

IM001 - INFLUENCE OF APOPTOSIS IN COMPARTMENTALIZED IMMUNE RESPONSE IN SPLEEN AND LIVER OF DOGS WITH VISCERAL *LEISHMANIASIS*

MOREIRA, P.R.R.; ROSSIT, S.M.; BANDARRA, M.B.; MUNARI, D.P.; VASCONCELOS, R.O.
FCAV / UNESP, JABOTICABAL, SP, BRASIL.
e-mail:pamela_rreina@yahoo.com.br

Apoptosis can be a useful mechanism or harmful to the host. The aim of this study was to evaluate whether deficient immune response in dogs with advanced disease might be related to apoptosis of lymphoid cells, as well as compare the response pattern of the liver and spleen. We evaluated 18 dogs symptomatic of the Center for Zoonosis Control Araçatuba (SP). The parasitic load was determined by immunohistochemistry, the use of dog sera positive for the disease (1:1000). For apoptosis anti-caspase 3 (1:200). The substrate reaction LSAB kit (Dako). The density of immunostained cells was determined by the nonparametric test of Sinal, to check the differences between the spleen and liver for parasites and apoptosis. Simple Pearson Correlation showed the degree of association between these variables ($P < 0.05$), through the statistical program SAS (SAS 9.1, SAS Institute, Cary, NC, USA). In the liver, the granulomas, when present, were type lepromatous. There was a marked difference in intensity of inflammation between the spleen and liver, with an intensity more discreet in the liver. In the spleen, the granulomas had multifocal to diffuse distribution with the presence of macrophages and rare lymphocytes and plasma cells. Statistical analysis found significant differences between spleen and liver for parasite load ($P = 0.0042$) and density of apoptotic cells ($P = 0.0075$). In Simple Pearson Correlation found a significant association between the parasite load of spleen and liver ($P = 0.03$), whereas the spleen had a mean of parasitized macrophages as to the liver. The density of cells undergoing apoptosis was no significant association ($P > 0.05$). It was concluded that apoptosis was significant and the parasitic load in the liver and spleen of symptomatic dogs. The highest means were observed in the spleen, featuring a higher susceptibility of this organ the multiplication of the parasite, compared to the liver. **Supported by:** FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo / Processo nº. 2009/15736-7).

IM002 - APOPTOSIS, PIT CELLS, TGF- β , MACROPHAGES AND THEIR INFLUENCE ON LIVER DEFENSE AGAINST VISCERAL *LEISHMANIASIS* IN DOGS

MOREIRA, P.R.R.; BANDARRA, M.B.; TOLEDO, G.N.; VASCONCELOS, R.O.
FCAV / UNESP, JABOTICABAL, SP, BRASIL.
e-mail:pamela_rreina@yahoo.com.br

The hepatic resistance to *Leishmania* chagasi multiplication seems to be a characteristic of canine infection. The aim of this study was analyze the liver of 30 dogs from an endemic area for canine visceral *Leishmaniasis*, classifying them in three groups: symptomatic (S), asymptomatic (A) and oligosymptomatic (O), considering the density of immunostained cells for apoptosis, macrophages, Pit cells, TGF- β and parasitic load, by immunohistochemistry. For analysis of parasitic load was used positive dog serum for the disease, for evaluation of apoptosis, the caspase 3 and for macrophages, the MCA, revelation was LSAB kit (Dako). For Pit cells was used CD56 with Advance HRP kit (Dako). For immunoblot of TGF- β , with AP Envision Kit (Dako). Mean immunostained cells were analyzed by variance analysis (ANOVA) and comparison between groups by Tukey test in Graphpad Prism statistical program ($P < 0,05$). In this study, it was observed a reduction of Pit cells population in the group of dogs infected and a predominance of Kupffer cells in the infected group when compared to control, also noting a difference between groups S and A ($P < 0.01$). Possibly, there is a cytotoxic action of Pit cell and microbicide action of Kupffer cells, which hinder the multiplication of the parasite. Apoptotic cells appeared immunostained predominantly in the inflammatory infiltrate, with positive lymphocytes for apoptosis. The parasite load did not differ significantly between groups. The TGF- β was significantly different between group S and control ($P < 0.05$). It was concluded that the more debilitated is the animal's immune system, the greater the parasitic load, independent of the clinical stage. In liver the intensity of inflammation and parasite density are mild to moderate, so liver is not the most favorable place for parasite multiplication. Possibly infection has capacity for self-resolution, belike under pit cells influence, predominantly Kupffer cells and TGF- β in group S dogs. **Supported by:** FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo / Processo nº. 2009/15736-7)

IM003 - Modulation of human macrophage surface molecules expression by *Leishmania amazonensis*.

COSTA, S.S.¹; SALAZAR-TERREROS, M.J.¹; FORNAZIM, M.C.²; NOWILL, A.E.²; GIORGIO, S.¹

1. INSTITUTO DE BIOLOGIA UNICAMP, CAMPINAS, SP, BRASIL; 2. CENTRO INTEGRADO DE PESQUISAS ONCOHEMATOLÓGICAS DA INFÂNCIA CIPOI (FCM) UNICAMP, CAMPINAS, SP, BRASIL.

e-mail: solangecost@gmail.com

Modulation of surface molecules expression in host cells is a strategy used by several infectious agents to escape or circumvent immune response. The aim of this study was to evaluate the regulation of cell markers expression in response to *Leishmania amazonensis* infection in macrophages derived from peripheral blood mononuclear cells from healthy human donors. Cell phenotyping was carried out by flow cytometry fluorescent-activated cell sorting (FACS) following infection of cell cultures with *L. amazonensis* over a range of 1:1 to 8:1 amastigotes to cell ratio. Initial results show that 6 out of 7 of the cell markers evaluated (monocyte specific LPS receptor CD14, Class II MHC, pathogen recognition receptors CD11b and CD11c and co-stimulatory molecules CD80 and CD86) were significantly down-regulated in cell cultures with the highest infection percentage (8:1 amastigote:macrophage ratio or 60% infected cell population) (Dunnett's Test $p < 0,05$). Preliminary tests using macrophages derived from the U937 cell line show the same result pattern. Down-regulation of these cell markers seems to be related with increasing number of infected cells in macrophage population. Differences in the expression of mannose receptor CD206 between infected and non-infected cells were also found, but there is no evidence of any relation with the parasitic load. The results suggest that *L. amazonensis* infection can alter the expression of macrophage surface markers. Biological relevance of these findings is under study. **Supported by:** CNPQ e FAPESP

IM004 - The experimental infection by *Trypanosoma cruzi* increases the development of atherosclerotic lesions in mice apoE^{-/-}

*FIGUEIREDO, V.P.¹; JÚNIOR, D.C.¹; LOPES, L.R.¹; AZEVEDO, M.A.¹; LIMA, W.G.¹; BAHIA, M.T.¹; PELLUZIO, M.D.C.G.²; DA SILVA PEDROSA, A.T.³.

1. UFOP, OURO PRETO - MG - BRASIL; 2. UFV, VIÇOSA - MG - BRASIL; 3. NÚCLEO DE PESQUISA EM CIÊNCIAS BIOLÓGICAS – NUPEB, LABORATÓRIO DE DOENÇA DE CHAGAS – UFOP, OURO PRETO - MG - BRASIL.

e-mail: vpf@nupeb.ufop.br

The atherosclerosis leads to clogged arteries by the accumulation of inflammatory cells and lipids on their walls, being the apolipoprotein E (apoE) an important transport glycoprotein in this process. Some infectious agents exert pro-atherogenic effects through the action of inflammatory mediators on the vascular wall. The *Trypanosoma cruzi* is a protozoan previously associated with cardiovascular inflammation and based on that, in this study we evaluated the interference of apoE in the development of cardiovascular lesions in C57BL/6 and C57BL/6 apoE^{-/-} mice infected with the Berenice-78 (Be78) and Colombian strains of *T. cruzi*. Daily parasitemia and weekly weight of animals were performed. Animals were euthanized 30 days post infection. Blood was collected for biochemical assays (total cholesterol, HDL cholesterol and triglycerides) and immunoassays (CCL2 and CCL5). Liver and feces were used for quantification of total lipids and aortic arch and heart tissue were collected for morphometric analysis. There was lower pre-patent period in the wild group compared to the apoE^{-/-} and no difference in the gain of weight between the groups. Total cholesterol and triglycerides were elevated in apoE^{-/-} group. Besides apoE^{-/-} infected with Be78 had increased fecal lipids, while the knockout mice infected with Colombian showed elevated levels of plasma triglycerides. There was high production of CCL2 and CCL5 in all infected animals. But in apoE^{-/-}, animals infected with Be78 showed low levels of plasma CCL5 than Colombian. A high inflammatory infiltration and parasites were observed in the cardiac tissues of group infected with Colombian strain. The apoE^{-/-} group infected with Colombian strain had increased atherosclerotic lesions and collagen content. These data suggest that the experimental infection with *T. cruzi* increases the development of atherosclerotic lesions in apoE^{-/-} mice as well as interferes in the production of inflammatory mediators and some types of lipid. **Supported by:** CAPES, UFOP, CNPq

IM005 - EVIDENCES OF SIMULTANEOUS MODULATORY EFFECTS IN A MICE MODEL OF CO-INFECTION WITH *Plasmodium yoelii* AND *Leishmania* sp.

**PINNA, R.A.¹; DE LUCA, P.M.; SILVA, L.A.; DÓRES, T.L.; TINOCO, N.A.B.; CYSNE-FINKELSTEIN, L.; OLIVEIRA-FERREIRA, J.; BANIC, D.M.
IOC/FIOCRUZ, RIO DE JANEIRO, RJ, BRASIL.
e-mail:rpinna@ioc.fiocruz.br**

Introduction: Malaria and Tegumentary *Leishmaniasis* are highly endemic in several regions of the world. Even though, there are no data on prevalence of co-infection between *Plasmodium* and *Leishmania* in Brazil. Considering that populations are naturally exposed to different pathogens, it is extremely important to study the interaction of parasites and possible changes in disease progress. Objectives: determine if concurrent infection with *Leishmania* spp. and *P. yoelii* influences the course and load of parasites in each disease. Material and Methods: BALB/C mice were divided into 7 groups (G1:*P. yoelii*; G2:*L. brasiliensis*; G3:*L. major*; G4:*L. amazonensis*; G5:*L. brasiliensis*+*P. yoelii*; G6:*L. major*+*P. yoelii* and G7:*L. amazonensis*+*P. yoelii*). Firstly, mice of G2-G7 were infected with one of the 3 *Leishmania* spp. (*L. brasiliensis*:1X10⁵ parasites; *L. major* and *L. amazonensis*:1X10⁴ parasites). *P. yoelii* 17XNL infection (1X10⁶ infected erythrocytes) was performed 3 days later on G1, G5-G7. Lesions sizes were monitored with a digital caliper for 11 weeks. The time of appearance and the number of ulcers in lesions was also observed. Parasite load of infected ears was determined by a quantitative limiting-dilution assay. Malaria evolution was monitored through blood strains stained with Giemsa. Results: Lesions of co-infected mice had a slower development compared to those of *Leishmania* spp. only infected mice. Ulceration occurred later and in a less number of lesions on these groups. The load of *Leishmania* spp. parasites was slower or stable in co-infected mice during malaria. The mean peak parasitemia of *P. yoelii* was slower on G5 and G6 and parasites were more persistent on G7. There was an increase in mortality on groups G6 and G7. Conclusions: Our data demonstrates that malaria infection influences the course of *Leishmaniasis* in mice, especially during parasitemic stage. In a minor grade, *Leishmaniasis* is also capable of influence the course of malaria in co-infected mice

Supported by:IOC, CNPq

IM006 - Evaluation of Usnic Acid in regulation of inflammatory response during *Trypanosoma cruzi* infection

***SALLES, B.C.S.¹; MOTA, R.A.T.¹; BAJRACHARYA, D.S.¹; MENDES, E.C.¹; SILVA, F.H.G.²; MOURA, S.A.L.³; DA SILVA PEDROSA, A.T.³.
1.UFOP, OURO PRETO, MG, BRASIL; 2.FIOCRUZ, RIO DE JANEIRO, RJ, BRASIL; 3.; 4.
e-mail:biatinass@bol.com.br**

The *Trypanosoma cruzi* infection induces a progressive cardiac inflammation and changes organ functionality and morphology leading to myocarditis, fibrosis and heart failure. This cardiac inflammation is associated with inflammatory cytokines and chemokines that regulate the recruitment of leukocytes and fibroblasts to the site of lesions. Usnic acid (UA) is a secondary lichen compound having inhibitory action on some microorganisms and presents anti-inflammatory and analgesic properties. The aim of this study is to evaluate the properties of the UA during acute inflammation using sponge model and then to analyze these properties in mice infected with Y strain of *T. cruzi*. In the first phase of our study, sponges were subcutaneously implanted in Swiss mice and subsequently, different doses (0, 25, 50, 100 and 200mg/kg) of UA was given orally for three days after implantation. In the second phase, Swiss mice were infected with Y strain of *T. cruzi* and then treated orally for 20 days after infection with similar described doses of UA. Inflammatory and angiogenic parameters were determined using biochemical assays (N-acetylglucosamine, myeloperoxidase, hemoglobin and collagen), immunoassays (CCL2, CCL5, TNF alpha, IL-10, IFN gamma, VEGF) and histological studies. In our preliminary studies in sponge model, it was found that the dosage of 25mg/kg was able to decrease infiltration of neutrophils, collagen deposition, angiogenesis and as well as inflammatory cell influx as evident by our histological studies. Similarly, same dose of UA in infected mice anticipated the pre-patent period with an increase in the peak of parasitemia and there was decreased plasma level of CCL2 and CCL5. Thus, our data suggest that the UA has an inhibitory effect on some inflammatory mediators which can be essential to study in experimental Chagas' disease. However, further studies are necessary to understand the real effects of the UA in the inflammatory mechanism in experimental Chagas. **Supported by:**CNPq,CAPES,ISID,TWAS,UFOP

IM007 - Pre-clinical trial for comparative analyses of immunogenicity and protection levels employing LBSap, Leishmune® and Leish-Tec® vaccines in BALB/c model.

MENDONÇA, L.Z.¹; LANNA, M.F.¹; RESENDE, L.A.¹; AGUIAR-SOARES, R.D.O.¹; ROATT, B.M.¹; REZENDE, S.A.¹; CASTRO-OLIVEIRA, R.A.¹; SILVEIRA-LEMOS, D.²; VIANNA, K.F.¹; REIS, A.B.¹; GIUNCHETTI, R.C.²

1.UFOP, OURO PRETO, MG, BRASIL; 2.UFMG, BELO HORIZONTE, MG, BRASIL.

e-mail:ludzanandreis@hotmail.com

In the last years, several researchers have been employed efforts to develop a vaccine against *Leishmania* infection using mice as a model for understanding the mechanisms of immunogenicity and protection against visceral *Leishmaniasis* (VL). In the present study, we performed a pre-clinical vaccine trial, including analysis of immunogenicity and protection levels, in different groups, as follows: LBSap (composed by *L. braziliensis* antigens plus saponina), Leishmune® and Leish-Tec®. BALB/c mice were inoculated with three doses of each vaccine and challenged with *L. chagasi*. Thirty days post-challenge all animals were submitted to euthanasia. At the times before of first dose (T0), after third dose (T1; 14 days after the last dose) and 30 days after *L. chagasi* challenge (T3) the blood was collected in order to evaluate the hemogram and *ex vivo* immunophenotyping whole blood leukocytes. The spleen and liver were collected at T3 for analyzing parasite load quantified by limiting dilution. Preliminary results demonstrated at T1 a increased of total lymphocytes in Leish-Tec® and LBSap groups. The *ex vivo* immunophenotypic analyzes demonstrated an increase ($P < 0.05$) in the CD3+ and CD4+ T-lymphocytes in LBSap group, besides tendency to increase in the CD4+ T-cells in the Leish-Tec® group. Moreover, LBSap group presented reduction ($P < 0.05$) in the levels of CD19+ B-cells. After *L. chagasi* challenge (T2) Leishmune® and LBSap groups displayed increased levels of CD3+ and CD4+ T-cells and reduction of CD19+ B-cells in all vaccine groups. The limiting dilution assay for quantifying *Leishmania* demonstrated reduction in the parasite load of the splenic tissue in the LBSap and Leishmune® groups. Furthermore, LBSap presented lower levels of parasite burden in liver compared to Leish-Tec®. We concluded that mice immunized by LBSap vaccine exhibited a strong immunogenicity profile reflecting in the reduction of parasite levels. **Supported by:** CNPq, FAPEMIG, CPqRR, UFMG, UFOP

IM008 - Immunotherapy with recombinant adenovirus vaccine carrying amastigote and trypomastigote protein coding sequences restores the heart tissue damage in experimental chronic chagasic cardiomyopathy

PEREIRA, I.R.¹; MARQUES, V.F.¹; PEREIRA, G.V.¹; CAETANO, B.C.²; MACHADO, A.V.²; ROMERO, O.B.³; RODRIGUES, M.M.⁴; GAZZINELLI, R.T.²; LANNES-VIEIRA, J.¹

1.FIOCRUZ, RIO DE JANEIRO, RJ, BRASIL; 2.UFMG, BELO HORIZONTE, MG, BRASIL; 3.UFSC, FLORIANÓPOLIS, SC, BRASIL; 4.USP, SÃO PAULO, SP, BRASIL.

e-mail:pereirair@ioc.fiocruz.br

Trypanosoma cruzi infection induces a severe disease that afflicts millions of Latin Americans. Despite the fact that 50% of *T. cruzi*-infected individuals remain in an indeterminate form of the disease, the other 50% develop the digestive or, mostly, the cardiac form of Chagas disease 10-30 years postinfection. There appears to be an emerging consensus that the pathology of the chronic chagasic cardiomyopathy (CCC) is associated with parasite persistence and imbalanced host immune response favoring chronic detrimental heart inflammation. Hence, immunotherapy surges as an alternative to stimulate protective immunity, delay progression and, even, reverse CCC. Here, this idea was challenged using the homologous prime-boost strategy with recombinant adenovirus (rAd) carrying coding sequences of the amastigote surface protein-2 (ASP2) and trans-sialidase (TS) proteins, previously shown to be effective in prophylactic protocols. Chronically Colombian-infected C57BL/6 mice were treated with rAdASP2+rAdTS in a prime-boost homologous protocol initiated at 120 days postinfection (dpi). Vaccine immunotherapy significantly reversed electrical alterations after the prime (analysis at 160 dpi) and the boost (analysis at 230 dpi) and increased survival. Moreover, when compared with pre-therapy (120 dpi) pos-therapy mice (230 dpi) had reduced heart connexin 43 loss, fibronectin deposition and CK-MB activity levels in serum, markers of cardiomyocyte lesion. Additionally, rAdASP2+rAdTS immunotherapy reduced response to polyclonal stimulus, but preserved specific IFN γ -mediated immunity, decreased frequency of potentially cytotoxic IFN γ +CD107a+ and CD107a+ CD8+ T-cells, while increased systemic IFN γ levels. Thus, recombinant adenovirus immunotherapy emerges as a rational alternative to interrupt progression and recover heart tissue injury in chronic Chagas disease. **Supported by:** CNPq/ INCTV

IM009 - THE PRO-INFLAMMATORY CYTOKINES INTERFERON-GAMMA AND TUMOR NECROSIS FACTOR FAVOR *TRYPANOSOMA CRUZI* INFECTION IN ASTROCYTES

SILVA, R.R.¹; DA SILVA, A.A.²; LANNES-VIEIRA, J.¹

1.IOC/FIOCRUZ-RJ, RIO DE JANEIRO, RJ, BRASIL; 2.UFF, NITERÓI, RJ, BRASIL.

e-mail:rafaelrosi@gmail.com

Introduction: The characterization of a nervous form during the chronic phase of Chagas disease remains a matter of debate among researchers. Acute infection in children under two years old and chronic infection of immunocompromised patients, with cancer and undergoing transplantation, as well as 75-80% cases of co-infected individuals who develop the acquired immunodeficiency syndrome (AIDS), have immunopathological changes in the central nervous system (CNS) with massive parasitism. Immunocompetent individual control growth, however parasite persists in The CNS in an apparent silent manner. Thus, it is necessary to investigate the mechanisms that operate in this process of invasion/establishment of *T. cruzi* infection in the nervous tissue, as well as understand the genesis of brain lesions and no progression to a chronic inflammatory form in the CNS, despite the development of chronic heart disease, the main disease form.

Objective: In this study, we evaluated the participation of astrocytes in the control of parasitism.

Methodology: We used primary cultures of C3H/He mice astrocytes untreated or treated with cytokines prior to infection with the Colombian strain of *T. cruzi*. Infection rates were measured.

Results: Interestingly, the astrocyte cultures treated with cytokines (IFN-gamma and tumor necrosis factor) present a number of intracellular amastigote and a percentage of infected cells significantly higher than untreated cultures. Further, these differences were dependent on the time of infection and the multiplicity of infection (MOI).

Conclusion: Our data indicate that an inflammatory milieu in the CNS may facilitate the invasion of astrocytes by *T. cruzi*. **Supported by:** CAPES, CNPq

IM010 - Cellular immune responses in dogs naturally infected by *Leishmania infantum* presenting different clinical forms

LEAL, G.G.A.¹; COURA-VITAL, W.²; ROATT, B.M.¹; AGUIAR-SOARES, R.D.O.¹;

GIUNCHETTI, R.C.¹; CARNEIRO, C.M.¹; CARVALHO, A.T.³; CARNEIRO, M.²; REIS, A.B.¹

1.UFOP, OURO PRETO, MG, BRASIL; 2.UFMG, BELO HORIZONTE, MG, BRASIL; 3.CPQRR, BELO HORIZONTE, MG, BRASIL.

e-mail:gleisianegomes84@hotmail.com

Molecular analysis, serology and immunophenotyping for T lymphocytes and their subsets, B lymphocytes and monocytes were performed on dogs naturally infected with *Leishmania infantum*. Herein, the canine visceral *Leishmaniasis* was re-classified clinically in asymptomatic dogs naturally infected by *L. infantum* using to serological and molecular diagnosis in two subgroups: Asymptomatic Dogs I (AD-I) with negative serological tests, but presenting positive *Leishmania* molecular diagnosis and Asymptomatic Dogs II (AD-II) animals with positive serology and molecular diagnosis for *Leishmania*. Detailed analysis of immune response cellular T-lymphocytes (CD5+, CD4+ and CD8+), B-cell (CD21+) and monocytes (CD14+) in *ex vivo* context was performed in comparison with the Oligosymptomatic Dogs (OD), Symptomatic Dogs (SD) and Control Dogs (CD). The results demonstrated that AD-I presented similar immunophenotypic features as those detected in CD group including the number of lymphocytes (CD5+), subpopulation (CD4+), B-cell (CD21+) and monocytes (CD14+) cells. High frequency of T (CD8+) lymphocytes was observed in AD-I in comparison to all group, whilst (CD5+ and CD4+) T-cells and B-cell (CD21+) were decreased in AD-II, OD and SD in comparison to the CD and AD-I groups. The analysis of monocytes (CD14 +) did not show difference in frequency of cells in the groups. Overall, the results supported the hypothesis that the asymptomatic-I dogs have a clinical spectrum able to influence the immunological status by T (CD8+) lymphocytes and this finding may be decisive in controlling the infection or promoting the clinical evolution of canine visceral *Leishmaniasis*. **Supported by:** FAPEMIG, CNPq, PPSUS/MS and DECIT/MS

IM011 - *Trypanosoma cruzi*-induced depressive-like behavior is independent of meningoencephalitis but responsive to parasiticide and TNF-targeted therapeutic interventions

PEREIRA, G.V.¹; DA SILVA, A.A.²; PEREIRA, I.R.¹; SILVA, R.R.¹; MOREIRA, O.C.¹; ALMEIDA, L.R.¹; SOUZA, A.S.³; ROCHA, M.S.³; LANNES-VIEIRA, J.¹
 1.FIOCRUZ, RIO DE JANEIRO, RJ, BRASIL; 2.UFF, NITERÓI, RJ, BRASIL; 3.UFRJ, RIO DE JANEIRO, RJ, BRASIL. e-mail:glauciavilar@yahoo.com.br

Inflammatory cytokines and microbe-borne immunostimulators have emerged as triggers of depressive behavior. Behavioral alterations affect patients chronically infected by the parasite *Trypanosoma cruzi*. We have previously shown that C3H/He mice present acute phase-restricted meningoencephalitis with persistent central nervous system (CNS) parasitism, whereas C57BL/6 mice are resistant to *T. cruzi* induced CNS inflammation. In the present study, we investigated whether depression is a long-term consequence of acute CNS inflammation and a contribution of the parasite strain that infects the host. C3H/He and C57BL/6 mice were infected with the Colombian (type I) and Y (type II) *T. cruzi* strains. Forced-swim and tail-suspension tests were used to assess depressive-like behavior. Independent of the mouse lineage, the Colombian-infected mice showed significant increases in immobility times during the acute and chronic phases of infection. Therefore, *T. cruzi*-induced depression is independent of active or prior CNS inflammation. Furthermore, chronic depressive-like behavior was triggered only by the type I Colombian *T. cruzi* strain. Acute and chronic *T. cruzi* infection increased indoleamine 2,3-dioxygenase (IDO) expression in the CNS. Treatment with the selective serotonin reuptake inhibitor (SSRI) fluoxetine abrogated the *T. cruzi*-induced depressive-like behavior. Moreover, treatment with the parasiticide drug benznidazole abrogated depression. Chronic *T. cruzi* infection of C57BL/6 mice increased tumor necrosis factor (TNF) expression systemically but not in the CNS. Importantly, TNF modulators (anti-TNF and pentoxifylline) reduced immobility. Therefore, direct or indirect parasite-induced immune dysregulation may contribute to chronic depressive disorder in *T. cruzi* infection, which opens a new therapeutic pathway to be explored. **Supported by:** faperj, capes cnpq

IM012 - *Leishmania (Viannia) braziliensis* ISOLATES DIFFERING IN ECTO-NUCLEOTIDASE ACTIVITIES OF PROMASTIGOTE STAGE DISPLAY DIFFERENT PATTERNS OF INFECTIVITY AND DISEASE CLINICAL OUTCOME

LEITE, P.M.¹; GOMES, R.S.¹; FIGUEIREDO, A.B.¹; SERAFIM, T.D.¹; TAFURI, W.L.²; SOUZA, C.C.²; MOURA, S.A.L.¹; RANGEL FIETTO, J.L.³; MELO, M.N.²; DIAS, F.R.⁴; OLIVEIRA, M.A.P.⁴; RABELLO, A.L.T.⁵; AFONSO, L.C.C.¹
 1.UFOP, OURO PRETO, MG, BRASIL; 2.UFMG, BELO HORIZONTE, MG, BRASIL; 3.UFV, VIÇOSA, MG, BRASIL; 4.UFG, GOIÂNIA, GO, BRASIL; 5.CPQRR - FIOCRUZ, BELO HORIZONTE, MG, BRASIL.
 e-mail:pauline@nupeb.ufop.br

Leishmania braziliensis is the species responsible for the majority of cases of human cutaneous *Leishmaniasis* (CL) in Brazil; usually it causes simple cutaneous ulcer, however, parasites may also metastasize and produce mucosal lesions. Factors leading to this diversity of clinical presentations are not well known, but parasite factors have lately been recognized as important. Since ecto-nucleotidases have a crucial role in metabolism of extracellular nucleotides, which can be correlated to parasitism and the development of infection, we evaluated the activity of these enzymes in promastigotes from 23 *L. braziliensis* isolates as a possible parasite-related factor that could influence the clinical presentation of the disease. Our results show the isolates differ in their ability to hydrolyze adenine nucleotides. One isolate with high (PPS6m) and another with low (SSF) ecto-nucleotidase activity were chosen for further studies. Mice inoculated with PPS6m show a delay in the development of the peak of lesion and present larger parasite loads than animals inoculated with the SSF isolate. In addition, PPS6m was able to modulate the host immune response by inhibiting dendritic cell activation and NO production by activated J774 macrophages. Furthermore, we observed a positive correlation between the time for peak of lesion development in C57BL/6J mice and enzymatic activity and clinical manifestation of the isolate. Interestingly, we found that *L. (V.) braziliensis* isolates obtained from mucosal lesions hydrolyze higher amounts of adenine nucleotides than isolates obtained from skin lesions. Finally, we observed that the amastigote forms from PPS6m and SSF isolates present low enzymatic activity that does not interfere with NO production and parasite survival in macrophages. Thus, our data suggest that the level of ecto-nucleotidase activity of the promastigotes may influence the disease outcome in *L. (V.) braziliensis* infection. **Supported by:** CNPq, CAPES, FIOCRUZ, UFOP, PRONEX/FAPEMIG, FAPEMIG

IM013 - Infectivity of *Leishmania (Viannia) braziliensis* isolates obtained from patients with different clinical forms of tegumentary *Leishmaniasis*

AMORIM, F.M.¹; RODRIGUES, Y.K.S.; NÉRIS, P.L.D.N.; CALDAS, J.P.A.; LEITE, J.A.;
RODRIGUES-MASCARENHAS, S.; OLIVEIRA, M.R.
UFPB, JOÃO PESSOA, PB, BRASIL. - e-mail:francianne.amorim@gmail.com

INTRODUCTION: *Leishmania (Viannia) braziliensis* is the main etiological agent of different clinical forms of tegumentary *Leishmaniasis* in Brazil. Although it is known that both the host and the parasite may influence the development of these different clinical forms, the real contribution of these factors is poorly understood. **OBJECTIVE:** To evaluate the *in vitro* infectivity of *L. (V.) braziliensis* isolates obtained from patients with different clinical forms of tegumentary *Leishmaniasis*. **MATERIALS AND METHODS:** Six human isolates of *L. (V.) braziliensis* were used: three obtained from patients with localized cutaneous *Leishmaniasis* (MHOM/BR/2010/JCTS, MHOM/BR/2009/MFTS and MHOM/BR/2011/JMTS), one from mucosal form (MHOM / BR / 2010/JCNS) and two from disseminated cutaneous *Leishmaniasis* (MHOM/BR/2010/JSL and MHOM/BR/2011/AF). Peritoneal macrophages from BALB/c mice were distributed into 24-well plates (5 x 10⁵ cells /mL) and incubated at 37°C at 5% CO₂. After 2 hours, stationary growth phase promastigotes (5 x 10⁶ cells) were added in the wells. After three hours the wells were washed to remove non-internalized promastigotes. After different times of incubation, coverslips were collected, stained and examined under light microscopy. Culture supernatants were used to analyze the production of the cytokines TNF- α , IL-6 and IL-10. **RESULTS:** The isolates presented similar infectivity, increased TNF- α production and decreased IL-6 production by infected macrophages. However, when evaluating the production of IL-10, it was found that macrophage infection with JMTS, JCNS and AF resulted in an increased production of this cytokine, whereas infection with the remaining isolates did not interfere with it. **CONCLUSION:** Differences observed among the *L. (V.) braziliensis* isolates might contribute to the clinical manifestations diversity presented post-infection by these parasites. **Supported by:** Coordenação de Aperfeiçoamento de Pessoal de Nível Superior

IM014 - Memory B cells and activated T cells from immunized BALB/c mice with irradiated tachyzoites of *Toxoplasma gondii*

ZORGI, N.E.¹; GALISTEO JUNIOR, A.J.²; SATO, M.N.³; ANDRADE JUNIOR, H.F.²; DE BRITO, C.A.³
1.ICB/USP, SÃO PAULO, SP, BRASIL; 2.IMTSP/USP, SÃO PAULO, SP, BRASIL;
3.HCFMUSP, SÃO PAULO, SP, BRASIL. e-mail:nanazorgi@gmail.com

Toxoplasmosis is usually an asymptomatic but causes a severe disease in specific hosts, as immune suppressed patients or fetus. It is transmitted by ingestion of oocysts from cat feces or cysts in raw or undercooked meat. Studies have shown that 255Gy irradiated tachyzoites do not cause infection in the host but induce the same partial protection as natural infection. However, studies are still necessary on immunological memory. Demonstration of adequate immune memory is the best way to evaluate this memory without challenge the host with infecting agent. Cell surface markers can evaluate cell commitment after stimulation with specific antigen, exposing memory cells. In this study we evaluated populations of spleen cells of immunized mice by flow cytometry after *in vitro* antigen stimulation for 48 hours. Mice were immunized by orally or parenterally route with three biweekly doses of irradiated 255Gy (Cobalt60) *T. gondii* tachyzoites. Two weeks after last dose, splenocytes were isolated and cultured in the presence of antigen. Specific markers were used to label cells to evaluate phenotypic, memory and activation of T cells (CD3, CD4, CD8, CD44) and of B cells (IgM, B220, CD23, CD27), analyzed by flow cytometry. BALB/c mice without immunization showed a population of helper T lymphocytes (CD3+CD4+) higher than the immunized BALB/c mice. The expression of CD44 both in CD4+ and CD8+ population was higher in immunized group These animals also showed a high population of B cells that express IgM, while increased memory B cells (CD19+B220+CD27+) was observed in the spleen of immunized mice. The increased population of memory B lymphocytes in immunized mice indicates that vaccination with irradiated parasites can induce an immune memory and a protection without the need to challenge the mice. The elucidation of mechanisms of immune response can help the design of a vaccine to interrupt the transmission chain of toxoplasmosis. **Supported by:** LIMHCFMUSP & CNPq. **Supported by:** CNPq

IM015 - EFFICIENCY OF IgG AND IgM ANTIBODIES DETECTION IN HUMAN TOXOPLASMOSIS BY FLUORESCENCE-LINKED IMMUNOSORBENT ASSAY (FLISA)

RODRIGUES, J.P.^{*1}; COSTA, A.²; GALISTEO JUNIOR, A.J.¹; RIBEIRO-PAES, J.T.³; ANDRADE JUNIOR, H.F.¹

1.IMT-SP, SÃO PAULO, SP, BRASIL; 2.ICB/USP, SÃO PAULO, SP, BRASIL; 3.UNESP, ASSIS, SP, BRASIL.

e-mail:jaque_polizeli@hotmail.com

Toxoplasmosis is a high prevalent parasitic infection with low morbidity, which results in significant affected people, mainly ocular disease, fetal infections and encephalitis in immune deficient patients. Diagnosis is mainly by specific antibody search, especially for acute infections, with different commercial tests and antigens, but the use of low thresholds and individual variation in patients lead to frequent inconsistencies. New fluorescence linked immunosorbent assays (FLISA) have been described using high performance fluorophores conjugates in microplates with direct and linear quantification of specific antibody. Those techniques allow high throughput protocols, as necessary in screening for antenatal diagnosis of toxoplasmosis. We devised to standardize FLISA anti-*T.gondii* IgG/IgM and, after this, analyze the efficiency of those techniques in 140 serum samples previously screened by the ELISA IgG/IgM. Compared to ELISA IgG, FLISA IgG demonstrated agreement of 92.8% (n=130) and kappa coefficients showing good concordance (K=0.7088;co-positivity=68.1%,CI=45,1%-86,1%;co-negativity=97.4%,CI=92,7%-99,4%). Correlation of serologic reactivity by the FLISA IgM provided results comparable to ELISA IgM with agreement of 95.7% (n=134) and good concordance Kappa index (K=0,6026;co-positivity=71,4%,CI=29,0%-96,33%;co-negativity=96,9%,CI=92,4%-99,1%). The agreement between the results of tests suggests that the FLISA can be used effectively for screening toxoplasmosis in large populations. Further improvement could allow more efficient tests for use either in solid supports as microplates or in liquid arrays systems as magnetic particles, allowing simultaneous detection of several classes of antibodies, such as IgG, IgM and IgA, useful in high throughput applications as antenatal care or epidemiological studies. **Supported by:**LIMHCFMUSP & CAPES.

IM016 - S20 ribosomal gene of *Leishmania (Viannia) shawi* delivered as DNA vaccine inhibits cellular immune response of BALB/c mice.

CAMPOS, B.L.S.^{*1}; BAHIA, D.²; SILVA, M.S.³; CARVALHO, K.I.¹; KALLAS, E.G.¹; MORTARA, R.A.²; CORBETT, C.E.P.¹; LAURENTI, M.D.¹; PASSERO, L.F.D.¹

1.FMUSP, SÃO PAULO, SP, BRASIL; 2.UNIFESP, SÃO PAULO, SP, BRASIL; 3.UNL, LISBOA, PORTUGAL.

e-mail:bruno.lsc@hotmail.com

Leishmaniasis is considered the second most important parasitic disease in the World. Effective prophylactic measures such as vaccines development must be thus adopted. Third-generation vaccines are interesting targets because they are highly immunogenic, stable and easy to produce. Thus, the present work aimed to investigate the immunogenicity of S20 ribosomal DNA vaccine. The S20 ribosomal gene was amplified from the genome of *L. (V.) shawi* and cloned into vector pVAX1. Competent *E. coli* were transformed with pVAX1-Ribo and grown to obtain large amounts of the pVAX1-Ribo DNA vaccine. BALB/c mice were immunized three times at regular intervals of 21 days with 100 µg DNA/dose intramuscularly with or without MPL as adjuvant. After 21 days of the last immunization, the animals were sacrificed for analysis of pVAX1-Ribo immunogenicity. The immunized group showed suppression of the CD3+CD4+TNF-α+ and CD3+CD8+IFN-γ+ cellular populations. Upon stimulation with specific antigen, CD3+CD4+IFN-γ+, CD3+CD4+IL-10+, CD3+CD8+IFN-γ+, and CD3+CD8+IL-10+ populations were suppressed, but there was an increase of CD3+CD8+TNF-α+ cells. The level of IgG2a was higher in immunized animals, and these antibodies recognized a protein of approximately 13kDa in whole *L. (V.) shawi* extract. Peritoneal macrophages of the immunized group were more susceptible to *L. (V.) shawi* infection and produced lower amounts of IL-12 compared to controls. Although IL-10-producing T cells were inhibited and the TNF-α-producing CD8+T cells were increased, this vaccine candidate showed to be suppressive, since it inhibited the effector function and the production of IL-12 from macrophages, as well as IFN-γ-producing T lymphocytes responsible for infection control. Acknowledge: LIM50-HCFMUSP and FAPESP (2011/51157-1) for grants. **Supported by:**FAPESP/LIM50-HCFMUSP

IM017 - Characterization of the mechanisms involved on Neutrophil Extracellular traps (NETs) formation induced by *Leishmania amazonensis*

ROCHAEL, N.C.^{*}; DO NASCIMENTO, M.T.C.; COSTA, A.G.B.; VIEIRA, T.S.S.; DE OLIVEIRA, M.P.; DE OLIVEIRA, M.F.; SARAIVA, E.M.
 UFRJ, RIO DE JANEIRO, RJ, BRASIL.
 e-mail:natyrochael@yahoo.com.br

Neutrophil extracellular traps (NETs) are composed by a DNA scaffold decorated with histones, cytosolic and granules proteins, which ensnare and kill microorganisms when extruded from neutrophils upon activation by microorganisms, such as *Leishmania*. This new microbicidal mechanism named netosis has been shown to be dependent on ROS generation by NADPH oxidase activity and also of the enzyme peptidyl arginine deaminase 4 (PAD4), which is involved in chromatin decondensation. Here we analyze the role of ROS and PAD4 in the netosis stimulated by *Leishmania amazonensis* (La) promastigotes, using human neutrophils isolated from healthy blood donors. ROS production was inhibited by treating neutrophils with several inhibitors before activation with La, and NET release quantified by measuring DNA in the cultures supernatants. Our results show that diphenylene iodonium, a flavoprotein inhibitor, significantly inhibited NET release induced by La. Similarly, the NADPH oxidase inhibitor apocynin reduced 34%, respectively the induction of NETs by La. Also, the antioxidant N-acetylcystein reduced NETs formation by 43%. To address the role of ROS produced by the mitochondria on NET formation we treated neutrophils with Rotenone, an inhibitor of mitochondrial complex 1, which inhibited 40% NETs release by La. Confirming the mitochondrial ROS participation on NET release, treatment with carbonylcyanide p-trifluoromethoxyphenylhydrazon, an uncoupler of mitochondrial oxidative phosphorylation, reduced 67% NETs formation by La. ROS inhibition by the different treatments was ensured by ROS measurements with specific probe. The PAD4 inhibitor Cl-Amidine reduced 50% the formation of NETs by La. Together, our results ensure that ROS and histones citrullination are involved in La-induced NET formation by human neutrophils, and suggest the involvement of mitochondrial-generated ROS in this process. We thank the Hemotherapy Service of HUCFF, UFRJ. **Supported by:** FAPERJ, CAPES and CNPq.

IM018 - Exploring the inflammatory role of the mast cell/kallikrein-kinin cell pathway in the modulation of T cell responses against *Trypanosoma cruzi*.

COSTA, L.J.M.^{1,2}; BORSOI, T.¹; PESQUERO, J.²; SCHARFSTEIN, J.¹; OLIVEIRA, A.C.¹
 1.IBCCF, UFRJ, RIO DE JANEIRO, RJ, BRASIL; 2.UNIFESP, SÃO PAULO, SP, BRASIL.
 e-mail:lucasjmc@gmail.com

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 immunostimulatory 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). 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 IFN γ 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. Preliminary studies 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. **Supported by:** CNPq, FAPERJ

IM019 - Immunization with the C-Terminal Domain Peptides of *Leishmania (L.) donovani* Nucleoside Hydrolase (NH36) aiming the identification of main epitopes

NICO, D.^{*1}; RODRIGUES, M.M.²; PALATNIK, M.¹; SOUSA, C.B.P.¹
 1.UFRJ, RIO DE JANEIRO, RJ, BRASIL; 2.UNIFESP, SÃO PAULO, SP, BRASIL.
 e-mail:dirlei@micro.ufrj.br

The C-terminal domain of NH36 (Nucleoside Hydrolase), nominated F3 (amino-acids 199-314), induced a main CD4+ T cell driven response. Immunization with F3 exceeds in 36.73±12.33% the protective response induced by NH36 and increases IgM, IgG2a, IgG1 and IgG2b antibodies. CD4+ T cell proportions, IFN-γ secretion, ratios of IFN-γ/IL-10 producing CD4+ and CD8+ T cells and percents of antibody binding inhibition by synthetic predicted epitopes and a 90.5-88.23% decreases in parasite load were detected in F3 vaccinated mice (NICO et al., Plos NTD, 2010). In order to map the domain which is the target of the adaptive immunity in F3 protein, we cloned three recombinant peptides: F31 (a.a. 199-241), F32 (a.a. 242-274) and F33 (a.a. 275-314) in pET28b system. The recombinant peptides were purified and then female Balb/c were vaccinated weekly, by the sc route, with three doses of 100µg of F3, F31, F32 or F33 recombinant proteins and 100µg of saponin (Sigma). Seven days after immunization, sera were collected and the intradermal response (IDR) to *Leishmania* antigen were performed. Mice were challenged with amastigotes of *Leishmania chagasi* and euthanized 15 days later. After immunization, the F31sap vaccine increased the IgG, IgG1 and IgM antibodies (p<0.05). But, after infection, only the IgG antibodies were increased (p<0.05) by the F31sap. After immunization, the IDR response at 24h and 48h after injection were significantly increased by the F31sap vaccine and after challenged at 24h the F31sap and F33sap vaccines were increased (p<0.05). F33sap was the only one that sustained the intradermal response at 48h. The F33sap determined the highest parasite load reduction (73.64%) followed by the F31sap (61.34%) (p<0.05). The F32sap showed the least response. These results agree with the prediction of algorithm program and confirm the presence of the important epitopes for antibodies generation in F31 and for CD4+ T cell in F31 and F33. **Supported by::**CAPES, FAPERJ, CNPQ

IM020 - TROPONIN T AUTOANTIBODIES CORRELATED WITH CHRONIC CARDIOMYOPATHY IN HUMAN CHAGAS DISEASE

NUNES, D.F.^{*1}; GUEDES, P.M.M.¹; ANDRADE, C.M.¹; OLIVEIRA, P.I.C.¹; CÂMARA, A.C.J.¹; CHIARI, E.²; GALVÃO, L.M.¹
 1.UFRN, NATAL, RN, BRASIL; 2.UFMG, BELO HORIZONTE, MG, BRASIL.
 e-mail:daniela_fnunes@yahoo.com.br

Myocardium damage during Chagas' disease results from parasite and immune response action. The molecular mimicry among *Trypanosoma cruzi* proteins and several self-antigens has been widely described, generating auto reactive CD8+ T cells and auto reactive antibodies. Autoantibodies generation and its role in Chagas disease immunopathogenesis are not elucidated. Here, troponin T and myosin auto-antibodies (IgG and isotypes) production were studied in ELISA using recombinant protein as antigen on sera of chagasic patients with indeterminate (IND/n=88), cardiac (CARD/n=48) and digestive/mixed (MIX/n=13) clinical forms of the disease, and samples from uninfected individuals (NI/n=7). The autoantibodies levels were correlated with left ventricular ejection fraction (LVEF). The results showed that sera from IND, CARD and MIX chagasic patients presented higher levels of total immunoglobulin G (IgG) specific troponin-T (p=0.0001) and myosin (p=0.0002) antibodies than NI. When grouped CARD patients in auto antibodies producers and not producers, and compared LVEF, we observed that anti-troponin T (p=0.042) and anti-myosin (p=0.013) producers displayed lower LVEF than patients that not produce autoantibodies. Moreover, anti-troponin T (p=0.0411) and anti-myosin (p=0.0034) IgG2 production was higher in CARD than IND chagasic patients. However, none difference in IgG1 production levels was observed in chagasic patients. Patients with IND, CARD and MIX clinical forms displayed a negative correlation between anti-troponin T/anti-myosin levels in serum and LVEF. These findings indicate that increased production of anti-troponin T and anti-myosin auto-antibodies is correlated with severity of Chagas cardiomyopathy. **Supported by::**CNPq; CAPES; FAPERJ

IM021 - PROTOCOL FOR PARASITISM QUANTIFICATION OF *Neospora caninum* BY FLUORESCENCE

MOTA, C.M.¹; FERREIRA, M.D.²; SILVA, M.V.¹; COSTA, L.F.¹; SILVA, M.J.B.¹; MINEO, J.R.¹; MINEO, T.W.P.¹

1. UFU, UBERLÂNDIA, MG, BRASIL; 2. USP, RIBEIRÃO PRETO, SP, BRASIL.

e-mail: carolinemartinsm@yahoo.com.br

Introduction: The techniques for measurement of parasite load have evolved throughout the years. Quantification of stained slides using a light optical microscope is the most common technique applied. However, this evaluation is labor-intensive procedure and somewhat subjective. Moreover, genetic manipulation technology is not a common procedure with *Neospora caninum*, turning results less reliable and reproductive. Thus, we aimed to develop a simple protocol for parasitism quantification of *N. caninum* through tachyzoite staining with fluorescent probes based on ester compounds - DDAO-SE CFDA-SE. Methods: Quantification protocols using traditional slide staining (Toluidine blue and Diff Quick) and fluorescence-based methods (CFDA-SE or DDAO-SE) were compared using HeLa cell line as host cells, infected with different rates of infection (MOIs - multiplicity of infection). The slide assays were read in optical microscopes, whereas the fluorescent probe-stained parasites were quantified by flow cytometry. Results: Notably, HeLa cells presented a dose-dependent parasite load, regardless of the technique used for quantification. However, parasite quantification by fluorescent probes CFDA-SE and DDAO-SE was performed within minutes, while slides had to be quantified along several days. Conclusion: This study described a simple and reproducible protocol for *N. caninum* quantification through parasite staining with fluorescent ester-based probes. **Supported by:** CAPES, CNPq, FAPEMIG, and FINEP.

IM022 - Troubleshooting of evaluation in the serological prevalence of toxoplasmosis in pigs of extensive breeding

DA SILVA, V.R.¹; GALISTEO JUNIOR, A.J.; ANDRADE JUNIOR, H.F.

1. IMTSP/USP, SAO PAULO, SP, BRASIL.

e-mail: vrodrigues_vanessa@hotmail.com

Toxoplasmosis is caused by an obligate intracellular protozoan *Toxoplasma gondii*, which affects warm blood animals and man. The transmission is by the consumption of contaminated food and water, raw or undercooked infected meat and transplacental infection. Meat-producing animals, such as pigs, are potential sources of transmission of *T. gondii* for humans. Prevalence in swine stocks varied according different regions or farming sanitation. Here, we study the prevalence of toxoplasmosis in pigs from extensive breeding farms near large São Paulo metropolitan area. We obtained 240 serum samples from adults pigs abated in municipal abattoirs. All samples were screened by ELISA or fluorescence-linked immunosorbent assay (FLISA) for detection of specific anti-*T.gondii* IgG antibodies. No parasitological approach was attempted to identify infected pigs. We observed discrepancies between the correlations of the tests probably due to the exponential ELISA results as compared to linear FLISA. Serological data from tests were analyzed using two cut-offs, the 99% confidence interval of the mean (LCO) and the classical mean plus 2 standard deviation (HCO). LCO sensitivity of 42% and a specificity of 74% were attained with a 0.1759 kappa index but HCO resulted in lower 36% sensitivity but higher 98% specificity 0.4239 kappa index. Those data results both in high prevalence of 52.5% (95%CI 46-59) with one of the two assays positive test using LCO or lowest prevalence of 1.7% (95%CI 0.5-4.5%) with both positive assays using HCO. We will discuss any other combination to show that the prevalence using presumptive test. Parasitological approach is mandatory in at least part of the sample in prevalence studies. Better improvement in FLISA could be found with chaotrope washing, but will remain the main troubleshooting problems. . Our results show the troubleshooting of receiving samples from an area without access to parasitological data from infected animals. **Supported by:** FUNDAP

IM023 - Immunotherapy against Visceral *Leishmaniasis* with Nucleoside Hydrolase peptides of *Leishmania (L.) donovani*

NICO, D.; FEIJÓ, D.F.; GOMES, D.C.; PALATNIK, M.; SOUSA, C.B.P.
 UFRJ, RIO DE JANEIRO, RJ, BRASIL.

e-mail:dirlei@micro.ufrj.br

Nucleoside hydrolases (NHs) show homology among parasite protozoa, fungi and bacteria. They are vital protagonists in the establishment of early infection and are therefore excellent candidates for the pathogen recognition by adaptive immune responses. The C-terminal domain of NH36 (Nucleoside Hydrolase), called F3 (amino-acids 199-314) induced a main CD4+ T cell driven response (NICO et al., Plos NTD, 2010). Three recombinant peptides covering the whole NH36 sequence were generated in the pET 28a and pET28b systems: F1 (amino acids 1-103, N-terminal domain); F2 (aa 104-198, central domain) and F3 (aa 199-314, C-terminal domain). In the present work we aimed to identify the domains that contain the relevant epitopes for immunotherapy against infection by *L. (L.) chagasi*. Female Balb/c were infected with 3x10⁷ amastigotes of *L.(L.) chagasi* and, 15 days later, they were vaccinated at weekly intervals, by the sc route, with 3 doses of 100µg of NH36, F1, F2 or F3 recombinant peptides and 100µg of saponin (Sigma). All the vaccines induced IgM, IgG, IgG1 and IgG2b antibodies higher than saline controls (p<0.0002). The intradermal reaction to the *Leishmania* lysate and the secretion of TNF-α, as well as the reduction of the hepatomegaly and the gain in corporal weight, were all strong correlates of protection to F3sap vaccine. We showed significant increase in the vaccines to CD4+/IL-2+ T cell (p<0.005). The F1sap and F3sap vaccines induced higher proportions by CD4+/IL-2+ T cells over the saline control (p<0.05). However, the F1sap vaccine, induced secretion of IFN-γ and IL-10 in the supernatants of the culture in vitro. Finally, the F3sap determined the highest parasite load reduction followed by de F1sap vaccine. The identification of the target of the immune response to NH36 represents a basis for the rationale development of a bivalent vaccine against *Leishmaniasis* and for multivalent vaccines against NHs-dependent pathogens. **Supported by:**CAPES, FAPERJ, CNPQ

IM024 - Evaluation of the role of immunoglobulins of immunized animals with *Toxoplasma gondii* irradiated tachyzoites to protect against brain cysts

FORGOSO, R.C.; GALISTEO JUNIOR, A.J.; ZORGI, N.E.; ANDRADE JUNIOR, H.F.
 IMTSP/USP, SAO PAULO, SP, BRASIL.

e-mail:raissa_forchar1@hotmail.com

Toxoplasma gondii is an intracellular protozoan of the phylum Apicomplexa, which causes toxoplasmosis, as definitive host has cats, whereas other species of mammals and birds are intermediate hosts. Infection with *T.gondii* naturally occurs through ingestion of raw or undercooked meat containing cysts or oocysts from cat faces. Vaccines containing gamma-irradiated parasites have been used successful in mice immunization. Mice immunized with irradiated tachyzoites intraperitoneally, have an antibody response similar to infected animals, showing the preservation of their characteristics and provide immunity to the animals similar to a natural infection. We studied the role of IgG antibodies from animals (C57Bl/6j mice) immunized with 10⁷ tachyzoites radiation-sterilized (255Gy/60Co) *T. gondii* RH stain intraperitoneal(i.p), with 3-biweekly doses against the formation of tissue cysts. Immunoglobulins were precipitated and incubated at 37°C for 3 hours with tachyzoites of *T. gondii* ME49 strain for the immune complex formation (antigen + antibody). The inoculum was conducted with two groups (n = 5) in C57BL/6J mice, which the first group received a dose of immune complex intraperitoneally and the other group (control) a dose of tachyzoites ME49 i.p. After thirty days, the brain and blood of the animals were taken for antibody detection by ELISA and tissue cysts by conventional optical microscopy. The extracted DNA was subjected to polymerase chain reaction (PCR). After the challenge was possible to observe by optical microscopy the absence of tissue cysts in animals that received the immune complex and was confirmed by PCR that showed low parasitemia with the absence in one sample. With these results we can suggest that antibodies against *T. gondii*, produced by animals immunized with irradiated parasites, are effective in protecting and plays an important role in the decrease of brain cysts in toxoplasmosis. **Supported by:** LIMHCFMUSP & CNPq.

IM025 - Immune complex dissociation ELISA for *Leishmaniasis*: Standardization of the assay in experimental models and preliminary results in canine and human samples.

CARVALHO, C.A.¹; HIRAMOTO, R.M.²; BORBOREMA, S.E.T.²; CARVALHO, R.P.³;

NASCIMENTO, N.D.⁴; MEIRELES, L.R.¹; ANDRADE JUNIOR, H.F.⁵

1.IMTSP/USP, SÃO PAULO, SP, BRASIL; 2.IAL/SES/SP, SÃO PAULO, SP, BRASIL;

3.CPS/SES, ARAGUAÍNA, TO, BRASIL; 4.IPEN/CNEN/SP, SÃO PAULO, SP, BRASIL;

5.UNIVERSIDADE DE SÃO PAULO, SAO PAULO, SP, BRASIL.

e-mail:hfandrad@usp.br

Leishmania (L) chagasi visceral *Leishmaniasis* is a chronic parasitic disease in humans and dogs. Finding the agent in bone marrow, lymph node or spleen aspirate is diagnostic, and specific IgG serology is used mainly for epidemiology, despite high level of serum immunoglobulin. There are few anecdotal reports of false negative serology in active disease ascribed to immune complexes. Dissociation of immune complexes could be performed by acid and we devised a simple test for dissociation of immune complexes in serum samples in routine ELISA, by acid treatment and neutralization in wells adsorbed with *Leishmania* antigen (dELISA). Confirmatory acid DOT ELISA was also developed for antigen detection by anti-*Leishmania* rabbit antiserum. In experimental hamster L.chagasi models, immune complexes interferes with ELISA mostly in the early stages of infection, as detected both by dELISA or antigen DOT-ELISA. In larger samples from endemic areas, dELISA increases seropositivity by 10% in negative dog samples (7/70) and 3.5% in negative human samples (3/85), both confirmed by antigen DOT-ELISA, showing that this test could be used in the serodiagnosis of visceral *Leishmaniasis*. This simple tool could be used as alternative approach to screening asymptomatic visceral *Leishmaniasis* patients with subclinical disease for invasive confirmatory tests. **Supported by:**:LIMHCFMUSP?CAPES

IM026 - Correlation between ectonucleotidasic activity and infective capacity of different clones of *Leishmania amazonensis*.

GOMES, R.S.¹; CARVALHO, L.C.F.¹; MELO, M.N.²; AFONSO, L.C.C.¹

1.UFOP, OURO PRETO, MG, BRASIL; 2.UFMG, BELO HORIZONTE, MG, BRASIL.

e-mail:rodrigosaar@nupeb.ufop.br

NTPDases are responsible for the hydrolysis of ATP to AMP and can control the immune response by reducing the inflammatory stimulus of ATP. Data from our group have shown that *L. amazonensis* expresses these enzymes on its surface. Here, we compared the infective capacity and ectonucleotidasic activity of different clones of PH8 *L. amazonensis* strain. For this, C57BL/6 mice were infected with 1×10^5 metacyclic forms of different clones. The clones 3Ia and 1IIIId lead to significantly smaller lesion compared to the original PH8 strain. Ectonucleotidasic activity was assayed by measurement of Pi released by incubation of parasites with ATP, ADP or AMP. Interestingly, the two clones presented an ATPase activity 40% lower than the original strain. Macrophages derived from the peritoneum of C57BL/6 mice were infected with the different clones in a rate of 3 parasites per cell. The evaluation was done by direct counting on optical microscopy. After 30 minutes, there was a 30% reduction in the percentage of macrophages with parasites attached when comparing the two clones with the original strain. Moreover, after 3 hours, we observed that the clones infected 40% less macrophages than the PH8 strain. After this time, the wells were washed and the cells stimulated with IFN- γ and LPS. After 48 hours, we observed that there was no reduction in the percentage of infected macrophages with the original strain. However, macrophages infected with clones were able to control parasite proliferation. Greiss method was used for measurement of NO and it was observed that, as compared to uninfected cells, only the original strain led to a 50% reduction in the NO production. Thus, the ectonucleotidasic activity of *L. amazonensis* is important for the infective capacity of the parasite and modulation the NO production by macrophage, favoring the survival and persistence of the parasite infection.

Supported by::CNPq; FAPEMIG; CAPES

IM027 - Use of meat exudate in the detection of IgG anti-*Toxoplasma gondii* for quality control of commercial meat cuts

MARCIANO, M.A.M.*; MECCA, J.N.; ANDRADE JUNIOR, H.F.; MEIRELES, L.R.
IMT, SAO PAULO, SP, BRASIL.
e-mail:mmm.bio@ig.com.br

Toxoplasmosis is a highly prevalent protozoan infection that causes serious problems in immunocompromised adults and congenitally infected children beyond economic losses due to reproductive problems in cattle herds, especially those destined to human consumption. The infection is mainly transmitted by ingestion of raw or undercooked meat, containing *T. gondii* cysts, characterizing this disease as one of the main zoonosis transmitted by food. In Brazil, there is no sanitary monitoring program for detection of *T.gondii* in commercial meat cuts. Detection laboratory methods for cysts as PCR or bioassay have limitations due to the long processing time and the uneven distribution of cysts in meat. It was demonstrated by standardized ELISA that the meat exudate from calves experimentally infected with *T. gondii* allows anti-*T. gondii* IgG detection in meat cuts, representing a promising approach to meat quality control. We decided to apply this method to research IgG in commercial beef cuts (n = 99) obtained at retail. Samples were tested at equivalents blood concentrations, determined by 540nm of absorbance. ELISA results showed a positivity of 38.38% (38/99) in the samples analyzed. This prevalence was similar to those described in the literature. Our results show the feasibility of this diagnostic approach, which could be very important and promising for food sanitation vigilance services or detection of prevalence of animal infection, contributing directly to the prevention of human infection and also to the elucidation of toxoplasmosis outbreaks transmitted by ingestion of contaminated meat.

IM028 - Immunotherapy against *Leishmania (L.) amazonensis* infection with Nucleoside Hidrolase (NH36) peptides of *Leishmania (L.) amazonensis*

NICO, D.*; FEIJÓ, D.F.; GOMES, D.C.; PALATNIK, M.; SOUSA, C.B.P.
UFRJ, RIO DE JANEIRO, RJ, BRASIL.
e-mail:danicg_rj@hotmail.com

The C-terminal domain of NH36 (Nucleoside Hydrolase), called F3 (amino-acids 199-314) induced a main CD4+ T cell driven response. Immunization with F3 exceeds in 36.73±12.33% the protective response induced by NH36. Increases in IgM, IgG2a, IgG1 and IgG2b antibodies. CD4+ T cell proportions, IFN- γ secretion, ratios of IFN- γ /IL-10 producing CD4+ and CD8+ T cells and percents of antibody binding inhibition by synthetic predicted epitopes and a 90.5-88.23% decreases in parasite load were detected in F3 vaccinated mice (NICO et al., Plos NTD, 2010). Three recombinant peptides covering the whole NH36 sequence were generated in the pET 28a and pET28b systems: F1 (amino acids 1-103, N-terminal domain); F2 (aa 104-198, central domain) and F3 (aa 199-314, C-terminal domain). In the present work we aimed to identify the domains that contain the relevant epitopes for immunotherapy against tegumentary *Leishmaniasis* by *L.(L.) amazonensis*. Female Balb/c were challenged with 1x10⁵ infective promastigotes of *L.(L.) amazonensis* and 6 weeks later they were vaccinated at weekly intervals, by the sc route, with 3 doses of 100 μ g of NH36, F1, F2 or F3 recombinant peptides and 100 μ g of saponin (Sigma). F3sap and NH36sap increased the IgA, IgM, IgG, IgG2a antibodies (p<0,05). The intradermal response to *L.(L.) amazonensis* lysate was higher in F3sap and NH36sap, at 24h and at 48h after injection. The secretion of the TNF- α was increased only by F3sap and F1sap vaccines (p<0.05). NH36sap and F3sap vaccines showed the smallest level of IL-10 in compared to saline control (p<0,05). The highest protection was from F3sap vaccine. The size of the lesion was reduced in 64% (p<0,001) compared to saline control and in 48% (p<0,05) to the F2sap vaccine. The identification of the target of the immune response to NH36 represents a basis for the rationale development of a bivalent vaccine against *Leishmaniasis* and for multivalent vaccines against NHs-dependent pathogens.
Supported by::CAPES, FAPERJ, CNPq

IM029 - Evaluation of Agglutination Tests of Anti - *Toxoplasma gondii* IgG as Diagnostic Methods for Quality Control of Meat

PINAGÉ DA SILVA, L.T.A.*; MARCIANO, M.A.M.; MECCA, J.N.; ANDRADE JUNIOR, H.F.; MEIRELES, L.R.
IMT, SAO PAULO, SP, BRASIL.
e-mail:mmm.bio@ig.com.br

Toxoplasma gondii is an obligate intracellular parasite that infects warm-blooded animals, causing severe disease in immunosuppressed individuals and economic losses in livestock. Ingestion of raw or undercooked meat containing viable cysts of the parasite is one of the main routes of its transmission. Laboratory methods for cyst detection as PCR or bioassay are impractical to perform on a large scale due to slow tests and random presence of cysts throughout the meat. It was demonstrated by standardized ELISA in experimental models that meat exudate allows anti-*T. gondii* IgG detection, with possible application in commercial cuts. In this work, we standardized agglutination tests as indirect hemagglutination (IH) and modified agglutination test (MAT) to evaluate the meat exudate efficiency in toxoplasmosis diagnosis as faster methods, without species-specific reagents and feasible in field situations. We used 77 meat exudate samples from rabbits and 89 samples from cattle previously analyzed by ELISA. Both IH and MAT demonstrated similar results to ELISA, our gold standard, and the tests were reproducible for rabbit samples, but for cattle samples the results were worse with some false negative results. These data show that the agglutination tests, using the meat exudate, should be strictly standardized and evaluated for each species before they are used as screening tests, since they can suffer nonspecific action of the exudate constituents as demonstrated in our work to cattle samples. However, techniques more sensitive as ELISA, do not seem to suffer this interference, since results were reproducible and reliable in all models of experimental infection with *T. gondii* evaluated in our work. Our ELISA results show the feasibility and importance of this diagnostic approach, using meat exudate samples as a tool for quality control of meat for marketing, contributing directly to the prevention of human infection.

IM030 - Proteomics of humoral response of experimental re-infections or reactivations in immunosuppressed mice infected and challenged with genetically distinct *Toxoplasma gondii* strains

MEIRELES, L.R.*; SANTOS, T.C.C.; ANDRADE JUNIOR, H.F.
IMTSP, SÃO PAULO, SP, BRASIL.
e-mail:lrmeirel@usp.br

Several strains of *T.gondii* have been described in recent years, with variable virulence that could be associated with human disease severity, including in the reinfection of a previous immune host. Although asymptomatic in most cases, the agent causes ocular disease or severe disease in immune compromised patients or fetus. For evaluate the effect of cross-protection between genotypes in reinfection or cyst reactivation, we study several protocols of infection and sequential challenge with genetically distinct strains of *T.gondii* (ME-49, type II strain or VEG, type III strain) in normal or dexamethasone treated mice. Infection was evaluated by strain specific humoral response and number of brain cysts. Specific IgG were determined by ELISA with peptides GRA6II (specific to ME49 strain) and GRA6I/III (specific to VEG strain). Groups of 10 BALB/C mice were infected orally with cysts of each strain and challenged with heterologous strain after 4 or 10 weeks of first infection, immunosuppressed or not by dexamethasone in the 8th and 11th week after primary infection. All effects were measured 14 weeks after primary infection. Brain cysts numbers appears not to be affected by challenge or immunosuppression. Humoral response was maintained specific against the strain of primary infection. Challenge strain boosts this primary humoral response without new specific antibodies production. Immunossuppression lowers antibody titers; despite similar brain cysts numbers. Challenge with heterologous strain usually protects from the disease observed in each strain alone, but without altering antibody profiles or tissue response, with ME49 infection protecting from VEG cyst burden. Studies on parasite burden by qrtPCR and identification of cyst origin by PCR RFLP are under progress. Experimental mice models could be interesting tools in the study of the origin of infecting organisms in *Toxoplasma* infections in immunosuppressed patients. **Supported by:** CNPq

IM031 - THE NOD-LIKE RECEPTORS ACCOUNT FOR INNATE AND ADAPTIVE IMMUNITY AGAINST *LEISHMANIA* INFECTION

LIMA-JUNIOR, D.S.¹; SILVA, E.L.¹; MARIM, F.¹; ULIANA, S.R.B.²; SILVA, J.S.¹; ZAMBONI, D.S.¹

1.FMRP/USP, RIBEIRAO PRETO, SP, BRASIL; 2.ICB/USP, SÃO PAULO, SP, BRASIL.

e-mail:djalmajunior@usp.br

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- κ B 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. Herein, we investigated the participation of Nod/Rip2 pathway in host response during *L. major* infection. Using bone marrow-derived macrophage (BMDMs) or –dendritic cells (BMDCs) from C57BL/6, Nod1^{-/-}, Nod2^{-/-} and Rip2^{-/-} mice, we observed that Nod1^{-/-}, Nod2^{-/-} and Rip2^{-/-} BMDMs had an impaired induction of NF- κ B–dependent products in response to infection and failed to restrict *L. major* replication. Moreover, IL-12p40 production and surface molecules expression were decreased in infected-Rip2^{-/-} BMDCs. *In vivo* infection demonstrated that Nod1 activation was crucial for efficient control parasite replication and resolve cutaneous lesions after 8 weeks of infection. Moreover, Rip2-dependent response was required for dendritic cells activation and induction of effective Th1 response *in vivo*. Analyzing the susceptibility and cytokines production in chimeras generated by irradiating recipient mice, we observe that Rip2-dependent signaling in radio-sensitive compartments was required for the control of the infection and induction of Th1 response. 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 in the induction of innate immune responses against *Leishmania* parasite, thus providing a novel function for Nod-like receptors family in parasite-host interactions. **Supported by::**FAPESP

IM032 - Saliva as a source of anti-*Toxoplasma gondii* IgG for enzyme immunoassay

SAMPAIO, B.F.C.^{*}; MEIRELES, L.R.; ANDRADE JUNIOR, H.F.

IMTSP, SÃO PAULO, SP, BRASIL.

e-mail:lrmeirel@usp.br

Toxoplasmosis that affects about one billion people worldwide is usually asymptomatic, despite ocular disease and severe and lethal disease in fetuses, AIDS patients and transplant recipients. Serology is the main approach for diagnosis and incidence determination is a difficult task due to high prevalence in most countries. Incidence studies are feasible in children but this age group is protected and difficult to approach by invasive methods as venipuncture. Saliva could be obtained by non-invasive procedure, acceptable for children, and it contains small amounts of IgG from mucosal and gingival crevicular fluid. Available antibody detection methods are focused in serum samples, with low sensitivity and few reports of alternative biological material, like saliva. Here, we standardized immunoassays with high sensitivity for detection of anti-*T. gondii* IgG in paired saliva and serum sample from 20 adult volunteers, which allows DOT-ELISA and a Protein A IgG capture assay. The sensitivity and specificity of the saliva DOT-ELISA were similar to sera ELISA. We also tested 100 saliva samples from university graduates in all assays, showing 19% (95%CI 12-28%) frequency of toxoplasmosis in this group, lower than reported for our area. Protein A IgG capture saliva assay was also efficient with similar results. Immunoassay with saliva IgG for toxoplasmosis is a very promising tool for use for the epidemiology of toxoplasmosis in children or other protected groups. **Supported by::**CAPES e LIM49/FMUSP

IM033 - Mast cells propagate inflammation in peripheral sites of *Trypanosoma cruzi* infection through the activation of the kallikrein-kinin cascade

NASCIMENTO, C.R.¹; ANDRADE, D.S.¹; DA SILVA, T.C.¹; DE ANDRADE, M.V.M.²; JULIANO, M.A.³; JULIANO, L.³; SCHARFSTEIN, J.¹; SVENSJO, E.¹

1.UFRJ, RIO DE JANEIRO, RJ, BRASIL; 2.UFMG, BELO HORIZONTE, MG, BRASIL;

3.UNIFESP, SÃO PAULO, SP, BRASIL.

e-mail:clarissarn@hotmail.com

In several heart disease models, there is evidence that myocardial remodeling is critically dependent on cardiac mast cells (MCs). Using plasma leakage as a read-out, we demonstrate that *T. cruzi* trypomastigotes (Dm28c strain) activate the Kallikrein-Kinin System (KKS) in MC-dependent manner. Our data showed that *T. cruzi*-evoked edema (i) is virtually abolished by a specific FXIIa inhibitor or by cromolyn, a stabilizer of MC granules (ii) was negligible in mast cell (c-kit)-deficient mice. Intravital microscopy in the hamster cheek pouch (HCP) yielded clues about the roles played by MCs and the activation of the intrinsic coagulation pathway/KKS. Addition of histamine (low concentrations) to the HCP strongly upregulated trypomastigote-induced plasma leakage, whereas treatment with cromolyn nullified these effects. We then found that histamine priming led to full-fledge plasma leakage in HCPs exposed to dextran sulfate 500 kDa, a negatively charged polymer which activates the KKS via FXII. Besides, the treatment with cromolyn significantly reduced the plasma leakage. Collectively, these results suggest that perivascular mast cells, acting as sensors of trypomastigotes, may link innate immunity to the KKS, generating high levels of vasoactive kinins and other inflammatory mediators in peripheral sites of infection. Ongoing studies may clarify whether the intrinsic pathway of coagulation might entrap parasites in fibrin meshes, perhaps creating a physical barrier that limits *T. cruzi* spread away from the primary foci of infection. **Supported by:** CNPq, FAPERJ, INBEB

IM034 - IMMUNOGENICITY OPTIMIZATION FROM RECOMBINANT VACCINE NUCLEOSIDE HYDROLASE (NH36) OF *Leishmania (L.) donovani* MADE BY A CHIMERA COMPOUND OF F1 AND F3 PEPTIDES

GOMES, D.C.¹; NICO, D.; FEIJÓ, D.F.; SOUSA, C.B.P.

UFRJ, RIO DE JANEIRO, RJ, BRASIL.

e-mail:danicg_rj@hotmail.com

Nucleoside Hydrolase (NH36) is the main molecule from FML complex of the vaccine Leishmune®, licensed for prophylaxis to canine visceral *Leishmaniasis*. Experiments performed with Balb/C mice immunized with the fragments F1 (1-103aa), F2 (104-198aa) and F3 (199-314aa) and saponin showed that protection against *L. (L.) chagasi* was related to its C-terminal domain (F3), mainly mediated by a CD4+ T cells response. Immunization with this peptide exceeded 36.73%±12.33 from the protective response induced by the cognate protein NH36, increasing: antibodies, CD4+ T cells, secretion of IFN-gama, ratio of CD4+ and CD8+ T cells producing IFN-gama/IL-10 and the percentages of inhibition of binding of antibodies by synthetic predicted epitopes. The increase in the Intradermoreaction (IDR) and in the ratio of CD4+ T cells producing TNF-alfa/IL-10 were strongly correlated with protection that was confirmed by the assay of in vivo depletion with monoclonal antibodies, by the epitopes for CD4 and CD8 cells predicted by the programs based algorithm Sette and by the pronounced decrease in parasite load. However, it was not detected any decrease in the parasitic load due to vaccination with the N-terminal domain (F1) of NH36, although there was an increase ratio of CD4+ T cells producing IFN-gama/IL-10 after challenge (NICO et al., 2010).

In this project, we aim to obtain a chimera of F1-F3 in pET28b system and compare its immunogenicity against the F1 and F3 peptides expressed independently. As an initial approach, Balb/C mice were immunized with 100µg of F1, 100µg of F3 or 100µg of F1 plus 100µg of F3 formulated with 100µg of saponin. We evaluated the immunogenicity of the vaccines by analysis of IDR and liver parasite load. After immunization, at 24h and 48h, the F3sap and F1+F3sap both showed the highest response. However, after challenge, the F1+F3sap vaccine was the highest IDR response against *Leishmania* antigen and a reduction of load parasite in 72,22%. **Supported by:** CAPES, FAPERJ, CNPq

IM035 - LBSap vaccine elicited long-lasting type 1 immune response in late period of *L. chagasi* challenge, displaying higher levels in both IL-12 and IFN- γ and lower production of IL-4

RESENDE, L.A.¹; ROATT, B.M.¹; AGUIAR-SOARES, R.D.O.¹; LANNA, M.F.¹; VIANNA, K.F.²; MENDONÇA, L.Z.¹; DA SILVA, J.G.L.¹; SILVEIRA-LEMOS, D.²; REIS, A.B.¹; GIUNCHETTI, R.C.²

1.UFOP, OURO PRETO, MG, BRASIL; 2.UFMG, BELO HORIZONTE, MG, BRASIL.

e-mail:lucilenearesende@yahoo.com.br

Dogs are the main reservoir of the *Leishmania infantum* (syn. *Leishmania chagasi*) and there is much interest in the understanding of mechanisms implicated in immunoprotection against canine visceral *Leishmaniasis* (CVL). In this context, the vaccine composed by *Leishmania braziliensis* promastigotes protein plus saponin as adjuvant (LBSap) has been investigated as a pre-requisite to understanding the mechanisms of immunogenicity against CVL. Cytokines (IL-4, IL-10, TNF- α , IFN- γ and IL-12) were evaluated in the in vitro context from supernatants of peripheral blood mononuclear cells (PBMC) cultures at the times previous vaccine protocol (T0), after third vaccine dose (T3) and at times 90 (T90) and 885 (T885) days after experimental challenge (dac). Our major results demonstrated that LBSap vaccine induced higher levels in both, IL-12 and IFN- γ , after vaccine protocol (T3) in PBMC stimulated with vaccine soluble antigen (VSA) and increased levels ($P < 0,05$) of IFN- γ in the presence of soluble *L. chagasi* antigen (SLcA). Furthermore, LBSap vaccine induced higher levels of IL-12, after early period of the *L. chagasi* challenge (T90) in PBMC stimulated with VSA and higher levels of IFN- γ in the presence of SLcA. Interestingly, after late period of the *L. chagasi* challenge (T885), LBSap group maintained higher ($P < 0.05$) levels of IL-12 (in the presence of VSA) and IFN- γ (in the presence of SLcA) and lower production of IL-4 (in the presence of VSA). We concluded that LBSap vaccine induced long-lasting type 1 immune response after challenge with *L. chagasi*, that was associated with immunoprotection. **Supported by::FAPEMIG**

IM036 - STUDY OF TOLL-LIKE RECEPTOR 9 AGONISTS IN DIFFERENT STRAINS OF THE PARASITE *TRYPANOSOMA CRUZI*

LOURDES, R.A.¹; ROPERT, C.²; GAZZINELLI, R.T.¹; BARTHOLOMEU, D.C.¹

1.UFMG, BELO HORIZONTE, MG, BRASIL; 2.CPQRR, BELO HORIZONTE, MG, BRASIL.

e-mail:ralourdes@gmail.com

It has been demonstrated that the *Trypanosoma cruzi* genomic DNA has TLR9-dependent immunostimulatory activity and that this receptor is essential for the parasite recognition and host resistance to experimental infections. We have previously identified immunostimulatory CpG motifs in the genome of *T.cruzi* CLBrenner. These sequences are not randomly distributed in the genome but instead are enriched in regions containing genes encoding surface proteins and other *T.cruzi* specific sequences. These regions are highly polymorphic in the two haplotypes of the hybrid CLBrenner strain, which led us to speculate that the abundance of these CpG motifs may vary in the genome of the distinct *T.cruzi* strains, thus contributing to a differential TLR9 activation. In this work, we studied the importance of TLR9 in the infections caused by distinct *T.cruzi* lineages. Our results indicate a lower importance of TLR9 in controlling infections with Colombiana, when compared with infections with CL Brener and Y strains. In these latter strains, TLR9 proved essential for controlling parasitaemia, resulting in a much greater survival rate of C57BL/6 when compared with TLR9-/-mice. We also performed immunostimulatory assays using dendritic cells and macrophages from C57BL/6 and TLR9-/-mice, incubated with genomic DNA of each strain, but no difference in their immunostimulatory capacity was observed. When live trypomastigotes were incubated with dendritic cells, we found that the ratio of the TNF- α production by cells from C57BL/6 and TLR9-/-mice was lower for Colombiana compared with those values for the other strains. These results correlated with the quantification of the regions enriched in CpG motifs in the genome of these strains. We speculate that Colombiana infection promotes a lower activation of the TLR9 receptor, leading to insufficient production of proinflammatory cytokines necessary for parasite clearance, thus leading to increased mortality of mice infected with this strain. **Supported by::CNPq, FAPEMIG e WHO**

IM037 - Characterization of immunogenic proteins derived from the total antigen of *Neospora caninum* fractionated by HPLC system and precipitation with ammonium sulphate

MIRANDA, V.S.^{*}; SPIRANDELLI DA COSTA, M.S.; CUNHA, A.O.; MACÊDO JÚNIOR, A.G.; SANTIAGO, F.M.; MINEO, T.W.P.; MINEO, J.R.
 UFU, UBERLÂNDIA, MG, BRASIL. e-mail:vanessa.smiranda@hotmail.com

Neospora caninum is an obligate intracellular parasite that has the dog as definitive host and other mammals, especially cattle, as intermediate hosts. The present study aimed to fractionate protein targets of *N. caninum*(Nc) by HPLC and ammonium sulfate precipitation. Proteins of the soluble antigen of Nc (NLA), were fractionated by HPLC using columns of exclusion by molecular weight; reversed phase C18 and ionic exchange(cationic and anionic). It was applied 0.545 mg of NLA, and the fractions were collected at each 750 µl in 0.5 ml/min flow. Fractions eluted were monitored absorbance at 280nm and analyzed by electrophoresis and reactivity by Western blotting (WB), using serum known positive by Nc. At the same time NLA aliquots were precipitated with ammonium sulfate at concentrations of 10 to 40% and after incubation were centrifuged being pellets and supernatants analyzed by electrophoresis and WB. The results obtained in fractionation by HPLC demonstrated significant power to fractionate NLA for all columns tested, except for the Anionic column, as the low pH promoted protein denaturation. Concerning WB analysis, it was detected the following immunogenic proteins: 27 to 52 kDa for Cationic column; 37kDa for C18; 32 and 37 kDa for Hydrophobic and 32 and 37kDa for gel filtration. When NLA was precipitated with ammonium sulfate at concentrations of 10 and 15%, it was observed seven proteins with apparent molecular weights from 32 to 50 kDa, whereas in other concentrations there was no difference of the protein profile comparing with total antigen. In conclusion, C18 column was more effective to fractionate the NLA, when compared with other columns, because showed in peak 2 only one protein of 37 kDa. When ammonium sulfate fractionating was carried out, the concentration of 15% showed the exclusion of high molecular weight proteins, which are the responsible to promote cross reactivity among other parasites from filo Apicomplexa, due to the presence of proteins in common. **Supported by::FAPEMIG**

IM038 - LBSapSal vaccine: analysis of biomarkers post vaccine protocol, and early and late period of the *L. chagasi* challenge

RESENDE, L.A.^{*1}; ROATT, B.M.¹; AGUIAR-SOARES, R.D.O.¹; LANNA, M.F.¹; PEREIRA, L.F.¹; MENDONÇA, L.Z.¹; CARDOSO, M.A.M.¹; SILVEIRA-LEMOS, D.²; REIS, A.B.¹; GIUNCHETTI, R.C.²

1.UFOP, OURO PRETO, MG, BRASIL; 2.UFMG, BELO HORIZONTE, MG, BRASIL.
 e-mail:lucilenearesende@yahoo.com.br

Dogs represent the most important domestic reservoir of *L. chagasi* (syn. *L. infantum*), and a vaccine against canine visceral *Leishmaniasis* (CVL) would be an important tool in the control of human visceral *Leishmaniasis* (HVL) by decreasing the infection pressure of *L. chagasi*. Herein, we investigated the immunogenicity of LBSapSal vaccination in dogs. In this sense, dogs were inoculated with: saline (C); sand fly gland extract (SGE) of *L. longipalpis* (Sal), *L. braziliensis* promastigotes protein plus SGE (LBSal), *L. braziliensis* promastigotes protein and saponin together with SGE (LBSapSal). Cytokines (IL-10, TNF-alfa, IL-4, TGF-beta, IL-12, IFN-gama and nitric oxide (NO) were evaluated in the in vitro context from supernatants of peripheral blood mononuclear cells (PBMC) cultures at four different times: previously the vaccine protocol (T0), after the third vaccine dose (T3) and at the times 90 (T90) and 885 (T885) days after experimental challenge (dac). Our major results demonstrated that LBSapSal group presented lower levels of IL-4 (T90) and TGF-beta (T90 and T885) after *Leishmania* stimulation. In addition, higher levels of IL-12 were observed in LBSapSal group (T3, T90 and T885). Likewise, increased levels of IFN-gama were related to LBSapSal (T3, T90 and T885) group. Cultures stimulated with SLcA of the group LBSapSal showed increased levels of NO (T885). These results obtained from the analysis of NO levels confirmed the hypothesis that this vaccine induces a potential resistance profile against *Leishmania* infection. In conclusion, the results of this study encourage the continuation of investigations related to parasitological and immunological events after challenge of this new vaccine against canine visceral *Leishmaniasis* and the establishment of new biomarkers of immunogenicity. **Supported by::FAPEMIG, CNPq, CAPES, FIOCRUZ, UFOP, UFMG.**

IM039 - STUDIES ON FOXP3+ REGULATORY T CELLS IN EXPERIMENTAL *Trypanosoma cruzi* INFECTION

CORDEIRO, S.N.S.*; MARINS-DOS-SANTOS, A.; REIS, R.F.; OLIVIERI, B.P.; HENRIQUES-PONS, A.; COTTA-DE-ALMEIDA, V.
IOC - FIOCRUZ, RIO DE JANEIRO, RJ, BRASIL.
e-mail:synara@ioc.fiocruz.br

Several studies have demonstrated that FoxP3+ regulatory T (Treg) cells are important controllers of immunity to infections. Patients with the indeterminate form of Chagas disease were shown to bear high numbers of circulating FoxP3+ Treg cells compared to patients that progressed to the cardiac form. However, antibody depletion studies in mice showed controversial results regarding a potential role of Treg cells in experimental *Trypanosoma cruzi* infection. Here, we searched for specific subgroups of Treg cells following the acute course of experimental *T. cruzi* infection. Our data show a significant decrease in the frequency of CD4+FoxP3+ cells in the spleen of infected animals, as compared to noninfected controls. These results prompted us to investigate the relative contribution of thymic-generated Treg (nTreg) cells compared to those induced in the periphery (iTreg). Thus, we analyzed the intracellular expression of the transcription factor Helios, which are regarded as a marker for the nTreg cells. Our data revealed that the frequency of both nTreg and iTreg cells are markedly decreased in infected animals. Moreover, analysis of absolute numbers show that Treg cells expand at lower level relative to the major expansion seen with the total CD4+ subset. Analyses of thymus of infected mice showed a decrease in Treg cells, although not as important as the decrease seen for other thymocyte subpopulations. In addition, we also demonstrated that treatment of infected mice with the trypanocidal drug benznidazole does not preclude the lower levels of splenic Treg cells. Altogether, these results indicate that Treg cells are critically targeted in *T. cruzi* infection, possibly affecting their differentiation pathways both in the thymus and in the periphery. **Supported by:**IOC, CAPES, FAPERJ

IM040 - SOCS2 is essential for modulation of innate immunity and cardiomyocytes function during *Trypanosoma cruzi* infection

ESPER, L.*; CAMPOS, D.R.; LARA, A.; COSTA, F.M.C.B.; TEIXEIRA, M.M.; GUATIMOSIM, S.; CRUZ, J.S.; MACHADO, F.S.
UFMG, BELO HORIZONTE, MG, BRASIL.
e-mail:lisiaesper@icb.ufmg.br

Background. During *Trypanosoma cruzi* infection, the innate immune response is crucial to control the pathogen growth and development of pathology. Lipoxin (LXA)₄ generation, through 5-Lipoxygenase (5-LO) activity, and induction of Suppressor of cytokine signaling (SOCS)2 is essential in this process. Here we investigated the effects of LXA₄ and SOCS2 in innate immune cells and cardiomyocytes function during *T. cruzi* infection. Methods and results. Splenic dendritic cells (DCs) and peritoneal macrophages (MO) were purified from WT and/or SOCS2 KO mice and pre-incubated with LXA₄ (15h) and then stimulated with tripomastigote forms of *T. cruzi* in presence or absence of IFN- γ *in vitro*. *T. cruzi* infection induced an increased TNF- α , IL-6 and IL-12 p40 mRNA expression, which was almost abolished when WT DCs was exposed to LXA₄. In addition, LXA₄ induced SOCS2 expression and inhibited the IL-12 p40 mRNA expression in WT MO infected with tripomastigote *in vitro*. Deficiency of SOCS2 results in elevated levels of TNF, IL-12, IL-6, IL-10, SOCS1 and SOCS3 expression by macrophages when compared with WT cells. Our results also demonstrated that stimulation with *T. cruzi* and IFN- γ , in the absence of SOCS2, results in reduction of AhR expression, a nuclear receptor for LXA₄, when compared with WT MO. The down modulation of AhR expression was also detected in *T. cruzi*-infected SOCS2 KO cardiomyocytes. Moreover, upon *T. cruzi* infection, the SOCS2 deficiency *in vivo* resulted in altered cardiac remodeling by increasing cardiac ventricular mass and reducing calcium and potassium levels in ventricular myocytes (demonstrated by electrophysiological and echocardiogram analyses). Conclusions. Taken together, the results indicated a LXA/AhR/SOCS2 role in the regulation of innate immunity and cardiomyocytes function during experimental *T. cruzi* infection. Keywords: SOCS2, innate immunity, *Trypanosoma cruzi*. **Supported by:**CNPq, FAPEMIG

IM041 - Evaluation of parasite burden and histopathological parameters in hamsters (*Mesocricetus auratus*) infected with two different strains of *Leishmania (Leishmania) infantum*

MOREIRA, N.D.⁺; SOUZA, J.V.; ROATT, B.M.; VIEIRA, P.M.A.; TREVISAN, .R.;
MATHIAS, F.A.S.; CARDOSO, J.M.O.; KER, H.G.; AGUIAR-SOARES, R.D.O.; CARNEIRO,
C.M.; LANA, M.; GIUNCHETTI, R.C.; REIS, A.B.
UFOP, OURO PRETO, MG, BRASIL.
e-mail:nadiabelladona@yahoo.com.br

The experimental infection in hamster model (*Mesocricetus auratus*), reproduces several typical aspects of the canine and human visceral *Leishmaniasis* which were closely related with the inoculum route. Considering the importance of this experimental model for the development and to test new therapeutics and immunoprophylaxis strategies for VL is particularly important the understood of the tissue parasitism evolution after experimental infection with *L. infantum* in hamster model. Herein we quantified the parasitism in the liver and spleen of hamsters experimentally infected by different routes (intradermal, intraperitoneal and intracardiac) and strains of *L. infantum* (MHOM/BR/74/PP75 and Wild) using two different methodologies to evaluate the tissue parasitism (*Leishman* Donovan Units and Real-Time qPCR). In addition we evaluated the histopathological picture. The animals were also followed at 1, 3, 6 and 9 months post-infection. Our major results showed in both strain that the inoculum by IC route lead to higher parasitism in the liver and spleen expressed by LDU analysis. Furthermore the qPCR showed higher sensitivity for the detection of animals with low parasitic burden. The installation of inflammatory process in the liver compartment occurred in the portal spaces with the evolution of infection with higher intensity in the IC group. Hypoplasia of lymphoid follicles was associated with the macrophages proliferation in red pulp and the destruction of tissue architecture splenic was more evident in the IC group. In conclusion the qPCR can be useful to access the parasitism in spleen and liver of hamster model infected with *L. infantum* independent of the inoculum route employed in the experimental infection and this technique may become an essential tool to assess the parasite density in the model experimental hamster after treatment or immunized with potential vaccine candidates. **Supported by::**CAPES

IM042 - Combination therapies with Enalapril plus Benznidazole increase plasma IL-10 levels during experimental infection with *Trypanosoma cruzi*

LEITE, A.L.J.⁺; COSTA, G.P.; BAJRACHARYA, D.S.; NASCIMENTO, Á.F.S.; MIRANDA, C.P.;
BAHIA, M.T.; DA SILVA PEDROSA, A.T.
UFOP, OURO PRETO, MG, BRASIL.
e-mail:aninhaleite_@hotmail.com

Trypanosoma cruzi infection triggers a chronic inflammatory process capable to develop functional and morphometrical alterations in cardiac tissue. In an attempt to reduce cardiac alterations caused by host-parasite relationship pharmacological therapies have been proposed aiming to reduce the inflammatory response such as therapies using inhibitors of angiotensin converting enzyme (ACE). Here, we evaluated treatment with Enalapril (ACE inhibitor) and Benznidazole (Bz) in a single or combination therapy during acute and chronic phase of experimental *T.cruzi* infection. C57BL-6 mice were infected with VL-10 strain of *T.cruzi* and treated during 20 days with different dosages of Enalapril (10, 15, 20,25mg/kg), Bz (40, 60, 80, 100mg/kg) and combinations of both (10+40;15+60;20+80;25+100mg/Kg). Biological samples (serum/heart) to immunoassay and histopathology were collected on the 30o and 120o days post infection. Preliminary data shown that treatment with Enalapril was not capable to reduce parasitemia, just Enalapril+Bz. Different doses of combinations were able to increase plasma IL-10 levels during acute and keeping them high during chronic phase. Using monotherapies we also observed an increase of plasma IL-10 levels in acute phase in comparison to untreated infected mice. However on 120o day it was observed maintenance on its levels using Bz in comparison with untreated infected mice and a decreasing levels using Enalapril (25 and 15mg/kg). In parallel, there was a reduction in TNF-alpha and CCL5 levels for those treated mice observed in acute phase. Considering the following up (acute to chronic phase) of these inflammatory mediators, treatments in combination were capable to increase, do not change and reduce IL-10, TNF-alpha and CCL5, respectively. In conclusion, we assume that treatment in combination using Enalapril and Bz may regulate the inflammatory process suggesting a potential protection against cardiac damages caused by experimental *T. cruzi* infection.

**IM043 - Therapies of restriction on Angiotensin II actions in experimental acute
Trypanosoma cruzi infection**

COSTA, G.P.¹; LEITE, A.L.J.¹; BAJRACHARYA, D.S.¹; MOTA, L.W.R.¹; LIMA, W.G.¹; GUEDES, P.M.M.²; BAHIA, M.T.¹; DA SILVA PEDROSA, A.T.¹

1.UFOP, OURO PRETO, MG, BRASIL; 2.UFRN, NATAL, MG, BRASIL.

e-mail:gpcosta@nupeb.ufop.br

Chagas heart disease (CHD) is the most important clinical manifestation of *Trypanosoma cruzi* infection and presents variable clinical course from asymptomatic to severe form of heart failure. Drugs previously used to improve functional capacity or mitigate cardiac remodeling in CHD (eg. inhibitors of angiotensin converting enzyme – ACE) have also presented actions on inflammatory mechanisms, a *sine qua non* condition for the pathogenesis of CHD. In this study, we evaluated the single and combined action of Enalapril (ACE inhibitor) and Losartan (angiotensin II receptor blocker) during the acute inflammatory phase of experimental Chagas disease. C57BL/6 mice were infected with Colombian strain of *T. cruzi* and treated for 20 days with Enalapril (25mg/Kg), Losartan (15mg/Kg), both (15mg/Kg each), Benznidazole (100 mg/Kg) and vehicle (untreated control). After 22 days of infection, the animals were euthanized. It was observed a reduction of blood and tissue parasites load in animals treated with Losartan or Enalapril, but not to the combination. Serum levels of inflammatory mediators TNF-alpha, CCL2 and CCL5 were reduced to those Losartan-treated animals which also showed an increase of IL-17 and IL-10 levels. Treatment with Enalapril leads to a reduction of TNF-alpha, IL-17 and CCL5, but maintained IL-10 and CCL2 serum levels. For the immunoassays, treatment with the combination showed similar results to those observed for Enalapril. All treatments showed a reduction in cardiac inflammation in histomorphometric analysis. Our data showed pleiotropic effects of treatments with the monotherapies of drugs which restrict the actions of Angio II through the interference of parasite replication and immune response modulation, culminating in the reduction of the inflammatory infiltrate in cardiac muscle tissue. Together, these findings suggest that these treatments can lead to a protection of heart damage mediated by immune response during acute experimental *T. cruzi* infection. **Supported by:** CNPq, FAPEMIG, CAPES, UFOP

IM044 - IL-17 expression in lesions of patients with different clinical forms of American Cutaneous *Leishmaniasis* caused by L.(*Leishmania*) *amazonensis* and L.(*Viannia*) *braziliensis*

MENEZES, J.P.B.¹; DE MORAIS, I.B.¹; RODRIGUES, F.M.D.¹; ALVES GAMA, M.E.²; LAURENTI, M.D.¹; CORBETT, C.E.P.¹; SILVEIRA, F.T.³; GOMES, C.M.C.¹

1.FMUSP, SAO PAULO, SP, BRASIL; 2.UFMA, SÃO LUIZ, MA, BRASIL; 3.IEC, BELÉM, PA, BRASIL. e-mail:joyce.menezes@uol.com.br

The immunopathogenesis of *Leishmania* infection was explained primarily through the balance Th1/Th2 immune response. However, recent studies point to the presence of Treg and Th17 cells playing a role in the immune response against parasite infections. Th17 cells are characterized by the production of IL-17, which constitutes a pro-inflammatory cytokine secreted primarily by activated TCD4+ and CD8+ cells. This study aimed to evaluate the profile of cells expressing IL-17 in skin lesions of patients presenting different clinical forms of American cutaneous *Leishmaniasis* (ACL) caused by L.(V.) *braziliensis* and L.(L.) *amazonensis*, addressing a better understanding on the role of Th17 cells in the immunopathogenesis of ACL in Brazil. 26 patients were examined: anergic diffuse cutaneous *Leishmaniasis* (ADCL):6; borderline disseminated cutaneous *Leishmaniasis* (BDCL):6, both by L.(L.) *amazonensis* (DTH-); localized cutaneous *Leishmaniasis* (LCL) also due to L.(L.) *amazonensis* with DTH-(8) and DTH+(5) and, LCL due to L.(V.) *braziliensis* with DTH+(6). Paraffin-embedded biopsies were submitted to immunohistochemistry using as primary antibody anti-IL-17 (SC7927), 1:250 dilution. For amplification and visualization of the reaction Novolink max polymer was used. The immunostained cells were counted in 5–10 fields (400x) in section by using an image analysis system (Zeiss). The comparison of IL-17+ cells density in the clinical-immunological spectrum of ACL showed a progressive increase in IL-17+ expression from the central LCL (DTH+) caused by L.(V.) *braziliensis* to the polar forms, ADCL and BDCL (DTH-) caused by L.(L.) *amazonensis*, as follows: (ADCLDHT-[506]>BDCLDHT-[384]>LCL/LaDTH-[318]>LCL/LaDTH+[251]>LCL/LbDTH+[289]). In conclusion, our results showed that cells expressing IL-17 seems to play an important role in the immunopathogenesis of these different clinical forms of ACL, characterized by a polarized immune response and different pathological expression. **Supported by:** Fapesp

IM045 - EXPRESSION OF CD39 AND CD73 IN *LEISHMANIA AMAZONENSIS* INFECTED MACROPHAGES

BAJRACHARYA, B.⁻¹; BAJRACHARYA, D.S.¹; DA SILVA PEDROSA, A.T.¹; GONÇALVES, R.²; AFONSO, L.C.C.¹

1.UNIVERSIDADE FEDERAL DE OURO PRETO, OURO PRETO, MG, BRASIL;

2.UNIVERSIDADE FEDERAL DE MINAS GERAIS, BELO HORIZONTE, MG, BRASIL.

e-mail:bjbajra@nupeb.ufop.br

Endogenous nucleotides produced by various group of cells under inflammatory conditions act as potential danger signal in vivo. Extracellular release of these nucleotides such as ATP is brief and is rapidly cleaved to adenosine (ADO) by series of ectonucleotidasic activities. ATP and ADO are strong immunomodulators that can affect immune cells and their functions which can be exploited by various pathogens including *Leishmania*. Macrophages (MØ) are the main host cells for *Leishmania* and are the principle effector cells that determine the fate of host and *Leishmania* infection. In this study, we investigated the expression of CD39 and CD73 in murine resident MØ infected with metacyclic forms of *Leishmania amazonensis* by flow cytometry. Our findings demonstrated that resident MØ express both CD39 and CD73 under ex-vivo conditions. The expression of both CD39 and CD73 in resident MØ has been found to decrease with incubation in vitro. We observed that this decrease was predominant in an uninfected population and expression was low at 96hrs of incubation. Interestingly, this decrease in the expression for both CD39 and CD73 was prevented when we infected resident MØ for further 24hrs after being rested for 72hrs of incubation prior to infection. In our study, LPS treatment, however, could not recover decrease in CD39 and CD73 expression. Furthermore, following the incubation and treatment, we analyzed the supernatant from the culture incubated under above conditions for CCL5, TNF-alpha and IL-10 production by ELISA. We observed that infection with *Leishmania* did not induce increased pro-inflammatory mediators (CCL5, TNF-alpha) contrary to what we observed for LPS treatment. There was no change in IL-10 production in all conditions in 24hrs of treatment following resting of the MØ. Our current efforts are to determine the role of CD39 and CD73 in *Leishmania*/ and MØ interaction and their role in host immune modulation. **Supported by:** TWAS, CNPq and FAPEMIG

IM046 - Ultrastructural aspects, morphological and analysis of cytokines expression in the interaction of *Leishmania (Leishmania) infantum chagasi* with peritoneal macrophages and microglia

LIMA, L.H.S.⁻¹; RAMOS, P.K.S.²; JUNIOR, J.A.P.D.²; SILVEIRA, F.T.²

1.UEPA/IEC, BELÉM, PA, BRASIL; 2.IEC, BELÉM, PA, BRASIL.

e-mail:luciahelenalima23@gmail.com

Visceral *Leishmaniasis* is an anthroponosis caused by protozoa of the genus *Leishmania*. In Brazil, the etiologic agent is *Leishmania (Leishmania) infantum chagasi* and the main transmitter specie is *Lutzomyia longipalpis*. This study has evaluated the ultrastructural, morphological aspects and the cytokines profile produced by macrophages and microglia in the interaction with *L. (L.) i. chagasi* in vitro. Macrophages and microglia cultures inoculated or not with *L. (L.) i. chagasi* were analyzed by 24, 48 and 72 hours. We used staining with Giemsa to count parasites, fluorescein diacetate/propidium iodide for cell viability, transmission electron microscopy to evaluate the ultrastructural aspects and enzyme immunoassay for quantification of TGF-β1, IL-10, IL-12, INF-γ, TNF-α and IL-1β. In macrophages with 24h of interaction, it was observed that 74% were infected with amastigotes, 66% with 48h and 58% with 72h. In microglia, with 24h of interaction, 86% of microglia were infected, 70% with 48h and 63% with 72h. The number of amastigotes per macrophage decreased during the interaction, however it remained constant with microglia. Cell viability in infected macrophages showed a variation between 24 and 72 h. In microglia, there was no variation (~85%). In control cultures in both cells there was no significant variation (var≥ 90%). In transmission electron microscopy, after 24h, it was observed the presence of promastigotes and amastigotes in both cells. In macrophages, there was a significant difference in the production of TGF-β1, however there was no significant production of proinflammatory cytokines. In microglia, a significant production of IL-12 was observed. There was also the production of proinflammatory cytokines TNF-α, IL-1β and of IL-10 anti-inflammatory, but they have not showed significant differences in times measured. Thus, we conclude that peritoneal macrophages and microglia did not stimulate an effective response against *L. (L.) i. chagasi*. **Supported by:** FAPESPA

IM047 - The evolution of *Leishmania (L.) amazonensis* infection co-inoculated with *Lutzomyia flaviscutellata* salivary gland in BALB/c mice.

FRANCESQUINI, F.C.¹; SILVEIRA, F.T.²; TOMOKANE, T.Y.¹; CARVALHO, A.K.¹; BARATA, I.R.²; BORDON, M.L.A.C.¹; CORBETT, C.E.P.¹; LAURENTI, M.D.¹
 1.FMUSP, SAO PAULO, SP, BRASIL; 2.IEC, BELÉM, PA, BRASIL.
 e-mail:thaisey@yahoo.com.br

The promastigote forms of *Leishmania* are transmitted by sand flies during blood feeding. Reports have shown that vector's saliva plays an important role in the modulation of immune response, helping the establishment of the infection. But most of these reports used the saliva of *L. longipalpis* or *P. papatasi* laboratory-reared and conflicting data have been described when the natural vector/parasite binomium is used. The aim of this study was evaluate the effects of wild-caught vector saliva of *Lutzomyia flaviscutellata* in *L.(L.)amazonensis* promastigotes infection in BALB/c mice. Animals were inoculated into the hind footpads with 10⁶ *L.(L.)amazonensis* promastigotes in the absence or presence of salivary gland lysate (SGL) from wild-caught *Lu. flaviscutellata*. The lesion size was evaluated weekly till 60th day PI when biopsies were collected for histological studies and determination of parasite burden, as well as draining lymph nodes to characterize T lymphocytes population and cytokines measure. Increase on the lesion size was observed with the evolution of the infection in all groups. Histological features were similar in all groups, characterized by mononuclear inflammatory infiltrate, macrophages heavily parasitized, few lymphocytes and neutrophils and focal areas of necrosis. The number of viable parasites was similar in the presence and absence of SGL from *Lu. flaviscutellata*. The infection leads to decrease of CD3⁺ T cells characterized specially by decrease on CD4⁺ subsets. At 8th week PI an increase on the CD8⁺ subsets was observed in both groups. The infection in the presence or absence of SGL increases the IL-4 and IL-10 level in the supernatant of lymph nodes cells culture under specific antigen stimulation. IFN-g was detected only in the absence of SGL. The results presented herein indicated that SGL from wild-caught *Lu. flaviscutellata* does not promote the enhancement of *L.(L.)amazonensis* infection in BALB/c mice. **Supported by::**CNPq, CAPES, LIM50/HC-FMUSP

IM048 - Effect of exercise on development of experimental *Leishmaniasis* by *Leishmania major*

ALVES, P.J.F.¹; TERRA, R.¹; SILVA, S.A.G.²; SALERNO, V.³; DUTRA, P.M.L.¹
 1.LIFE - DMIP - FCM - UERJ, RIO DE JANEIRO, RJ, BRASIL; 2.DMIP - FCM - UERJ, RIO DE JANEIRO, RJ, BRASIL; 3.EEFD - UFRJ, RIO DE JANEIRO, RJ, BRASIL.
 e-mail:pedro.falci.alves@gmail.com

Leishmaniasis represents a group of diseases caused by protozoan from the genus *Leishmania*. Following infection, the cellular immune response (Th1) influences the control of extent of the infection while the humoral response (Th2) relates to the progression of the disease. Several studies suggest that moderate exercise influences the immune system by stimulating the Th1 response that controls parasitic infections. We evaluated the impact of moderate aerobic exercise on the progression of infection by *Leishmania major* in mice through three cohorts (N=8): control, exercise, treatment using Glucantime®. Infection with *Leishmania* was initiated by an inoculation with 2 x 10⁶ promastigotes into the plantar cushion. Exercise consisted of swimming for 30 min, three times per week while loaded with a progressive weight that was related to the corporal index. Training and treatment with a therapeutic dose of Glucantime® (8 mg/kg) began one week after infection. After 12 weeks, *Leishmanial* lesions did not develop in trained and treated groups, showing a significant difference from the fifth week in relation to the infected group. Only the trained group showed a positive DTH. Parasitic load was about 10,000 fold less than the infected group. The cytokines IL-12 and IFN-γ were measured in infected legs and in popliteal lymph node cells incubated with medium and challenged with total antigen of *L. major*. The trained group showed a significant difference, in relation to the infected group, in IL-12 and IFN-γ concentrations in both lymph node cells challenged by the antigen and infected legs. These data suggest that exercise modulates the Th1 response in BALB/c mice infected with *L. major* providing a protective response against this parasitosis. **Supported by::**CNPq, FAPERJ and PIBIC-UERJ

IM049 - TLR9 CONTRIBUTES TO EFFICIENT CONTROL OF INFECTION WITH THE PROTOZOAN PARASITE *LEISHMANIA INFANTUM CHAGASI* THROUGH NEUTROPHILS RECRUITMENT

SACRAMENTO, L.A.*; NASCIMENTO, M.S.L.; LIMA-JUNIOR, D.S.; COSTA, D.L.; SILVA, J.S.; PEREIRA, V.C.

FMRP - USP, RIBEIRÃO PRETO, SP, BRASIL. e-mail:laisamorim.s@hotmail.com

The protozoan *Leishmania infantum chagasi* (Lic) is the causative agent of visceral *Leishmaniasis* (VL) in Brazil and South America, causing high morbidity/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. 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 4th and 6th 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-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 proinflammatory cytokines release. 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. **Supported by::**CAPES, CNPq, FAPESP

IM050 - CHARACTERIZATION OF T REGULATORY CELLS (CD4⁺CD25⁺FoxP3⁺) IN *L. (L.) amazonensis* AND *L. (V.) braziliensis* MURINE INFECTION

CARVALHO, A.K.¹; CARVALHO, K.I.¹; FRANCESQUINI, F.C.¹; PASSERO, L.F.D.¹; SANTOS, B.A.N.¹; GOMES, C.M.C.¹; SILVEIRA, F.T.²; CORBETT, C.E.P.¹; KALLAS, E.G.¹; LAURENTI, M.D.¹

1.FMUSP, SAO PAULO, SP, BRASIL; 2.IEC, BELEM, PA, BRASIL.

e-mail:anakely@gmail.com

New World *Leishmania* species, as *L.(L.)amazonensis* and *L.(V.)braziliensis*, are able to initiate different type of immune response in the vertebrate host. T regulatory cells (T-reg) are important to limit damage caused by exacerbate cellular immune response against pathogens. The aim of this study was evaluate the T-reg cells in experimental infection caused by *L.(L.)amazonensis* and *L.(V.)braziliensis*. BALB/c mice were inoculated into the hind footpad with 10⁶ promastigotes of both parasite species and the infection was monitored during 8 weeks. At 4th and 8th weeks PI, biopsies from skin and the draining lymph nodes were collected and processed to determine the parasite load and the lymph node cells were processed to determine the number of T-reg (CD4⁺CD25⁺FoxP3⁺) and T lymphocyte producing IL-10 (CD3⁺CD4⁺IL-10⁺). An increase in the lesion size as well as in the tissue inflammation and parasitism was observed in *L.(L.)amazonensis* infection. There were no differences in the number of T-reg compared to the control group, neither at 4th nor at 8th week PI in *L.(L.)amazonensis* infection. However, in *L.(V.)braziliensis* infection, a high number of T-reg cells were observed at 4th week PI when tissue parasitism and histological changes were evident in skin and lymph nodes. A regression in the number of T-reg cells was present at 8th weeks PI when the control of the infection was observed. A positive correlation was observed between the numbers of CD3⁺CD4⁺IL-10⁺ and CD4⁺CD25⁺FoxP3⁺ cells. T-reg cells seems to have a weak participation during *L.(L.)amazonensis* infection, while in *L.(V.)braziliensis* infection these cells seems to have a regulatory role aiming to suppress high inflammatory response. The results show the ability of the parasites species in develop an appropriate cellular immune response in the vertebrate host which lead to the control of *L.(V.)braziliensis* infection and the spreading of *L.(L.)amazonensis* infection. **Supported by:** CAPES, FAPESP and LIM50-HCFMUSP

IM051 - Evaluation of cross reactivity profile between *Neospora caninum* and *Toxoplasma gondii*

MACÊDO JÚNIOR, A.G.¹; FERREIRA, F.B.; SILVA, M.V.; OLIVEIRA, A.C.M.; SANTIAGO, F.M.; OLIVEIRA SILVA, D.A.; MINEO, J.R.; MINEO, T.W.P.
 UFU, UBERLÂNDIA, MG, BRASIL. e-mail:agmacedojr@gmail.com

Neospora caninum and *Toxoplasma gondii* are closely-related apicomplexan parasites able to infect a wide range of warm-blooded hosts. A large number of manuscripts have described serological tests for the detection of antibodies against *N. caninum* and *T. gondii*, but diagnostic based on native antigenic extracts are still deficient in order to exclude serological cross-reactivity. In that sense, we aimed to determine the cross-reactivity profile in species of mammalian hosts and trace which antigenic fractions of *N. caninum* and *T. gondii*. First, we searched for serological cross reactivity in serum samples derived from different hosts. With the exception of cattle, which did not present a single sample positive for *T. gondii*, all other species (Humans, Sheep, Goat, Dogs) presented a high percentage of double positive samples in a assay based in parasitic soluble antigens. In order to understand whether that phenomenon was due to co-infections or serological cross-reactivity, experimental infections with *N. caninum*, *T. gondii*, or both parasites, were performed in BALB/c mice. Serum samples were collected following infection kinetics and submitted to ELISA and Western Blotting (WB) against distinct antigens extracts of *T. gondii* and *N. caninum*. By ELISA, cross reactivity was clearly observed between soluble antigen extracts of both parasites, and was minimized in secreted/excreted antigenic fractions. Surface antigens presented the highest specificity among all tested; however the amounts of specific antibodies against those antigens were small, if compared to the other fractions. By WB, we observed that serological cross reactivity was present in antigens with molecular weight over 40 kDa in soluble parasitic extracts, while was observed only in proteins with over 70 kDa in secreted/excreted fractions. The knowledge of the antigen recognition profile by serum antibodies may allow us to identify potential targets to discriminate the infections by both parasite species. **Supported by:** CAPES, CNPq, FAPEMIG, FINEP

IM052 - ROLE OF PROTEIN KINASE R IN THE KILLING OF *Leishmania major* BY MACROPHAGES IN RESPONSE TO NEUTROPHIL ELASTASE AND TLR4

FARIA, M.S.^{1,2}; CALEGARI-SILVA, T.C.¹; DIAS, B.T.¹; LOPES, U.G.¹; MOTTRAM, J.C.²; LIMA, A.P.C.A.¹

1.IBCCF/UF RJ, RIO DE JANEIRO, RJ, BRASIL; 2.WELLCOME TRUST CENTRE FOR MOLECULAR PARASITOLOGY, GLASGOW, ESCOCIA.

e-mail:mariliasf@biof.ufrj.br

Leishmania major multiplies inside macrophages, residing in a specialized vacuole where it evades killing by the host cell. This protozoan has three genes encoding ecotin-like serine peptidase inhibitors, *ISP1-3*. We reported that the expression of *ISP2* enables *L. major* to survive and grow in macrophages. *ISP2* inhibits neutrophil elastase (NE) at the surface of macrophages, preventing the activation of Toll-like receptor 4 (TLR4) that results in parasite death. Here, we describe that the activation of macrophage dsRNA-activated kinase (PKR) downstream of NE-TLR4 is responsible for the killing of *L. major* mutants lacking *ISP2* and *ISP3* ($\Delta isp2/3$). PKR activation in macrophages has been associated with the survival of *L. amazonensis* through the production of IL10. In contrast, we show that in RAW cell lines stably expressing dominant-negative (DN) PKR or in macrophages from PKR knockout mice, $\Delta isp2/3$ survival and growth do not require the inactivation of NE or neutralization of TLR4. Parasite killing was reversed by 2-aminopurine, a kinase inhibitor and the treatment of RAW cells with the synthetic double-stranded RNA poly[I:C] promoted the death of wild type *L. major* while it did not further enhance killing of $\Delta isp2/3$. Both WT and $\Delta isp2/3$ *L. major* increased the levels of the p65 subunit of NF- κ B, but its levels were reduced more rapidly in RAW infected with $\Delta isp2/3$. Assays for promoter activation by NF- κ B using luciferase as a reported gene showed that, in contrast to WT, $\Delta isp2/3$ does not induce gene expression. In qPCR assays, $\Delta isp2/3$ induced increased expression of TNF- α and iNOS, but not of IL10 and IFN- γ . Consistently, $\Delta isp2/3$ promoted secretion of TNF- α and nitric oxide by RAW, but not by DN-PKR RAW. Neutralizing antibodies to TNF- α reverted parasite killing. We propose that, in the absence of *ISP2*, the activation of PKR downstream of NE and TLR4 in macrophages infected with *L. major* leads to inflammatory innate responses that contribute to parasite killing. **Supported by:** CNPQ, Wellcome Trust, FAPERJ

IM053 - PI3Kgamma CONFERS RESISTENCE TO INFECTION BY *TRYPANOSOMA CRUZI* AND IS IMPORTANT FOR THE MICROBICIDAL ACTIVITY OF MACROPHAGES.

SILVA, M.C.^{*}; SESTI-COSTA, R.; DIAS, F.C.; SILVA, G.K.; CUNHA, F.Q.; SILVA, J.S.; CUNHA, T.M.

FMRP, RIBEIRAO PRETO, SP, BRASIL.

e-mail:mariaclaudiasilva@usp.br

The phosphatidylinositol-3-kinases (PI3Ks) are a family of lipid kinases that plays an important role in a several number of cellular processes including survival and proliferation in different cell types. Among PI3Ks, PI3Kgamma is 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 PI3Kgamma during experimental infection by *T. cruzi*. For this purpose, C57BL/6 and PI3Kgamma^{-/-} mice were infected with 10³ trypomastigotes forms of *T. cruzi* Y strain and, eighteen days after infection, mice were terminally anesthetised and heart tissue was harvested for assessment of inflammation, lesion and parasitism level. The parasitemia and survival rate were observed daily, during about 35 days. The involvement of PI3Kgamma in the killing of the parasite and NO production was assessed in bone marrow differentiated macrophages. The infection by *T. cruzi* causes an increase in the PI3K activation in the heart tissue. Although there is no difference in the parasitemia in all times evaluated, all knockout mice died at day 25 after infection, whereas control WT mice were alive after 35 days. PI3Kgamma^{-/-} mice also showed greater inflammation, parasitism and lesion in the heart tissue. Interestingly, after infection, knockout's mice heart tissue express five times higher levels of iNOS enzyme compared with WT mice, but the levels of arginase I expression are twenty times higher in knockout mice compared with WT mice. *In vitro*, macrophages from PI3Kgamma^{-/-}, when stimulated with IFN-gamma, fail to produce NO and killing the parasite. These results indicate that PI3Kgamma is critical for the host to control *T. cruzi* parasitism. In the heart tissue, PI3Kgamma is not involved in the leukocyte migration, but it probably helps in the microbicidal mechanisms of macrophage by mediation of NO production, which is important to kill intracellular parasite. **Supported by::**FAPESP, Cnpq

IM054 - Purification and characterization of chitinase enzyme present in total soluble antigen of *Toxoplasma gondii* tachyzoites

CUNHA, A.O.^{*}; FERREIRA, F.B.; KNYCHALA, L.M.; MARTINS, C.A.; SANTIAGO, F.M.; MINEO, J.R.

UFU, UBERLÂNDIA, MG, BRASIL.

e-mail:amanda.olicunha@hotmail.com

Toxoplasma gondii is an obligatory intracellular parasite that has the cat as definitive host and a great varied of other mammals 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). After obtaining the total antigen of *T. gondii* (STAG), it was subjected to electrophoresis 12% according to REISFELD for proteins with basic characteristics. Then, Ch-Tg was withdrawn from the gel, pooled, and added to Ambic buffer, pH 7.4, centrifuged at 14.000g 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 25°C 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, 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, 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). It can be concluded that 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.

IM055 - CORRELATION BETWEEN PARASITE LOAD AND INFLAMMATION IN THE EAR SKIN OF DOGS NATURALLY INFECTED BY LEISHMANIA INFATUM

CARDOSO, J.M.O.*; PONTES, D.T.; VIEIRA, P.M.A.; ROATT, B.M.; COURA-VITAL, W.; GIUNCHETTI, R.C.; REIS, A.B.; CARNEIRO, C.M.
 UFOP, OURO PRETO, MG, BRASIL.
 e-mail:ja_mirelle@yahoo.com.br

Canine visceral *Leishmaniasis* (CVL) is a severe zoonotic disease of the dog caused by protozoa of the *Leishmania donovani* complex that represent an important public health problem in the tropical and subtropical regions of the world. The skin is the first point of contact with organisms of the genus *Leishmania* by sandy fly vectors and skin lesions are the most usual manifestation of CVL. Based on this, the aim of this study is investigate the histological pattern and parasite load (Real time PCR) in the skin of dogs naturally infected with *Leishmania*. For this, thirty six dogs naturally infected were categorised as asymptomatic (n=12), oligoymptomatic (n=12) and symptomatic dogs (n=12) and these were compared to control dogs (n=8). These animals were euthanized and ear skin samples were collected. All animals showed an inflammatory reaction ranging from mild to intense, and the cellular exudate consisting mainly of mononuclear cells. There was no formation of granulomas and neither the presence of giant cells in the skin in any of the dogs studied. The skin of symptomatic animals presented an increase of inflammatory infiltrate and a higher parasite load when compared to all other groups and the observed cellular exudate was composed mainly by plasmocytes, macrophages and lymphocytes. In the analysis of extracellular matrix components, it was observed that the more intense the inflammation, like the presented by the symptomatic dogs, lower area of collagen was noted. Since this compartment is formed mainly by collagen fibers can be assumed that the presence of amastigotes may be involved in the degradation of extracellular matrix. These data suggest that symptomatic animals have higher skin lesion associated with an increased parasite load on these animals. In this sense, the skin should be considered as a good place for parasitological analysis, being a reliable indicator for the severity of clinical disease in the CVL. **Supported by:** FAPEMIG, CNPq and UFOP.

IM056 - CHARACTERIZATION OF IMMUNODOMINANT B CELL EPITOPE IN GRA2 PROTEIN FROM TOXOPLASMA GONDII

BASTOS, L.M.*; SANTOS, F.A.A.; OLIVEIRA, A.C.M.; MACÊDO JÚNIOR, A.G.; MOTA, C.M.; SILVA, M.V.; SANTIAGO, F.M.; GOULART FILHO, L.R.; MINEO, T.W.P.; MINEO, J.R.

UFU, UBERLÂNDIA, MG, BRASIL.
 e-mail:anacarolm@yahoo.com.br

Toxoplasma gondii is an obligate intracellular parasite of the Apicomplexa phylum that infects up to one third of the world population. Due to the persistent nature of infection, immunochemical characterization of *T. gondii* proteins may lead to the discovery of epitopes that are hallmarks of infection and could be used for the development of new diagnostic immunoassays. Dense Granule Protein 2 (GRA2) has been assessed as a potential marker of infection since early 1990's, however its structure and respective immune epitopes remain undetermined. In this study, we aimed to characterize B cell epitopes within dense granule GRA2 protein of *T. gondii*. For that strategy, we have used several proteomic techniques. We have first analyzed GRA2 protein and predicted potential B cell epitopes through analysis of its linear amino acid sequence. Since it was observed that such protein presented potentially well-defined immunoepitopes, we have raised monoclonal antibodies (MAb) against it, which specificity was confirmed by bi-dimensional electrophoresis and Western blotting, and later by mass spectrometry. Next, in order to identify we used the MAb in phage display experiments, using a random commercial conformational library of 7-mer random peptides, which mimic true epitopes of the parasite. After selection, twenty distinct clones were identified, and we observed that all clones presented linear alignments with a consensus sequence of the previously predicted immunodominant region of GRA2 protein. We conclude that the definition of immunodominant epitopes within parasitic proteins, by multiple approaches, is promising due to the recent evolution of proteomic techniques and algorithms for in silico predictions. As we showed here, such approach may be useful in order to determine such targets for *T. gondii*, and may help to predict infection status in new standardized diagnostic immunoassays. **Supported by:** CNPq, CAPES, FAPEMIG, FINEP.

IM057 - The Aryl Hydrocarbon Receptor (AhR) activation and its effects in development of immune response, myocarditis and reactive oxygen species production during *Trypanosoma cruzi* experimental infection

BARROSO, A.*; ESPER, L.; COSTA, F.M.C.B.; ARAUJO, R.R.S.; CARNEIRO, M.B.H.; VIEIRA, L.Q.; RACHID, M.A.; MACHADO, F.S.

UFMG, BELO HORIZONTE, MG, BRASIL. e-mail: deiabarroso86@hotmail.com

The immune response balance is essential to control parasite growth and pathology development during *Trypanosoma cruzi* infection. Lipoxins(LXA)₄, an anti-inflammatory eicosanoid, by 5-lipoxygenase enzyme activity, is important in the regulation of inflammatory cytokines production during this infection. The role of aryl hydrocarbon receptor(AhR), a LXA nuclear receptor, during *T.cruzi* infection is not known. 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 the heart. Deficiency of AhR resulted in higher resistance to infection, associated with 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. In absence of AhR was also observed 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. Collectively, our data suggests that AhR activity is essential to modulate innate and adaptative immune response and development of myocarditis during *T. cruzi* infection. **Supported by:** CNPq and FAPEMIG

IM058 - Immune mechanisms and biomarkers associated with anemia in *Plasmodium vivax* malaria.

MOURÃO, L.C.*¹; GOMES, T.C.¹; DE ALMEIDA, Z.B.¹; FONTES, C.J.F.²; HORTA, M.F.M.¹; ANDRADE, H.M.¹; BRAGA, É.M.¹

1.UFMG, BELO HORIZONTE, MG, BRASIL; 2.UFMT, CUIABÁ, MT, BRASIL.

e-mail: luizacmourao@yahoo.com.br

Over recent years, *Plasmodium vivax* has been linked with a number of severe manifestations similar to those reported to *P. falciparum* infection. Among these complications, severe anemia remains the least understood and one of the major causes of mortality and morbidity among young children and pregnant women in endemic areas. In this direction, there is a need to better understand the mechanisms involved in *P. vivax* anemia. The present study aimed to identify possible immunological biomarkers associated with anemia. We first compared the total IgG antibody response among 64 subjects that were stratified into three different groups based on malaria infection and also according to hemoglobin and hematocrit values: healthy control (n = 10), non-anemic (n = 22) and anemic (n = 32), both with patent *P. vivax* infection. The anemic group presented significantly higher concentrations of total IgG when compared with the other groups (P < 0.0001). In order to investigate whether the antibodies from the anemic group were self-reacting anti-red blood cells (RBC), we tested the reactivity of the IgG from the three groups against a O+ RBC membrane protein extract from healthy donors using Western blotting. We observed that both non-anemic and anemic groups showed a higher production of antibodies against RBC membranes compared to the control group. However, the number of protein recognized tended to be higher in anemic patients. Similarly, and corroborating these findings, we showed that healthy RBC were more susceptible to complement-mediated lysis when treated with sera from anemic than non-anemic patients suggesting that these self-reacting antibodies could bind to uninfected RBC. All these results suggest a possible link between antibody-mediated RBC lysis and RBC loss during *P. vivax* infection. Proteomic studies are currently being carried out in order to identify the RBC proteins that were differentially recognized by antibodies from anemic and non-anemic patients. **Supported by:** CNPq e FAPEMIG

IM059 - METACYCLIC OR BLOOD *TRYPANOSOMA CRUZI* TRYPOMASTIGOTES TRIGGERED CHANGES IN SERUM LEVELS OF CYTOKINES

SILVA, E.M.V.¹; NOGUEIRA, C.¹; FRANCISCO, A.F.¹; MENESES MACHADO, E.M.¹; CARVALHO, A.T.²; MARTINS-FILHO, O.A.²; VIEIRA, P.M.A.¹; TAFURI, W.L.¹; CARNEIRO, C.M.¹

1.UFOP, OURO PRETO, MG, BRASIL; 2.IRR-FIOCRUZ, BELO HORIZONTE, MG, BRASIL.

e-mail:edithvaladares@yahoo.com.br

The recent increase in the number of immigrants with Chagas disease in non-endemic countries lead to a higher probability of contamination by blood transfusion which makes important to conduct studies on the impact of infection with blood forms in the course of Chagas disease. Based on this, the aim of this study was to evaluate the kinetic of cytokines serum levels in Swiss mice (n=30) infected with metacyclic (MT) or blood trypomastigotes (BT) of Be-78 strain during the acute phase. Data were assessed by one-way analysis of variance (ANOVA); when interactions were significant, the Tukey test was used to determine the specific differences between mean values. The kinetic evaluation of serum levels of inflammatory cytokines demonstrated that there was an increase in the levels of TNF- α in 14 and 28 days after infection in animals of BT and MT groups, respectively. IFN- α presented a profile similar to observed in TNF- α , with a higher production of these cytokines associated with the peak of parasitaemia in both groups. However, infection by MT forms leads to an earlier production of IFN- γ at day 14o after infection. Therefore, this early production of IFN- γ in the MT group could be correlated with lower parasitemia observed in this group when compared to animals of BT group. These results showed that the initial interaction between MT forms and the vertebrate host induces an immune response profile different from that observed in BT infection. Thus, the infection by MT forms is more silent and promotes an immune response capable of controlling not only the number of parasites during the acute phase of infection, but also could limit the development of lesions associated with Chagas disease. **Supported by:**CNPQ

IM060 - New Type of Delayed Hypersensitivity with Infiltration of Eosinophil, Lymphocytes and Th1 Profile Induced by LaAg plus Saponin Vaccine.

PEREIRA, J.C.; GONÇALVES, C.S.; PACHECO, M.D.C.; ROSSI-BERGMANN, B.; GUEDES, H.L.M.

UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO, RJ, BRASIL.

e-mail:herbert@biof.ufrj.br

Leishmania amazonensis is the main agent of anergic diffuse cutaneous *Leishmaniasis*. Our previous studies demonstrated that LaAg vaccine plus Saponin induced a partial protection against *L. amazonensis* infection observed by partial reduction of lesion growth and parasite load. In this study, we investigated the initial immune response after challenge to characterize the protective response. Prior to footpad infection with *L. amazonensis*, BALB/c mice were twice vaccinated by the intramuscular route with 25 ug of LaAg containing 100 ug of Saponin. We found that vaccinated mice developed delayed hypersensitivity peaking at 15-18 hour similar to Jones-Mote reaction. Histological studies demonstrated a lymphocyte and eosinophil infiltration and reduction of neutrophil, macrophage and mast cells infiltration in comparison to non-vaccinated mice. Eosinophil and lymphocyte (Th1) cells are related to parasite control and Neutrophils and Mast Cells are associated to susceptibility of *L. amazonensis* infection, suggesting that vaccine induced a cellular infiltrate to corroborate to parasite control. Indeed, We observed by mRNA quantification in the peak of hypersensitivity an increase of T-bet and GATA-3, but not FOXP3 on popliteal lymph node cells and demonstrated in footpad an increase of IL-4, IL-10, IFN- γ and IL-12 production and TGF- β reduction demonstrating a Th1 and Th2 profile. However, we observed an increase of iNOS levels in the footpad of vaccinated mice. The increase of iNOS in infected footpad is related to Th1 profile and associate with parasite elimination. Our result demonstrated a new-type of delayed hypersensitivity associated with Th1 response different of classical DTH reaction and suggests the involvement of this new-type of delayed hypersensitivity to parasite control in vaccinated mice against *L. amazonensis* infection. **Supported by:**CNPq

IM061 - Immunological evaluation of canine cardiac tissue face immunosuppression and inoculum by different infective forms of *Trypanosoma cruzi* during the acute phase

SOUZA, S.M.¹; OLIVEIRA, D.T.¹; VELLOSO, J.P.L.¹; VIEIRA, P.M.A.¹; FONSECA, K.S.²; LANA, M.¹; REIS, A.B.¹; TAFURI, W.L.¹; CARNEIRO, C.M.¹

1.UNIVERSIDADE FEDERAL DE OURO PRETO, OURO PRETO, MG, BRASIL; 2.UFOP, OURO PRETO, MG, BRASIL. e-mail:Shelerufop@yahoo.com.br

The dog proved to be a good model for Chagas' disease because presents some aspects related to human disease, such as susceptibility to infection and manifestations of acute and chronic phase. In this context, this study explores the aspects of immunosuppression in canine model, comparing the infection by different infective forms of *T. cruzi*. Thus, it was made morphometric quantification of cardiac inflammation and relative expression of cytokines by real time polymerase chain reaction in cardiac tissue. The group infected with metacyclic trypomastigotes showed a lower inflammatory process that seen in dogs infected with blood trypomastigotes as well as the group infected by metacyclic trypomastigotes and immunosuppressed. The groups infected with blood forms and immunosuppressed had no inflammation increased in relation to non- infected animals. The expression of cytokines IL-12p40, IFN- γ and TNF- α were increased in the groups infected with metacyclic trypomastigotes and immunosuppressed, blood trypomastigotes and blood trypomastigotes and immunosuppressed. The enzyme iNOS increased in groups inoculated with metacyclic forms and infected with metacyclic forms and immunosuppressed. The cytokines IL-10 and TGF- β 1 were increased in animals infected with blood trypomastigotes and in both groups that underwent immunosuppression. However, the expression of IL-4 cytokine was increased in the group infected with metacyclic trypomastigotes and immunosuppressed. Thus, the group infected with metacyclic trypomastigotes showed a more preserved histopathology compared to other groups, with less inflammation and the ratio of cytokines showed a Th1 immune response. Previous studies from our laboratory showed the presence of CD8+ T cells and CD14+ macrophages in metacyclic tripomastigotes group inoculated, these data combined with the results presented here confirm the importance of these cells as well as the IL-12 in experimental dog *T. cruzi* infection. **Supported by::**FAPEMIG, CNPQ and UFOP

IM062 - Differentiation between *Toxoplasma gondii* and *Neospora caninum* infections in serum samples from blood donors from Catalão - GO.

SPIRANDELLI DA COSTA, M.S.¹; FERNANDES, L.V.; MIRANDA, V.S.; SANTIAGO, F.M.; MINEO, T.W.P.; MINEO, J.R.

UFU, UBERLÂNDIA, MG, BRASIL.

e-mail:myllaspirandelli@hotmail.com

Neospora caninum is a cyst-forming protozoan parasite closely related to *Toxoplasma gondii* due to the existence of similar components. Considering dogs as definitive host to *N.caninum*, humans are exposed which can lead neosporosis be considered as a public health concern. In respect of toxoplasmosis it may be acquired by ingestion of water/food contaminated with oocysts, and the transmission has been confirmed by blood transfusion and organ transplants from infected people. The present work aimed the diagnosis for toxoplasmosis and neosporosis in serum samples from blood donors from Blood Center of Catalão. After obtaining the soluble antigen of *N.caninum* (NLA) and *T.gondii* (STAg) it was carried out sensitization of plates for ELISA immunoassays being 20 μ g/mL for *N.caninum* and 10 μ g/mL for *T.gondii*. For both assays the serum samples were used at a dilution of 1/100 and the human IgG conjugated with peroxidase in a proportion of 1/250. For immunoblot assay nitrocellulose membranes containing NLA or STAg were eletrotransferred and incubated with serum and conjugates, under the same conditions used in ELISAs. For the characterization of immunogenic proteins from *N.caninum*, two-dimensional eletrophoresis (SDS-PAGE-2D) was performed followed by Immunoblot. The results obtained by ELISA showed that 65% of blood donors were positive for *T.gondii* and confirmed by the presence of proteins TgSAG-1 and TgSAG-2 by Immunoblot. For *N.caninum*, it was observed 27% of positivity in serum samples by ELISA but only 13% were confirmed positive by Immunoblot based on visualization of immunodominant proteins from this parasite ranging from 35 to 45 kDa. Immunoblots carried out after SDS-PAGE-2D demonstrated that only the 37 kDa protein from *N.caninum* was stained. It can be concluded that the occurrence of *N.caninum* and *T.gondii* infections needs to be confirmed by Immunoblot in order to differentiate false positive results due to cross reactivity among proteins from these parasites.

IM063 - Neospora caninum limits host cell ROS production through Dectin-1 innate recognition pathway

SILVA, M.V.¹; MACÊDO JÚNIOR, A.G.¹; FERREIRA, F.B.¹; SANTIAGO, F.M.¹; OLIVEIRA SILVA, D.A.¹; SEREZANI, C.H.C.²; SILVA, M.J.B.¹; MINEO, J.R.¹; MINEO, T.W.P.¹
 1.UFU, UBERLÂNDIA, MG, BRASIL; 2.INDIANA UNIVERSITY, INDIANAPOLIS, ESTADOS UNIDOS.

e-mail:murilo.ufu@gmail.com

Neospora caninum has been associated with cattle abortions since early 1990's. The mechanisms underlying host resistance against this pathogen remains unclear and it has been the subject of intense study by our group. To unravel the initial host-parasite interactions, we recently demonstrated the importance of pathogen recognition receptors (PRRs) in *N. caninum* innate immune recognition. However, the signaling programs induced by the interaction between *N. caninum* and PRRs and the consequences of such interaction remains unclear. Here, we investigated the role of Reactive Oxygen Species (ROS) following stimulation by *N. caninum* in different cell types. We observed that soluble antigens of *N. caninum* (NLA) induces ROS production in peripheral blood mononuclear cells (PBMC) of naïve calves and murine splenocytes. The relative importance of ROS in controlling protozoan infection was further verified in NADPH oxidase enzyme (NOX2^{-/-}) deficient mice. To determine the specific receptor involved in ROS generation during *N. caninum* infection, we evaluated the role of Dectin-1 receptor in this context. We found that Dectin-1 downregulates ROS production by spleen cells exposed to live parasites and NLA. Furthermore, treatment of WT mice with Laminarin (selective Dectin-1 antagonist) increased ROS production and limited parasite replication during acute phase of infection, along with lower brain parasitism and inflammation during chronic (latent) infection. Together, our results demonstrate that *N. caninum* limits oxidative stress during infection through Dectin-1 recognition. These findings suggest that PRR-mediating ROS inhibition is a potential target for the development of therapeutic and prophylactic measures against neosporosis. **Supported by:** CAPES, CNPq, FAPEMIG, FINEP

IM064 - Identification of a novel panel of *Trypanosoma cruzi* antigens with potential application for serodiagnosis of Chagas disease

CUNHA, J.L.R.^{2,1}; RIBEIRO, D.R.S.¹; MENDES, T.A.O.¹; LOURDES, R.A.¹; ROCHA DOS SANTOS, A.R.¹; CÂMARA, A.C.J.²; GALVÃO, L.M.²; GILMAN, R.¹; GAZZINELLI, R.T.³; FUJIWARA, R.T.¹; BARTHOLOMEU, D.C.¹

1.UFMG, BELO HORIZONTE, MG, BRASIL; 2.UFRN, NATAL, RN, BRASIL; 3.UNIVERSIDAD CAYETANO HEREDIA, LIMA, PERU.

e-mail:jaumlrc@yahoo.com.br

In the chronic phase of Chagas disease, due to the low parasitemia and high anti-*T. cruzi* IgG titers, its diagnosis is majorly based in serological tests. These assays may present low specificity due to the occurrence of cross reactivity with other parasite infections, and low sensibility caused by the extreme polymorphism of the *T. cruzi* strains. Therefore the identification of new *T. cruzi* specific antigens conserved in the different parasite strains is still required. In this work, we have performed B-cell epitope prediction on proteins derived from single copy genes represented by pair of alleles in the CL Brener genome. We assumed that, because CL Brener is a recent hybrid between TcII and TcIII lineages, it is likely that conserved epitopes in its pair of alleles could also be conserved in the parental genotypes. We have excluded those peptides also presented in *Leishmania major*, *L. infantum* and *L. braziliensis* to minimize the chance of cross-reactivity. A peptide array containing the putative epitopes with the highest prediction scores was synthesized and the reactivity of the peptides tested by immunoblot using pool of sera of C57BL/6 mice chronically infected with Colombiana (TcI), CL Brener (TcVI) or Y (TcII) strain. Peptides recognized by the three pools of mice sera were synthesized as soluble peptides and tested by ELISA using individual sera from infected mice. The potential use of these antigens to identify human infection was evaluated by ELISA using sera of chagasic patients from Brazil and Peru. The occurrence of cross reactivity with sera from patients with *Leishmaniasis* and from animals infected with *T. rangeli* was also evaluated. We identified at least three *T. cruzi* specific peptides that were recognized by sera from Brazilian and Peruvian chagasic patients. We are currently expressing the three recombinant proteins containing each one of these peptides in *Escherichia coli* as well as a chimeric protein in which all three peptides were fused. **Supported by:** INCTV, FAPEMIG, CNPq, CAPES

IM065 - Effects of obesity on the heart inflammation in experimental infection with *Trypanosoma cruzi*

LOPES, L.R.^{*}; ARMACHE, N.N.; FIGUEIREDO, V.P.; RIBEIRO, S.M.L.T.; SILVA, M.E.; BAHIA, M.T.; DA SILVA PEDROSA, A.T.
 UFOP, OURO PRETO, MG, BRASIL.
 e-mail:laisroquete@hotmail.com

Trypanosoma cruzi infection induces progressive inflammatory manifestation capable to affect the structure and the function of heart. Host nutritional status appears to exert essential role in the immune response, besides contribute to other physiological or metabolic functions. Therefore, hypercaloric diet contributes to the establishment of the obesity, another inflammatory disease capable to reduce the life quality in individuals in different ages. The aim of this study is to evaluate possible alterations and/or damage caused by obesity on the systemic and cardiac inflammatory response in acute (30 days) and chronic (90 days) phases of experimental *T.cruzi* infection. Fisher rats were submitted to a standard or HC diet and then infected (or not) with Colombian strain of *T.cruzi* and parasitological, biochemical and immune parameters evaluated using heart tissue, serum and adipocytes. Uninfected rats received both diets as control group. In our preliminary data, animals under HC diet presented an increasing weigh gain during acute and chronic phases of the infection followed by reduction of the parasitemia. However, there was no difference in global cholesterol among groups receiving both diets. Serum levels of triglycerides were higher in those infected animals feed with standard diet in acute phase, however during chronic phase there was an elevation in this parameter in the infected and obese rats. Inflammatory mediators TNF- α and CXCL3/Fractalkine presented high levels in all infected rats, independently of the diet. Histopathological analysis in heart tissue demonstrated an increase of leukocyte recruitment the group infected animals with HC diet. Besides, the group feed with HC diet had greater inflammation than animals receiving the conventional diet. Based on this preliminary data, we suggest that HC diet may affect the inflammatory status in *T.cruzi* infected animals possible interfering in the course of this infection. **Supported by:** CNPq, UFOP.

IM066 - Immunopathological aspects of chronic infection with blood or metacyclic trypomastigotes of *Trypanosoma cruzi* in mice

BITENCOURT, F.C.O.⁻¹; MARQUES, F.S.¹; VIEIRA, P.M.A.¹; NOGUEIRA, C.¹; FONSECA, K.S.¹; CARVALHO, A.T.²; MARTINS-FILHO, O.A.²; MENESES MACHADO, E.M.¹; TAFURI, W.L.¹; CARNEIRO, C.M.¹
 1.UFOP, OURO PRETO, MG, BRASIL; 2.FIOCRUZ, BELO HORIZONTE, MG, BRASIL.
 e-mail:flacbo@yahoo.com.br

Previous results of our group emphasize the importance of taking into account the inoculum source of *Trypanosoma cruzi*, since vectorial or transfusional routes of *T. cruzi* infection may trigger distinct parasite-host interactions during the acute phase that may influence relevant biological aspects of chronic Chagas disease. Based on this, the aim of this study was to evaluate changes related to immunopathological parameters during the chronic phase of experimental infection in Swiss mice by metacyclic trypomastigotes or blood trypomastigotes of Berenice-78 strain. For this purpose, 10 animals in each group, control (non infected animals) and infected by metacyclic trypomastigotes (MT) or blood trypomastigotes (BT), were euthanized at 180 days after infection and all statistical analyses of the data were assessed by one-way analysis of variance (ANOVA) and Tukey test being considered significant at $p < 0.05$. To investigate whether the infection by MT or BT forms could interfere with the circulating leukocytes in the blood, differential counts were done on these cells and the results showed decrease in the number of White Blood Cells, monocytes and lymphocytes in animals inoculated with MT forms. The phenotypic analysis of peripheral blood mononuclear cells showed an increase in NK cells, B lymphocytes and in subpopulations of T lymphocytes (CD4⁺ and CD8⁺) in the BT group. Morphometric analyses of the heart showed no inflammatory cells in both infected groups, but animals infected with BT forms showed a larger area of collagen deposition. Based on these results we can conclude that the initial interaction between the metacyclic trypomastigotes and the vertebrate host induces a histopathological profile different from that observed in infection by blood trypomastigotes, since the latter induces an intense collagen deposition which may result in a worse disease progression during the chronic phase. **Supported by:** FAPEMIG, CNPq e UFOP

IM067 - Standardization of a Co-culture System for in vitro Analysis Immunoprotective Vaccines against Canine Visceral *Leishmaniasis*

VIANNA, K.F.*; AGUIAR-SOARES, R.D.O.; ROATT, B.M.; SILVEIRA-LEMOS, D.; RESENDE, L.A.; MOURA, S.A.L.; MENDONÇA, L.Z.; REIS, A.B.; GIUNCHETTI, R.C.
 UFOP, OURO PRETO, MG, BRASIL.
 e-mail:rodrigodan@gmail.com

Canine visceral *Leishmaniasis* (CVL) is a parasitic disease affecting several regions of the world. The vaccination against *Leishmania* infection is considered the major rational method for controlling this disease. In this context, the screening of potential antigens using in vitro methods would be useful for evaluation vaccine candidates against CVL. In this context, herein, we established the ratio of CD4+ and CD8+ T lymphocytes: canine monocytes-derived macrophages (M ϕ) in co-culture systems. In this sense, we employed magnetic columns and microbeads for purifying CD4+ and CD8+ T cells and co-cultured with canine M ϕ (previously infected with *L. chagasi* promastigotes) of healthy dogs. In this in vitro analysis, purified cells were used in the ratios: 1 CD4+ or 1 CD8+ + 5 M ϕ (1:5), 1:2, 1:1 and 2:1. The results indicated that 1:2 ratio of lymphocytes (CD4+ or CD8+) and M ϕ is sufficient to analyze functional activity of lymphocytes (IFN- γ , TNF- α , IL-12, IL-10 and IL-4 and microbicidal activity of M ϕ) using culture supernatant after 72 hours of *L. chagasi* infection. Also, 1:1 and 2:1 ratios of CD8+ and CD4+ T cell presented high cytotoxic activity in *L. chagasi* infected M ϕ . In this sense, the results indicated that canine CD4+ T cells also induced cell lysis in infected M ϕ and further studies are required to analyze this mechanism. In addition, this co-culture system using purified lymphocytes and *L. chagasi* infected M ϕ will be further analyzed in cells from vaccinated dogs, aiming to validate this methodology as tool for immunoprotection studies against CVL. **Supported by:** FAPEMIG, CNPq, CAPES, FIOCRUZ, UFOP and UFMG.

IM068 - American Tegumentary *Leishmaniasis* caused by *Leishmania* (*Viannia*) in dogs from the municipality of Itupeva, São Paulo

BATISTA, L.F.S.¹; ROSSI, C.N.¹; ASCHAR, M.²; DE LIMA, A.C.S.²; ROSA, M.F.³; TOMOKANE, T.Y.²; CORBETT, C.E.P.²; MICHALANY, N.S.⁴; LARSSON, C.E.¹; LAURENTI, M.D.²

1.FMVZ-USP, SÃO PAULO, SP, BRASIL; 2.FMUSP, SÃO PAULO, SP, BRASIL; 3.UNIFESP, SÃO PAULO, SP, BRASIL; 4.LABORATÓRIO PAULISTA DE DERMATOPATOLOGIA, SÃO PAULO, SP, BRASIL.

e-mail:fabibatista@usp.br

Municipality of Itupeva, near to Campinas, State of São Paulo, presents human cases of American Tegumentary *Leishmaniasis* (ATL) as well as species of vectors of the ATL, however it has been classified only as area of canine visceral *Leishmaniasis* transmission. This study reported three dogs living in the same ownership and sharing the same clinical profiles. Although none of the three dogs show signs of visceral disease, the presence of cutaneous ulcers with raised edges on the upper lip was common to the three dogs. One of the dogs showed an extension of the lesion to the mucosal of the left nostril consistent to ATL caused by *Leishmania* (*Viannia*). Antibodies anti-*Leishmania* was detected in the serum of the three dogs by ELISA, furthermore strong cellular immune response was detected by the delayed hypersensitivity skin test and lymphocyte proliferation assay in vitro both employing specific antigen of the genus *Leishmania*. Microscopically the lesions were characterized by a chronic mononuclear inflammatory infiltrate with no evidence of parasites by immunohistochemistry. Nevertheless, DNA of G6PD of *Leishmania* (*Viannia*) was detected in paraffin biopsy of the cutaneous lesion in one of the three dogs by real time PCR, whereas were not identified DNA from *Leishmania* (*Leishmania*) *infantum chagasi* in the buffy coat in any of the three dogs. The results suggest the potential role of *Canis familiaris* as reservoir of ATL caused by *Leishmania* (*Viannia*) in the municipality of Itupeva (SP). **Supported by:** LIM50/HCFMUSP

IM069 - THE ADJUVANT RETINOIC ACID IMPROVES THE ANTILEISHMANIAL LaAg INTRANASAL VACCINE EFFICACY AND INCREASES THE CD4⁺ FOXP3⁺ POPULATION IN THE NASAL MUCOSA

BEZERRA, I.P.S.⁻¹; SOUZA, B.L.S.C.¹; CARNEIRO, G.²; FERREIRA, L.A.M.²; GUEDES, H.L.M.¹; ROSSI-BERGMANN, B.¹

1.UFRJ, RIO DE JANEIRO, RJ, BRASIL; 2.UFMG, BELO HORIZONTE, MG, BRASIL.

e-mail:izabella@biof.ufrj.br

Intramuscular vaccination with *Leishmania amazonensis* promastigotes total antigen (LaAg) worsens disease progression, but oral or intranasal vaccination with LaAg confers protection in mice against *L. amazonensis* infection. Protection afforded by LaAg when given by mucosal routes could be related to tolerance induction to *Leishmania* antigens. Retinoic acid (RA), a metabolite from retinol, is important for FoxP3⁺ regulatory T cell generation in the intestinal mucosa, which has been associated with oral tolerance. Based on these findings, we observed that dietary retinol is necessary for the LaAg oral vaccine efficacy. Little is known about tolerance induction in nasal mucosa. In this study, the role of RA as an adjuvant to the LaAg intranasal vaccine was investigated. Since RA is insoluble in aqueous solvents and may be irritating, we used RA encapsulated in solid lipid nanoparticles at 0.1% (RA-SLN). Thus, BALB/c mice were intranasally immunized with LaAg alone or associated with RA-SLN. After immunization, cervical lymph node cells were phenotyped by flow cytometry and cytokine expression on these organs were evaluated by real time PCR. 7 days after immunization, mice were infected with 2x10⁵ *L. amazonensis* and the lesion development was monitored. 60 days post infection, the parasite burden and the cytokine profile in the lesion were evaluated. LaAg associated with RA-SLN promoted a lower lesion development, a decreased parasite burden and a higher level of mixed cytokines in the lesion. Moreover, LaAg associated with RA-SLN increased the CD4⁺ FoxP3⁺ regulatory T cell population in cervical lymph nodes and enhanced IL-10 expression on these organs. These results indicate that the adjuvant RA-SLN improved the LaAg intranasal vaccine efficacy. The presence of RA in the nasal mucosa during immunization may induce regulatory T cells responsive to *Leishmania* antigens in the vaccine and these cells may be involved in post-infection protective events. **Supported by::CAPES**

IM070 - *Leishmania braziliensis* overexpressing SL-RNA modulates macrophage functions

PINZAN, C.F.⁺; DE TOLEDO, J.S.; CRUZ, A.K.

FMRP, RIBEIRÃO PRETO, SP, BRASIL.

e-mail:capinzan@gmail.com

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 polyadenylation 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]). 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-1 β 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. 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. **Supported by::FAPESP, CNPq**

IM071 - PATHOGENESIS OF EXPERIMENTAL CEREBRAL MALARIA: ROLE OF AHR
COSTA, F.M.C.B.^{*}; DA SILVA, A.A.; ESPER, L.; BARROSO, A.; VAL, C.H.; OLIVEIRA, B.C.;
RACHID, M.A.; TEIXEIRA, A.L.; MACHADO, F.S.
UFMG, BELO HORIZONTE, MG, BRASIL.
e-mail:fatimacbrant@gmail.com

Introduction: Human cerebral malaria (CM) is the most important complication of *Plasmodium falciparum* infection especially among children in sub-Saharan Africa. Mice infected with *P. berghei* ANKA (PbA) faithfully recapitulate many of the characteristics of human. Although the pathogenesis of CM is not completely understood, could not be explained by only one mechanism. The Aryl Hydrocarbon receptor (AhR) is an intracellular receptor activated by several ligands and is important to modulate the inflammatory response. However, the involvement of AhR in CM is not known. Objective: Investigate the role of AhR in the regulation of immune response and development of CM. Methods: C57Bl/6 (WT) and AhR^{-/-} mice were infected with PbA and the parasitemia and survival were monitored periodically. The production of cytokines in the serum, brain and spleen was assessed by ELISA and flow cytometry. The expression of SOCS1/SOCS2/SOCS3 was assessed by Real Time PCR. Leukocyte recruitment in the brain was evaluated by intravital microscopy. Nitric oxide (NO) was assessed by the Griess method in the brain. Histopathology analysis was also performed in brain and liver. Results: The parasitemia was dramatically higher in PbA-infected AhR^{-/-} mice when compared with WT. In the brain and spleen of PbA-infected AhR^{-/-} mice there was a significant decreased expression of TNF- α /IL-1 β /IFN- γ /IL-12/IL-10 when compared with WT counterparts. Additionally, there was an increased levels of TGF- β /IL-6/IL-17 and high expression of FOXP3 in the brain of infected AhR^{-/-} mice when compared with WT infected mice. In the absence of AhR, there was decrease expression of SOCS3 in brain after infection, an increased level of alanine aminotransferase (ALT), evident liver damage, as well as lower hematocrit during the infection. Moreover, PbA-infected AhR^{-/-} mice had increased BBB permeability. Conclusions: These findings indicate, for the first time, the role for AhR in the immunopathogenesis of CM. **Supported by::**CAPES, CNPq, FAPEMIG

IM072 - P21-His6, based on *Trypanosoma cruzi* P21, as a potential chemotactic compound.

RODRIGUES, A.A.^{*}; CLEMENTE, T.M.; MACHADO, F.C.; SANTOS, P.C.F.; MINEO, T.W.P.;
SILVA, C.V.
UFU, UBERLÂNDIA, MG, BRASIL.
e-mail:pcesar6@gmail.com

Trypanosoma cruzi P21 was recently characterized and its activity during parasite internalization in host cell has been studied. Previously, our group observed that the recombinant protein, P21-His6, induce its biological activities via binding to CXCR4 chemokine receptor. This way, this study aimed to analyze P21-His6 chemotactic potential by kinetic of cellular recruitment *in vivo* and *in vitro*. For these porpouse, C57BL/6 animals were intraperitoneally injected with 40 μ g/mL of the protein, *in vitro* cell migration assay, using transwell plates and flow cytometry method, to characterize the cell population recruited, using molecular markers for T, B, NK and NKT lymphocytes, neutrophils, monocytes and dendritic cells. Our results showed that P21-His6 induced higher cell recruitment in comparison to the positive control, thioglycollate injection, especially at 72 hours. However, distinctly to thioglycollate P21-His6 only initiated its cellular recruitment 24 hours after intraperitoneal inoculation. Flow cytometry of peritoneal recruited cells showed a predominance of neutrophils and monocytes. The *in vitro* chemotaxis assay showed that P21-His6 was able to recruit higher number of neutrophils than the macrophage inflammatory protein-2 (MIP-2) and had its chemotactic effect blocked in the presence of bacterial Pertussis toxin (PTX), responsible for inhibiting G protein-coupled receptors, such as as CXCR4 receptor. All together, we suggest that P21-His6 can mimic SDF1- α upon biding to CXCR4 receptor, inducing leukocyte recruitment. All the experiments were approved by ethics committee on animal research. **Supported by::**FAPEMIG/CAPES/CNPq

IM073 - MUSHROOM AGARICUS BLAZEI MURRIL TREATMENT AND MODULATION OF IMMUNE RESPONSE DURING PLASMODIUM BERGHEI ANKA INFECTION

VAL, C.H.¹; COSTA, F.M.C.B.¹; MIRANDA, A.S.¹; OLIVEIRA, B.C.¹; ESPER, L.¹; RODRIGUES, F.G.¹; SANTOS, E.A.²; REGIS, W.C.B.³; MACHADO, F.S.¹

1.UFMG, BELO HORIZONTE, MG, BRASIL; 2.PUC, BELO HORIZONTE, MG, BRASIL;

3.MINAS FUNGI, BELO HORIZONTE, MG, BRASIL.

e-mail:fatimacbrant@gmail.com

Introduction: The *Agaricus blazei* Murrill (AbM) is a Brazilian originated mushroom, and it is being used as an alternative medicine and functional food, which preventing various diseases, such as infection, allergy, and cancer. AbM was described to contain bioactive compounds related to an immunomodulatory activity. Malaria is a disease caused by *Plasmodium* species reaching 106 countries, affecting approximately 216 million people and leading to deaths of 655,000, and that has been exacerbated by the emergence of drug-resistant parasites. The most important complication of *Plasmodium falciparum* infection in human is the development of cerebral malaria (CM). Here, we investigated the effect of AbM in modulation of immune response and development of cerebral malaria (CM) in mice infected with *P. berghei* ANKA (PbA), this parasite strain faithfully recapitulate many of the characteristics of human CM. Material and Methods: C57Bl/6 mice were pretreated (3 days) with extract or fraction of AbM and than infected with 1x10⁵ infected red cells, followed by treatment with AbM (extract or fraction) or chloroquine, and the parasitemia, survival, body weight, development of CM and immune response were evaluated. Results: Mice treated with extract or fraction of AbM demonstrated lower parasitemia, longer survival, reduced weight lost and protection against CM development associated with reduced levels of pro- and anti-inflammatory cytokines production (TGF- β /IL-10, IFN- γ , IL-1 β , IL-6 and IL-17) when compared with untreated PbA-infected mice. Main Conclusions: These findings indicate, for the first time, that AbM has an important protective role in the development of experimental cerebral malaria and modulation of immune response during PbA infection. **Supported by**::CNPq, FAPEMIG

IM074 - STUDY OF MORPHOMETRY AND IMMUNE MODULATION DURING EXPERIMENTAL CHAGAS DISEASE BY DIFFERENT PHARMACOLOGICAL THERAPIES

BAJRACHARYA, D.S.¹; ROCHA, P.P.¹; SILVA, F.H.G.²; SALLES, B.C.S.¹; GONÇALVES, K.R.¹; COSTA, G.P.¹; NOVAES, R.D.³; BAJRACHARYA, B.¹; PENITENTE, A.R.¹; BAHIA, M.T.¹; DA SILVA PEDROSA, A.T.¹

1.UNIVERSIDADE FEDERAL DE OURO PRETO, OURO PRETO, MG, BRASIL; 2.INSTITUTO OSWALDO CRUZ, FIOCRUZ, RN, BRASIL; 3.UNIVERSIDADE FEDERAL DE VIÇOSA, VIÇOSA, MG, BRASIL. e-mail:deenabajra@gmail.com

Chagas heart disease, an inflammatory characterized illness caused by *Trypanosoma cruzi*, triggers acute and chronic infection driving cardiomyocytes toward an aggressive and progressive damage process with consequent fibrosis and loss of functionality. Enalapril, an angiotensin converting enzyme (ACE) inhibitor, and simvastatin, a powerful lipid-lowering drug, are used in the treatment and the prevention of cardiovascular disease. The purpose of this study was to analyze morphometric parameter, immune modulation and remodeling of heart in the mice infected with colombiana strain of *T. cruzi* and treated with these drugs. C57BL/6 mice were infected and then treated for 20 days with simvastatin (20mg/kg), enalapril (25mg/kg) and benznidazole (100mg/kg). Animals receiving vehicle (phosphate buffer) were used as a control group. Mice were euthanized on day 24 after infection. Morphometric analyses, immunoassay, biochemical assay, immunohistochemistry were performed to study morphological and immunological modifications of the heart. In our preliminary results, both simvastatin and enalapril treated animals presented increase in the area of intracellular amastigote nests in the heart in comparison to untreated mice whereas no amastigote nest was found in benznidazole treated animals. In addition, along with marked inflammatory infiltration in the heart, simvastatin presented low CCL5 and CCL2 plasma level whereas only CCL5 level was low in enalapril treated animals. The plasma cytokine levels in benznidazole treated mice were similar to control group. According to our current findings, pharmacological therapies interfere in host systemic immune parameters and based on our morphometric analysis, we suggest that the high percentage of amastigote nests present a partially dependence on host immune response and may exert a close relation with the persistence of heart inflammatory process during the chronic phase of *T. cruzi* infection. **Supported by**::FAPEMIG, CAPES, TWAS, CNPq, ISID, UFOP

IM075 - Inflammatory angiogenesis induced by antigens of the Y strain *Trypanosoma cruzi*

*SILVA, F.H.G.¹; BAJRACHARYA, D.S.²; LIMA, L.C.³; SALLES, B.C.S.²; MOURA, S.A.L.²; ANDRADE, S.P.³; BARCELOS, L.S.³; BAHIA, M.T.²; DA SILVA PEDROSA, A.T.².
1.IOC/FIOCRUZ, RIO DE JANEIRO, RJ, BRASIL; 2.UFOP, OURO PRETO, MG, BRASIL;
3.UFMG, BELO HORIZONTE, MG, BRASIL.
e-mail:hildemagnaguedes@hotmail.com

Angiogenesis and inflammation are persistent features of several pathological conditions induced by pathological agents. Particularly, glycoproteins derived from the protozoan *Trypanosoma cruzi* are suggested to induce this inflammatory angiogenesis. In this study, we investigated the effects of total antigen from 108 trypomastigote forms of *T. cruzi* (Y strain), inoculated in sponge implantes of mice, on angiogenesis, inflammatory cell pattern and endogenous production of inflammatory and angiogenic mediators on days 1, 4, 7 and 14 post implant. There was an elevation of hemoglobin content on the 14th day, assessed by hemoglobin of the implants with difference between those sponges stimulated with T.cruzi antigen or vehicle (PBS). However, parasite antigens induced high production of vascular endothelial growth factor (VEGF) and inflammatory mediators TNF-alpha, CCL2 and CCL5 on the 7th day in sponges when compared to the unstimulated group. Accumulation of neutrophils and macrophages was determined by measuring of myeloperoxidase (MPO) and N-acetyl-b-D-glucosaminidase (NAG) enzymes activity, respectively. MPO activity was unaffected by T.cruzi antigen, but macrophage accumulation was increased after stimulation with antigens initiating at day 4 and peaking at day 7. Morphometric analysis of the sponges suggested that inflammatory and angiogenic mediators, particularly induced by macrophages, increased blood vascularization in the matrix of the sponges peaking at 14th day. Together, our results suggest that T.cruzi molecules are the pivotal reason to the magnification of inflammatory angiogenesis in the sponge model and this process might be trigger by T.cruzi-induced inflammatory mediators. **Supported by:** CAPES, CNPq, FAPEMIG, Twas

IM076 - Antigenic determination in Plasmodium vivax Merozoite Surface Protein 7 (PvMSP7) using Spot-Synthesis technique.

SALVIANO ALVES, T.M.¹; OLIVEIRA, I.C.¹; FERREIRA, G.M.F.¹; MOURÃO, L.C.¹; AVILA, R.A.M.¹; OLÓRTEGUI, C.C.¹; FERNANDES FONTES, C.J.²; BRAGA, E.M.¹.
1.UFMG, BELO HORIZONTE, MG, BRASIL; 2.UFMT, CUIABA, MT, BRASIL.
e-mail:tryciamar@gmail.com

MSP7 (Merozoite Surface Protein 7) is a protein expressed by Plasmodium parasites during the blood-stage stage and it is located at the surface of the invasive merozoite. It forms a complex with MSP1 and MSP6, which is believed to participate in the initial interaction among the merozoite and the erythrocyte. Thus, this complex has been proposed as a potential vaccine candidate. On the basis of this, an important step that precedes the use of these molecules as biological tools is obtaining information at the amino acid level to characterize the possible epitopes of these proteins which are targets for antibodies. However, few studies have been performed for the identification and mapping of the epitopes in PvMSP7. So, the present work aimed to describe, for the first time, the epitope mapping of the full-length PvMSP7, using the Spot-synthesis technique. Moreover, we also tried to associate the pattern of antibody reactivity with morbidity parameters, which were evaluated here, by anemia and thrombocytopenia. B-cell linear epitope mapping was done by the synthesis of an overlapping 15-mer peptide library covering the entire extension of PvMSP7. The peptides were synthesized in a solid phase, by the spot method, using Fmoc strategy. Pooled serum from five different groups: non-infected, non-anemic and non-thrombocytopenic, anemic and non-thrombocytopenic, and finally, anemic and thrombocytopenic were tested against 453 in the MSP7G, 377 in the MSP7A and 311 in the MSP7K peptides. According to spot-image intensities, we identified 07 antigenic determinants in the complete sequence of PvMSP7A, 13 in the complete sequence of MSP7K and, finally, 11 spots in the PvMSP7G. The next step will focus on the analysis and comparison among antibody reactivity from the different study groups as an attempt to identify the possible biomarkers involved in malaria clinical manifestations like anemia and thrombocytopenia. **Supported by:** CNPq FAPEMIG

IM077 - Inflammation and cytokine profiles in the esophagus of dogs experimentally infected with *Trypanosoma cruzi* is associated with the phase of infection and / or strain used

FONSECA, K.S.¹; NOGUEIRA, C.¹; LOPES DE CASTRO, L.M.¹; VIEIRA, P.M.A.¹; VELOSO, V.M.¹; BAHIA, M.T.¹; GUEDES, P.M.M.¹; SA, R.G.¹; CARNEIRO, C.M.²

1.UFOP, OURO PRETO, MG, BRASIL; 2.UFRN, NATAL, RN, BRASIL.

e-mail:katia.fonseca@gmail.com

The evaluation of inflammatory cells and cytokines produced by them, may allow a better understanding of the genesis of chronic injuries in Chagas disease. In this study, Beagle dogs were infected with Y or Berenice-78 (Be-78) strains. These animals were necropsied and esophagus were collected to perform inflammation quantification, immunohistochemistry to detect T CD4+ and CD8+ lymphocytes and real-time PCR for quantification of cytokines mRNA IFN-g, IL-6, TNF-a, IL-12, IL-4, IL-10, TGF- β and iNOS. The acute inflammatory process was predominantly mononuclear and higher in the infected groups. Immunohistochemistry analysis of animals infected by both strains showed that the majority of inflammatory cells were positive for CD4+ or CD8+ in similar proportions. However, in the chronic phase, the inflammatory infiltrate was reduced and it was observed only in animals infected with Be-78 strain. During acute phase was observed an increase in mRNA expression of proinflammatory cytokines TNF-a and IFN-g in animals infected with the Y strain compared to the other groups. In the chronic phase there was an increase in mRNA expression of IL-6, IL-12, TNF-a and iNOS in animals infected with Be-78 strain in relation to the other groups. Regarding anti-inflammatory cytokines, there was a increase in mRNA expression of IL-10 in animals infected with the Y strain compared to the group of uninfected animals in the acute phase. Thus, both infections promote an acute inflammatory response, predominantly lymphocytic with similar proportions of T cells CD4+ and CD8+, restricted, in the chronic phase, to the Be-78 strain. In addition, in animals infected with the Y strain the acute inflammation was associated with expression of proinflammatory cytokines, whereas in animals infected with Be-78 strain is not associated with expression of proinflammatory cytokines evolving to chronic inflammation associated with expression of these cytokines and iNOS **Supported by:** Fapemig, CNPq e ufop

IM078 - Secreted Products Of Metacyclic Trypomastigotes Forms Of *Trypanosoma cruzi* Confers an Increased Ability to Resist Complement Mediated Lysis

NETO, L.L.¹; OSSES, I.E.¹; ARAYA, M.I.R.
FIOCRUZ, RIO DE JANEIRO, RJ, BRASIL.

e-mail:quimica.leandro@gmail.com

During the first contact parasite host cells, the insect-derived metacyclic trypomastigote (M.T) forms of *T. cruzi* have to avoid the lysis by the complement system and invade host cells. Our previous data support that resistance to complement killing is not a strict characteristic of M.T of *T. cruzi*; rather, there are strains sensitive and resistant to complement killing by normal human serum (NHS). We have hypothesized that M.T. could be releasing serine protease inhibitors (SERPINS) that are able to inhibit complement activation. We found inhibitory activity in secreted products present on supernatants from M.T. but not on supernatants of epimastigotes. We made a complement-lysis assay using epimastigotes parasites in presence of 50 % NHS diluted in RPMI (a condition that lysed 100 % of the parasites at 37 C in 30 min) or in the supernatant obtained at 28, 37°C from epimastigotes and M.T cultures. We have found that supernatant derived from M.T culture at 28 and 37 °C showed an inhibitory effect ranging from 50-80 % of complement-mediated lysis of epimastigotes. However the supernatant derived from epimastigotes at 28 °C did not show any inhibitory effect. In complement-lysis assay using NHS and NHS treated with EGTA (an inhibitor of classical and Lectin pathway) we have seen 30 % of inhibition at the alternative pathway when the M.T supernatant was used. Moreover when epimastigotes forms were treated with M.T supernatants an increased deposition of MBL, C3 and H ficolins complement factors were detected at the cell surface. This suggests that inhibitor products are somehow affecting the complement system. Furthermore we have detected that the inhibitory effect is dose dependent and is different between strains. We have detected the presence of genes coding for SERPINS at *T. cruzi* genome. We have cloned and overexpress in epimastigotes. The ability of the parasite to resist the complement lysis and the expression level of these genes are under investigation. **Supported by:** Capes

IM079 - P2Y RECEPTORS ACTIVATION INDUCES *TOXOPLASMA GONDII* EGRESS IN INFECTED MACROPHAGES

MOREIRA-SOUZA, A.C.A.¹; VOMMARO, R.C.; SILVA, R.C.
UFRJ, RIO DE JANEIRO, RJ, BRASIL. e-mail:alinec.biologia@gmail.com

Introduction: The G-protein coupled P2Y receptors are involved in several cellular events such as, PLC activation and calcium release from intracellular stores. Our group has been studying the implications of the P2Y receptors in the context of infections with pathogens, which subvert the host response. *Toxoplasma gondii* is a protozoan parasite known to escape from lysosomal fusion, obtain nutrients from the host favoring its replication; the parasite disrupts the host cell membrane and initiates a new cycle of infection causing damage to the affected tissue.

Objective: To study the role of P2Y receptors activation in *T. gondii* infection of macrophages.

Methodology: Experiments were carried out using peritoneal macrophages (MΦ) from Balb/c mice. The cells were plated for 48 h, infected with *T. gondii* (RH strain) in the ratio of 3:1 for 2 h and incubated with 100μM UTP or UDP for 30 minutes. These cells were used for analysis of parasite burden after 16 hours or immediately after treatment and processed for scanning electron microscopy. Infected culture supernatant was collected to quantify free parasites. The free parasites obtained from the supernatant were incubated with a fresh non infected macrophage culture, for 24 h in order to test the viability of the parasites from the treatment with UTP or UDP.

Results: We found that UTP treatment reduced the parasite burden compared with controls. We observed a higher amount of tachyzoites in the culture supernatant which received the nucleotides. In addition, it was observed by the scanning electron microscopy that after treatment, the tachyzoites with their apical pole towards the extracellular medium, confirming the egress of parasites from the host cells. We observed that the tachyzoites from supernatant of treated macrophages with UTP and UDP were less infective than fresh non treated tachyzoites.

Conclusion: P2Y receptors might be involved in host immune response against *T. gondii* infection **Supported by**::CNPq; CAPES; PRONEX; INPeTAm

IM080 - DIFFERENT INFECTIVE FORMS TRIGGER DISTINCT IMMUNE RESPONSE IN CARDIAC TISSUE

PERIN, L.H.¹; OLIVERI, L.M.R.¹; VIEIRA, P.M.A.¹; FONSECA, K.S.¹; NOGUEIRA, C.¹;
MENESES MACHADO, E.M.¹; MARTINS-FILHO, O.A.²; REIS, A.B.¹; TAFURI, W.L.¹;
CARNEIRO, C.M.¹

1.UFOP, OURO PRETO, MG, BRASIL; 2.CPQRR, BELO HORIZONTE, MG, BRASIL.

e-mail:luisa_op@yahoo.com.br

Metacyclic trypomastigotes (MT) and blood trypomastigotes (BT) infections are associated with distinct cytokine profile in the spleen during the acute phase of Chagas disease. Thus, the objective of this study was to evaluate the cytokine profile in heart tissue of mice infected by different infective forms throughout the acute phase of infection and assess whether this organ present the same pattern observed in the spleen. Five mice of each group (non-infected, infected with MT forms and infected with BT forms) were euthanized before infection (0) and at 7, 14, 28 and 42 days after infection and the heart were removed for quantification of cytokines mRNA IFN-γ, TNF-α, IL-12, IL-10 e TGF-β by Real Time-PCR. To the cytokine IL-12 it was observed in both infected groups an increase of mRNA expression on day 28 after infection, being this increase maintained at 42 day only in BT group. The animals in the BT group showed an increase in mRNA expression of IFN-γ on day 7 after infection and from day 28 after infection. On the other hand, in MT group was observed increased expression of mRNA for IFN-γ on days 14 and 42 after infection. Thus, can be noted a decrease in the expression of IFN-γ on the peak of parasitemia at days 14 and 28 for BT and MT, respectively. Regarding the anti-inflammatory cytokines, there was an increased expression of mRNA for TGF-β in the MT group, thus demonstrating that in these animals appears to occur an immunoregulatory profile of cytokines in the heart, while the BT group showed an increase in IL-10 only at 28 days after infection. In this sense, animals infected by MT forms were able to induce an immunoregulatory response in heart and a decreased in cardiac inflammation at the end of the acute phase, when the parasitemia has already come under control. Moreover, animals in the BT group failed to present this kind of response in early infection, which leads to an exacerbation of the heart inflammatory process. **Supported by**::Fapemig, CNPq e ufop

IM081 - Silicon effect on *Artemisia annua* and *Toxoplasma gondii* proliferation
ROSTKOWSKA, C.¹; MOTA, C.M.; SANTIAGO, F.M.; OLIVEIRA, T.C.; OLIVEIRA, L.A.;
KORNDORFER, G.H.; OLIVEIRA SILVA, D.A.; MINEO, J.R.
UFU, UBERLÂNDIA, MG, BRASIL.
e-mail:cristinacarmo@yahoo.com.br

Toxoplasmosis is an important zoonotic disease due to ability of its causal agent, *Toxoplasma gondii*, to infect large number of vertebrates and to be associated with congenital infection or opportunistic disease in immunosuppressed patients. Knowing that traditional treatment has shown adverse effects, low-toxicity compounds like artemisinin have been researched as well the *Artemisia annua* infusion. This study aimed to investigate the effects of applying Silicon (Si) to the soil, on *A. annua* plant physiology and the role of the infusion obtained from plants grown under different Si treatments on *T. gondii* replication in cell culture. The experimental design consisted of planting *A. annua* seedlings under five treatments with and without Si applied to the soil (0, 200, 400, 800 and 1600 kg/ha). Analysis of foliar macronutrients showed a significant increase only for Nitrogen with the highest Si concentration (1600 kg/ha), but this effect had no influence in plant height and artemisinin content. The highest artemisinin content in plant leaves and in the infusion was determined by high performance liquid chromatography (HPLC). Parasite proliferation assays showed that both cell treatments with the infusion (obtained with 400 kg/ha Si and without Si) after infection decreased parasite proliferation in a dose-dependent manner. In conclusion, the Si treatment in the soil had positive effect on the glandular trichome area and artemisinin content, but this outcome was not associated with a better efficacy of *A. annua* infusion on *T. gondii* replication suggesting that other components rather than artemisinin could be contributing to this effect. **Supported by::**CNPQ, FAPEMIG, CAPES

IM082 - NATURALLY ACQUIRED ANTIBODIES INDUCED TO POLYMORPHIC AND CONSERVED DOMAINS OF *Plasmodium vivax* MEROZOITE PROTEIN 1 IN SUBJECTS WITH LIMITED EXPOSURE TO MALARIA IN THE BRAZILIAN AMAZON
ALMEIDA, Z.B.¹; MOURÃO, L.C.¹; FERREIRA, M.U.²; BRAGA, E.M.¹
1.UFMG, BELO HORIZONTE, MG, BRASIL; 2.USP, SÃO PAULO, SP, BRASIL.
e-mail:zelia.b.almeida@hotmail.com

Malaria remains a major public health problem in tropical and subtropical countries, and often appears as the leading cause of morbidity and mortality. *Plasmodium vivax* is the most prevalent species in Brazil, accounting for about 80% of malaria cases reported in the country. Antigens expressed on the merozoite surface of *P. vivax*, such as MSP1 are considered potential targets for a possible antimalarial vaccine. PvMSP1 contains six highly polymorphic domains, which is a major problem for its inclusion in a subunit vaccine. It is not well known if such protein polymorphism is associated or not with a variant-specific humoral immunity. Thus, this work aimed to evaluate the naturally acquired antibody response against three recombinant antigens representing different regions of PvMSP1: two polymorphic regions (block 10) and a conserved one (MSP-1₁₉). For this, 503 subjects living in a rural community located in Acre state, Brazilian Amazon region, were followed up for 17 months. Sera were obtained during three distinct periods: March 2010, April to May 2010 and July 2011. Three PvMSP1 recombinant proteins corresponding to polymorphic domains (10BP13 and 10BR07) and conserved one (MSP-1₁₉) were used. Our results showed that the frequency of individuals with IgG antibodies induced to the conserved domain (MSP-1₁₉) was higher than to other antigens and similar across the three cohorts evaluated (57%, 69% and 54%, respectively), corroborating the high immunogenicity of MSP-1₁₉. Levels of antibodies anti-MSP-1₁₉ were significantly different between 2nd and 3rd cohorts (0,001<p< 0,01). In relation to polymorphic domains, the levels of anti-10BP13 and anti-10BR07 antibodies were significantly different among all cohorts and there was a decreasing trend during the follow-up. Our next step is to associate the humoral immune response with the malarial clinical and epidemiological aspects as an attempt to validate those specific antibodies as potential exposure biomarkers. **Supported by::**CNPq

IM083 - Vitamin D deficient mice increase resistance to *L. amazonensis* infection

BRAGA, S.D.F.S.¹; PRATTI, J.E.S.; ROSSI-BERGMANN, B.; GUEDES, H.L.M.
UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO, RJ, BRASIL.
e-mail:herbert@biof.ufrj.br

Vitamin D (VitD) has several effects on immune system such as the participation in the resolution of infection with *M. tuberculosis* thought of induction antimicrobial peptides and the increase of susceptibility with *L. major* infection by induction of Th2 response and decrease Th1 response. *Leishmania amazonensis* is the main agent of anergic diffuse cutaneous *Leishmaniasis*. The infection in mice, a mixed of Th1 and Th2 response is present in susceptible and resistant animal. Our aim is to investigate the role of VitD on *L. amazonensis* infection. BALB/c and C57BL6 mice with one month were submitted on a diet without VitD for 45 days and mice with normal diet. Afterward, mice were infected with 5×10^5 (low dose infection) or 3×10^6 (high dose infection) of promastigotes on footpad. Lesion development was evaluated by paquimeter and the parasite load by limited-dilution. Using low dose infection, deficient BALB/c presented a delay in the lesion development and to decrease parasite load in day 60 of infection. Using high dose infection, no difference was observed on lesion development on BALB/c. However, in VitD deficient mice it still had a reduction of parasite load. C57BL6 infected using low-dose-infection demonstrated that deficient mice has a similar acute phase with the same lesion development until day 40. Nevertheless, VitD deficient mice start to resolute the infection after this date and in normal diet mice the lesion still growing until day 50. The lesion resolution after maximum lesion size in deficient mice is in 2 week and the resolution on normal diet mice is in 4 weeks demonstrating the increase of resolution on infection. The increase of resistance in deficient VitD mice (BALB/c and C57BL6) is involved with the increase of Th1 cytokines in infected footpad. All these finding together demonstrate the participation of VitD on disease development in *L. amazonensis* infection. **Supported by:** CNPq, FAPERJ

IM084 - SAG2A protein or soluble tachyzoite antigen (STAg) entrapped in cationic liposomes, but not with BSR4 molecule enhances mice protection against *Toxoplasma gondii* infection

SANTANA, S.S.¹; OLIVEIRA SILVA, D.A.¹; PAJUABA, A.C.¹; ANDRADE, L.A.¹; VAZ, L.D.¹; SILVA, N.M.¹; PICOLO, B.U.¹; SILVA, M.J.B.¹; MINEO, T.W.P.¹; PIROVANI, C.P.²; MINEO, J.R.¹; JÚNIOR, J.P.C.¹

1.UFU, UBERLANDIA, MG, BRASIL; 2.UESC, ILHEUS, BA, BRASIL.

e-mail:silastro@yahoo.com.br

INTRODUCTION: *Toxoplasma gondii* is an obligate intracellular parasite that infects all nucleated cells of animals endothermic and causes the disease known as toxoplasmosis. Several efforts have been undertaken with the aim of finding a vaccine against the parasite, although there is no effective preparation available nowadays. **OBJECTIVE:** The goal of present study was to evaluate the immune response of C57BL/6 mice to immunization with recombinant antigens (SAG2A and BSR4) and soluble antigen from *Toxoplasma gondii* (STAg) incorporated into cationic liposomes. **MATERIALS AND METHODS:** Cationic liposomes (Lip) consisting of phosphatidylethanolamide/phosphatidylcholine/cholesterol were prepared with proteins (rSAG2A, rBSR4, rSAG2A plus rBSR4 or STAg). Mice were immunized three times at 15 days with Lip-rSAG2A, Lip-rBSR4, Lip-rSAG2A plus rBSR4 or LipSTAg, Lip or PBS. **RESULTS:** The levels of total IgG were higher in the group immunized with the Lip-BSR4, Lip-SAG2A/BSR4. The highest levels of IgG1 and IgG2a were found in the group immunized with Lip-BSR4, although higher levels have been also observed in the group immunized with Lip-BSR4/SAG2A. After challenge, mice immunized with Lip-STAg, Lip and Lip-SAG2A had lower mortality in comparison to other groups. Furthermore, animals from Lip-STAg and Lip groups did not succumb during acute infection, suggesting a immunoregulatory role of cationic liposome adjuvant since early phase of *T. gondii* infection. Interestingly, animals from group immunized with Lip-BSR4 showed a evident reduction of mice protection. Additionally, animals immunized with Lip-SAG2A or Lip-SAG2A/BSR4 had lower parasitism scores in brain tissue sections, whereas it was observed that the Lip-BSR4 group showed the greatest parasitism score. **CONCLUSION:** SAG2A or STAg entrapped with liposomes display an immunostimulatory effect enhancing mice protection, while the vaccination with lip-BSR4 apparently produces a impairment of immune response against *T.gondii*. **Supported by:** CNPQ, CAPES e FAPEMIG

IM085 - The role of TLR 9 on *L. amazonensis* infection and in LaAg intranasal vaccine against murine *Leishmaniasis* on C57BL6 background.

PRATTI, J.E.S.¹; BRAGA, S.D.F.S.¹; ROSSI-BERGMANN, B.¹; GUEDES, H.L.M.
 UFRJ, RIO DE JANEIRO, RJ, BRASIL.
 e-mail:juliana_pratti@hotmail.com

TLR9 is involved in immunity against several diseases and it has been used as target to induce immune response against vaccines, major in nasal mucosa vaccines where it has a great expression. LaAg intranasal vaccine induces protection against *L. amazonensis* infection, the etiologic agent of diffuse cutaneous *Leishmaniasis*. Genomic DNA of *L. amazonensis* has capacity to activate TLR9. LaAg vaccine is composed by the whole antigens of *L. amazonensis* promastigotes, including DNA. In this work, we investigate the role of TLR9 on *L. amazonensis* infection and if DNA present on LaAg participate as adjuvant in intranasal vaccine. C57BL6 mice (WT) and C57BL6 deficient in TLR9 (TLR9 ^{-/-}) were infected with 5 x 10⁵ promastigotes in footpad. The lesion development was evaluated by paquimeter and the parasite load by limited-dilution. WT and TLR9 ^{-/-}, male and female, displayed the same profile of lesion development. However, TLR9 ^{-/-} shown an increase in lesion after day 45. WT started the resolution of the lesion after day 45 and deficient mice after day 75. In the chronic phase, parasite load of TLR9 ^{-/-} was higher than WT, suggesting the importance of TLR9 to parasite control. Besides, LaAg vaccinated WT shown resistance since the beginning of infection, with smaller lesion meanwhile day 32. LaAg vaccinated TLR9 ^{-/-} did not induce protection in the lesion development, however, accelerated the process of lesion control, which was not observed in the no-vaccinated TLR9 ^{-/-} mice. Parasite load after lesion resolution in chronic phase demonstrated the reduction of parasite load on vaccinated mice WT and TLR9 ^{-/-} compared to controls no vaccinated. These results indicate the role of TLR9 on immunity against *L. amazonensis* and to suggest the use of TLR9 agonist (CPG) as adjuvants for intranasal vaccines against *L. amazonensis*.

IM086 - Disruption of Nod2 results in impaired Th1 response during experimental *N. caninum* infection

FERREIRA, M.D.^{*1}; FONSECA, D.M.¹; LIMA-JUNIOR, D.S.¹; MOTA, C.M.²; SILVA, M.V.²;
 MACÊDO JÚNIOR, A.G.²; ZAMBONI, D.S.¹; SILVA, J.S.¹; MINEO, T.W.P.²
 1.FMRP/USP, RIBEIRÃO PRETO, SP, BRASIL; 2.UFU, UBERLÂNDIA, MG, BRASIL.
 e-mail:tiagomineo@icbim.ufu.br

Neospora caninum is an intracellular parasite that causes major economic impact on cattle raising farms, and infects a wide range of warm-blooded hosts worldwide. Cytosolic Nod-like receptors (NLRs) represent an important family of microbial sensor proteins that have been identified as key host molecules in innate immune recognition and the inflammatory response to microbial products. However, the role of NLR in the induction/regulation of adaptive immune response against intracellular protozoan infection is unclear. In this study, we evaluated the role of NLRs in host response to *N. caninum* infection. For that purpose, Nod2^{-/-} and WT mice were infected with *N. caninum* tachyzoites to evaluation of acute phase parasitism, inflammatory cell migration and cytokine production. Nod2^{-/-} mice exhibited higher parasite burden in the peritoneal exudate and lungs compared to WT mice. Inflammatory cell migration was impaired in both compartments, as Nod2^{-/-} mice presented decreased migration of dendritic cells, B and T lymphocytes to the peritoneal cavity. Mononuclear cell infiltrates were also significantly reduced in the lungs of Nod2^{-/-} mice, as compared to WT. In parallel, we observed that dendritic cells and macrophages from Nod2^{-/-} mice presented lower MHCII expression, fact that was associated to lower IFN- γ production in spleen cell antigenic recall, and in lung and brain homogenates. However, no difference in IL-10 production was observed. Surprisingly, Nod2^{-/-} mice demonstrated increased survival if compared to WT mice. Based on the results herein presented, we proposed that Nod2 has an important role in TH1 programming during initial immune responses to *N. caninum*. This additional activation of TH1 response appears to be important to parasite clearance, but could contribute to pathogenesis and mortality during *N. caninum* infection. **Supported by:** CAPES, CNPq, FAPESP, FAPEMIG, FINEP

IM087 - Production, characterization and applications for *Toxoplasma gondii*-specific polyclonal chicken egg yolk immunoglobulins

JÚNIOR, Á.F.^{*1}; SANTIAGO, F.M.¹; SILVA, M.V.¹; FERREIRA, F.B.¹; MACÊDO JÚNIOR, A.G.¹; MOTA, C.M.¹; FARIA, M.S.²; SILVA FILHO, H.H.¹; OLIVEIRA SILVA, D.A.¹; JÚNIOR, J.P.C.¹; MINEO, J.R.¹; MINEO, T.W.P.¹

1. UFU, UBERLÂNDIA, MG, BRASIL; 2. UNIPAC, UBERLÂNDIA, MG, BRASIL.

e-mail: flaviabatistaf@yahoo.com.br

Toxoplasma gondii may cause abortions, ocular and neurological disorders in warm-blood hosts. Immunized mammals are a wide source of hyperimmune sera used in different approaches, including diagnosis and the study of host-parasite interactions. Unfortunately, mammalian antibodies present limitations for its production, such as the necessity for animal bleeding, low yield, interference with rheumatoid factor, complement activation and affinity to Fc mammalian receptors. IgY antibodies avoid those limitations; therefore they could be an alternative to be applied in *T. gondii* model. In this study we immunized hens with soluble tachyzoite antigens of *T. gondii* (STAg) and purified egg yolk antibodies (IgY) by an inexpensive and simple method, with high yield and purity degree. IgY anti-STAg antibodies presented high avidity and were able to recognize a broad range of parasite antigens, although some marked differences were observed in reactivity profile between antibodies produced in immunized hens and mice. Interestingly, IgY antibodies against *Neospora caninum* and *Eimeria spp.* did not react to STAg. We also show that IgY antibodies were suitable to detect *T. gondii* forms in paraffin-embedded sections and culture cell monolayers. We conclude that, due to its cost-effectiveness, high production yield and varied range of possible applications, polyclonal IgY antibodies are useful tools for studies involving *T. gondii*. **Supported by:** CAPES, CNPq, FAPEMIG, FINEP

IM088 - Experimental in vivo infection by low virulent *Trypanosoma cruzi* strain is rapidly controlled by IFN- γ

RODRIGUES, A.A.^{*}; LUISI, C.M.J.; MACHADO, F.C.; DOS SANTOS, M.A.; TEIXEIRA, T.L.; SILVA, C.V.

UFU, UBERLÂNDIA, MG, BRASIL.

e-mail: juniorcml@yahoo.com.br

Background: *T. cruzi* strains have been divided into six discrete typing units (DTUs) according to their genetic background. These groups are designated *T. cruzi* I to VI. In this context, amastigotes from G strain (*T. cruzi* I) are highly infective *in vitro* and show no parasitemia *in vivo*. Here we aimed to understand why amastigotes from G strain are highly infective *in vitro* and do not contribute for a patent *in vivo* infection.

Methodology/Principal Findings: Our *in vitro* studies demonstrated the first evidence that IFN- γ would be associated to the low virulence of G strain *in vivo*. After intraperitoneal amastigotes inoculation in wild-type and knockout mice for TNF- α , Nod2, Myd88, iNOS, IL-12p40, IL-18, CD4, CD8 and IFN- γ we found that the latter is crucial for controlling infection by G strain amastigotes. *In vivo* Treatment with anti-Asialo GM1 antibody is under development in order to demonstrate if NK cells are the primary source of IFN- γ .

Conclusions/Significance: Our results showed that amastigotes from G strain are highly infective *in vitro* but did not contribute for a patent infection *in vivo* due to its susceptibility to IFN- γ production by host immune cells early in infection. These data are useful to understand the mechanisms underlying the contrasting behavior of different *T. cruzi* groups for *in vitro* and *in vivo* infection. **Supported by:** FAPEMIG, CAPES, CNPq

IM089 - Assessment of the immunogenicity mechanism induced by LACK DNA intranasal vaccine protective against murine *Leishmaniasis*

SOUZA, B.L.S.C.^{*}; BEZERRA, I.P.S.; MELLO, M.F.; GUEDES, H.L.M.; ROSSI-BERGMANN, B.
UFRJ - IBCCF, RIO DE JANEIRO, RJ, BRASIL.
e-mail:blscosta@yahoo.com.br

We have previously shown the effectiveness of using LACK DNA intranasal vaccine against cutaneous *Leishmaniasis* in BALB/c mice (Pinto, E.F. et al., 2004) and visceral *Leishmaniasis* in both BALB/c mice (Gomes, D.C.O. et al., 2007) and hamsters (Gomes, D.C.O. et al., 2011). In this work, we proposed to evaluate the immunogenicity induced by LACK DNA intranasal vaccine. For this, BALB/c mice were immunized twice by the intranasal route with 30 µg of LACK DNA. Thirty-six hours after boost immunization the mRNA expression of transcripts factors and cytokines related to Th1, Th2 and Treg responses in the cervical lymph nodes were quantified by real time PCR. The LACK DNA intranasal vaccine induced an increase in the mRNA of transcription factor Tbet, a reduction in the mRNA of FoxP3 and did not induce changes in the transcripts of GATA-3. The quantification of cytokine mRNA showed an increment in the transcripts of IL-12 and a reduction in the transcripts of IL-4. No differences were found in the transcripts of IL-10 and TGF-β. Seventy-two hours after boost immunization, the cervical lymph nodes were collected and assessed for their population of CD4+FoxP3+ cells. Despite the immunization by mucosal route, the protection induced by this vaccine seemed to be independent of FoxP3+ regulatory T cells. These data have shown that intranasal vaccination with LACK DNA leads to a Th1 response that can be associated with protection in challenges with cutaneous and visceral infection. **Supported by:** CNPq/FAPERJ
Supported by: CNPq - FAPERJ

IM090 - ACTIVATION AND INFECTION OF NEUTROPHILS IS *LEISHMANIA* SPECIES DEPENDENT

BATISTA, M.A.^{*}; DE CASTRO, M.R.R.; FERREIRA, Z.G.; LEITE, J.C.; AFONSO, L.C.C.;
SILVEIRA-LEMOS, D.
UFOP, OURO PRETO, MG, BRASIL.
e-mail:azevedomauricio@yahoo.com.br

Neutrophils are key components of the inflammatory response and are one of those primary immune cells that interact with *Leishmania*. Neutrophils are recruited within hours to the site of parasite inoculation altering the expression of cell surface integrins and Toll-like receptors (TLRs). The development of pathogen specific immune response may depend on the activation and migration of the neutrophils thereby determining the fate of host and *Leishmania* interaction. In our study, we evaluated the infectivity of *L. amazonensis*, *L. brasiliensis* and *L. major* in neutrophils harvested from bone marrow of 8-12 weeks old C57BL/6 mice and then purified by Percoll gradient centrifugation. Bone marrow cell suspended in Hank's balanced salt solution were laid on top of a three-layer Percoll gradient by 3 mL each of 1.095, 1.085 and 1.070 g/mL Percoll solution. After centrifugation the lowest band and the upper part of the 1.095 g/mL layer were collected as the neutrophils fraction. We also analyzed the expression of CD49d, CD62L, TLR2 and TLR4 by flow cytometry. Purified neutrophils were infected with the stationary phase promastigotes labeled with carboxyfluorescein diacetate succinimidyl ester (CFSE) in a ratio of 5:1 at 37°C for 3hrs. Our results showed that infectivity of *Leishmania* parasite is species dependent. We observed that *L. brasiliensis* had higher infectivity than *L. amazonensis* and *L. major* ($p < 0,01$). In addition, results from CD49 and TLR2 analysis showed higher expression in all groups of infected neutrophils compared to uninfected population ($p < 0,005$ and $p < 0,001$, respectively). However, the expression of TLR2 was only higher in infected neutrophils with *L. brasiliensis* when compared to other two species ($p < 0,001$). Apparently, there was no change of CD62L and TLR4 expression in control and infected population. Thus, our results demonstrated that infectivity depends of the parasites species may further determine their survivability within the host.

Supported by: FAPEMIG/CAPES/CNPq/UFOP

IM091 - Intracellular replication-deficient *Leishmania donovani* induces cross-protective immunity against cutaneous *Leishmaniasis* caused by *Leishmania (Viannia) braziliensis*

MENEZES-SOUZA, D.¹; FIUZA, J.A.¹; NAKDASI, H.²; SELVAPANDYAN, A.²; PASSOS, L.A.¹; GAZZINELLI-GUIMARÃES, P.H.¹; GAZZINELLI-GUIMARÃES, A.C.¹; BARTHOLOMEU, D.C.¹; FUJIWARA, R.T.¹; BUENO, L.L.¹

1.UFMG, BELO HORIZONTE, MG, BRASIL; 2.FDA-DHHS, BETHESDA, ESTADOS UNIDOS.

e-mail:daniel.ufop@gmail.com

A vaccine for *Leishmaniasis* has been a goal for a century, but there are still no effective vaccines available for American Cutaneous *Leishmaniasis* caused by *Leishmania braziliensis*. This study addresses whether a live attenuated centrin gene-deleted *L. donovani* (*LdCen1*^{-/-}) parasite can persist and be both safe and protective in animals. *LdCen1*^{-/-} has a defect in amastigote replication both *in vitro* and *ex vivo* in human macrophages. In this study, hamsters (*Mesocricetus auratus*) were immunized with 1x10⁷ promastigotes of *LdCen1*^{-/-} intraperitoneally. The animals were divided into three groups: Group 1 – Control; Group 2 – Immunized with a dose of *LdCen1*^{-/-}; Group 3 - Immunized with three doses of *LdCen1*^{-/-} at 10 days intervals. After a period of 30 days, the animals were challenged with 1x10⁷ *Leishmania braziliensis* (M2903) promastigotes in the dorsal skin region. The first analysis was done 30 days after the first immunization where six animals from each group were euthanized and evaluated cytokine expression in the splenocytes. We observed an increase (p<0.05) in proinflammatory cytokines IFN- γ , TNF- α and IL-12 in animals immunized with *LdCen1*^{-/-} when compared to the control group, and an increase in the group with a dose when compared to the group that received three doses of *LdCen1*^{-/-}. Preliminary data suggest a protective profile in immunized animals with *LdCen1*^{-/-}. The animals will be euthanized 8 weeks after challenge and evaluated the expression of cytokines and iNOS in the spleen, skin and lymph nodes, as well as, NO and the levels of total IgG anti-*Leishmania* in serum. Will be evaluated also the parasite load and the rate of proliferation of splenocytes. The data from this study can guide the development of a safe and effective vaccine candidate against cutaneous *Leishmaniasis*. **Supported by:**FAPEMIG/CAPES/UFMG

IM092 - Immunological and histopathological analysis of naturally infected dogs with *Leishmania (Leishmania) chagasi* treated with liposome-encapsulated meglumine antimoniate associated to allopurinol

AMORIM, I.F.G.¹; DA SILVA, S.M.; FIGUEIREDO, M.M.; CASTRO, R.S.; PINTO, A.J.W.; MADEIRA, I.M.V.; CARMO, D.D.; BARBOSA, V.S.; SOUZA, C.C.; PINHEIRO, L.J.; FREZARD, F.; MICHALICK, M.S.M.; TAFURI, W.L. UFMG, BELO HORIZONTE, MG, BRASIL. e-mail:izabelafg@yahoo.com.br

This study aimed to evaluate the efficacy of leishmanicidal liposome-encapsulated drug in combination with leishmaniostatic agent, since this represents a good strategy in the treatment of canine visceral *Leishmaniasis*. Mongrel dogs naturally infected with *L. chagasi* (n=46) were treated with six doses of liposomal formulation of meglumine antimoniate (LMA) (6.5mg Sb/kg of b.w./dose) given at 4-day intervals, plus allopurinol (Allo) (20mg/kg/24 h per os) for 140 days. Comparison was made with groups treated with LMA, Allo, empty liposomes (Empl) plus Allo, Empl and saline. Dogs remained without treatment from day 140 to 200 after the start of treatment. Blood samples were collected before the beginning of treatment for the immunophenotypic evaluation. For this, density gradient separation was used to enrich for peripheral blood mononuclear cells (PBMC) and flow cytometry was carried out on PBMC cells using mouse anti-human monoclonal antibodies anti-CD14, anti-TLR2 and mouse anti-canine anti-CD11b, anti-CD4 and anti-CD8. At day 201, another blood collection and skin, liver and spleen samples were collected for histological and parasitological examination. The group Allo, with emphasis LMA plus Allo, showed a clinical improvement and significant reduction of tissue parasite load. However, no frequency modulation of lymphocyte population CD4/CD8 and expression of CD11b (CR3) and TLR-2 in CD14+ monocytes were observed during the treatment. Dogs treated with Allo, considering the various associations, and dogs treated with LMA, when compared to Empl, showed lower numbers of intralobular hepatic granulomas than other groups. In spleens the treatment was not able to reduce the T-dependent area depletion of the white pulp. Thus, the animals under the treatments, despite clinical and parasitological improvement, should be accompanied by a longer term, since histological changes such as degeneration and fibrosis were found mainly in livers of LMA and LMA plus Allo groups. **Supported by:**CAPES; FAPEMIG; CNPq

IM093 - Immunogenicity profile induced by Live Attenuated *Leishmania donovani* Centrin Knock out parasites vaccination in dogs

FIUZA, J.A.¹; MENEZES-SOUZA, D.¹; NAKDASI, H.²; SELVAPANDYAN, A.²; DUNCAN, R.²; DEY, R.²; RICCI, N.¹; BUENO, L.L.¹; BARTHOLOMEU, D.C.¹; CORRÊA-OLIVEIRA, R.¹; SANTIAGO, H.C.¹; FUJIWARA, R.T.¹

1.UFMG, BELO HORIZONTE, MG, BRASIL; 2.FDS-DHHS, BETHESDA, ESTADOS UNIDOS.
e-mail:daniel.ufop@gmail.com

Canine visceral *Leishmaniasis* (CVL) is a major veterinary and public health problem in large parts of Southern Europe, the Middle East and South America. Control of animal reservoirs is still based on elimination of seropositive dogs in endemic areas. Treatment of infected dogs is not considered appropriate as this can lead to emergence of resistance since the same drugs are used to treat human infections as well. Therefore vaccination against CVL remains the best alternative in control of the animal reservoirs. In this study, we present data on the immunogenicity profile of a live attenuated parasite *LdCen*^{-/-} in a canine infection model. The immunogenicity of the *LdCen*^{-/-} parasites was evaluated by antibody secretion, production of intracytoplasmic cytokines in plasma and supernatant of cultures of PBMCs, activation and proliferation of T cells, and other phenotypic markers. Vaccination with *LdCen*^{-/-} resulted in high immunogenicity as revealed by the higher IgG, IgG1, and IgG2. Furthermore, these animals presented higher lymphoproliferative response, CD4⁺ and CD8⁺ T cell activation, IFN- γ production by T CD8⁺ T cells, TNF- α and IL-12/IL-23p40 secretion. These results contribute to the understating of immunogenicity elicited by using live attenuated *L. donovani* parasites and to the development of effective vaccines against visceral *Leishmaniasis*. **Supported by::** FIOCRUZ/UFMG/CNPq/FAPEMIG/NIH/FDA

IM094 - Predictive Value of Transforming Growth Factor-beta1 in Chagas Disease

WAGHABI, M.C.¹; SARAIVA, R.M.²; ARAUJO-JORGE, T.C.¹

1.IOC, RIO DE JANEIRO, RJ, BRASIL; 2.IPEC, RIO DE JANEIRO, RJ, BRASIL.
e-mail:mwaghabi@hotmail.com

Introduction: Up to 30% of patients with Chagas disease progress to the cardiac phase, which has high mortality. The mechanisms underlying this progression are still poorly understood. Transforming growth factor-beta1 (TGF- β 1) may be implicated in the development of Chagas heart disease and our group has already reported high TGF- β 1 values in the serum of patients with Chagas heart disease. Materials and Methods: We retrospectively analyzed the outcome of patients whose TGF- β 1 serum values were determined in a previous publication of our group. Results: Sixty-eight patients (49,10 years old) were followed for a mean of 10.0 \pm 3.6 years. There were a total of 22 patients at the indeterminate phase (32.3%), 20 patients at stage A (29.4%), 13 at stage B1 (19.1%), 8 at stage B2 (11.8%) and 5 at stage C (7.4%). All-cause mortality occurred in eleven patients. Left ventricular (LV) end-systolic diameter was an independent predictor of all-cause mortality (HR 2.8; CI 95% 1.5 to 5.0; p=0.0007), while TGF- β 1 was not. The optimal cutoff for LV end-systolic diameter to identify patients who died was 4.1 cm (area under the curve [AUC]=0.77, p=0.002, sensitivity 73%, and specificity 80%). However, in patients with normal to mild LV systolic dysfunction, TGF- β 1 was higher among patient who died than in survivors (49.5 \pm 15.5 ng/ml vs. 17.6 \pm 3.1 ng/ml, p=0.003). TGF- β 1 (HR 1.02; CI 95% 1.0 to 1.04; p=0.01) and LV ejection fraction (HR 0.91; CI 95% 0.83 to 0.99; p=0.02) were independent predictors of all-cause mortality. The optimal cutoff for TGF- β 1 to identify patients who died among those with normal or mild LV systolic dysfunction was 12.9 ng/ml (AUC=0.82, p=0.003, sensitivity 100%, and specificity 57%) and for LV ejection fraction was 53% (AUC=0.74, p=0.009, sensitivity 50%, and specificity 90%). Main Conclusions: TGF- β 1 was an independent predictor of all-cause mortality only in the group of patients with normal to mild LV systolic dysfunction. **Supported by::** cnpq

IM095 - CD8+ T-CELLS EXPRESSING INTERFERON GAMMA OR PERFORIN PLAY ANTAGONISTIC ROLES IN HEART INJURY IN EXPERIMENTAL *TRYPANOSOMA CRUZI*-ELICITED CARDIOMYOPATHY

SILVERIO, J.C.¹; PEREIRA, I.R.¹; CIPITELLI, M.C.¹; VINAGRE, N.F.¹; RODRIGUES, M.M.²; GAZZINELLI, R.T.³; LANNES-VIEIRA, J.¹

1.FIOCRUZ, RIO DE JANEIRO, RJ, BRASIL; 2.UNIFESP, SAO PAULO, SP, BRASIL; 3.UFMG, BELO HORIZONTE, MG, BRASIL. e-mail:lannes@ioc.fiocruz.br

In Chagas disease, CD8+ T-cells are critical for the control of *Trypanosoma cruzi* during acute infection. Conversely, CD8+ T-cell accumulation in the myocardium during chronic infection may cause tissue injury leading to chronic chagasic cardiomyopathy (CCC). Here we explored the role of CD8+ T-cells in *T. cruzi*-elicited heart injury in C57BL/6 mice infected with the Colombian strain. Cardiomyocyte lesion evaluated by creatine kinase-MB isoenzyme activity levels in the serum and electrical abnormalities revealed by electrocardiogram were not associated with the intensity of heart parasitism and myocarditis in the chronic infection. Further, there was no association between heart injury and systemic anti-*T. cruzi* CD8+ T-cell capacity to produce interferon-gamma (IFN γ) and to perform specific cytotoxicity. Heart injury, however, paralleled accumulation of anti-*T. cruzi* cells in the cardiac tissue. In *T. cruzi* infection, most of the CD8+ T-cells segregated into IFN γ + perforin (Pfn)neg or IFN γ negPfn+ cell populations. Colonization of the cardiac tissue by anti-*T. cruzi* CD8+Pfn+ cells paralleled the worsening of CCC. The adoptive cell transfer to *T. cruzi*-infected cd8-/- recipients showed that the CD8+ cells from infected ifn γ -/-pfn+/+ donors migrate towards the cardiac tissue to a greater extent and caused a more severe cardiomyocyte lesion than CD8+ cells from ifn γ +/-pfn-/- donors. Moreover, the reconstitution of naïve cd8-/- mice with CD8+ cells from naïve ifn γ +/-pfn-/- donors ameliorated *T. cruzi*-elicited heart injury paralleled IFN γ + cells accumulation, whereas reconstitution with CD8+ cells from naïve ifn γ -/-pfn+/+ donors led to an aggravation of the cardiomyocyte lesion, which was associated with the accumulation of Pfn+ cells in the cardiac tissue. Our data support a possible antagonist effect of CD8+Pfn+ and CD8+IFN γ + cells during CCC. CD8+IFN γ + cells may exert a beneficial role, whereas CD8+Pfn+ may play a detrimental role in *T. cruzi*-elicited heart injury. **Supported by:** CNPQ, FAPERJ

IM096 - Inducible nitric oxide synthase in heart tissue and nitric oxide in serum of *Trypanosoma cruzi*-infected rhesus monkeys: association with heart injury

CARVALHO, C.M.E.¹; LANNES-VIEIRA, J.²

1.UCDB, CAMPO GRANDE, MS, BRASIL; 2.FIOCRUZ, RIO DE JANEIRO, RJ, BRASIL. e-mail:lannes@ioc.fiocruz.br

The factors contributing to chronic Chagas' heart disease remain unknown. High nitric oxide (NO) levels have been shown to be associated with cardiomyopathy severity in patients. NO produced via inducible nitric oxide synthase (iNOS/NOS2) is proposed to play a role in *Trypanosoma cruzi* control. However, the participation of iNOS/NOS2 and NO in *T. cruzi* control and heart injury has been questioned. Here we explored the participation of iNOS/NOS2-derived NO in heart injury in *T. cruzi* infection. Rhesus monkeys and C57BL/6 and Nos2-/- mice were infected with the Colombian *T. cruzi* strain. Parasite DNA was detected by polymerase chain reaction, *T. cruzi* antigens and iNOS/NOS2+ cells were immunohistochemically detected in heart sections and NO levels in serum were determined by Griess reagent. Heart injury was assessed by electrocardiogram, echocardiogram, creatine kinase heart isoenzyme (CK-MB) activity levels in serum and connexin 43 (Cx43) expression in the cardiac tissue. Chronically infected monkeys presented conduction abnormalities, cardiac inflammation and fibrosis, which resembled the spectrum of human chronic chagasic cardiomyopathy (CCC). Chronic myocarditis was associated with parasite persistence. Cx43 loss and increased CK-MB activity levels were primarily correlated with iNOS/NOS2+ cells infiltrating the cardiac tissue and NO levels in serum. Studies in Nos2-/- mice reinforced that the iNOS/NOS2-NO pathway plays a pivotal role in *T. cruzi*-elicited cardiomyocyte injury and in conduction abnormalities that were associated with Cx43 loss in the cardiac tissue. *T. cruzi*-infected rhesus monkeys reproduce features of CCC. Moreover, our data support that in *T. cruzi* infection persistent parasite-triggered iNOS/NOS2 in the cardiac tissue and NO overproduction might contribute to CCC severity, mainly disturbing of the molecular pathway involved in electrical synchrony. These findings open a new avenue for therapeutic tools in Chagas' heart disease. **Supported by:** CNPQ, FAPERJ

IM097 - Oral infection of mice with *Neospora caninum* bradyzoites induced by in vitro stage interconversion

KNYCHALA, L.M.*; VIEIRA, P.C.A.; SILVA, M.V.; MACÊDO JÚNIOR, A.G.; FERREIRA, F.B.; MOTA, C.M.; OLIVEIRA, A.C.M.; BARROS, P.S.C.; SANTIAGO, F.M.; MINEO, J.R.; MINEO, T.W.P.

UFU, UBERLÂNDIA, MG, BRASIL.

e-mail:tiagomineo@icbim.ufu.br

Neospora caninum is a an intracellular protozoan parasite with worldwide distribution, which has been associated to abortions since the early 1990's, and is estimated to cause major economic impact in cattle raising farms. Additionally, it has been described to infect a wide range of warm-blooded hosts worldwide. The mechanisms underlying host resistance against this pathogen remains unclear and has been the subject of study by our group. Experimental models that assess host responses against the parasite are usually based on parenteral infections. In that sense, this work aimed to establish an infection protocol using oral inoculation of *N. caninum* tissue cysts, simulating natural conditions. For that purpose, stage interconversion of tachyzoites to bradyzoites was induced in vitro, through continuous selective pressure by a nitric oxide donor compound (Sodium Nitroprusside - SNP). Stage interconversion was confirmed by RT-PCR, where it could be observed that parasites submitted to culture in the presence of SNP decreased mRNA expression of tachyzoite related SAG1 gene, opposed to an increment of bradyzoite specific SAG4 gene expression. Additionally, cell cultures containing tissue cyst like forms were positively stained with parasite specific antibodies and Dolichos biflorus agglutinin, a lectin with affinity to carbohydrates contained in tissue cyst walls. Different groups of mice were inoculated orally with in vitro induced tissue cysts, tachyzoites, tachyzoites after pre-blockage of gastric pH, along with intraperitoneal controls. As observed by IgG seroconversion, mice infected with tissue cysts produced similar responses to animals infected by intraperitoneal tachyzoites. More experiments are underway in order to confirm the success of oral infection protocol using in vitro induced bradyzoites. If confirmed, this protocol will be a promising tool to be applied in studies involving host-parasite relationship of *N. caninum* infection. **Supported by:** CAPES, CNPq, FAPEMIG, FINEP

IM098 - Reduced phagocytic capacity and upregulation of major phagocytosis-related cell surface molecules induces an inflammatory cytokine profile in monocytes from patients with tegumentary *Leishmaniasis*

SILVA, M.F.C.E.*¹; GOMES, L.I.¹; SILVA, A.O.¹; SILVA, R.R.¹; GONTIJO, R.C.¹; FREIRE, J.M.¹; QUARESMA, P.F.¹; ANDRADE, D.P.¹; MELO JUNIOR, O.A.O.¹; MELO, M.N.²; MARTINS-FILHO, O.A.¹; GONTIJO, C.M.F.¹; MAGALHAES, V.P.¹; CARVALHO, A.T.³

1.CPQRR, BELO HORIZONTE, MG, BRASIL; 2.UFMG, BELO HORIZONTE, MG, BRASIL;

3.CPQRR/FDA-NIH, BELO HORIZONTE, MG, BRASIL.

e-mail:mfernandes@cpqrr.fiocruz.br

The distinct ability of phagocytes to present antigens, produce cytokines and provide co-stimulatory signals may contribute to the severity of the outcome of tegumentary *Leishmaniasis*. In this work, we evaluate the phenotypic features of phagocytes along with the cytokine signature of circulating T-cells from individuals presenting positive Montenegro skin test (MST+, n=09) and patients with tegumentary *Leishmaniasis* (TL, n=18) as compared to control group (CT, n=09). These individuals are indigenous resident in Xakriabá community, northern of Minas Gerais State, Brazil, where the prevalence of the disease is around 9.0%. Our data demonstrated that monocytes from TL patients displayed an impaired ability to phagocytize *Leishmania braziliensis*. This impaired phagocytic capability did not reflect on the expression of the MHC-I, CD23, CD80, TLR-2, TLR-4 and TNF-alpha molecules that are upregulated in TL as compared to MST. The lymphocyte-derived cytokine signature demonstrated that a down-regulated synthesis of IL-10 or IL-4 is associated with lower *L. braziliensis* phagocytic index and lower nitric oxide levels produced by monocytes. The analysis of skin biopsies in the lesions from TL evidenced, in patients with recent injuries (less than 90 days), increased expression of IL-10. Moreover, it was observed that patients with late lesions (more than 90 days) show increased expression of TNF-alpha and IL-12. Our findings demonstrate that a differential phenotypic features found in TL may be triggering distinct immune response related to high morbidity in tegumentary *Leishmaniasis*. **Supported by:** CPqRR-FIOCRUZ, FAPEMIG, CAPES, CNPq/Brazil, European Community and CBER-FDA/USA