduction: Chromoblastomycosis (CBM) is a chronic, suppurative, granulomatous mycosis of the skin and subcutaneous tissues, whose main etiologic agent is the dimorphic fungus *Fonsecaea pedrosoi.* However, the literature lacks of a suitable animal model for the disease. With this in mind, the current study was carried out aiming the immunopathology characterization of experimental CBM, caused by the fungus *F. pedrosoi* in different murine strains.
Methods and Results: The ATCC 46428 strain of *F. pedrosoi* was cultivated for 15 days under shaking and then filtered to obtain fungal propagules for infection and its total soluble proteins (chromo-g). For a comparative study between the current murine models of the disease, BALB/C, C57BL/6 and B10A mice were infected subcutaneously with 10^6 fungal cells in its footpads. Morphometric analysis of the footpads was performed in every 5 days post infection. Every 15 days the animals were sacrificed and their footpad tissue collected for CFU and histopathological analysis, as well as extraction of serum for cytokine and nitric oxide quantification. Lymph node cells were collected from draining popliteal lymph node for immunophenotyping and in vitro lymphoproliferative response assays. All mice strains developed edema, fibrosis, necrosis and suppurative granulomatous lesions in the footpad just like those found in humans, showing also the presence of muriform cells, the parasitic form of the fungus. The highest values of fungal burden were given 15 days post infection, showing gradual reduction up to 60 days post infection, when the cure was reached. BALB/C mice showed an efficient recruitment and proliferation of T lymphocytes after stimulation with *F. pedrosoi* and its secreted proteins.
Conclusion: Taken together our previous data suggest that all strains, specially BALB/C and C57BL/6 or in a less degree B10A, showed a similar immunological and histopathological profile to that found in CBM patients, although it appears to have a greater participation of cellular immunity, which can explain the acute aspects of the disease in current murine models.

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Leishmania EFFECT ON HEME CYTOTOXICITY

NIVEA FARIAS LUZ[1,2]; THEO ARAUJO-SANTOS [1,2]; ROQUE P. ALMEIDA[3]; MARCELO TORRES BOZZA[4]; VALERIA MATOS BORGES[1,2]

[(1)] Centro de Pesquisas Gonçalo Moniz/Fundação Oswaldo Cruz, Salvador-Brazil; [(2)] Universidade Federal da Bahia, Salvador-Brazil; [(3)] Medicine Department, Hospital Universitário, UFS, Aracaju, Brazil; [(4)]Departamento de Imunologia, Instituto de Microbiologia, Universidade Federal do Rio de Janeiro-Brazil.

Introduction: Visceral leishmaniaisis (VL) is a major public health problem worldwide. This disease is highly associated with chronic inflammation and with hematological manifestations, such as anemia, hemolysis and spontaneous bleeding. Given this scenario, mechanisms related to hemolysis and release of heme may be involved with the pathogenesis of VL. Heme is highly cytotoxicity to the host, and thought to participate in be the pathogenesis of infectious immune-mediated inflammatory conditions, i.e., malaria and sepsis. Our previous data argue that Heme oxygenase-1 (heme degradation enzyme) has a critical role in the *Leishmania chagasi* infection and is strongly associated with the inflammatory imbalance during VL (The J. of Immunology 188: 4460-4467, 2012). Herein, we evaluated the role of heme in the infection by *L. chagasi*, the causative agent of VL cases in Brazil.

Methods and Results: Monocyte-derived macrophages (MDM) and promonocytic THP-1 cell line were infected *in vitro* with *L. chagasi* (LSH) and cultured with 30μM heme. The release of Lactate Dehydrogenase (LDH) was measured 12hours post treatment, in cell-free culture supernatant by a cytotoxicity detection kit. Intracellular reactive oxygen species (ROS) levels were measured 2hours post treatment with a fluorescent probe staining following analysis by flow cytometer. Heme induced LDH release (12.45±3.179, CTR vs. 96.13±17.6 Heme), and ROS generation (70.23±4.861, CTR vs. 115.5±9.534 Heme) in MDM and THP-1 cells, besides Annexin-V-PI staining showed the induction of a significant number of necrotic cells (6.747±4, CTR vs. 55.35±4.995 Heme). Interestingly, *L. chagasi* infection abrogated both heme-induced cytotoxicity (96.13±17.6, Heme vs. 32.45±12.52 LSH+Heme) and ROS production (271±3.28 Heme vs. 207±10.87LSH+Heme) in THP-1 cells. Finally, we evaluated serum samples obtained from patients with VL (n=49) and

endemic controls (n=39) from an endemic area in the Northeast of Brazil. Patients with VL presented higher concentrations of total heme in the serum (62.25μM±5.739) compared to health individuals (23.86μM±2.273). **Conclusion**: Taken together, our data suggest that heme contributes to the pathogenesis of VL and reinforces the idea that *L. chagasi* parasite modulates host immune responses.

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ROLE OF STRONGYLOIDES VENEZUELENSIS INFECTION IN THE EVOLUTION OF EXPERIMENTALLY INDUCED COLITIS IN MICE

Rodrigues, VF1; Bahia, MPS1; Cândido, NR1; Pereira, CAJ1; Araújo, ES1; Rodrigues, JL1; Corrêa, O1; Negrão-Corrêa, D1. 1 – Universidade Federal de Minas Gerais

Introduction: The infection with intestinal nematode, such as Strongyloides, induces a modified type-2 immune response, in which cytokines of type 2 and regulatory are produced. Epidemiologic studies suggested that nematode-induced immune response also has the ability to modulate unrelated inflammation such as inflammatory bowel disease (IBD), a chronic inflammatory disorder that manifests in a dysregulated mucosal immune response against intestinal bacteria. However, the mechanisms involved in this modulation process are not well established and is probably dependent on the nematode life cycle. In this experimental work, we intent to verify the effect of Strongyloides venezuelensis infection, a nematode that establishes into the small intestine mucosa and it is spontaneously eliminated after 2 weeks, infection on the evolution of Dextran sodium sulfate (DSS)-induced colitis in mice. **Methods and Results**:For this proposal, Balb/c female mice were subcutaneously infected with 700 L3 of S. venezuelensis and after 5 days they received drinking water containing 4 % of DSS for 7 days. Groups of Balb/c only infected or only treated with DSS were kept as control. Colitis score was determined based on weight loss, fecal blood and diarrhea, and clinical appearance. Upon autopsy, at 12 days of infection, parasite burden and the length of the colon were determined. Samples of small and large intestine were recovered for cytokine quantification and histopatologic analysis. The data showed that DSS-induced colitis did not interfere with the parasite burden, but S. venezuelensis infection significantly reduced the colitis score. Compare to DSS-treated mice, the infected and treated animals also showed lower cellular infiltration and mieloperoxidase activity, but higher eosinophil peroxidase in colon. At the time of autopsy, the colon of mice infected and DSS-treated mice had higher levels of type-2 cytokines (IL-4 and IL-13) but similar levels of IL-10 and type-1 cytokine compare to DSS-treated mice. Conclusion:Our data indicates that S. venezuelensis infection is able to modulate DSS-induced colitis in mice. The results also suggested that the modulation was due to the increase of type-2 response in colon.

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GRANULOMAS AND CYTOKINES MODULATIONS IN *SCHISTOSOMA MANSONI AND PARACOCCIDIOIDES BRASILIENSIS* CO- INFECTED MICE

Maria Claudia Noronha Dutra Menezes[(1)]; Francisco José de Abreu(1); Marina Okuyama Kishima[(1)]; Tania Longo Mazzuco[(1)]; Jefferson Crespigio[(1)]; Paula Leonello Álvares e Silva[(1)]; Eiko Nakagawa Itano[(1)].

[(1)] State University of Londrina, UEL, Londrina, PR, Brazil.

Introduction: The helminth *Schistosoma mansoni* (Sm) and fungus *Paracoccidioides brasiliensis* (Pb) are two important pathogens that cause chronic granulomatous disease, both endemic in Brazil. They are the agents of schistosomiasis mansoni and paracoccidioidomycosis (PCM) that has the Th2 and Th1 immune response as the main defense, respectively. The present study investigated the immunopathological impact in co-infection by these two agents in experimental murine model. **Methods and Results**: It was used BALBc mice simultaneously infected with both or *P. brasiliensis* as secondary infection: a) infected with *S. mansoni* and *P. brasiliensis* during 70 days (Sm70Pb70); b) infected first with *S. mansoni* and 28 days before the final period with *P. brasiliensis* (Sm70Pb28) and as controls *S. mansoni* alone for 70 days (Sm70) or *P. brasiliensis* alone for 70 (Pb70) or for 28 (Pb28) days. The liver histopathology and liver cytokines (IL-4, IL-10, IL-13 and IFN-γ) or serum cytokines (IL-4 and IFN-γ) levels were performed in all groups. The co-infection resulted in a marked decreased *S. mansoni* and increased *P. brasiliensis* granulomas numbers in relations to controls ($p< 0.05$). Increased liver IL-4 or IL-13 (Sm70Pb28 > Sm70Pb70) and IL-10 (Sm70Pb28) levels and increased serum IFN-γ level than control Sm70 were observed. In relation to Pb28 or Pb70 controls groups decreased liver IL-4, IL-10 and INF-γ (Sm70Pb70) and decreased INF-γ and increased IL-4 (Sm70Pb70) serum levels were observed. Additionally, it was observed granulomas lacking the peripheral halo in close contact between *P. brasiliensis* and *S. mansoni* eggs/ worm and also detected presence of the *S. mansoni* and or *P. brasiliensis* in other organs in co-infected group by histopathology analysis. **Conclusion**: In conclusion, our data show, for the first time that co-infection with *S.mansoni* and *P.brasiliensis*, induce the liver granulomas and cytokines modulations to each other and possible induction of the severity diseases in experimental BALB/c mice.

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TREATMENT WITH *Harpagophytum procumbens*: ANTI- EOSINOPHILIC ACTIVITY EVIDENCE IN THE *Toxocara canis* INFECTION

SANDRA REGINA PEREIRA DE OLIVEIRA[1]; JOICE MARGARETH DE ALMEIDA RODOLPHO[1]; RICARDO DE OLIVEIRA CORREIA[1]; LAÍS CRISTINA DE SOUZA[1]; NAIARA NAIANA DEJANI[4]; DÉBORA MEIRA DEJANI [1]; PAULO CEZAR VIEIRA[2], LUCIA HELENA FACCIOLI[4]; CLAUDIO ALBERTO TORRES SUAZO[3], FERNANDA DE FREITAS ANIBAL[1].

DMP, Laboratório de Parasitologia, UFSCar[1]; DQ, UFSCar[2]; DEQ, UFSCar[3] Faculdade de Ciências Farmacêuticas de Ribeirão Preto, USP[4].

Introduction: Helminthic infection causes a series of immune responses: eosinophilia, IgE serum and mastocytosis tissues. The of Visceral Larva Migrans (VLM) is a Syndrome worldwide occurrence, and *Toxocara canis* is the etiologic agent. Presence of antigen-secreting eggs in tissue initiates immunological response of Th2-type that is marked by eosinophilia, high levels of IgE. Natural products have long been used in medicine as alternative treatment for various diseases, including inflammatory processes. *Harpagophytum procumbens* (Hp) has been used as treatment for a variety of illnesses, including, arthritis and diseases of the digestive tract. The present study examined the role of *Harpagophytum procumbens* in eosinophil recruitment and interleucina-5 (IL-5) levels during *T. canis*-infection model. **Methods:** Balb/c mice were divided on groups: control, Tc and Tc+Hp. Infected animals treated or not received 500 eggs/animal *T. canis* by gavage. After18 days of infection the animals were euthanized and the fluid was extracted for further evaluation of eosinophlis/mm^3. The level of IL-5 was determined by ELISA from plasma samples in period described. **Results:** The results showed that the treatment with *Hp* down modulated the number of eosinophlis in blood and IL-5 levels. We observed significant reduction these cells and IL-5 levels when compared with animals only infected and untreated. **Conclusion:** Thus, these data suggest that, Hp natural extract may modulate the immune responses during the VLMS by interfering with the migration of EO decreasing the IL-5 levels of in these compartments analyzed.

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PPARγ IN DENDRITIC CELLS DURING IMMUNOSUPPRESSION SEPSIS-INDUCED

Molinaro, R*, Navarro-Xavier, R.A.*, Vieira-de-Abreu, A*, Ribeiro A. S.*, Castro-Faria-Neto H.C.*, Benjamim C.F.[#], Bozza, P.T.*

*Immunopharmacology Laboratory, IOC/FIOCRUZ/RJ/BRAZIL;[#]Inflammation Laboratory, UFRJ/RJ/BRAZIL

INTRODUCTION: Sepsis is a main cause of death in intensive care unit and even the high mortality during sepsis episode, survival patients can develop an immunosuppression. However, the modulatory mechanisms involved are not well understood. Lipids play a crucial role in immunomodulatory response. Recent studies demonstrated that high content of lipids in dendritic cells (DC) play an important role on DC function. PPARγ are lipid-activated nuclear receptor have major roles in regulation of lipid metabolism, LD biogenesis and inflammation. Our aim was investigate the role of PPARγ in DC during sepsis and immunosuppression state. **METHODS AND RESULTS:** Mice were subjected to cecum ligation and puncture (CLP) or false-operated (Sham). BMDC were analyzed for LD quantification, cytokines and gene expression. After 24 hours, total lung cells and pulmonary DC from septic mice showed more LD compared with sham group. After 15 days, lungs from post-septic mice showed higher gene expression of PPARγ and ADRP compared with sham group. Lungs from infected post-septic mice did not exhibit increase levels of PPARγ as infected post-septic sham mice. We observed that infected septic and post-septic BMDC showed decreased numbers of LD and pro-inflammatory cytokines (TNF-α, MCP-1 and IL-1ß) and increased IL-10 production compared with infected from Sham group. Moreover, post-septic BMDC showed increase phagocytic activity but the nitric oxide was decreased rather than Sham group. **CONCLUSION**: Our results shown that infected post-sepsis BMDC have lower number of LD, increased PPARγ expression and anti-inflammatory profile of cytokine production, suggesting that. Further experiments will be necessary to characterize the PPARγ in DC functions during the immunosuppression state.

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