IM.01 – IDENTIFICATION OF NOVEL *Leishmania infantum chagasi* ANTIGENS THAT HELP THE SERODIAGNOSIS IN VISCERAL LEISHMANIASIS

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Visceral leishmaniasis (VL) is a chronic and potentially fatal disease caused by the *Leishmania* genus. Rio Grande do Norte is endemic for VL, where the etiological agent is *Leishmania infantum chagasi* (*Lic*). Treatment requires toxic drugs and diagnosis depends of parasitological confirmation on bone marrow. There is neither vaccine nor gold standard for immunodiagnosis, therefore, purified antigens are essential to be used like infection marker. Thus, our aim was to test the effectiveness of four *Lic* amastigote antigens in the detection of specific antibodies in humans and dogs resident in Rio Grande do Norte. The antigens were expressed, purified, tested by ELISA and Western blot, and compared to *r*K39 (Burns, 1993) and soluble *Leishmania* antigens (SLA). Antigens termed 314, 319, 503 and 648 had sensitivity and specificity, respectively: 95.24%-19.35%, 90.48%-51.61%, 95.24%-22.58%, 28.57%-90.32% when compared to SLA, and 90.48%-11.54%, 80.95%-30.77%, 95.24%-15.38%, 33.33%-84.62% when compared to *r*K39. With human serum, the antigens showed sensitivity and specificity, respectively, 76.14%-78.52% (314), 90.91%-57.78% (319), 69.32%-71.11% (503), 95.40%-58.54% (648) when compared to *r*K39, and 46.56%-75.76% (314), 62.96%-45.45% (319), 46.56%-63.64% (503), 69.68-39.39% (648) when compared to SLA. The relationship between the presence of infected dogs in the neighborhood and human infection shows that, the diagnosis with 503, 64.44% of asymptomatic dogs that had positive serology were related with positive humans too. This data illustrate the necessity to monitor the dogs for prevent the parasite spread. Western blot with human serum showed a better recognition of 648 antigen, whereas with the canine serum 503 stood out. The Western blot showed to be a more sensitive test for the diagnosis, and 648 and 503 the best antigens for diagnosis of human and canine infection, respectively.
Supported by CNPQ, CAPES, NIH.

IM.02 – IMMUNOLOGICAL PROFILE INDUCED BY *LEISHMANIA MAJOR* INFECTION IN A MODEL OF PARAFFIN IMPLANTATION

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A model of monocytic inflammation using a subcutaneous implantation of paraffin tablets is able to produce a chronic inflammatory reaction. When paraffin implantation is coupled with *Leishmania major* infection, both genetically susceptible (BALB/c) and resistant (C57BL/6) strains of mice have large amounts of parasites associated with inflammatory macrophages at 21 days post infection. At the present study we have determined cytokines produced in inflammatory capsule. This model was carried out by implantation of paraffin tablets under the dorsal skin of Balb/c or C57BL/6 mice. Mice were then infected with *L. major* and sacrificed 21 days after infection. Inflammatory capsule was collected for histopathology and cytokines measures by RT PCR. Cytokines IL-12, TNF-alfa and IFN-gamma associated to resistant phenotype and IL-4 and IL-10 associated to susceptive phenotype to *L. major* infection were determined. Otherwise, chemokines related to monocyte-macrophage and lymphocyte recruitments were also investigated. RT-PCR analysis has shown that BALB/c mice showed strong IL-4 and IL-10 mRNA expression than controls with very little expression of IFN-gamma. In contrast, both IFN-γ and IL-10 mRNA was found in higher levels in C57BL/6 animals. Moreover, in C57BL/6 mice the expression of chemokines mRNA of CCL3/MIP-1 alpha was more highly expressed than CCL2/MCP-1, suggesting differential and important role in recruitment and activation of distinct immune cells which can define a permissive profile at the site of *L. major* infection. We conclude that the Th1 immune response C57BL/6 did not change to a Th2 response. Supported by CAPES

IM.03 – EVIDENCE FOR ENDOGENOUS INTERLEUKIN-10 DURING NOCICEPTION IN THE EAR MODEL OF *L. MAJOR* INFECTION

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Cutaneous leishmaniasis (CL) has been experimentally reproduced by inoculating low dose parasite loads into an intradermal site (ear). *L. major* infected BALB/c mice presented severe lesions and Th2 response. IL-4-/- C57BL/6 and BALB/c mice showed healing lesions and Th1 response. Th1 and Th2 cells secrete pro- and anti-inflammatory cytokines, respectively. Classical description of syndromes produced by CL does not include pain complaints, and some studies reported painless lesions. Using the ear model, we investigated variations in nociception along 12 weeks post infection and its relationship to IL-10 production in BALB/c susceptible mice and in IL-4 -/-C57BL/6 and BALB/c resistant mice. The infection induced hyponociception in BALB/c after wk 9, followed by a decrease of IL-10tissue levels. C57BL/6 showed a short-lived hypernociception in wk 2, followed by an IL-10 local increase. IL-4 -/- BALB/c mice showed a sustained hypernociception from wk 1 associated to an IL-10 increase at wk 12. Recently, we have showed that cytokines such as IL-6, TNF-α and KC contribute to afferent nerve sensibilization. However, exogenous IL-10 has been demonstrated to prevent the development of dynorphin-induced allodynia, presumably by inhibiting pro-inflammatory cytokines. In the other hand, our results suggest that endogenous IL-10 may be involved in nociception induction in this infection model. In support to our observations, recent data indicate the involvement of endogenous IL-10 in nociception. Regardless the mechanisms, these results suggest that further studies on IL-10 and other cytokines in nociception are needed. Financial Support: FAPEMIG(CBB-1048/05), CAPES and CNPq.

IM.04 – EFECTIVENESS OF IMMUNOENZYMATIC METHOD FOR RESEARCH INTO PROTOZOAN CYSTS IN HIV PATIENTS

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Diarrheal diseases associated with parasitic infections are highly prevalent in HIV patients. Among the protozoa, *Cryptosporidium* and *Giardia duodenalis* are major etiological agents of diarrheal infections. The enzyme immunoassays have replaced the microscopic routine procedures in hospitals and health services. These tests are sensitive and specific and allow analysis of numerous samples. The aim of this study was to evaluate the efficiency of the immune ELISA method to detect antigens of *Giardia duodenalis* and *Cryptosporidium* spp in fecal specimens of HIV patients in comparison with conventional techniques of microscopy, to demonstrate the importance of employment in a routine laboratory method more sensitivity from a single stool sample. Methods were employed to parasitological flotation, Ziehl-Neelsen modified and enzyme immunoassays for *Giardia* and *Cyptosporidium* Test II. The samples were collected in three alternate days and stored in 10% formalin and sent to the Laboratory of Parasitology /DBS/UEM where they were examined. We collected 132 samples of faeces. 45 (34%) were positive for some intestinal parasite. Through the parasitological methods have been diagnosed *Entamoeba coli* (40%), *Giardia duodenalis* (20%) *Strongyloides stercoralis* (13%), *Cystoisospora* sp (13%), *Blastocystis hominis* (6,6%) and detection of *Cryptosporidium* sp negative. The enzyme immunoassays were negative for *Cryptosporidium* and *Giardia* sp (63%) positive. It is observed in this sample there is no correlation of parasitological methods and immunoassay for the diagnosis of *Giardia* sp and there may be cross reactivity between commensal protozoa. For the diagnosis of *Cryptosporidium* concordance of methods suggesting that the immunoenzymatic method could be used routinely because it is easy to perform and interpret compared to the method of Ziehl-Neelsen modified. Supported by Fundação Araucária

IM.05 – INCREASED TRL2 AND TLR4 EXPRESSION IN MONONUCLEAR CELLS FROM DOGS NATURALLY INFECTED BY LEISHMANIA SP. AFTER IMMUNOMODULATOR TREATMENT

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Visceral leishmaniasis in dogs causes immune system unbalance. In some advanced stages of visceral leishmaniasis there is an increase in the production of anti-leishmania antibodies and leishmania antigen-specific lymphoproliferative unresponsive; there is a decrease of gama IFN production with a concomitant increase of IL-10. The chemotherapy is not effective and reoccurrence is often observed after treatment due to imperfect elimination of the parasite. Furthermore, the use of antimony in dogs can select resistant strains to these drugs. It is therefore, essential to study new alternatives for the treatment of infected dogs, which may reduce the incidence of the disease in epidemic areas. The immunmodulator, P-MAPA, a proteinaceous aggregate of ammonium and magnesium phospholinoelate-palmitoleate anhydride derived from Aspergillus Oryzae, has been shown to induce immunity. However, the process in innate immunity is unknown. Mononuclear blood cells of infected dogs were assessed for cell surface expression of TRL2 and TRL4 following incubation with 2,5; 5; and 10 ug/ml of P-MAPA, these receptors were measured using monoclonal antibody by flow cytometry. P-MAPA 2,5 and 10 ug/ml showed higher TRL2 surface expression when compared with the baseline expression (65,42% baseline ; 75,45% P- MAPA -2,5 µg/ml; 81,10% P- MAPA -10 µg/ml). Similarly, 10ug/ml P-MAPA increased TRL4. Our findings showed that P-MAPA increased TRL2 and TRL4, which enhanced innate immune response. The sequential effect of P-MAPA is being investigated. Financial Support FAPESP

IM.06 – REAL TIME PCR FOR DETECTION OF LEISHMANIA SPP IN SPLEEN TISSUE FROM DOGS NATURALLY INFECTED

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Leishmania spp. is intracellular protozoan parasites that cause a wide spectrum of diseases in humans and dogs worldwide. The performance of the less expensive SYBR-Green-based PCR assay, for quantifying *Leishmania chagasi* in spleen tissue samples was to monitor the efficacy of antileishmanial drugs or vaccines. The assay was performed with the LightCycler system using SYBR Green and primers 150 and 152 that amplifying a 120-bp fragment from minicircles of the kinetoplast DNA (kDNA). Twenty dogs with clinical manifestation of visceral leishmaniasis and positive serology for anti-Leishmania antibody were included in study group. DNA was extracted from spleen samples using Hight Pure PCR Template Preparation Kit (ROCHE). There was no evidence of PCR inhibition when the DNA was isolated from spleen sample. The standard curve designed for quantitation of parasite showed linearity with a correlation coefficient of 0,99 and slope -3,058. All dogs with symptoms and positive serology showed positive real-time PCR. Real Time PCR based on SYBR Green may therefore be an appropriate and inexpensive alternative for detection Leishmania spp in dogs.
Financial Support : FAPESP

IM.07 – APOPTOSIS IN THE SPLEEN AND PERIPHERAL BLOOD IN DOGS NATURALLY INFECTED BY *LEISHMANIA (L.) CHAGASI*

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Dogs are the main domestic reservoirs of *L. (L.) chagasi*. In naturally infected dogs changes in the white pulp of the spleen and decrease of lymphocytes in peripheral blood are frequently observed. To investigate whether if apoptosis is responsible for such features T cell apoptotic were quantified from the spleen and peripheral blood of dogs naturally infected with *L. (L.) chagasi*, with clinical manifestation and compared with healthy dogs. A total of 13 adult symptomatic dogs, were serum positive for *L. (L.) chagasi* by indirect ELISA. A group of 6 healthy dogs, from a non-endemic area were included in the study as negative controls. These animals were serum negative for *L. (L.) chagasi*, by indirect ELISA. Samples of spleen from both groups were removed by surgical excision after sedation. The mononuclear cells were simultaneous labeled with CD3 monoclonal antibody (Serotec, UK) and apoptosis (Anexin V kit and Mutilcaspase kit - Guava, Hayward, CA). The procedure of the test was in accordance with the manufacturer's instructions. Data were acquisition in EasyCyte mini ® (Guava, Hayward, CA), the analysis of the data was held in the Software Express Plus ® Guava. Data clearly indicated that T lymphocytes from PBMC and spleen tissue of infected dogs showed a significantly higher level of apoptosis compared with that observed in healthy controls ($p<0.05$, Wilcoxon test). We showed that the apoptosis level in T cell from the spleen and peripheral blood were higher in infected dogs when compared to that of healthy ones showing that the presence of *L. (L.) chagasi* induces apoptosis in T cell. Since the progression of infection is related to the impairment of cell mediated immunity the detection of T cell apoptosis could contribute to inefficient cell immune response during *L. chagasi* infection.
Financial Support FAPESP

IM.08 – QUANTITATION OF REGULATORY T CELL IN SPLEEN AND PERIPHERAL BLOOD IN *L. (L.) CHAGASI* NATURALLY DOGS INFECTED

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Dogs are the main domestic reservoirs of *L. (L.) chagasi*. Once in the vertebrate host, the parasite may cause visceral leishmaniasis, which can also be transmitted to humans. The infected dogs showed increase of anti-Leishmania antibodies and decrease of cellular immunity. Regulatory T cells (T reg) have been shown to be involved in the direct induction of immunosupression of effector cellular immune response. To investigate the possible involvement of T reg cells during *Leishmania* infection, the presence of T reg from the spleen and peripherical blood mononuclear cells (PBMC) of dogs naturally infected with *L. (L.) chagasi*, with clinical manifestation, were quantified. A total of 15 adult dogs from the Zoonosis Control Center of Araçatuba, S.P Brazil, and serum positive for *L. (L.) chagasi* by indirect ELISA and positive rK39 and 05 adult healthy dogs from non endemic area were included in the study. Samples from spleen and PBMC were used for quantification of T reg by flow cytometry using monoclonal antibodies for CD4 and Foxp3. In the spleen T reg levels in infected dogs were lower than in control groups ($P<0.05$), whereas in PBMC no differences were observed between two groups. These results suggest that T reg population is involved in *Leishmania* infection and may have a possible role in promoting parasite persistence and establishment of chronic infection. Financial Support FAPESP

IM.09 – CCL2, CCL4 AND CCL5 CHEMOKINES INDUCE THE MONOCYTES AND PLAMOCYTES MIGRATION IN DERMAL OF DOGS NATURALLY INFECTED BY *LEISHMANIA INFANTUM*

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The immune response in the skin tissue of dogs infected by *L. infantum* and its association with the clinical progression during canine visceral leishmaniasis (CVL) is poorly understood and limited studies are available. In this work, a detailed analysis of the chemokines expression (CCL2, CCL4, CCL5, CCL13, CCL17, CCL21, CXCL8 and CCL24) using Real-Time PCR, as well as, the histopathology study focusing in the dermal inflammatory infiltrate (neutrophils, eosinophils, macrophages, basophils and lymphocytes) of 35 naturally infected dogs presented different clinical status of CVL. Infected dogs were subdivided according as follows: Asymptomatic (AD;n=10), Oligosymptomatic (OD;n=10) and Symptomatic (SD;n=15). Sixteen non-infected dogs (CD) were used as control group. Our results demonstrated that severe forms (OD and SD) of the CVL are characterized by the appearance of numerous clinical signs in the skin (localised or generalized alopecia, dermatitis and cutaneous lesions) and a positive correlation with skin parasite density was observed (r=0.4409/p=0.0080). Enhance of parasite load also was detected in the skin dogs showing the maximum clinical score (SD) when compared with AD (p<0.05). Skin of the SD group presented increase of CCL2 and CCL4 expression when compared with CD (p<0.05). Moreover, OD and SD presented increase of CCL5 expression in relation CD (p<0.05). Assessment of the skin inflammatory cells revealed increase of macrophages (%) and reduction of lymphocytes, eosinophils and basophils according to the clinical progression of CVL (p<0.05). In agreement with these results, increase of cellular infiltrate composed mainly by mononuclear cells was correlated with clinical evolution (r=0.5400/p=0.0004). In conclusion, our date indicate that higher expression of CCL2, CCL4 and CCL5 chemokines is associated with the severe disease and contribute for the orchestrate the cell migration (monocytes and plamocytes) to compose the intense dermal inflammatory infiltrate trying to control the infection. Supported by: Pronex 2007 (CNPq/FAPEMIG); CAPES; IRR/FIOCRUZ and UFOP

IM.10 – FUNCTIONAL ALTERATIONS OF MACROPHAGES CULTURED IN HYPOXIA AND INFECTED WITH *LEISHMANIA AMAZONENSIS*

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Regions of low oxygen tension (hypoxia) are common features of inflamed/infected tissues. Macrophages exposed to hypoxia and infected with *Leishmania amazonensis* amastigotes are able to reduce intracellular parasitism. However, the mechanisms contribute to the resistance of macrophages to *L. amazonensis* infection under hypoxia are not known. In this study we investigated modifications of infected macrophages in hypoxia, such as NO and ROS synthesis, cytokines production, exo/phagocytosis, ATP release, HIF-2α expression, and whether apoptosis occurred in intracellular amastigotes. Our results indicate that hypoxia does not induce the synthesis of NO in macrophages infected with *L. amazonensis* as well as iNOS expression, and iNOS knockout macrophages lacking NO synthesis are still able to reduce infection when cultured in a hypoxia. Although noninfected macrophages produce more ROS in hypoxia than in normoxia, *L. amazonesis*-infected macrophages show similar levels of ROS in normoxic and hypoxic conditions. Antioxidants NAC (ROS scavenger) and Ebselen (glutathione peroxidase mimic) inhibit the leishmanicidal effect of hypoxia, indicating that ROS is important to the effect of hypoxia on leishmanial infection. The cytokines TNF-α, IL-12 and IL-10 releases are similar in normoxia and hypoxia by infected macrophages. Although hypoxia inhibits the phagocytosis of inert particles or fixed parasite, it does not affect *L. amazonensis* entry into macrophages. Hypoxic treatment does not induce the exocytosis of internalized particles by macrophages.Also Infected macrophages show similar levels of ATP in normoxia and hypoxia and apoptosis-like death in intracellular amastigotes does not occur in hypoxic conditions. HIF-2α immunoreactivity is elevated in nuclei of macrophage infected with *L. amazonensis.* Thus, with exception of ROS, NO, cytokines, phago/exocytosis and energetic metabolism of macrophages are not related to the anti-*Leishmania* activity of hypoxia. Furthermore, we can speculate if HIF-2α could be involved in the phenotype changes of infected macrophages in hypoxia. Supported by FAPESP and CNPq

IM.11 – *LEISHMANIA INFANTUM* INFECTION IN THE GOLDEN HAMSTER MODEL

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The hamster is considerate a good experimental model to study visceral leishmaniasis (VL), considering the parasite visceralization and the disease progression that is similar to observed in humans and dogs. However, little is known in relation the evolution of the natural history during the experimental infection by *L. infantum* in hamsters as well as the use of this model to evaluate vaccine and/or drugs in pre-clinical therapeutic study. In the present work, different infection routes (intradermal, intracardiac and intraperitoneal) were tested to evaluate the disease progression. The outcome of the infection was assessed by clinical signs, serum levels of total-IgG, nitric oxide and parasite load in spleen tissue during 1, 3 and 6 months post infection with *L. infantum* promastigotes. Our majors results demonstrate that the infection culminate in the parasites dissemination regardless of the infection route. Splenomegaly was observed in 50% of intracardiac group and the histopathology analysis of the spleen reveled hyperplasia and hypertrophy in the white and red pulp, red pulp congestion and higher reactivity with exacerbation of white pulp in three groups during 1, 3 and 6 months after infection. These findings were most pronounced in the animals inoculated by intracardiac route. The increase of NO levels was detected in three groups in the months (1, 3 and 6) when compared to control group, regardless of the infection route. All groups of hamsters experimentally infected produced higher levels of IgG after infection when compared with the control. The animals infected by intracardiac route developed an intense polyclonal activity resulting higher production of IgG levels in sixth months after infection. Given the parallelism existing between the outcomes of *Leishmania* infection in hamsters, dogs and humans, we believe that our data illustrate that the hamster presented similar disease evolution confirming their capability as experimental model to study VL. Supported by: CAPES, FAPEMIG, FIOCRUZ and UFOP

IM.12 – 1631 Murine infection by *Leishmania amazonensis* after oral treatment with pyrazole carbohydrazide derivatives: Histopathology study of cutaneous lesion

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Leishmaniasis is found in group of diseases known as Neglected Tropical Diseases. Since there is no antileishmanial vaccine in clinical use, control of Leishmanisis relies almost exclusively on chemotherapy. Synthetic new compounds pyrazole carbohydrazide were tested and produced no toxicity on peritoneal cells of mice and demonstrated *in vitro* activity in promastigotes of *Leishmania sp*. Furthermore, we observed significant therapeutic effect in experimental murine infection with *L. amazonensis* resulting in lower parasite load and reducing size of a skin lesion without causing any toxic effect. Our objectives were to analyze the inflammatory cells and evaluate the histology of skin lesions of mice infected with *L. amazonensis* and treated with pyrazole carbohydrazide. CBA mice were infected in the foot with *L. amazonensis*. The animals were orally treated from the second to sixth week after infection with 1.5mg/Kg/day of pyrazole carbohydrazide. At 12 weeks post-infection the animals were anaesthetized and sacrificed for histological examination. In immunohistochemistry were used monoclonal antibodies to identify B and T lymphocytes, macrophages and neutrophils. Histopathological study revealed that changes in the dermis are correlated to the macroscopic size of the lesion. In footpad of mice infected with *Leishmania* was observed an intense presence of vacuolated macrophages infected with richly diverse intracellular and extracellular amastigotes. In addition to dermal macrophages, we observed a mixed inflammatory infiltrate containing lymphocytes and neutrophils. CBA mice infected and treated with pyrazole carbohydrazide minimized skin lesions and the structures of the epidermis and dermis were found preserved with little inflammatory infiltrate. Although the dermis of treated animals were found vacuolated macrophages with intracellular parasites, they were far less numerous than in untreated animals. In conclusion, therapeutic activity of pyrazole carbohydrazide influence in reducing infiltration of macrophages, neutrophils, B and T lymphocytes in skin lesions during experimental murine cutaneous leishmaniasis. Supported by PROPPI/UFF, FAPERJ, CAPES, FIOCRUZ

IM.13 – COMPARTMENTALIZED IMMUNE RESPONSE IN SPLEEN OF DOGS IMMUNIZED WITH LBSAP AND LBSAPSAL VACCINES AFTER EXPERIMENTAL CHALLENGE WITH *LEISHMANIA CHAGASI*

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The spleen provides an appropriate environment for the priming/activation of T cells by antigen presenting cells and microbicidal mechanisms by *Leishmania*-infected macrophages. Herein, we evaluated a detailed immunological analysis of spleen trying to understand the involvement of the cellular immune response, particularly in these vaccines phase I and II trials. In this context, two new vaccines against canine visceral leishmaniasis (CVL) were tested. LBSap vaccine, composed of *Leishmania braziliensis* antigen (LB) and saponin (Sap), and LBSapSal vaccine, composed of LB, Sap and sand fly gland extract (Sal). We evaluated the cellular immune response in spleen considering *ex vivo* and *in vitro* analysis by immunophenotyping and cytokine levels in the supernatant of *Leishmania*-stimulated cultures at 885 days after *Leishmania chagasi*-challenge (dac). Our major results showed that LBSapSal vaccine elicited increased levels of $CD5^+$ and $CD4^+$ T-splenocytes. In addition, *in vitro* immunophenotypic analysis of splenocytes in non-stimulated cultures showed higher counts of $CD8^+$ T-cells in dogs vaccinated with LBSap vaccine and also in splenocytes stimulated with soluble *L. chagasi* antigen (SLA) of dogs vaccinated with LBSapSal. Furthermore, the LBSap group exhibited a negative correlation between $CD8^+$ T-cells and IL-10 when stimulated by SLA (P=0.0075; r=–0.9286). Additional the analysis in LBSap group displayed a negative correlation (P=0.0074; r=–0.9287) between $CD4^+$ T-cells and TNF-α following stimulation with SLA. Similarly, LBSapSal group exhibited a negative correlations between $CD4^+$ T-cells and IL-10 (P=0.0038; r=–0.6929) and between $CD4^+$ T-cells and TNF-α (P=0.0033; r=–0.7074). In conclusion, our data suggested that the potential organ-specific resistance profile elicited in splenocytes in dogs immunized by both vaccines (LBSap and LBSapSal) at 885 dac. This immunological feature observed in spleens of LBSap or LBSapSal immunized dogs is compatible with effective control of the etiological agent of CVL.
Supported by PRONEX–FAPEMIG, CNPq, PAPES-V and UFOP.

IM.14 – CYTOKINES LEVELS AND PARASITE LOAD IN THE BONE MARROW OF DOGS IMMUNIZED WITH LBSAP VACCINE AND CHALLENGED WITH *LEISHMANIA CHAGASI*

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A dog vaccine may be the most practical and effective method by which to reduce the incidence of human visceral leishmaniasis. In this sense, the vaccine composed by *Leishmania braziliensis* promastigotes protein plus saponin as adjuvant has been investigated as a pre-requisite to understanding the mechanisms of immunogenicity against canine visceral leishmaniasis (CVL). In this study, dogs were immunized with saline (C); *L. braziliensis* promastigotes protein (LB), Saponin (Sap), *L. braziliensis* promastigotes protein and saponin (LBSap). Cytokines (IL-10 and TNF-α) in the supernatants of peripheral blood mononuclear cells (PBMC) cultures and parasite load in bone marrow were evaluated at times 90, 435 and 885 days after challenge (dac). Our major results demonstrated that LBSap group displayed significant decrease of IL-10 in the presence of both stimuli (vaccine soluble antigen-VSA or soluble *L. chagasi* antigen-SLcA) during 885 dac. Parasitological analysis in bone marrow was not displayed parasite load until 885 dac. 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. In this context, we are analyzing IL-4, TGF-β and IFN-γ cytokines and the *Leishmania* DNA load by Real Time PCR in different tissues for efficacy vaccine evaluation. Supported by PRONEX–FAPEMIG, CNPq, PAPES-V and UFOP.

IM.15 – NEUTROPHILS ARE REQUIRED TO THE EARLY CONTROL OF *LEISHMANIA AMAZONENSIS* INFECTION IN BALB/C MICE

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Neutrophils provide the first line of defense against infection and contribute to the initiation of inflammation. However the role of neutrophils during infection with *Leishmania* is not clear. Moreover, most studies were performed in *Leishmania major* model of infection. Hence the aim of this work was to investigate the role of neutrophils during infection with *Leishmania amazonensis*. Our results showed that during the first hours after infection there was a massive migration of neutrophils to the site of infection in both BALB/c and C57BL/6 mice, however the presence of neutrophils was more prominent in BALB/c mice. We also demonstrated that the presence of neutrophils at the site of infection was essential for the expression of IL-1β on the first 24 hours post-infection. In the absence of neutrophils there was an exacerbation of lesions during the first week of infection in BALB/c mice, but not in C57BL/6 mice. However, the final outcome of the disease was not affected. The larger lesions were associated with higher activity of arginase at the infection site and higher parasite loads in the ears and draining lymph nodes one week post-infection. Also, there was increased secretion of IL -10 by draining lymph node cells of infected mice depleted of neutrophils. Neutrophil have also proven to be important for the migration of cells to the site of infection and draining lymph nodes. There was an increase in the numbers of dendritic cells in the ears one day post-infection followed by a reduction in the percentage of B cells and regulatory T cells and an increase in T cells at seven days after infection in depleted mice. In conclusion, our results indicate that neutrophils are involved in the early control of *L. amazonensis* infection in BALB/c, but not in C56BL/6 mice. Supported by CAPES, CNPq and FAPEMIG.

IM.16 – MODULATION OF DENDRITIC CELL RESPONSE BY DIFFERENT SPECIES OF *Leishmania* AND THE PARTICIPATION OF EXTRACELLULAR-ATP AND ADENOSINE ON THIS PROCESS

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Dendritic cells (DC) play an essential role in the modulation of innate and adaptive immune response and several studies have evaluated the interaction between *Leishmania* and DC. Extracellular-ATP exhibits pro-inflammatory properties whereas adenosine is an important anti-inflammatory mediator. In this work, we investigated the effects of *Leishmania sp* infection on DC response and the participation of ATP and adenosine on this process. C57BL/6 bone marrow DC infected with metacyclic promastigotes of *L. amazonensis*, *L. braziliensis* or *L. major* showed decreased expression of CD86 and MHCII, and increased expression of 5'-nucleotidase. *L. amazonensis* was more infective than other species. In addition, we examined the proliferation of T CD4+ cells of *L. amazonensis* infected C57BL/6 mice as well as T cells from BALB/c mice (MLR) after co-culture with infected DC. *L. amazonensis* infected DC presented a reduced ability to induce cellular proliferation in both situations. On the other hand, *L. braziliensis* or *L. major* infections had no such effect. In order to evaluate the mechanisms by which these parasites can modulate the DC response, we used the most infective specie as model. IL-10 production was not altered after *L. amazonensis* infection. DC infection in the presence of suramin (an inhibitor of CD39 activity) enhanced CD86 and MHCII expression. Treatment with adenosine receptor antagonists was also able to increase the expression of the activation markers. Moreover, we demonstrate that the presence of suramin or adenosine receptor antagonists at the time of infection recovered DC ability to induce T cell proliferation. In conclusion, *Leishmania* promastigotes impair DC response and this process may be influenced by ATP hydrolysis and activation of adenosine receptors.
Supported by FAPEMIG and CNPq.

IM.17 – RESTRICTION OF *LEISHMANIA AMAZONENSIS* INFECTION BY THE INFLAMMASOMES

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Pattern recognition receptors such as Nlrc4, Naip5, Nlrp3 and Nlpr1, belongs to the family of the Nod like receptors (NLRs), and plays a critical role in the activation innate immune cells in response to pathogen-associated molecular patterns (PAMPs) and danger-associated molecular patterns (DAMPs). Once activated, these receptors trigger activation of a molecular platform composed by the protease caspase-1, so-called inflammasome. Although it is well established that inflammasomes play a critical role in inflammation and resistance against bacterial infections, there is virtually no information on the role of inflammasomes for recognition and restriction of protozoan parasite infections. Here, we investigated the role of the inflammasomes in the recognition and restriction of the infection by the protozoan parasite *Leishmania (L) amazonensis*. By investigating different inflammasomes, we found that Nlrc4-triggered inflammasome is important for recognition and restriction of *L. amazonensis* infection in macrophages and in vivo. Macrophages obtained from Nlrc4$^{-/-}$ and caspase-1$^{-/-}$ -deficient mice showed a diminished leishmanicidal activity in response to IFN-γ or IFN-γ+TNF-α activation. Furthermore, Nlrc4$^{-/-}$ and caspase-1$^{-/-}$ infected-mice developed more severe lesions containing higher parasite burdens. These features were accompanied by an impaired production of IL-1β in the spleen and draining lymph node and a diminished NOS2 expression in the lesion and lymph node. By searching for the mechanisms involved in the Nlrc4/caspase-1-dependent restriction of *L. amazonensis* infection, we found that IL-1β production was key for NO-dependent restriction of *L. amazonensis* multiplication in macrophages. Conversely, *L. amazonensis*-infected Nlrc4$^{-/-}$ and caspase-1$^{-/-}$ macrophages showed a reduction of NOS2 expression and NO production. Collectively, our data shows that inflammasome-derived IL-1β is a key mediator in the induction of effector immune responses toward *L. amazonensis* infection, primarily via NOS2 expression and nitric oxide generation. Importantly, this study shows for the first time that the inflammasomes effectively participate of the recognition and induction of innate immune responses against a protozoan parasite. Supported by FAPESP, CNPq, PEW and WHO/TDR.

IM.18 – LBSAP-VACCINE; *IN VITRO* IMMUNE RESPONSE IN VACINATED DOGS AFTER CHALLENGE THROUGH INTRADERMAL INOCULUM USING PROMASTIGOTES OF *LEISHMANIA CHAGASI* PLUS SAND FLY SALIVA

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The control of *L. chagasi*/*L. infantum* infection in dogs is essential to interrupt the current spread of human visceral leishmaniasis. In this context, a vaccine against canine visceral leishmaniasis (CVL) would be an important tool in the control of human visceral leishmaniasis (VL). Recently our group developed and evaluated the immunogenicity of a vaccine against CVL composed by *L. braziliensis* antigens plus saponin (LBSap). Herein we show the results of the LBSap immunogenicity until 435 days after challenge (dac) by intradermal inoculum using 1x10^7 late-log-phase promastigotes of *L. chagasi* and saliva of *Lutzomyia longipalpis*. Dogs vaccinated with LBSap showed increased levels of numbers of both CD4$^+$ and CD21$^+$ lymphocytes and higher counts of circulating CD14$^+$ monocytes. Moreover, the circulating CD5$^+$ and CD8$^+$ T lymphocytes presented positive correlation with MHC-II expression by lymphocytes in the LBSap group. The evaluation of the *in vitro* immune response into LBSap vaccinated dogs including: lymphoproliferative reaction and immunophenotyping of peripheral blood mononuclear cells (PBMC) after antigenic stimuli using VSA (vaccine soluble antigen) and SLcA (soluble *L. chagasi* antigen). Our major results displayed in PBMC VSA-stimulated intense cell proliferation and an increased count of SLcA-CD4$^+$T-lymphocytes at 90dac in the LBSap group. Furthermore, higher cells proliferations were observed at 435dac in PBMC of LBSap vaccinated dogs after both VSA and SLcA stimuli. These results indicate an effective *Leishmania*-specific immune response even after challenge. In addition, the LBSap group showed at 90dac, higher expression of MHC-II in the lymphocytes cells populations following *in vitro* stimulation with SLcA. LBSap group exhibited positive correlations between CD4+ T-cells and MHC-II expression in cultures stimulated by SLcA or VSA. Taken together, these findings supported the hypothesis that the vaccination using LBSap vaccine elicited strong immunogenicity after challenge with *L. chagasi* maintaining potentially and compatible effective control of the etiological agent of CVL. *Supported by:* FAPEMIG, UFOP, FIOCRUZ, CNPq and CAPES.

IM.19 – THE ROLE OF *LEISHMANIA*'S NTPDASES IN THE PROCESS OF PARASITE ADHESION TO MACROPHAGES

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ENTPDases are enzymes that have the ability to hydrolyze di and triphosphate nucleotides under the stimulus of bivalent ions. Recent studies demonstrate the important role of this enzyme in the process of parasite adhesion and internalization into host cells. Our study aims to understand more deeply the mechanism by which this enzyme favors infection by parasites of the genus *Leishmania*. To achieve the objectives of this study, experiments were performed in order to evaluate adherence rate (30 minutes) and internalization (3 hours) of *Leishmania amazonensis* by peritoneal macrophages using optical microscopy. After treatment of macrophages with recombinant ENTPDase, we noted a reduction in the percentage of cells with adhered parasites and the rates of infection after three hours. This result indicates that the macrophage may present a ligand responsible for adhesion of the enzyme that is important in the process of parasite uptake. The same results were obtained after incubation of parasites with a polyclonal antibody anti-ENTPDase for 30 minutes. Furthermore, the increased expression of the enzyme induced by growth of the parasite with suramin (ectonucleotidases inhibitor) improved the rate of adherence and internalization of the parasites. On the other hand addition of adenine to culture medium reduced the expression of the enzyme also reducing the uptake rates. Interestingly, in all cases there were no differences in numbers of adhered parasites or the number of amastigotes per macrophage, which suggests a limited number of the ligand on the macrophage membrane and an "all or nothing" expression of this molecule by the host cell. In conclusion, our results suggest that parasite ENTPDase is an important molecule in the adhesion of *Leishmania amazonensis* promastigotes to host cells. We are currently investigating the macrophage ligand involved in this association. Supported by FAPEMIG and CNPq

IM.20 – 2271 DISTINCT PATTERN OF IMMUNOPHENOTYPIC FEATURES OF INNATE AND ADAPTIVE IMMUNITY AS A PUTATIVE SIGNATURE OF THE CLINICAL AND LABORATORIAL STATUS OF PATIENTS WITH LOCALIZED CUTANEOUS LEISHMANIASIS

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Introduction and Objectives: American Tegumentary Leishmaniasis is a protozoan disease with distinct clinical manifestations depending on the infecting *Leishmania* species and on the pattern of the host immune response. In this study, we analyzed the phenotypic features of innate and adaptive immunity in patients with localized cutaneous leishmaniasis (LCL), categorized according to their clinical/laboratorial status, including: the number of lesion (L1 and L2-4), the days of illness duration (≤60 and >60) and the reactivity in the Montenegro skin test (MT^- and MT^+), aiming to identify immunological biomarkers applicable in clinical studies. **Results:** Our findings highlighted several phenotypic features observed in all LCL patients (↑HLA-DR in neutrophils, ↑$CD8^+$HLA-DR^+/$CD4^+$HLA-DR^+ T-cells and ↑HLA-DR in B-lymphocytes, ↑CD23 in neutrophils, monocytes and B-cells and ↑seric $NO2^-$+$NO3^-$ levels). Selective changes were associated with distinct clinical/laboratorial status, with L1 displaying enhanced cellular immunity (↑HLA-DR in neutrophils, ↑$CD8^+$HLA-DR^+/$CD4^+$HLA-DR^+ T-cells and ↑seric $NO2^-$+$NO3^-$ levels) and L2-4 mostly marked by increased humoral immune response (↑$CD5^-$ and $CD23^+$ B-cells). Patients from ≤60 presented mixed profile of innate and adaptive immunity (↓CD28 in neutrophils and ↑$CD4^+$ T-cells, without compensatory leishmanicidal mechanisms) whereas patients from >60 showed most changes in the adaptive compartment with prominent activation of $CD8^+$ T-cells and ↑seric $NO2^-$+$NO3^-$ levels. Patients from MT^+ displayed increased putative leishmanicidal capacity including (↑HLA-DR and ↑CD23 in neutrophils, ↑CD23 in monocytes, ↑$CD8^+$HLA-DR^+/$CD4^+$HLA-DR^+ T-cells and ↑seric $NO2^-$+$NO3^-$ levels). In conclusion we summarize a range of immunological biomarkers (↓CD28 in neutrophils, ↑CD23 in monocytes, ↑HLA-DR in CD8+ T-cells and ↑seric $NO2^-$+$NO3^-$ levels) and discuss their significance in clinical studies of LCL. **Financial Support:** CAPES, FAPEMIG, CNPq and FIOCRUZ.

IM.21 – STUDY OF THE INTERACTION BETWEEN CANINE NEUTROPHILS AND *LEISHMANIA CHAGASI*

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Visceral leishmaniasis (VL) is one of the most important emerging diseases with high prevalence in Latin American. Dogs have an important role in public health, been the principal reservoir for this intracellular parasite in the urban zone. Neutrophils are the major population of circulating leukocytes and are quickly recruited to an inflamed site participating in pathogens killing by diverse mechanisms. One of such killing mechanism, NETosis, occurs with the release of neutrophil chromatin associated to granule proteins in a web shape. Here we study NET release by *L. chagasi* promastigotes in neutrophils of healthy dogs. Our results showed that *L. chagasi* induce NET release in dogs' neutrophils and these structures are composed of histone, elastase and DNA. Quantification of NETs showed 3 times more NET released after parasite interaction than control canine neutrophils [NØ = 2.653 ± 1893 ng/mL; NØ + parasite = 6.031 ± 2.386ng/mL]. Incubation of *L. chagasi* with neutrophils resulted in 44% of promastigote killing compared with parasites alone. We also measured myeloperoxidase (MPO), another NET component. Diminished MPO activity was seen in neutrophils after parasite interaction [NØ = 0.472 ± 0.6 mU/10^5céls.; NØ + parasites = 0.413 ± 0.581 mU/10^5céls; NØ + *E. coli* = 2.427 ± 1.906 mU/10^5céls]. Our results demonstrate that canine neutrophils are able to release NETs after *L. chagasi* interaction and that these webs are toxic to the promastigotes. Interestingly, our results suggest that promastigotes modulate MPO in the NETs.

Supported by Faperj and CNPQ.

IM.22 – ECTO-NUCLEOTIDASIC ACTIVITIES IN PROMASTIGOTES OF *Leishmania (Viannia) braziliensis* STRAINS

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Leishmania braziliensis is responsible for the majority of cases of human cutaneous leishmaniasis in Brazil. This parasite has been associated with a broad range of clinical manifestations ranging from a simple cutaneous ulcer to a very destructive form of leishmaniasis with mucosal involvement. In this context, ecto-nucleotidases have been implicated in a crucial role in metabolism of extracellular nucleotides, which can be correlated to parasitic adhesion to target cells and parasite virulence. In this study, the ecto-nucleotidase activity of various *Leishmania (V.) braziliensis* strains, isolated from lesions of patients from different locations in Brazil presenting diverse clinical manifestations were characterized and compared using whole-promastigotes. Moreover, we also examined if parasite ecto-nucleotidase activity can be correlated with infectivity in C57BL/6 mice. For enzyme activity evaluation, we measure the amount of inorganic phosphate released after incubation of live parasites with ATP, ADP and AMP. Furthermore, we evaluated lesion size in C57BL/6 mice after inoculation of promastigotes forms of *L. braziliensis* isolates in the footpad. Tissue parasitism in the footpad and draining lymph node was evaluated by limiting dilution assay. The isolates obtained from patients with mucocutaneous leishmaniasis (MCL) hydrolyze higher amounts of adenine nucleotides than isolates obtained from patients with cutaneous leishmaniasis (CL). Corroborating the hypothesis about the correlation between ecto-nucleotidase activity and virulence, the lesions caused by PPS6 (MCL isolate) in the footpad of C57BL/6 mice were larger and persisted longer than most of the CL isolates. Increased lesion development was accompanied by increased parasite load in both footpad and draining lymph node. We suggest that the enzymes involved in metabolism of extracellular nucleotides may have an important role in the clinical manifestations of patients with mucocutaneous and cutaneous leishmaniasis in the same or different geographical region. Financial Support: CNPq, CAPES, FIOCRUZ, UFOP, PRONEX/FAPEMIG, FAPEMIG

IM.23 – PHENOTYPIC FEATURES OF THE IMMUNE RESPONSE IN SERONEGATIVE DOGS NATURALLY INFECTED BY *LEISHMANIA INFANTUM* WITH INAPPARENT DISEASE CONFIRMED BY PCR

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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 including humoral (IgG, IgG1, IgG2, IgM, IgA and IgE) and cellular T-lymphocytes ($CD5^+$, $CD4^+$ and $CD8^+$), B-cell ($CD21^+$) and monocytes ($CD14^+$) in *ex vivo* context was performed in comparison with the Symptomatic Dogs (SD) and Control Dogs (CD). The results demonstrated that AD-I presented similar immunophenotypic features as those detected in CD group including isotype profile as well as the number of monocytes ($CD14^+$) cells. Moreover, equivalent biomarkers in AD-II and SD groups was observed such as higher levels of IgG, IgG2, IgM and IgA immunoglobulins and elevated number of eosinophils. High frequency of T ($CD5^+$) lymphocytes and the $CD4^+$ T-cells was observed in both AD-I and AD-II groups in comparison to the SD group, whilst ($CD8^+$) T-cells was higher only in AD-II in comparison to the SD group. The analysis of B-lymphocytes revealed an increased frequency of this cell-type only in AD-II animals in comparison to the SD group. Overall, the results supported the hypothesis that the asymptomatic dogs have a dichotomous clinical spectrum able to influence the immunological status and this finding may be decisive in controlling the infection or promoting the clinical evolution of canine visceral leishmaniasis. Supported by PRONEX–FAPEMIG, CNPq and UFOP, DECIT/MS.

IM.24 – *IN VITRO* IMMUNOGENICITY IN DOGS VACCINATED WITH LBSAPSAL VACCINE AFTER CHALLENGE WITH *LEISHMANIA INFANTUM*

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The development of new vaccines is a priority for the control of canine visceral leishmaniasis (CVL) since therapy dogs is ineffective and no vaccine against CVL is accepted by the Ministry of Health (Brazil). We investigated the *in vitro* immune response in dogs immunized with LBSapSal vaccine and challenged intradermally with 1 x 10^7 promastigotes of *L. (L.) infantum plus* salivary gland extract of *Lutzomyia longipalpis* following by 435 days after challenge (dac). Previous studies showed that LBSapSal vaccine was able to increase the absolute number of $CD5^+$T-lymphocytes circulating, reflected by higher $TCD4^+$ and T $CD8^+$ counts in 435dac, besides an increase of $CD8^+$T-lymphocyte during throughout the period after the challenge, indicating the establishment of protective immunity against *Leishmania* infection. Consistent with this hypothesis, our results showed an increase lymphoproliferative activity after antigenic stimulation with SLcA (soluble *L. chagasi* antigen) in 90 and 435dac as well as an increase frequency (stimulation index) of SLcA-specific $CD5^+$ and $CD8^+$T-lymphocytes during 435dac in the LBSapSal group. We also observed an increase in the rate of proliferation MHC-II-lymphocyte stimulation with VSA (soluble vaccine antigen) accompanied by a positive correlation between $CD21^+$B cells and $CD8^+$T-lymphocytes with MHC-II expression in 90dac. The correlations between cell proliferations in the LBSapSal group 435dac showed a similar profile in both stimuli employing VSA or SLcA. In this sense, positive correlations were observed between MHC-II expression with $CD8^+$T and $CD5^+$T-lymphocytes VSA/SLcA-specific, and negative correlations with $CD21^+$B-lymphocytes VSA/ SLcA-specific in 435dac.Thus, this study indicated that LBSapSal vaccine presents an *in vitro* profile consistent with immune protection against CVL. Further investigation focusing the parasitological and molecular analysis to evaluate the efficacy of dogs immunized with the LBSap vaccine has been assessed in our lab. Supported by: FAPEMIG, CNPq, FIOCRUZ and UFOP.

IM.25 – RELATIONSHIP BETWEEN DENDRITIC AND CD4$^+$/CD8$^+$T CELLS IN THE SKIN OF BALB/C MICE INFECTED WITH *L. (L.) AMAZONENSIS* AND *L. (V.) BRAZILIENSIS*

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The role of the Langerharns and dermal dendritic cells in the development of cellular immune response is still contradictory. In BALB/c mice, *L. amazonensis* infection is characterized by an uncontrolled replication of parasites, whereas *L. braziliensis* infection is characterized by self-healing lesions with reduction in the number of parasites. In order to evaluate the relationship between the cellular immune response and dendritic cells in the skin, BALB/c mice were inoculated into the hind footpads with 10^6 promastigotes of both parasite species, control was inoculated with PBS. The infection was monitored during 8 weeks. At 4th and 8th weeks PI, biopsies of skin inoculation site were collected to determine the parasite load by limiting dilution and the density of CD207$^+$, CD11c$^+$, CD4$^+$ and CD8$^+$ cells by immunohistochemistry. Concerning to the density of Langerharns (CD207$^+$) and dermal dendritic (CD11c$^+$) cells, BALB/c infected with *L. amazonensis* showed significant increasing in density of positive cells at 4th week PI compared with control group and BALB/c mice infected with *L. braziliensis* at 8th week of infection. Already, the density of CD4$^+$ cells in BALB/c infected with *L. amazonensis* increased at 4th and 8th weeks PI in relation to the control; and BALB/c infected with *L. braziliensis* at 8th week PI in relation to control and *L. amazonensis* group. By the other side, the density of CD8$^+$ cells was higher only in BALB/c infected with *L. braziliensis* at the 8th week PI. The results showed that an efficient CD4 and CD8 immune response occurs in BALB/c mice infected with *L. braziliensis* at 8th week of infection when a significant increase on the number of dendritic cells was observed, suggesting differences on the antigen presentation to the host immune system between parasite belong to the sub-genus *Leishmania* and *Viannia.* Supported by FAPESP, CAPES and LIM-50 HC-FMUSP.

IM.26 – THE PATTERN RECOGNITION RECEPTORS NOD1 AND NOD2 ARE RECRUITED TO *LEISHMANIA*-CONTAINING VACUOLES AND ACCOUNT FOR THE RESTRICTION OF *LEISHMANIA AMAZONENSIS* MULTIPLICATION IN MACROPHAGES

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Leishmaniasis, caused by parasites of the *Leishmania* genus, is the second-largest parasitic induced disease in the world. The immunity against leishmaniasis is based on the development of IFN-γ-producing Th1 lymphocytes and on the production of nitric oxide by activated macrophages. Thus, an early generation of a Th1 response is a key event for an effective immune response. Th1 differentiation requires effective recognition of the parasites by professional phagocytes such as macrophages and dendritic cells. These cells express a series of pattern recognition receptors (PRR), such as toll-like receptors (TLRs) and nod-like receptors (NLRs). Among NLRs are Nod1 and Nod2, which are cytosolic proteins, know to participate of the recognition of intracellular bacteria. Up to date there is no information regarding the recognition of *Leishmania* by Nod1 and Nod2. Here we evaluated the role of Nod1 and Nod2 receptors for recognition and killing of *L. amazonensis in vitro* and *in vivo*. Initially, we used chinese hamster ovary (CHO) cells transfected with vectors encoding Nod1 or Nod2 to evaluate the recruitment of Nod1 or Nod2 proteins to the *Leishmania*-containing vacuole (LCV). By confocal microscopy we demonstrated that both Nod1 and Nod2 are readily recruited to the LCV. To evaluate the role of these proteins in macrophage resistance we infected macrophages obtained from C57BL/6 (wild-type), Nod1$^{-/-}$, Nod2$^{-/-}$ or Rip2$^{-/-}$ mice and found that the NLR-deficient macrophages presented higher proportion of *L. amazonensis* infected cells and high amounts of intracellular parasites per cell. By performing *in vivo* infections, we confirmed the importance of Nod/Rip2 pathway for restriction of the infection as Nod1$^{-/-}$, Nod2$^{-/-}$ or Rip2$^{-/-}$ mice show a robust lesion development. These data suggest that Nod1 and Nod2 receptors are important for sensing of *L. amazonensis* infection and may act as key components of the innate immune response against leishmaniasis. Supported by FAPESP, CNPq, PEW and WHO/TDR.

IM.27 – TREATMENT WITH BONE MARROW CELLS INFLUENCE IN CHRONIC CUTANEOUS LESION OF MICE (TNFRp55$^{-/-}$ BL/6) INFECTED BY *LEISHMANIA MAJOR*

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Data from our group suggest that TNFRp55$^{-/-}$ C57BL/6 mice might be a good model to study chronic lesions caused by *Leishmania* infection and to test therapies, for example, cell therapy with stem cells. We showed that when infected with *L. major*, TNFRp55$^{-/-}$ mice develop chronic lesions and can control parasite growth at the site of infection when compared to wild type mice, but maintain the intense inflammatory infiltrate. The aim of this study was to verify the efficacy of using preparations of purified mononuclear bone marrow cells (MO-BMC) as a treatment for chronic lesions in TNFRp55$^{-/-}$ mice infected with *L. major*. After 15 weeks of infection groups of mice were treated with MO-BMC (intravenously) and analyses were performed 4 weeks after the treatment. After the treatment with purified MO-BMCs the lesions were reduced and the histological analysis showed evidence of healing in the treated mice in comparison with animals treated with PBS. MO-BMC GFP^{+} cells were transferred to infected mice and after 24 hours or 7 days, no GFP^{+} cells were located at the site of infection but were found in the draining lymph nodes. The analysis of cytokines in the draining lymph nodes of treated mice showed increased levels of IL-10, after recall response with soluble antigen of *L. major,* when compared to animals treated with PBS. Our work suggests that the treatment with MO-BMCs can influence the course of chronic cutaneous lesions in TNFRp55$^{-/-}$ mice infected with *L. major*. Supported by CAPES, CNPq and FAPEMIG.

IM.28 – 2871 THE BALANCE BETWEEN ARGINASE I AND iNOS EXPRESSION IS RELATED TO THE INITIAL CONTAINMENT OF THE LESION AND PARASITE REPLICATION IN IFN-γ DEFICIENT MICE DURING LEISHMANIA AMAZONENSIS INFECTION

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The resistance to *Leishmania major* is associated with the development of a Th1 immune response, with IFN-γ as the key effector cytokine activating macrophages to kill intracellular parasites, via the induction of NO production. The susceptibility to *L. major* is due to the development of Th2 response. Although this Th1-Th2 dichotomy is well established in the *L. major* infection model, it may not adequately explain the pathogenesis of infection by *L. amazonensis*; for which susceptibility is not associated with a polarized Th2 response. Surprisingly, IFN-γ can induce the replication of these parasites *in vitro*. In addition, IFN-γ-/- mice present higher susceptibility at later time points after infection. Since data on the role of this cytokine *in vivo* is lacking, the aim of this work was to investigate the role of IFN-γ during infection by *L. amazonensis*. Our results showed that lesions in the footpads of IFN-γ-/- mice displayed less parasites 8 weeks after infectionas compared to the same size of lesionin the footbeds of as C57BL/6 mice. The decreased parasite numbers were associated with lesser expression of arginase I in the footpad and IL-10 in the lymph nodes. Interestingly, these mice showed the same expression of iNOS in the lesion, suggesting an IFN-γ-independent mechanism of induction of this enzyme. After 16 weeks of infection, IFN-γ-/- showed exacerbated lesions and higher parasite loads. Also, there was an increase in arginase I activity, and a dramatic decrease in iNOS expression. We also demonstrated that IFN-γ is essential for the development of immunity conferred by Leishvacin. In conclusion, our results indicate that it is possible to induce iNOS in the absence of IFN-γ and this induction maybe important for the initial containment of the lesion progress.
Supported by CAPES, CNPq, FAPEMIG and INCT/Redoxoma.

IM.29 – SIGNALING PATHWAYS INVOLVED ON NEUTROPHIL EXTRACELLULAR TRAPS TRIGGERED BY *LEISHMANIA,* PHORBOL ESTER AND N-*FORMIL*-METIONIL-LEUCIL-FENILALANIN

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A new mechanism of neutrophil death, NETosis, was recently described where neutrophils die releasing fibrous traps of DNA, histones and granule proteins, named NETs (Neutrophil Extracellular Traps), which can kill bacteria and fungi. Moreover, NETosis is activated by microorganisms, synthetic and microbial products such as PMA (Phorbol Myristate Acetate) and fMLP (N-*formil*-metionil-leucil-fenilalanin). Our group demonstrated that the protozoan parasite *Leishmania* stimulate neutrophils to release NETs, and are killed by these structures. NETs were also detected in biopsies of patients with cutaneous leishmaniasis. Since then, our group is interested in studying, comparatively, the cell signaling pathways involved on NETs induction by three different inducers – PMA, fMLP and promastigotes of *L.amazonensis.* Thus, human neutrophils, isolated by a density gradient from blood of healthy donors, were incubated with inhibitors of protein kinase C (Bisindolylmaleimide I, BIS), phospholypase C (U73122), protein G (Pertussis Toxin), or NADPH oxidase (diphenylene iodonium, DPI), before the addition of PMA, fMLP or parasites. After 2 hours, NETs released in the supernatant were quantified by the Picogreen assay, which detects NETs DNA. Our results demonstrated that NET induced by PMA and fMLP were inhibited (ranging between 10 and 50%) by all inhibitors tested. DPI and U73122 inhibited 50% of the NET induction by fMLP. The highest inhibition of PMA induced NETs was obtained with BIS (35% inhibition). Interestingly, NETs triggered by *L. amazonensis* were not affected by none of the tested compounds. Our results suggest that different from *Leishmania,* the signaling pathways involved in NET release by PMA and fMLP are linked to protein G, activation of PKC, and dependent of reactive oxygen species. We thank the Hemotherapy Service of Hospital Clementino Fraga Filho (UFRJ), and the support from FAPERJ, CNPq, PIBIC-UFRJ.

IM.30 – HIGH NOS2 EXPRESSION IN MACROPHAGES IS ASSOCIATED WITH LOW PARASITE BURDEN IN SPLEEN OF DOGS NATURALLY INFECTED WITH *L. (L.) CHAGASI*

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Leishmania is intracellular parasite of macrophages responsible for visceral leishmaniasis in dogs. Dogs are the main reservoir for human leishmaniasis. Recent studies suggest that asymptomatic dogs develop a Th1 immunological profile. In protective immune response, amastigotes are phagocytosed and destroyed by activated macrophages, in which in vivo studies suggest iNOS plays an important role as regulating and effector molecule preventing the multiplication of amastigotes. The aim of this study was evaluate the expression of NOS2 in macrophages of spleen from dogs with visceral leishmaniasis in order to identify a possible involvement of this molecule on the parasite control. Twenty *Leishmania* positive dogs, 10 symptomatic and 10 asymptomatic from the Center of Zoonosis Control of Araçatuba city were submitted to euthanasia and biopsies of spleen were collected and processed by immunohistochemistry using mouse anti-*Leishmania* and rabbit anti-human NOS2 (Santa Cruz) polyclonal antibody and LSAB kit (DAKO). Quantitative analysis was performed using the image analysis system in order to quantify the number of amastigotes and the number of NOS2+ cells. Biopsies of dogs from non-endemic area of visceral leishmaniasis were used as control. Correlation between the number of parasites and the number of cells expressing NOS2 was assessed using Spearman test. High expression of NOS2 in macrophages was related with low number of amastigotes in all cases ($r= -0.508$, $p= 0.04$). The results suggest that NOS2 expression by macrophages plays an important role in the control of *Leishmania* parasites in the dog tissue.Supported by FAPESP and LIM50 HC-FMUSP

IM.31 – SERIAL STUDY OF HUMAN CASES OF CUTANEOUS LEISHMANIASIS: CLINICAL AND EPIDEMIOLOGICAL ASPECTS

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The northern region has the highest incidence of leishmaniasis in the country, estimated to be about more than 2,000 new cases a year only in the state of Amazonas. The clinical manifestations are related to different species of *Leishmania* and the host immune response. The objective of this preliminary study was to correlate the clinical human cutaneous leishmaniasis with cellular response by analysis of the cells population by hemogram and detection of the peripheral T-cells (CD4+ and CD8+ lymphocytes). The select cases diagnosed as ATL were analyzed. This study of serial cases was conducted from June 2009 to June 2010, coming from five cities of Amazonas State. Eleven patients with skin lesions and aged between 21 and 58 years, evolution time of the injury about a month in 83% of cases, with predominance of activities in the forest area, presented lymphocytosis in 73% of cases, monocytosis 63%, eosinophilia 45%, neutropenia 45% and anemia 9% in the patients before the treatment with pentavalent antimony N-methylglucamine. Two patients had a low platelet values.The predominant parasite species was Leishmania (V.) guyanensis in 36% of cases, L.(L.) amazonensis and L.(V.) naiffi in 9,5% of cases, respectively. Was observed high levels of CD4+ T-cells (increase of 82%) and decreasing of 91% CD8+ in all of the cases studied. The presented data contribute to understanding the epidemiology of leishmaniasis, immune status and clinical form that the mechanisms involved in the infectious process can be better understood. The determination of Th1 and Th2 response can also be influenced by the strains of *Leishmania*, parasite inoculated dose, by the inoculation site and vector sand fly saliva, besides the immunological aspects and genetic predisposition of the host. This work is in progress. Supported by: Capes, Fapeam/PPSUS.

IM.32 – VACCINATION WITH THE LEISHMUNE®´S NUCLEOSIDE HYDROLASE MAPS THE C-TERMINAL DOMAIN AS THE TARGET OF THE PROTECTIVE IMMUNE RESPONSE

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Nucleoside hydrolases (NHs) of protozoa emerged vital protagonists of pathways for parasite replication and establishment of early infection. Immune protection against NHs would prevent disease at the early infection of several pathogens. We identified the domain of the NH of *L. donovani* (NH36) responsible for its immunogenicity and protective efficacy as a potential candidate of a multivalent synthetic vaccine. Methods: three recombinant proteins of NH36 [aminoacids 1-103 (F1), 104-198 (F2) and 199-314 (F3)] were generated in the pET28b system and used with saponin for vaccination of Balb/c mice further challenged with *Leishmania chagasi or Leishmania amazonensis*. The protective response was evaluated by a cytokine-ELISA assay, inhibition of antibody binding by synthetic predicted epitopes, intracellular staining (ICS), DTH to leishmanial antigen, *in vivo* depletion with anti-CD4+/anti-CD8+ antibodies, parasite load evaluation, increase of footpads lesions and RTPCR. Results: protection against *L. chagasi* is related to a mainly CD4+ T cell driven response with a lower contribution of CD8 + T cells and to antibodies directed to its C-terminal domain of NH36 (amino-acids 199-314). This was mediated by an increase in specific antibodies, DTH and the ratios of IFNγ/IL-10 and TNFα-IL-10 CD4+ and CD8+ producing cells and confirmed by *in vivo* depletion with monoclonal antibodies, algorithm predicted CD4 and CD8 epitopes and a pronounced decrease in parasite load (90.5-88.23%; p=0.011). No decrease in parasite load was detected after vaccination with the N-domain of NH36, despite the induction of IFN-γ/IL-10 expression by CD4+ cells after challenge. Both peptides reduced the size of footpad lesions, but only the C-domain reduced the parasite load of mice challenged with *L. amazonensis* (p=0.039). Conclusions: The identification of the target of the immune response to NH36 represent a basis for the rationale development of a bivalent vaccine against leishmaniasis and for multivalent vaccines for NHs-dependent pathogens.
Support: by CNPQ and FAPERJ

IM.33 – COMPARISON THE PROPHYLACTIC POTENTIAL OF THE NUCLEOSIDE HYDROLASE GENETIC VACCINE ON VISCERAL LEISHMANIASIS ADMINISTERED IN MICE THROUGH THE INTRAMUSCULAR OR THE INTRANASAL ROUTE

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In this work we assayed the potential development of a "needle free" vaccine using the Nucleoside hydrolase gene of *Leishmania donovani* cloned in the VR1012 plasmid (VR1012-NH36). Methods: 100µg of either the VR1012NH36 vaccine or empty plasmid were administered through the intramuscular (*im*) or the intranasal (*in*) mucosal route in BALB/c mice further challenged with *L. chagasi*. Antibodies were measured in an ELISA assay against the recombinant NH36. The cell immune response evaluated by DTH and intracelluar staining of splenocytes and the parasite load evaluated in LDU of liver. Results: After immunization, the DNA vaccine *im* induced a signifficant decrease of IgG1 and the vaccine *im* and *in* determined an increase of IgG3 antibodies. After infection only the im vaccine induced a significant increase of IgG2b antibodies. The DTH response against leishmanial lysate was significantly increased by both vaccines, both before and after infection. Before infection both vaccines were equally potent in DTH induction. This response was 70% higher for the *im* group after infection (ANOVA $p=0.000$;Tukey´s HSD $p<0.05$). The ICS analysis disclosed no differences in proportions of IFN-gamma CD4 T cells ($p=0.166$) . The TNF-alpha-CD4_ T cells ($p=0.012$) and IFN-gamma-CD8+ T cells were significantly increased ($p=0.036$) only in the *im* group and the IL-10-CD4+ cells were increased for the empty plasmid *im* control group ($p=0.012$). The reduction of parasite load showed significant variations (ANOVA $P=0.002$; Kruskall Wallis $p=0.0069$). We observed a 72,01% reduction generated by the vaccine administered through the *im* route in contrast with a 31.90% reduction by the vaccine administered by the *in* route (ANOVA $p=0.002$; $p<0.05$). Both vaccine protected more than the saline and empty plasmid controls. Despite the lower protection, a needle free vaccine using the gene of Nucleoside hydrolase might be developed with the aid of adjuvants.
Support: by CNPQ and FAPERJ

IM.34 – EFFECT OF ADMINISTRATION OF A LIPOSOME FORMULATION OF MEGLUMINE ANTIMONIATE BY INTRAPERITONEAL OR INTRAVENOUS ROUTES AS A TREATMENT OF VISCERAL LEISHMANIASIS

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New treatments for visceral leishmaniasis are very important because of disease severity. Some conventional therapies are not completely efficient, and there are various cases of drug resistance. The aim of this study is to test the best route of administration of a new medicine formulation, meglumine antimoniate-containing liposomes. The effect of this drug is attributed to the sustained release of liposomes and to their natural tendency to be cleared from the circulation by the fixed macrophages of the liver, spleen and bone marrow, which are the major sites of parasite infection. Therefore the liposome formulations improve the use of antimonials, enabling a reduction in drug dose and therapy duration. In this study Balb/c mice were infected by *Leishmania (Leishmania) chagasi*, and treated with drug by two routes: intraperitoneal and intravenous administration, in a unique dose of 30mg Sb/Kg. The liposome formulation was prepared by the dehydration–rehydration method, allowing the encapsulation of 40% of meglumine antimoniate in lipid vesicles with a 400 nm mean diameter. A quantitative limiting dilution assay was performed to determine its impact on the reduction of parasitic load, and the hematoxilin-eosin staining technique was performed to analyze the histological alterations. We have observed a significant reduction of liver and spleen parasite burdens in animals treated with this drug by both routes, when compared to animals treated with free meglumine antimoniate, empty liposomes or PBS. In some cases the parasite was not found after treatment. The histological changes were observed by the reduction of hepatic granuloma formation or complete absence of them. In conclusion we can affirm that both routes were effective to reduce the parasite load and to prevent granuloma formation. Supported by CAPES.

IM.35 – CD1a$^+$, FACTOR XIIIa$^+$ Dermal Dendrocytes, CD4$^+$ And CD8$^+$ T-cells EXPRESSION IN AMERICAN CUTANEOUS LEISHMANIASIS

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The dendritic cells, including Langerhans cells (LCs), have been regarded as a pivotal link between T lymphocytes and macrophages, presenting *Leishmania* antigens and stimulating specific CD4$^+$ and CD8$^+$ T-cells proliferation. The role of LCs in the immunopathogenesis of human and experimental cutaneous leishmaniasis has been well studied, however, there are few articles addressing the involvement of Factor XIIIa$^+$ dermal dendrocytes (FXIIIa$^+$ DD) in such diseases. Factor XIIIa$^+$ DD is a bone marrow monocytic lineage derived cell and member of the skin immune system. The aim of this study was to determine the CD1a$^+$, Factor XIIIa$^+$ DD, CD4$^+$ and CD8$^+$ T-cells expression in the cellular infiltrate of skin lesions of twenty-two cases of localized cutaneous leishmaniasis (LCL) from Buriticupu municipality, pre-Amazonian region of Maranhão State, Brazil. In addition, the skin biopsies performed for immunohistochemistry were also submitted to a Polymerase Chain Reaction (PCR) for characterizing the *Leishmania* species. The paraffin-embedded biopsies were submitted to immunohistochemistry using monoclonal antibodies for CD1a$^+$ (1/20, clone 010[1]/DAKO), Factor XIIIa$^+$ DD (1/100, CM377C/Biocare Medical), CD4 (1/400, OPD4/DAKO) and CD8 (1:100, CD8/144 DAKO) T-cells. For amplification and visualization of the reaction Novolink max polymer was used. The immunostained cells were counted in 5–10 fields (400x) in each section by using an image analysis system (Zeiss). The Factor XIIIa$^+$ DD (546 mm^2) expression was higher (P< 0.05) than that of CD1a$^+$ (296 mm^2) and the CD8$^+$ (2374 mm^2) was also higher (P< 0.05) than that of CD4$^+$ (1268 mm^2) in the lesions of these patients. The PCR results confirmed that all parasites associated to these LCL cases were classified as *Leishmania* (*Viannia)* spp. Considering that all these LCL cases came from a typical *L. (V.) braziliensis* endemic area and that this *Leishmania* parasite is a good modulator of a cellular immune response, we conclude that our findings are strongly suggesting a correlation between the Factor XIIIa$^+$ DD expression with that of CD8$^+$ T-cells, which might explain the high delayed-type hypersensitivity reaction found in this group of patients. Supported by: LIM-50/HCFMUSP, FAPESP, CAPES.

IM.36 – *LEISHMANIA MAJOR* ENCODED INHIBITORS OF SERINE PEPTIDASES (ISPS) MODULATE THE UPTAKE AND RELEASE OF PARASITE FROM MURINE NEUTROPHILS

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The successful establishment of Leishmania infection is associated with the capability of the parasite to evade microbicidal responses by professional phagocytes. Neutrophils are the first cells to be recruited to the site of infection and to be parasitized. *Leishmania* is able to avoid killing by neutrophils, and the phagocytosed parasites reside temporarily as viable metacyclics, before being released to infect macrophages. Alternatively, parasitised apoptotic neutrophils are taken up by standby macrophages. In a previous work, our group showed that *L. major* has three genes similar to bacterial ecotins, which are inhibitors of trypsin-fold serine peptidases, termed ISPs. Recombinant ISP2 inhibits neutrophil elastase with moderate affinity and *L. major* mutant lines lacking *ISP2* and *ISP3* (Δ*isp2/isp3*) are more efficiently phagocytosed by peritoneal macrophages than wild type (WT) parasites, in a mechanism dependent on neutrophil elastase activity. On the other hand, approximately half of intracellular Δ*isp2/isp3* die within the first 15 hours. Since neutrophils produce large amounts of tryspin-fold serine peptidases that contribute to their microbicidal activity we set out to evaluate the possible role of ISPs in the interaction of neutrophils with *L. major* by using Δ*isp2/isp3* null mutants as a tool. We show that Δ*isp2/isp3* are internalized more efficiently by bone-marrow purified neutrophils from C57/BL6 mice when compared to WT parasites and are also released in higher numbers within 12 hours after phagocytosis, contrary to the observed in macrophages. The passage of parasites by neutrophils influences their subsequent infectivity to macrophages. Moreover, peritoneal macrophages infected with Δ*isp2/isp3* were able to recruit neutrophils in vitro more efficiently than those infected with WT or with parasites re-expressing *ISP*2 and *ISP*3. Our results suggest that the regulation of host SP activity by *L. major* ISPs influences the parasite interaction with professional phagocytes, possibly contributing to the establishment of the infection. Supported by Wellcome Trust, FAPERJ and CNPq.

IM.37 – COLLAGEN, FIBRONECTIN AND LAMININ ALTERATIONS OF THE CERVICAL LYMPH NODE IN CANINE VISCERAL LEISHMANIASIS

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The aim of this work was study the extracellular matrix alterations in liver, spleen and cervical lymph nodes in dogs naturally infected with *Leishmania (Leishmania) chagasi* correlating with clinical aspects, histological, parasitological and immunological. This study was carried out with 30 dogs, divided at three groups: ten not infected animals (group control) and twenty infected animals. All them was mongrel dogs with undefined age, obtained from the municipality of Belo Horizonte, MG, metropolitan area. Infected animals were divided in two groups: asymptomatic group composed by ten animals without clinical signs of the disease; group denominated symptomatic: composed by ten animals with classical clinical signals of the disease as skin lesions (alopecia, eczemas and ulcers), loss weight and lymphopathy. During necropsy cervical lymph nodes fragments were collected and fixed in buffer formaldehyde solution to 10% for histological analyses. Paraffined sections were stained by Hematoxylin-Eosin (HE); Gomori's Ammoniacal Silver staining for reticular fibers and strepto-avidin peroxidase Immunohistochemical method for tissue *Leishmania* amastigotes detection. Frozen tissue sections were stained by strepto-avidin peroxidase Immunohistochemical method for laminin (LN) tissue characterization and immunofluorescence technique for fibronectina (FN). The tissue images were transferred to a computer video screen by means of the software KS300 and relayed to a computer-assisted image analysis system (Kontron Elektronic/Carl Zeiss, Germany) for morphometrical analysis. Significant increase collagens deposition in cervical lymph nodes of infected dogs when compared to controls animals. There was significant difference between symptomatic and asymptomatic dogs collagen deposition in organs. Positive correlation between the parasite load and collagen deposition in cervical lymph nodes of infected animals. In fact, symptomatic animals showed increase collagen deposition in these organs, it's can be associate to parasite burden. Adhesive fibers LN and FN expression in cervical lymph nodes was higher in symptomatic animals than in asymptomatic. No significant statistical difference when we compare LN expression in cervical lymph nodes between symptomatic and asymptomatic groups. Our results demonstrate that in canine visceral leishmaniasis induces fibrogenesis in lymph nodes attached the parasite load and degenerative processes. **Key words**: Lymph node, extracellular matrix, dog, canine visceral leishmaniasis

IM.38 – IDENTIFICATION OF AN ANTIGENIC TARGET ASSOCIATED WITH THE PROGRESSION OF THE EXPERIMENTAL *Leishmania* (*Leishmania*) *amazonensis* INFECTION

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Previously we isolated an active and antigenic ATP diphosphohydrolase isoform from *Leishmania* (*Leishmania*) *amazonensis* promastigotes, and described cross-immunoreactivity with potato apyrase, suggesting that the parasite and vegetable proteins share antigenic conserved epitopes (Parasitology 135:327, 2008). Seven domains were identified as highly conserved among plant apyrases and putative NDPases from *L. major*, *L. infantum* and *L. braziliensis* found in the genomes of these parasites (Parasitology 135:943, 2008). A recombinant fragment (rDomA) designed based on domain A was cloned into pQE30 for heterologous expression in *E. coli* BL21 as a 6xHis fusion protein. The rDomA was used as a probe to evaluate, along a period of 90 days, the reactivity of sera from BALB/c mice (n= 5) subcutaneously infected by injecting 10^4 *L.* (*L.*) *amazonensis* amastigotes in the left hind footpad. The primary lesion kinetics in amastigote-infected mice showed progressive large lesions at 20, 40, 60 and 90 days after infection. Histopathological analyses of additional animals showed, at 20 days after infection, discreet inflammatory infiltrates at the site of inoculation that increased progressively during the course of the infection and, at 90 days post-infection, the dermis presented necrotic tissue. Analysis by ELISA, using rDomA as coating antigen and sera diluted 1:100, showed significantly higher ($P<0.001$) IgG antibody levels at 20 (0.084 ± 0.027) and 40 (0.754 ± 0.227) days post infection, when compared to the levels found prior to infection (0.033 ± 0.017). At 60 (0.269 ± 0.179) and 90 (0.354 ± 0.171) days post-infection, IgG antibody levels significantly ($P<0.001$) decreased as compared to the levels found at 40 days post-infection. Taken together, these results suggest that an antigenic NDPase in *L. amazonensis* species contributes to modulate the host immune response, and appoint the rDomA as a new biomolecule to be tested in protocols for investigation in murine *Leishmania* models. Supported by FAPEMIG, CNPq, CAPES, IOC/FIOCRUZ and UFJF.

IM.39 – *L. amazonensis* NDPase: A NOVEL MEMBER OF THE ATP DIPHOSPHOHYDROLASE FAMILY WHICH SHARES A HIGHLY CONSERVED ANTIGENIC DOMAIN

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A particular domain A was shown as a conserved functional region in plant apyrases and putative NDPases found in the Leishmania genomes, suggesting a clear association between structure and antigenicity (Parasitology 135:943, 2008). These results prompted us to search for the NDPase gene from *L. (L.) amazonensis*. Genomic DNA was extracted from promastigotes (MHOM/BR/1973/M2269 strain) and used as template in a PCR amplification with oligonucleotides that were designed based on the nucleotide sequence of both putative *L. major* and *L. infantum* NDPases. The amplified 1152 pb fragment (LaNDPase) obtained by PCR was cloned into pCRII vector. Positive clones were sequenced using M13 universal primers and the sequences of nucleotides were identical between them. Analysis of the multiple alignments between the deduced protein sequence and its orthologues revealed that LaNDPase displayed the five conserved regions described for the ATP diphosphohydrolase family and it is highly homologue (79-87% identity and 85-92% similarity over 361 amino acids) to the putative NDPases found in the *L. infantum*, *L. major* and *L. braziliensis* genomes. High homology was also found between the domain A from the LaNDPase and its counterpart within putative *Leishmania* NDPases (80-90% identity and 85-97% similarity) and potato apyrase (52% identity and 60% similarity). High level of homology exists between the predicted 3-dimensional structures of these proteins, and the domains A are exposed and have high score for antibody binding. IgG antibody from promastigote *L. amazonensis*-infected BALB/c mice had high reactivity against a recombinant fragment (rDomA), designed based on this domain A and obtained as a 6xHis fusion protein. These results identify, for the first time, an NDPase gene in *L. amazonensis* species and revealed that it codifies to a protein that maintains the same functional antigenic domain A, which could be explored in future studies of the leishmaniasis. Supported by FAPEMIG, CNPq, CAPES and UFJF.

IM.40 – IDENTIFICATION OF A NEW TOOL FOR THE STUDY OF CANINE VISCERAL LEISHMANIASIS

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We identified an active ATP diphosphohydrolase (EC 3.6.1.5) isoform, which shares conserved epitopes with potato apyrase, in promastigote of *L. (L.) chagasi*, the causative agent of canine visceral leishmaniasis. Thus, sera (dil. 1:100) of healthy (HEA) and infected (INF) dogs domiciled in endemic area were analyzed by ELISA, using potato apyrase as antigen. The IgG antibody level in HEA (n=18; 0.154 ± 0.058; 78% seropositivity) or INF (n=38; 0.159 ± 0.057; 76% seropositivity) was significantly (P<0.001) higher than that found in healthy dogs domiciled in non endemic area (Control; n=30; 0.049 ± 0.030), suggesting that HEA group was pre-sensitized with the same epitopes. The IgG antibody seropositivity was elevated in the INF group clinically classified as asymptomatic (AD; n=11; 73%), oligosymptomatic (OD; n=12; 75%), and symptomatic (SD; n=15; 80%), and no significant difference was observed among them. Analysis of the amino acid sequence from a *L. infantum* putative NDPase (ATP diphosphohydrolase) revealed high identity with a conserved domain (DomC) belonging to potato apyrase. A recombinant fragment (rDomC) was designed based on this domain and cloned into pQE30 for heterologous expression in *E. coli* BL21. The rDomC was obtained as a 6xHis fusion protein. By ELISA, the IgG antibody reactivity (serum diluted 1:100) against rDomC from INF (0.366 ± 0.083) was elevated, and significantly (P<0.001) higher than that found in HEA group (0.271 ± 0.044). When compared to the HEA, the total IgG antibody level was significantly higher in the AD (0.311 ± 0.045; P<0.05), OD (0.354 ± 0.073; P<0.01) and SD (0.414 ± 0.087; P<0.001) groups. These results suggest that in visceral leishmaniasis the conserved domain is a target for the host immune response. Additionally, this work appointed the rDomC as a new biomolecule to be tested in protocols of studies of the visceral leishmaniasis. Supported by FAPEMIG, CNPq, CAPES and UFJF

IM.41 – INFLUENCE OF ECOTIN-LIKE SERINE PEPTIDASE INHIBITORS OF *LEISHMANIA MAJOR* IN PARASITE INTERNALIZATION AND SURVIVAL IN MURINE DENDRITC CELLS

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Survival of *Leishmania major* in professional phagocytes is associated with the downmodulation of microbicidal and inflammatory responses. We described three genes in *L. major*, *ISP*1, *ISP*2 and *ISP*3, that share similarity to ecotin, a bacterial inhibitor of serine peptidases (SP) such as trypsin, cathepsin G and neutrophil elastase (NE). We reported that *L. major* mutants deficient in *ISP*2 and *ISP*3 (Δ*isp*2/3) are internalized more efficiently by macrophages but have diminished capacity to survive and multiply inside those cells. Increased phagocytosis of Δ*isp*2/3 requires the CD11b, a sub-unit CR3, TLR4 and unregulated activity of NE, present at the surface of macrophages. Infected dendritic cells are very efficient in initiating the parasite specific T-cell response in *L. major* infections and this is related to the ability of the parasite to modulate the parasithophorous vacuole-lysosome fusion. In this work, we investigated if ISPs influence the interaction of *L. major* purified metacyclic promastigotes with bone marrow-derived dendritic cells (BMDC) from BALB/c mice. Δ*isp2/3* mutants were internalized by BMDC less efficiently than wild type (WT) parasites, a phenotype that was reversed by the re-expression of both genes in the mutant. The uptake of Δ*isp2/3* by BMDC returned to WT levels upon addition of the serine peptidase inhibitor aprotinin, of recombinant ISP2 or upon pre-incubation of BMDC with neutralizing antibodies to TLR4. Internalized parasites survive inside BMDCs for at least 24 h post-infection. In contrast to the observed in suceptible BALB/c, Δ*isp2/3* were internalized more efficiently than WT by BMDC of resistant C57/BL6 mice, and this was likewise reversed by aprotinin. Those results suggest that host SP and TLR4 influence the interaction of *L. major* with DCs, which is subject to modulation by parasite ISPs. Furthermore, the consequences of SP inhibition by ISPs to parasite uptake by DCs might vary between susceptible and resistant mice. Supported by CNPq, Wellcome Trust, FAPERJ.

IM.42 – IDENTIFICATION OF A DOMAIN FROM *L. braziliensis* NDPase AS TARGET FOR THE HUMAN IMMUNE RESPONSE

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Domains of high identity between potato apyrase and the putative *L. braziliensis* NDPase found in the genome of this parasite were observed by alignment of their primary amino acid sequences and by hypothetical three-dimensional models, suggesting that these conserved domains may be exposed and available for antibody binding. These results prompted us to test the antigenicity of a specific homologue domain. Potato apyrase was purified from *Solanum tuberosum* and Lb preparation was obtained from *Leishmania (V.) braziliensis* promastigote forms (MHOM/BR/1975/M2903 strain). Synthetic peptides belonging to the conserved domain from both *L. braziliensis* NDPase (LbB1LJ) and its potato apyrase counterpart (potB1LJ) were obtained by solid-phase synthesis. Patients with American cutaneous leishmaniasis (ACL) were diagnosed by positive parasitological examination, Montenegro skin test and polymerase chain reaction (DNA standard obtained from MHOM/BR/1975/M2903 strain). Potato apyrase was recognized in Western blots by IgG antibody from ACL patients, suggesting that the parasite and vegetable proteins share antigenic conserved epitopes. Serum samples (dil. 1:50) from healthy individuals from non-endemic area for leishmaniasis (n= 10) and ACL patients (n= 20) were tested by ELISA ($OD_{492\ nm}$). The IgG antibody levels against Lb (C, 0.095 ± 0.027; ACL, 0.193 ± 0.078; cutoff, 0.149), potato apyrase (C, 0.111 ± 0.022; ACL, 0.198 ± 0.049; cutoff, 0.155), LbB1LJ (C, 0.045 ± 0.050; ACL, 0.214 ± 0.111; cutoff, 0.145) and potB1LJ (C, 0.108 ± 0.040; ACL, 0.202 ± 0.103; cutoff 0.188) were significantly ($P < 0.01$) higher than that found in healthy individuals, with 65%, 90%, 80% and 50% seropositivity, respectively. These results are in accordance with the existence of shared antigenic epitopes between potato apyrase and *L. braziliensis* NDPase, and demonstrate that in leishmaniasis infection the conserved domain is a target for the human immune response.
Supported by FAPEMIG, CNPq, CAPES and UFJF.

IM.43 – EVALUATION OF CD11B, MHC CLASS II AND TLR-2 IN PERIPHERICAL BLOOD MONONUCLEAR CELLS OF NATURALLY INFECTED DOGS WITH *LEISHMANIA (LEISHMANIA) CHAGASI* TREATED WITH MEGLUMINE ANTIMONIATE ENCAPSULATED IN NANOMETRIC LIPOSOMES AND ALLOPURINOL

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Novel liposome formulation of meglumine antimoniate (LMA) associated with allopurinol are being evaluated in mongrel dogs with visceral leishmaniasis. Group I (n=16) was treated with six doses of LMA at 6.5mg Sb^{5+}/kg, dose given with 4-day intervals; Group II (n=16) received six doses of antimony-free lipossomes (FL) given at the same dose as that for group I; Group III (n=8) and IV (N= 12) received six doses of saline. On the first day of protocol, eight dogs in groups I, II and III were also co-treated with allopurinol, 20 mg/kg/s.i.d., for 180 days. Group IV remained as control. Thus, to study the activation state of monocytes, peripheral blood of dogs (20ml) was collected from the jugular vein into EDTA tubes. Density gradient separation was used to enrich for peripheral blood mononuclear cells. The analysis was performed selecting a region of side scatter (SSC) *versus* forward scatter (FSC) (gate R1) in flow cytometer. Within in R1, the mean fluorescence intensity (IMF) of each of the markers was determined in a second histogram. The analysis made 60 days after initiation of treatment revealed that the IMF of CD11b, in $CD14^+$ monocytes, was significantly higher in dogs treated with FL associated with allopurinol compared to group LMA associated with allopurinol ($p<0,01$). For the IMF of TLR2, within the population of monocytes $CD11b^+CD14^+$, the values were statistically higher in FL group associated with allopurinol ($p<0,01$); allopurinol ($p<0,01$) and control ($p<0,05$) in comparison to LMA. When comparing the IMF of MHC Class II wasn't observed statistical difference between the groups. In the literature, the role of empty liposomes in the immune response has been reported. Thus, we are looking forward to analyze these results. So far the data obtained is the preliminary results of the period of 60 days after initiation of treatment protocol assays. Supported by CNPq and FAPEMIG and CAPES

IM.44 – HISTOPATHOLOGICAL, PARASITOLOGICAL AND LYMPHOCYTES $TCD4^+FOXP3^+$, $CD8^+FOXP3^+$ STUDY OF THE JEJUNE AND COLON OF INFECTED DOGS WITH *Leishmania (Leishmania) infantum*

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Canine visceral leishmaniasis is a worldwide zoonosis. Gastrointestinal tract disorders occur in response to human and canine visceral leishmaniasis. The aim of this study was to provide a systematic immunopathological study. Populations of T lymphocytes $CD4^+FOXP3^+$ and $CD8^+FOXP3^+$ of seven dogs naturally infected with *L. infantum* were characterized. Samples of skin ears, liver, spleen and lymph nodes were obtained to confirm *Leishmania* infection. Samples of gastrointestinal tract - jejune and colon were prepared for histological, morphometrical analysis and immunological assays. Suspensions cells obtained form the lamina propria were obtained and analyzed by flow cytometry. Macroscopic observation revealed no severe alterations of the mucosa however, 42% of animals contained hyperemia. A chronic cellular exudate was mainly observed in the lamina propria layer. It was composed plasma cells, lymphocytes and macrophages parasitized or not with amastigotes forms of *Leishmania*. Correlation between inflammatory reaction and parasitism was higher in the colon ($r^2=0,68$) and skin ($r^2=0,59$). In comparison to these parameters between the organs of single dog, the inflammatory reaction was statistically different ($p<0,0016$), where the abdominal lymph nodes and jejune were the anatomical site more reactive. However in the parasitic load was not verified difference statistics. The analysis of lymphocytes demonstrated that the population of $LTCD4^+FOXP3^+$ in colon, jejune e lymph node mesenteric it is greater that of $LTCD8^+FOXP3^+$ ($p=0,0411$; 0,0085 and 0,0115, respectively). However, we did not observe statistical difference between the population of $LTCD4^+FOXP3^+$ and $CD8^+FOXP3^+$ of organs of one same dog, (p=07165 and 0,7562, respectively). The correlation between parasitism and $LTCD4^+FOXP3^+$ was only positive ($r^2=0,5545$) in the colon. In conclusion, we observed a high parasite burden throughout the mucosa jejune and colon without severe tissue alterations.
Support by CNPq and FAPEMIG

IM.45 – ACTIVATION OF PLATELETS BY *LEISHMANIA MAJOR* PARASITES TO ATTRACT A POPULATION OF KILLER MONOCYTES TO THE SITE OF INFECTION

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Leishmania spp are intracellular parasites that reside in macrophages where they can multiply and cause disease. During steady state, resident tissue macrophage homeostatically arise from local tissue-resident progenitor stem cells which constitutively give rise to mature tissue macrophages. During inflammation, however, monocytes from the blood represent the major source of macrophages. Monocytes rapidly exit the blood and within a few days transform into cells that are morphologically and functionally indistinguishable from macrophages. There are two major sub-populations of monocytes, based on their morphology, physiology and by differential surface "marker" expression. One of the populations expresses the granulocyte marker called GR1 and it is considered as the inflammatory subset. In this study we examined the kinetics of leukocyte recruitment into lesions following *Leishmania major* infection of mice, and observed the rapid migration of a population of F4/80+CD11b+GR1+ monocytes that rapidly engulf and efficiently kill *L. major*. We demonstrate that platelet activation by complement-opsonized parasites is responsible for the recruitment of these killer monocytes into the lesions. Activated platelets adsorb to *L. major* in the presence of complement and secrete PDGF. PDGF induces the rapid release of MCP-1 from mesenchymal cells to recruit GR1+ monocytes. In conclusion, this work shows the role of platelets in host defense, which involves the rapid recruitment of a sub-population of inflammatory monocytes from the blood to tissue where they rapidly engulf and kill intracellular parasites. Supported by National Institutes of Health (NIH) –USA.

IM.46 – THE EFFICACY DURATION OF THE INTRANASAL pCI-neo-LACK VACCINE AGAINST LEISHMANIA CHAGASI INFECTION IS CONCOMITANT WITH THE PERIPHERAL EXPRESSION OF LACK TRANSCRIPTS.

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We previously demonstrated that intranasal (i.n.) immunization with a plasmid DNA encoding the p36/LACK leishmanial antigen (pCI-neo-LACK) effectively protects susceptible mice against both cutaneous and visceral leishmaniasis. In the present study, systemic expression after nasal uptake, and the duration of protective immunity was addressed. By using reverse transcriptase (RT)-PCR, we detected the expression of mRNA-LACK transcripts in the spleen, brain, cervical and popliteal lymph nodes of BALB/c mice on day seven through 3 months after vaccination with two i.n. doses of 30 μg of pCI-neo-LACK, declining afterwards. RNA expression coincided with an enhanced cutaneous hypersensitivity to skin-injected parasite antigens, and to protection against visceral leishmaniasis. Mice that were infected with *Leishmania chagasi* after 7 days or 3 months, but not 6 months of vaccination had significantly lower parasite loads than non-vaccinated controls. An examination of the responsiveness of their spleen cells to parasite antigens revealed an enhanced blastogenesis and increased production of IFN-γ and IL-4 cytokines, but decreased IL-10. On the other hand, in animals infected after 6 months of vaccination, the IL-10 response was as high as in non-vaccinated controls. Together, these data show that the 3-month duration of the protective immunity against *L. chagasi* infection conferred by intranasal pCI-neo-LACK is associated with the concomitant systemic expression of mRNALACK. Financial support: CNPq. *Key Words:* Leishmaniasis; *Leishmania chagasi*; intranasal; vaccine; LACK.

IM.47 – EFFICACY OF INTRANASAL VACCINATION WITH LACK-DNA AGAINST VISCERAL LEISHMANIASIS IN EXPERIMENTAL HAMSTER MODEL

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LACK (*Leishmania* analogue of the receptor kinase C) is a conserved protein in the protozoan of the genus *Leishmania*, that is associated with the immunopathogenesis and susceptibility of BALB/c mice to *L.major* infection. Previously, we demonstrated that intranasal immunization with a plasmid carrying the LACK gene of *Leishmania infantum* (LACK-DNA) promotes protective immunity in BALB/c mice against *Leishmania amazonensis* and *Leishmania chagasi.* In the present study, we investigated the protective immunity given in hamsters intranasally vaccinated with 2 doses of LACK-DNA (30 µg). Compared with the controls (PBS and pCI-neo), animals vaccinated with LACK-DNA showed a significant reduction in parasite load in the spleen and liver, increased lymphoproliferative response and increased nitric oxide (NO) production by splenocytes stimulated with parasite antigens. Furthermore hamsters vaccinated with LACK-DNA showed high IgG and IgG2a serum levels as compared to control animals comparable not only predictive of clinical outcome following vaccination, but also with the protection observed. Our results showed that intranasal vaccination with LACK-DNA promoted protective immune response in hamsters and shows the broad spectrum of intranasal LACK DNA in different host species as previously demonstrated in murine visceral leishmaniasis. Financial support: CNPq. Key words: Leishmaniasis; *Leishmania chagasi*; hamster model; intranasal administration; LACK-DNA; mucosal vaccine

IM.48 – PAF RECEPTOR IS REQUIRED TO ANTI-LEISHMANIAL ACTIVITY MILTEFOSINE MEDIATED

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Visceral leishmaniasis, which can be caused by *Leishmania donovani* in India or *L. chagasi* in Brazil, is considered by WHO of the six main public health problems worldwide and is fatal if untreated. Miltefosine (hexadecylphosphocholine), which was first used as treatment in cancer patients has been used as an effective oral drug in visceral leishmaniasis. There is a structural similarity between Miltefosine and platelet activating factor (PAF). PAF acts by binding to the receptor of PAF (PAFR) present in target cells. The mechanism by which the drug works is not well established, our hypothesis is that due to structural similarity between Miltefosine and PAF, the drug uses this receptor to enter in the cell. To test this hypothesis, BALB/c and $PAFR^{-/-}$ mice were orally treated from the 14th day after infection for 7 consecutive days at doses of 20mg/kg/day and killed on day 28. Our data showed that treatment of BALB/c led to a reduction in parasite load in the liver and spleen of these mice. Interestingly, we did not find the same treatment effect in $PAFR^{-/-}$ mice. Therefore, these mice continued to show higher parasite load in these organs. To investigate an immunomodulatory function of the drug, we measured the levels of IL-4, IL-10, IFN-γ and TNF-α in the serum of these mice. We found no differences in the amount of released cytokines between BALB/c mice treated or not. However, peritoneal macrophages from wild type mice infected and treated showed reduction in Arginase I activity. Thus, our results indicate that the drug uses the PAF receptor to enter in target cells where, in addition to direct cytotoxic effect on Leishmania, showed immunomodulatory mechanism by inhibiting the activity of Arginase I in macrophages. Supported by CAPES, CNPq and FAPEMIG.

IM.49 – EVALUATING THE EFFECTIVENESS OF TRANSGENIC TOBACCO (NICOTIANA TABACUM L.) EXPRESSING THE PARASITE ANTIGEN LACK IN ORAL VACCINATION AGAINST CUTANEOUS LEISHMANIASIS IN MICE

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LACK (analogue of the receptor for activated protein kinase C (PKC)) is a well conserved parasite protein. Our group has shown that oral and nasal vaccination with whole parasite antigen LaAg or nasal vaccination with its component LACK in the form of DNA confer protection against visceral and cutaneous leishmaniasis. In this work, we evaluated the potential of a transgenic tobacco expressing the LACK antigen (LACK +/+) to serve as an edible vaccine against cutaneous leishmaniasis. The fresh leaves were crushed to powder in liquid nitrogen and then lyophilized for one day. BALB/c mice received 2 doses of the vaccines with a 1 week interval: a) tobacco LACK+/+ or control LACK-/- (20 mg,); b) LaAg (100 ug). One week after the second dose, mice were s.c. infected with $2x10^6$ promastigotes of *L.amazonensis*. The growth of the lesions was followed for three months, when the animals were sacrificed for quantification of parasite load in the footpad by Limiting Dilution Assay. The results showed a slower development and lower parasite burden compared to PBS, LACK-/- tobacco and LaAg. Vaccination with the LACK -/ - tobacco did not affect growth of the lesion, however, the parasite load in this group was significantly lower than PBS. These results indicate that the immunomodulatory effect of oral vaccination with the transgenic tobacco is reflected in protection, even if partial, against infection by *L.amazonensis*, proving to be even more effective than LaAg. Ongoing experiments are comparing the effect of intragastric gavage versus ad libitum intake, as well as evaluation of the use of transgenic tobacco LACK +/+ in protection against visceral leishmaniasis. Supported by CNPq.

IM.50 – SALIVARY GLAND HOMOGENATE FROM *PSYCHODOPYGUS WELLCOMEI* DID NOT ENHANCES *LEISHMANIA (VIANNIA) BRAZILIENSIS* INFECTION IN BALB/c MICE.

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The promastigote forms of *Leishmania* are transmitted by sand flies during blood feeding. Some reports have shown that vector's saliva plays an important role in the modulation of immune response, helping the establishment of the infection. But the most of these reports used *L. longipalpis* or *P. papatasi* laboratory-reared as a source of saliva to infect different models with different species of parasite; and conflicting data have been described when the natural vector/parasite binomium is used. Thus, the present work has the main aim evaluate, in the natural vector/parasite binomium, the effects of wild-caught vector saliva of *Psychodopygus* **wellcomei *in* L. (V.) braziliensis *promastigotes*** infection in BALB/c mice***. The animals*** were inoculated into the hind footpads with 10^6 *L. (V.) braziliensis* promastigotes without (P group) or with salivary gland homogenate from wild-caught *P.* **wellcomei** (P+W-SGH group). Control groups received only salivary gland homogenate or PBS in the hind footpads. After 10 weeks post infection, fragments from skin and lymph nodes were collected to analyze the main histopathological changes, parasite burden, as well as cytokine and nitrate amounts in supernatant of draining lymph nodes cells cultures. Both groups, P and P+W-SGH, showed similar footpad swelling with a peak at 5 weeks pi, followed by progressive regression. In addition, no significant difference has been found in histological pictures as well as in parasite load in skin and lymph nodes. Concerning to the cytokines production in the supernatant of the lymph nodes cells culture, IL-4 did not show significant differences between the groups, however P+W-SGH group produced decreased IFN-γ levels compared to P group ($p<0.05$). NO levels were similar between the groups. The results presented herein indicated that *P. wellcomei* salivary gland homogenate from wild-caught vector does not promote the enhancement of the *L. (V.) braziliensis* infection in BALB/c mice.
Supported by LIM50 HC-FMUSP.

IM.51 – VITAMIN A-DEFICIENCY IMPAIRS INTESTINAL CD4$^+$ FOXP3$^+$ REGULATORY T CELL EXPANSION AND THE EFFICACY OF THE ORAL LAAG VACCINE AGAINST *LEISHMANIA AMAZONENSIS* INFECTION

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Oral immunization with *L. amazonensis* promastigotes total antigen (LaAg) induces protection in mice against *L. amazonensis* infection (Pinto *et al*, 2003). Retinoic acid (RA), a vitamin A metabolite, is a cofactor required for efficient TGF-β-mediated naive T cell differentiation to Foxp3$^+$ regulatory T cells in the intestinal mucosa (Sun *et al*, 2006). In this study, the RA influence on the oral LaAg vaccine immunogenicity and efficacy was investigated in vitamin A-deficient mice. Pregnant BALB/c were fed with vitamin A-containing (Vit A+) or vitamin A-free (Vit A-) pelleted food from days 7-10 of gestation and during lactation. After weaning, pups were kept under their mother's diet throughout the experiment. At six weeks of age, they were orally vaccinated with two doses of LaAg (100 μg) with seven days interval. Two days after the last dose, Foxp3 expression on CD4$^+$ mesenteric lymph node (MLN) cells was assessed by flow cytometry. To evaluate the vaccine efficacy, 7 days after the last dose, mice were infected subcutaneously in the footpad with *L. amazonensis* and lesion development was monitored for 70 days. On day 70 post infection, the parasite burden and the cytokine profile were evaluated in the infection site. Vaccination increased the percentage of CD4$^+$ Foxp3$^+$ cells only in Vit A+ mice (from 5.9% to 10.9%). The faster lesion development and increased parasite burden indicated that Vit A- are more susceptible to infection than Vit A+ animals. Oral LaAg was only effective in Vit A+ animals, increasing IFN-γ and decreasing IL-4 production in the infected footpads. These results show that vitamin A/RA is required for an efficient response against peripheral *L. amazonensis* infection and suggest that vitamin A/RA–dependent expansion of CD4$^+$ Foxp3$^+$ regulatory T cells in the intestinal mucosa dictates the efficacy of the oral LaAg vaccine. Supported by CNPq.

IM.52 – TGF-β *VERSUS* ENZYME INDUCIBLE OXIDE NITRIC SYNTHASE (INOS) EXPRESSION IN AMERICAN CUTANEOUS LEISHMANIASIS DUE TO *LEISHMANIA (VIANNIA) BRAZILIENSIS* AND *LEISHMANIA (LEISHMANIA) AMAZONENSIS*

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TGF-β is a potent regulatory cytokine that suppresses the expression of iNOS and IFN-γ. In contrast, little is known about the expression of iNOS in human ACL. The aim of this study was to determine the TGF-β and iNOS expression in lesions of ACL due to the major pathogenic leishmanial parasites found in Brazil: *Leishmania (Viannia) braziliensis* and *Leishmania (Leishmania) amazonensis*. 26 patients were examined: anergic diffuse cutaneous leishmaniasis (ADCL) and borderline disseminated cutaneous leishmaniasis (BDCL), both due to *L. (L.) amazonensis* and with DTH$^-$ (five cases); localized cutaneous leishmaniasis (LCL) also due to *L. (L.) amazonensis* with DTH$^-$ (five cases) and DTH$^+$ (three cases) and, LCL due to *L. (V.) braziliensis* with DTH$^+$ (eight cases). Paraffin-embedded biopsies were submitted to immunohistochemistry using the polyclonal anti-TGFβ1 (sc146) and anti-iNOS (N-20, sc-691) antibodies. 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 TGFβ1+ cellular expression (mm^2) has shown a progressive increase from the reactive LCL central form due to *L. (V.) braziliensis* to the non-reactive ADCL and BDCL extremity forms due to *L. (L.) amazonensis* (LCL/*Lb*$^{DTH+}$[492] < LCL/*La*$^{DTH+}$[562] < LCL/*La*$^{DTH-}$[600] < BDCL$^{(DHT-)}$[944] < ADCL$^{(DHT-)}$ [1534]). In contrast, the iNOS expression has shown a decreasing profile in the same way of this spectrum (LCL/*Lb*$^{DTH+}$[1284] > LCL/*La*$^{DTH+}$[946] > LCL/*La*$^{DTH-}$[606] > BDCL$^{(DHT-)}$[460] > ADCL$^{(DHT-)}$[243]), characterizing an inverse association between these modulators of cellular immune response. In conclusion, our results allow a logical understanding on the role of TGF-β and iNOS expression in the genesis of these different clinical forms of ACL, emphasizing the importance of the antigen-specific ability of *L. (V.) braziliensis* or *L. (L.) amazonensis* as primordial factor for the development of these clinical forms of ACL.

Supported by: LIM50/HC FMUSP; FAPESP (06/56319-1)

IM.53 – STUDY OF REGULATORY T CELLS (TREGS) FUNCTION IN HUMAN CUTANEOUS LEISHMANIASIS DUE TO *LEISHMANIA BRAZILIENSIS* INFECTION

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In *L. braziliensis* cutaneous leishmaniasis (CL), despite the patients present strong Th1 responses, related to protection, they develop the characteristic skin ulcers. However, sub-clinical patients, that control parasite replication without developing lesions, have milder Th1 responses and higher immunoregulatory cytokines production. This suggests that excessive or non-modulated Th1 responses may be related to tissue damage. Since Tregs are important modulators of Th1 responses in murine leishmaniasis, our objective is to characterize the role and participation of Tregs in human infection with *L. braziliensis*. PBMC from CL patients or noninfected donors (ND) were phenotyped by flow cytometry. $CD4^+CD25^+$ populations from ND and CL patients' PBMC were isolated and their suppressive functions on PBMC evaluated. Also, patients' Montenegro skin reaction measurements were correlated with FoxP3, IL-10 and IL-10R mRNA expressions in the lesions. CL patients' frequency of cells with Treg characteristics was higher, although not significantly different from ND. CL patients and ND $CD4^+CD25^+$ cells inhibited more efficiently proliferation of ND than CL patients PBMC, but inhibited IFN-γ production of CL patients and ND PBMC stimulated with anti-CD3 similarly. They also suppressed IFN-γ production in CL patients PBMC stimulated with parasite antigens. TNF-α production was suppressed by CL or ND $CD4^+CD25^+$ cells when patients' PBMC were stimulated with parasite antigen, while no suppression occurred in CL and HD PBMC stimulated with anti-CD3. We observed a not significant positive correlation between the expressions of FoxP3, IL-10 and IL-10R and DTH responses. These results suggest that CL patients' Tregs are as effective as ND Tregs in suppressing effector T cell responses, however, CL patients effector cells seem to be less susceptible to suppression. Future experiments will focus on cells isolated from the lesions, where most strongly activated cells are located and Tregs may play major role in controlling tissue damage. Support: FAPESP, CAPES, NIH.

IM.54 – *LEISHMANIA (VIANNIA) BRAZILIENSIS* ISOLATED FROM CUTANEOUS LESIONS INDUCES EARLY DISEASES IN IFNγ KNOCKOUT MICE THAN ISOLATES OBTAINED FROM MUCOSAL LESIONS.

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Leishmania (Viannia) braziliensis is the major parasite that causes tegumentary leishmaniasis in Brazil. The clinical manifestations of the disease caused by this pathogen include the cutaneous and mucosal forms. The control of the parasite is dependent on the activation of macrophages by IFNγ. In that sense, IFNγ knockout are highly susceptible to the infection. To evaluate the virulence of the parasites isolated from patient with cutaneous (CL) or mucosal (ML) leishmaniasis, IFNγ knockout mice were inoculated with doses among 100 to 100.000 amastigotes obtained originally from two CL (JCJ8c and RPL5c) or ML (PPS6m and JBC8m) patient. The isolates were characterized as *L. (V.) braziliensis* by PCR using specie specific primers to glucose-6-phosphate dehydrogenase. The lesion in the infected foot pad of mice and the metastasis in the contra lateral paw were followed weekly by a gauge. Infections with doses of 100.000 parasites induced similar lesions for all isolates, reaching in the fourth week the sizes of 1.9±0.8 mm (JCJ8c); 2.7±1.1 mm (RPL5c); 2.5±1.5 mm (PPS6m) and 2.6±0.5 mm (JBC8m). At the same time, the dose of 100 parasites of the CL isolates induced lesions that reached the size of 1,7±0,7 mm (JCJ8c) and 0.9±0.19 mm (RPL5c), however, the ML isolates were unable to induced any lesion until this time. Metastasis in the non inoculated paw was observed for all isolates, and the CL isolates induced measurable metastasis six weeks after infection in all doses. ML isolates induced measurable metastasis only with the highest doses. These data suggest that ML parasites grow slowly than CL isolates *in vivo*. This behavior can induce a weaker immune response and consequently a better survival of the parasites in the host. Supported by CAPES, CNPq, FUNAPE and FAPEG

IM.55 – EVALUATION OF TUMOR NECROSIS FACTOR AND INTERLEUKIN 10 PRODUCTION IN WHOLE BLOOD CULTURES OF AMERICAN TEGUMENTARY LEISHMANIASIS PATIENTS

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The role of tumor necrosis factor (TNF) and interleukin 10 (IL-10) in American Tegumentary Leishmaniasis (ATL) is not completely understood, mainly in infections caused by *Leishmania* (*Viannia*) complex. The aim of this study was to evaluate the production of TNF and IL-10 *in vivo*, and *ex vivo* in whole blood cultures of ATL patients. ATL patients (22 cutaneous leishmaniasis, CL; 8 mucosal leishmaniasis, ML) and healthy controls were evaluated. Biopsy fragments were used to determine *Leishmania* subgenus using kDNA-PCR and PCR-RFLP. Whole blood cultures were incubated with *Toll*-like receptor agonists (LPS; Pam_3Cys), and *L. (V.) braziliensis* antigen. TNF and IL-10 were measured by using immunoenzymatic assay. West-Central Region contributed with 64.3% of ATL cases and *L.* (*Viannia*) sp was detected in 21 biopsy fragments. TNF levels were higher in serum and in cultures of ATL patients than in controls (serum: 198.5, from 50 to 1,976.0 pg/mL *vs* 114.9, from 50 to 450.7 pg/mL, $n = 25$, $p < 0.05$; LPS: 2,308.0, from 652.7 to 4,262.0 pg/mL *vs* 1,292.0, from 312.4 to 3,954.0 pg/mL, $n = 27$; $p < 0.05$). There was a positive correlation between number of lesions (CL) and TNF concentrations in activated cultures (LPS, $r = 0.62$, $p < 0.01$, $n = 21$). Serum levels of IL-10 did not significantly differ between patients and controls. Whole blood cultures of patients and controls produced similar amounts of IL-10. A positive correlation was detected between TNF and IL-10 in serum of CL patients ($r = 0.54$, $p < 0.05$, $n = 17$), but not in ML patient serum. Our data showed that patients infected with *L.* (*Viannia*) sp parasites produce high levels of TNF and similar levels of IL-10 compared to healthy controls. Data suggest that TNF can be related to severity of CL. Financial support: FAPEG, CNPq, CAPES

IM.56 – IMPROVED INTRANASAL VACCINE EFFICACY OF LACK-DNA AND LaAg LEISHMANIAL ANTIGENS BY ENCAPSULATION IN CHITOSAN MICROPARTICLES AND COMBINED DELIVERY.

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We have previously shown the effectiveness of intranasal vaccination against cutaneous leishmaniasis in mice using both LACK DNA and free particulate leishmania antigen (LaAg) (Pinto E.F. et al., 2004). Chitosan microparticles (CMP) are biocompatible, cationic and mucoadhesive delivery systems potentially suitable for mucosal delivery of negatively charged antigens. In this work, we proposed to optimize LACK DNA and LaAg efficacy by absorption onto CMPs, and combined delivery. For this, CMPs with mean diameter of 5 um and + 30,0 mV of surface charge were constructed and adsorbed with LACK DNA or LaAg at 1:50 and 2:1 Ag:polymer (w/w), respectively. BALB/c mice were immunized twice by the intranasal route with suboptimal doses of LACK DNA plus LaAg in their free forms or complexed with CMPs and then challenged with fluorescent *L. amazonensis*-GFP promastigotes. Animals immunized with a combination of both antigens complexed with CMPs presented slower lesion growth ($p \leq 0{,}001$) and reduced parasite burden ($p \leq 0{,}001$), as compared to PBS controls. On day 97 of infection, those mice were producing higher levels of IFN-γ in the infected footpads as compared to animals receiving free antigens ($p \leq 0{,}001$) or PBS ($p \leq 0{,}001$), whereas local IL-4 production was not significantly affected. These data show that i.n. vaccination with LACK DNA and LaAg adsorbed onto CMPs led to Th-1 type immunity at the infection site, and increased the effectiveness of the combined vaccination. Supported by CNPq.

IM.57 – BALB/c and C57BL/6 MACROPHAGES UNDERGO APOPTOSIS DURING *IN VITRO* INFECTION OF *LEISHMANIA AMAZONENSIS*, BUT NOT DURING INFECTION OF *L. MAJOR* OR *L. GUYANENSIS*

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Apoptosis of host cells has been widely demonstrated as a key event on the outcome of several infectious diseases, downregulating immune responses against infection. Leishmaniasis is a wide-spectrum illness caused by species of the protozoan parasite *Leishmania*, which replicates within macrophages of the mammalian host. In the murine model, *Leishmania amazonensis* can cause a growing lesion at the site of infection, which can either lead to death of some strains of mice, such as BALB/c, or to the control of infection, as C57BL/6. *Leishmania major* and *L.guyanensis* are also responsible for the cutaneous form of the disease, but have different host susceptibility/resistance patterns in the murine model. Using PI permeabilization assay we have demonstrated previously that there is a viability reduction of BALB/c or C57BL/6 peritoneal macrophage infected *in vitro* with *L.amazonensis* when compared to non-infected cells. Macrophages from both strains infected with *L. amazonensis* presented a typical DNA fragmentation pattern, seen in agarose gels and TUNEL assay, starting within the first hours of infection, indicating that macrophage death occurred through apoptosis. These results were corroborated with the increase of hypoploydic nuclei in macrophage cultures infected with *L.amazonensis* when compared to the non-infected control. In addition, using flow cytometry, we have shown that macrophages infected with *L. amazonensis* also expose phosphatidylserine, as demonstrated by the increased binding of AnnexinV to the host cells. On the other hand, the typical ladder pattern of DNA fragmentation was not observed in BALB/c macrophages infected with *L.major* or *L.guyanensis* Together, our results show that macrophages infected *in vitro* with *L. amazonensis* die through apoptosis. They also suggest that *L.major* or *L.guyanensis* do not induce apoptosis in mice macrophages. It seems therefore that apoptosis of host cells does not correlate with susceptibility or resistance of the host, but rather with strain-specific features. Financial support:FAPEMIG, CNPq

IM.58 – IMMUGENICITY AND EFFICACY OF *LEISHMANIA AMAZONENSIS* EXTRACELLULAR SERINE PROTEASES FRACTION (LASP-EX) AND OLIGOPEPTIDASE B2 AS ANTILEISHMANIAL VACCINES

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Leishmania amazonensis is the main agent of anergic diffuse cutaneous leishmaniasis. Our previous studies demonstrated that contrary to intramuscular (i.m.) immunization with whole *Leishmania amazonensis* antigens (LaAg) that enhances mouse susceptibility to cutaneous leishmaniasis, intranasal (i.n.) vaccination confers protection. Using a single-step aprotinin-agarose chromatography, serine proteases were partially purified from extracellular extracts of *L. amazonensis* promastigotas (LaSP-Ex). Their effectiveness in i.m. and i.n. vaccination was compared using a protocol similar to used with LaAg. BALB/c mice were twice vaccinated by the i.m. or i.n route with 25μg of LaSP-Ex, prior to footpad infection with *L. amazonensis*-GFP. We found that i.m. immunization with LaSP-Ex promoted increased susceptibility to subsequent infection, irrespective of the presence of saponin as adjuvant. When using the i.n. route, LaSP-Ex induced a strong protective immunity, as seen by the significantly smaller lesion sizes and parasite burden at day 125 after infection. These findings indicate that similarly to observed with LaAg, LaSP-Ex displays opposing effects when used by the i.m. or i.n. routes. I.n. vaccination with LaSP-Ex induced TGF-β production, but not IL-10 in cervical lymph nodes. At PID 7, decreased IL-10 and increased TGF-β were produced in lesion-draining popliteal lymph nodes, suggesting the involvement of T regulatory cells both in the mucosa and the periphery. At PID 125, protection was accompanied by elevated IFN-γ and IL-12 in the infected footpads of LaSP-Ex vaccinated mice in relation to non-immunized mice, indicating expansion of Th1 cytokines in infected site. A possible active component of this fraction is oligopeptidase B2 (OPB2), a new serine oligopeptidase described in *Leishmania amazonensis* (de Matos Guedes et al, 2008). I.n. vaccination with recombinant OPB2 induced protection as observed for LaAg and LaSP-Ex. This study proposes that LaSP-Ex and recombinant OPB2 are further explored as a potential defined adjuvant-free mucosal vaccines against cutaneous leishmaniasis.
Supported by FAPERJ and CNPq.

IM.59 – METACYCLIC OR BLOOD *TRYPANOSOMA CRUZI* TRYPOMASTIGOTES TRIGGERED CHANGES IN NK AND T-CELLS DERIVED CYTOKINES IN SPLEEN

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We previously demonstrated that infection by different infective forms of *T. cruzi* causes different immune responses in peripheral blood. Herein we have further focus in the cytokines produced by NK and T cells in the spleen following infection with metacyclic or blood trypomastigotes of Be-78 strain. Our results demonstrate that in animals infected with metacyclic forms there is a decrease in the percentage of NK cells in 42 days after infection in the spleen. Moreover, regardless of the infecting form there is an increase of $CD8^+$ lymphocytes in the spleen of animals at 28 and 42 days after infection. Regarding the production of cytokines, we observed an earlier production of IFN-γ by CD8 cells in mice infected with metacyclic trypomastigotes on day 7 post infection, whereas in animals infected with blood forms have an early production only of TNF-α, by both $CD4^+$ and $CD8^+$ T lymphocytes and NK cells. But this increase in IFN-γ and in TNF-α returns to baseline levels at 42 days after infection in animals infected with metacyclic forms, however in animals infected with blood forms, levels for these cytokines do not return to baseline remaining elevated until 42 days after infection. For immunomodulatory cytokine IL-10, there is an increase of the levels in animals infected with metacyclic forms in 28 and 42 days after infection, whereas animals infected with blood forms there is a decrease in the production of this cytokine at day 7 after infection by CD4 and NK cells. These results re-emphasize the importance of the inoculum source triggering distinct aspects of the immune response, showing that the infection with blood forms leads to an exacerbation in the production of inflammatory cytokines, with higher levels even after controlling parasitemia. Supported by FAPEMIG (PPM, Redes Toxifar e Bioterismo), CNPq and UFOP.

IM.60 – IMMUNOHISTOCHEMICAL STUDY OF CELLS INFILTRATING THE MYOCARDIUM OF DOGS EXPERIMENTALLY INFECTED WITH METACYCLIC OR BLOOD TRYPOMASTIGOTE FORMS OF *TRYPANOSOMA CRUZI*

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Similarly to that observed in humans, *Trypanosoma cruzi* experimentally infected dogs showed a mononuclear inflammatory infiltrate during the acute phase of infection. The identification of these cells is an important strategy to understand the immunopathological mechanisms triggered by the parasite during the infection. Therefore, the cells involved in acute experimental infection of dogs by metacyclic (MT) or blood trypomastigotes (BT) of Berenice-78 *T. cruzi* strain were quantified by morphometric analysis in right atrium and interventricular septum. Quantification of cell phenotype showed that $CD4^+$ T lymphocytes are predominant in the inflammatory infiltrate of animals infected with both forms and both tissue sections. On the right atrium the $CD8^+$ T lymphocytes were more present in the infected groups in comparison to the control group. However, $CD14^+$ macrophages cells were prevalent only in animals infected with MT when compared to the control group. In the interventricular septum the $CD8^+$ T lymphocytes and the $CD14^+$ macrophage cells were found in large numbers in the MT group when compared to control and BT group. Neutrophils are present in less number if compared to the other cells analyzed, but were found in greater numbers in the right atrium of both infected groups. The better preserved histopathological picture observed in the animals infected with MT may be directly related to the interaction between $CD8^+$ T lymphocytes and $CD14^+$ macrophages because they are potent producers of interleukin-12, a cytokine that stimulates the production of interferon-gamma by $CD8^+$ T lymphocytes. This cytokine is crucial in the process of infection resistance by *T. cruzi* during the acute phase via stimulation of nitric oxide synthesis by macrophages. The results showed that the MT forms was more efficient and less harmful for the host.
Supported by FAPEMIG (PPM, Redes Toxifar and Bioterismo), CNPq and UFOP.

IM.61 – QUANTIFICATION OF CARDIAC INFLAMMATION IN BALB/c MICE WITH *TRYPANOSOMA CRUZI* CLONES MIXED INFECTION SUBJECTED TO TREATMENT WITH BENZNIDAZOLE

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Analysis of *Trypanosoma cruzi* reservoirs, vectors and the man has revealed that the presence of mixed infections (MI) is frequent in nature. Moreover, experimental studies demonstrated that in these cases there is not only an overlap of effects of mixing clones, but an interaction between fundamental biological properties. This shows the importance of mixed infections studies and our group has assessed MI with *T. cruzi* clones belonging to genotypes main 19, 20, 39 and 32, which are widely distributed in American continent. Female BALB/c were inoculated intraperitoneally with 5,000 blood trypomastigotes of each clone (Gamba cl1 and OPS21 cl1 – 19 genotype, P209 cl1 and Cuica cl1 - genotype 20, Bug2148 cl1 and SO3 cl5 – 39 genotype, SO3 cl5 - genotype 39, IVV cl4 and MAS cl1 – 32 genotype) combined in pairs and treated with benznidazole, during 20 consecutive days. We evaluated the cardiac inflammatory response and the impact of treatment on MI. Two animals of each combination were necropsied 30-35 dpi, (acute phase-AP) and others two animals between 120-125 dpi (Chronic phase-CP). The results were compared with their respective untreated groups. The hearts samples were fixed in formalin, processed, submitted to microtomy and staining with hematoxylin-eosin for inflammatory process quantification. The inflammatory response was different between the monoclonal and mixed infections in almost all cases, and the mixed infection triggered inflammatory response more intense than expected, mainly in the untreated group. In general, the treatment reduces heart inflammation. Only in monoclonal infections and in acute phase occurred increased inflammation after treatment and this was not correlated with any specific genotype. These data corroborate with previous studies from our group in relation to virulence and drug susceptibility, demonstrating that in mixed infections, the cardiac lesions development is difficult to be predicted given the behavior of individual clones.
Supported by FAPEMIG (PPM, Redes Toxifar and Bioterismo), CNPq, CAPES and UFOP.

IM.62 – IMPACT OF GALECTIN-1 ON THE EVOLUTION OF ACUTE EXPERIMENTAL *TRYPANOSOMA CRUZI* INFECTION.

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Galectin-1 (Gal-1) is a β-galactoside-binding protein and participates in several biological processes, including modulation of immune response. In the literature, there are several reports about the potential therapeutic use of Gal-1 for autoimmune processes, degenerative and infectious diseases. However, there are few reports on the involvement of Gal-1 in Chagas disease. Thus, this work was conducted to study the participation of endogenous Gal-1 in acute experimental infection by *T. cruzi*. Galectin-1-deficient mice (KO - Gal-$1^{-/-}$) or wild type (WT - Gal-$1^{+/+}$) mice were used to perform the *in vivo* experiments. The animals were infected with trypomastigotes of *T. cruzi* (strain Y), intraperitoneally. The biological parameters analyzed were parasitemia and survival; histopathology of heart tissue; leukocyte immunophenotyping by flow cytometry; cytokine detection by ELISA. Infected-Gal-$1^{-/-}$ mice showed the lowest levels of parasitemia. Interestingly, all infected-KO mice survived after the infection, whereas the infected-WT mice showed a drastic reduction in survival. The absence of endogenous Gal-1 promoted a reduction on inflammatory cells infiltrate in the cardiac muscle of infected mice. The number of circulating TCD8+ cells in the infected-WT mice were higher, and the sera of infected-WT mice showed higher levels of IFN-γ and IL-12, compared with the infected-KO mice. Taken together, these results suggest that the absence of endogenous Gal-1 promoted immunological profiles that cooperate to the resolution of acute experimental infection by *T. cruzi*. Supported by CAPES.
Keywords: Galectin-1, *Trypanosoma cruzi*, infection, lectin, immunoregulation

IM.63 – THE ROLE OF Rip2 IN THE SIGNALING PATHWAY DOWNSTREAM OF NOD1 IN RESPONSE TO *Trypanosoma cruzi* INFECTION

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Chaga's disease, caused by *Trypanosoma cruzi*, is a serious parasitic disease in Latin America, affecting approximately 18 million people. It was described that the immune response against *T. cruzi* is initially triggered by pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs). However the participation of non-TLR PRR remains largely obscure. We have recently demonstrated that Nod1, a member of the Nod-like receptors (NLRs) family, accounts for host response and resistance against *T. cruzi* infection in macrophages and in a murine model of Chagas disease. Here, we aimed to investigate the signaling pathway downstream of Nod1 that operates in response to T. cruzi infection. It is well established that Nod1 signals via the Rip2 kinase to trigger mitogen-activated protein kinase (MAPK) and NF-kB activation. Thus, we used macrophages from C57BL/6 (WT), $Nod1^{-/-}$ or $Rip2^{-/-}$ mice to investigate the role of Rip2 for macrophage response against the infection. Macrophages were pre-treated with IFN-γ and infected with *T. cruzi*. The number of intracellular amastigotes was analyzed 48 h after infection by Giemsa staining. In addition we evaluated the release of trypomastigotes from cells infected for 3, 4 and 5 days. The, activation of MAPK Erk1/2 and p38 was evaluated by western blot in infected macrophages. We found that as compared to WT, macrophages from $Nod1^{-/-}$ and $Rip2^{-/-}$ contained a higher number of intracellular amastigotes and released higher numbers of trypomastigotes to the tissue culture supernatant. Besides, we found that Erk1/2 and p38 were activated regardless to the presence of Nod1 and Rip2. These results suggest that activation of MAPK is not dependent on Nod1/Rip2 pathway. Nonetheless, Rip2 seems to be at least partially involved in the signaling pathways downstream to Nod1 for restriction of intracellular parasitism by macrophages.
Supported by FAPESP, CNPq, PEW, WHO/TDR and FAEPA.

IM.64 – PRODUCTION OF MONOCLONAL ANTIBODIES AGAINST *Trypanosoma cruzi* VIRULENCE FACTOR NUCLEOSIDE TRIPHOSPHATE DIHOSPHOHYDROLASE 1 (NTPDase-1)

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Trypanosoma cruzi is the etiological agent of Chagas Disease, a tropical neglected disease that remains as a Public Health problem in many countries including Brazil. NTPDase-1 is an ecto-nucleotidase from apyrase/CD39 family that was previously demonstrated as a virulence factor and infectivity facilitator in in vitro and in vivo *T. cruzi* infections. Because of this NTPDase-1 is considered as a good target in rational drug design to Chagas disease chemotherapy. Monoclonal antibodies can be applied as chemotherapeutic agent and used in biological and biochemical assays. The main goal of this work is to produce specific monoclonals against *T. cruzi* NTPDase-1. To do this we expressed the NTPDase-1 in bacterial heterologous system pET21b. The recombinant protein was purified by Ni-NTA affinity chromatography. The purified protein was used to immunize mouse. Specific antibodies were detected by indirect ELISA and Dot-ELISA assays using the recombinant *T. cruzi* NTPDase as antigen. Spleen cells were isolated and fused with tumor cells to produce stable hybridomas. We isolated seven stable hybridomas and 51 isolated clones producing monoclonals. Many of them were not able to recognize *Leishmania* NTPDases. These monoclonal will be analyzed about its ability to inhibit nucleotidase activity of *T. cruzi* NTPDase and the in vitro *T. cruzi* infectivity.
Supported by: UFV, FAPEMIG, CAPES

IM.65 – NEUROLOGICAL MANIFESTATION IN EXPERIMENTAL *Trypanosoma cruzi* INFECTION: DEPRESSION AND MEMORY

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Since the discovery of the American trypanosomiasis by Carlos Chagas in 1909, it is known that the protozoan parasite *Trypanosoma cruzi* affects the central nervous system (CNS). Neuropathological lesions and parasites inside glial cells of the CNS are detected in humans, mainly, during the acute phase of infection. In the chronic phase, cases of neuropathological alterations are rare, while more frequent and severe in immunocompromised individuals with co-infections (eg, HIV), in transplanted and cancer patients. In Chagas disease, psychiatric disorders, cognitive and behavioural alterations are detected during the chronic phase, probably related by cardiac features and/or by *T. cruzi*-elicited direct or indirect CNS injuries. Experimentally, C3H/He infected mice develop a severe meningoencephalitis during the acute phase that self resolves in the chronic infection, although parasite antigens persist in the CNS. Further, drug-induced immunosuppression results in reactivation of the CNS inflammation. Therefore, we investigated the presence of neuropsychiatric changes, in special, depression and memory impairment during the experimental *T. cruzi* infection. Adopting *T. cruzi*-infected C3H/He mice during the acute phase (30 days post-infection) and chronic phase (90 days post-infection), we performed forced swimming and tail suspension tests to assess depression. Additionally, object recognition test was elected to investigate memory status. Acute and chronically infected mice showed high immobility time when submitted to forced swimming and tail suspension tests. Importantly, when compared of non-infected control mice, the infected C3H/He mice did not show difference in the open field test, specific to assess locomotor alterations. In memory test, chronically infected C3H/He mice showed reduced time of exploration of a novel object, revealing mnemonic deficit. In conclusion, depression and memory deficit are present during experimental infection by *T. cruzi*.
Supported by: IOC-Fiocruz, CAPES, FAPERJ.

IM.66 – CD8 + T CELL FUNCTION IN THE PATHOGENESIS OF CHRONIC CHAGASIC CARDIOMYOPATHY

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Persistence of *Trypanosoma cruzi* associated with inflammation at target tissue is proposed to be the main immunopathogenic mechanism leading to chronic cardiomyopathy (CCC) in Chagas disease. CD8+ T cells comprehend the majority of inflammatory cells invading the heart tissue in CCC. Although human CD8+ T cells expressing cytokines and cytolytic effector molecules in a segregated manner were detected in different infectious diseases, less is known about the phenotype and function of CD8+ T cell subsets in *T. cruzi* infection. Herein, we approached the role of CD8+ T cell subsets, segregating pro-inflammatory (interferon-gamma-IFN-g+) and cytotoxic (perforin-pfp+) phenotypes, particularly recognizing parasite antigens, in the immunopathogenesis of *T. cruzi*-elicited experimental CCC. *T. cruzi*-infected C57BL/6 mice present parasitemia and cardiac parasitism during the acute infection peaking at 42-45 days post-infection (dpi). Specific IFN-g+ and cytotoxic (CTL) CD8+ T lymphocytes recognizing ASP-2 peptide were first detected at 15 dpi, while high frequency of these subsets were detected at 30 dpi, preceding parasitemia peak, and persisted in high percentage after parasite control (120 dpi). The persistence of high frequencies of peripheral CTL and IFN-g+ CD8+ T cells during the chronic phase paralleled the increase in numbers of these cells in the heart tissue and the increase in CK-MB levels in serum and a disorganized expression of connexin 43, markers of cardiomyocyte lesion, as well as significant electrocardiographic abnormalities. All these cardiac alterations were first detected at 30 dpi paralleling the entrance of these CD8+ T cell subsets in the heart tissue. Furthermore, higher frequency of inflammatory (IFN-g+) than cytotoxic (pfp+) CD8 + T cells is detected in the spleen of infected mice, however CD8+pfp+ present a more prone migratory phenotype with higher frequency of CCR5+LFA-1+ cells. Presently, we are studying the real migration potential and effector function of these CD8+ segregated subsets in *T. cruzi*-elicited cardiomyopathy.
Financial support: Universal-2006/CNPq, IC/CNPq, IMTEV/INCT de Vacinas, Bolsa de Produtividade/CNPq, CNE-FAPERJ

IM.67 – TREATMENT WITH THE IMMUNOMODULATORY AGENT PENTOXIFYLLINE DURING THE CHRONIC PHASE OF EXPERIMENTAL CHAGAS DISEASE DECREASES CARDIAC ALTERATIONS

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Chagas disease, caused by *Trypanossoma cruzi* infection, is one of the main causes of death due to heart failure in Latin America. Pentoxifylline (PTX), a methylxanthine derivate, has shown to display immunomodulatory, anti-inflammatory and anti-tumor effects. In the present study we used a C57BL/6 mouse model of chronic Chagas disease to investigate the effects of PTX treatment during the chronic phase, targeting disease progression. PTX treatment did not alter the survival rate, parasitemia and heart parasitism during the chronic *T. cruzi* infection. However, the heart of PTX-treated mice showed a significantly reduced deposition of fibronectin and impaired connexin 43 loss compared with saline-treated mice, resembling non-infected controls. More importantly, PTX treatment of *T. cruzi*-infected animals decreases serum levels of CK-MB, one of the markers of myocardial injury. Furthermore, PTX treatment improves the cardiac function revealed by electrocardiographic alterations. These results demonstrated that treatment with PTX during the chronic phase of *T. cruzi* infection prevents the development of severe chronic cardiomyopathy. Therefore, PTX might be considered a potential adjunct therapy for Chagas disease.
Supported by CNPq

IM.68 – SPLENECTOMY INCREASES TISSUE PARASITISM IN MURINE *TRYPANOSOMA CRUZI* INFECTION

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The spleen is a secondary lymphoid organ that harbors a variety of cells such as T and B lymphocytes and antigen presenting cells important to immune response development. In this study we evaluated the impact of spleen removal on the cardiac and liver inflammatory response in these animals to *T. cruzi* infection. C57BL/6 mice were infected with Y strain. Animals were submitted to necropsy during the acute phase of infection. Fragments of heart and liver were fixed in 4% paraformaldahyde (pH7.2), dehydrated in alcohol and embedded in paraffin. Sections were stained with Haematoxylin-Eosin (HE) for standard histological procedures. At day 9 after infection, we observed a higher number of amastigotes in hearts of splenectomized mice but there was no difference in the heart inflammatory infiltrates between splenectomized and control group. No difference was observed either at day 16 in heart parasitism. In mice without spleens, liver sections displayed a higher number of amastigotes 9 and 16 days post infection, but presented more inflammatory infiltrates in the liver only at day 9 after infection when compared to the control group. These results demonstrate that most of the parasites were lodged in tissues and not in the bloodstream at day 9 post infection and this could contribute to mice death. In addition, *T. cruzi* infection is more severe in splenectomized mice as a result of impaired immune responses that are essential for protective immunity against the parasite. Supported by UFMG and CNPq.

IM.69 – REGULATORY EFFECTS OF IL-18 ON CYTOKINE PROFILES AND DEVELOPMENT OF MYOCARDITIS DURING *TRYPANOSOMA CRUZI* INFECTION

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Trypanosoma cruzi causes Chagas disease, an important cause of heart disease in South and Central America. Resistance to *T. cruz*i infection is associated with the production of cytokines such as TNF-α, IFN-γ and the production of nitric oxide. The imbalance of cytokine production is thought to contribute to the pathogenesis of *T. cruzi* infection in the mouse model. IL-18 plays an important role in the immune system by enhancing T-cell responses, regulating IFN-γ production and promoting the development of Th1 immune responses. Here, we investigated the role of IL-18 in the modulation of cytokine production and development of myocarditis during murine *T. cruzi* infection. C57BL/6 and IL-18$^{-/-}$ mice were infected with trypomastigotes of *T. cruzi* (Colombian or Y strain) and parameters such as parasitemia, immune response (by FACs, Real-Time PCR, ELISA) and histopathology were assessed. Infected IL-18$^{-/-}$ mice displayed significantly higher levels of IL-12 and IFN-γ and reduced levels of IL-10 when compared with infected wildtype and Y strain infected mice. The number of T helper type-1 IFN-γ-producing CD4 and CD8 T-cells was significantly elevated in infected IL-18$^{-/-}$ mice and the percentage of cells expressing CD4^{+}CD25^{+high} and FOXP3 in the spleen of infected mice was reduced. We also found reduced expression of iNOS and a reduction in the infiltration of leukocytes in the heart at the early onset of chronic disease in IL-18$^{-/-}$ mice when compared with wildtype counterparts. Taken together, our results indicate that IL-18 production, during experimental infection only with the Colombian but not the Y strain contributes to the balance of IFN-γ, IL-12, TNF-α and IL-10 production and correlates with the modulation in the development of infection-induced myocarditis. #Contributed equally. Supported by CNPq, FAPEMIG, CAPES and Pró-Reitoria de Pesquisa-UFMG (Programa de Auxílio à Pesquisa de Doutores Recém-Contratados).

IM.70 – 3642 EFFECTS OF ZILEUTON TREATMENT DURING *TRYPANOSOMA CRUZI* EXPERIMENTAL INFECTION

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Chagas' disease is an illness caused by the protozoan *Trypanosoma cruzi* and it is the major cause of heart disease in the endemic areas. The vigor of immune response against *T.cruzi* is directly related with the development of chagas' cardiomyopathy. Lipoxin(LX)A_4 production by the activation of the enzyme 5-lypoxigenase(5-LO) down-modulate and promote the resolution of inflammatory processes. The beneficial effects of LXA administration in models of inflammatory pathology, along with the fact that administration of Zileuton leads to the inhibiton of 5-LO activity, has suggested therapeutic promise for specific harnessing of the biological activities of LXA. Herein, we investigated if manipulation of lipoxins production by Zileuton during *T.cruzi* infection could be a potential therapeutic toll. WT mice were infected with *T.cruzi* and treated with Zileuton (30mg/kg) and at different time point after infection the parasitemia, pathology and the expression levels of cytokines were verified in the heart, spleen, liver and serum. Our results showed that treatment with Zileuton results in decreased levels of IFN-γ production by splenic CD4 and CD8 T cells when compared with untreated counterparts. In addition, we found reduction of mRNA expression levels for AhR (an nuclear lipoxin receptor), IL-6, IL-10 and IFN-γ in the heart of treated mice. Moreover, the treatment with Zileuton results in the lower parasitemia and lower leukocyte infiltration into the heart when compared with untreated mice. In addition, our electrophysiological analyses, using the patch-clamp technique, demonstrated that the treatment with Zileuton during *T.cruzi* infection results in the protection of cardiomyocytes activities, including decreased in the repolarization time. In summary, our results suggest that the treatment with Zileuton could be a "powerful toll" in the therapeutic field to modulate the development of cardiomyopathy in Chagas' disease. Supported by CNPq, FAPEMIG, PRPq-UFMG, and FUNDEP-SANTANDER.

IM.71 – INFLAMMATORY ANGIOGENESIS INDUCED BY *Trypanosoma cruzi* ANTIGENS IN MURINE SPONGE MODEL

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Chagas heart disease is an inflammatory illness caused by *Trypanosoma cruzi* and responsible for thousand deaths/year around the world. *T. cruzi* antigens (glycoproteins) appear to be the main trigger for the acute and chronic inflammation driving cardiomyocytes toward a progressive damage process with consequent fibrosis and loss of functionality. Angiogenesis, the formation of blood vessels, has emerged as an essential key for repair process in distinct chronic inflammatory process, especially cardiovascular diseases. Here, we analyzed the angiogenic and inflammatory components of the fibrovascular tissue induced by sponge implants in Swiss mice (n=10) and the modulation of these components induced by total antigens from Y strain of *T. cruzi*. Angiogenesis was assessed by hemoglobin content and VEGF by production in the implants and inflammation determined by measuring the levels of myeloperoxidase (MPO), N-acetylglucosaminidase (NAG), chemokines (CCL2 and CCL5) and regulatory/inflammatory cytokines (IL-10, IFN-g and TNF-a). Both parameters were evaluated at days 1, 4, 7 and 14 post-implantation and corroborated by histological evidence. In our preliminary challenges with antigens (100ul from 10^8 parasites injected inside the sponge) at day 1st post-implant, showed an early accumulation of neutrophils (MPO) at day 4th in contrast with a predominance of macrophages (NAG) population at 7th day post-implant. Surprisingly, even inducing early inflammation, *T. cruzi* antigens inhibited angiogenesis evidenced by decreasing in hemoglobin and VEGF contents as well as by histological sections, when compared with non-challenge animals. Our data suggest that inflammatory angiogenesis is directly affected by *T. cruzi* antigens and it might be a close genetic-related process.
Financial support: CNPq, CAPES, ISID, CNPq-TWAS, UFOP.

IM.72 – 4721 THE ROLE OF SOCS-2 IN THE REGULATION OF IMMUNE RESPONSE DURING *TRYPANOSOMA CRUZI* INFECTION

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Infection with *Trypanosoma cruzi* results in a robust inflammatory reaction in many organs which limits parasite proliferation and results in tissue damage. The regulation of parasite-induced inflammation is multifactorial involving many complex pathways including the lipoxin pathway. Lipoxin (LX) A4 production is mediated by the enzyme 5-lipoxigenase (5-LO) which induces suppressor of cytokine signaling (SOCS)-2 expression which in turn down-regulates pro-inflammatory cytokine production. To investigate the contribution of lipoxins in *T. cruzi* infection we infected wild type (WT), SOCS-2 and 5-LO null mice with the Y strain. When compared with WT mice, the parasitemia was significantly reduced in SOCS-2 and increased in 5-LO null mice. The expression of IFN-γ, TNF-α, IL-6, SOCS-1 and SOCS-3 was reduced in the spleen of SOCS-2 infected mice. The expression of IFN-γ and TNF was reduced in the hearts of *T. cruzi*-infected SOCS-2 and 5-LO null mice compared with WT mice. Infection of 5-LO null mice resulted in lower expression of SOCS-2 in the spleen and heart compared with WT mice. In peritoneal macrophages isolated from WT and SOCS-2 null mice pre-incubated with LXA and then infected there was an increased nitric oxide production and trypanocidal activity in infected SOCS-2 null macrophages simultaneously stimulated with IFN-γ when compared with WT macrophages. SOCS-2 null macrophages exhibited low trypanocidal activity when incubated without IFN-γ. The inhibition of parasite growth by IFN-γ was significantly blocked when WT cells, but not SOCS-2 null cells, were pre-incubated with LXA. The observations indicate a role for the 5-LO/LXA/SOCS-2 pathway in the regulation of the inflammatory response during experimental *T. cruzi* infection. Supported by CNPq, FAPEMIG and Programa de Auxílio à Pesquisa de Doutores Recém-Contratados-Pró-Reitoria de Pesquisa (UFMG).

IM.73 – ESSENCIAL ROLE OF CASPASE-1 FOR THE HOST INNATE IMMUNE AGAINST PARASITE *TRYPANOSOMA CRUZI*

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Chagas disease is caused by the protozoan parasite *Trypanosoma cruzi.* Several studies have demonstrated that IL-1β is important in the experimental infection against this parasite. A new family of pattern recognition receptors, Nod-like receptors (NLRs) has been described. Among NLRs, NLRC4 and Nalp3 form an intracellular multimolecular complex with active caspase-1, called inflammasome, which is necessary for cleavage and secretion of the active forms of the IL-1β. In this study we aimed to determine the role of caspase-1 in the innate immune response against *T. cruzi*. First we investigated if *T. cruzi* is able to trigger caspase-1 activation in murine bone marrow-derived macrophages (BMMs). We found that C57BL/6 (WT) and NLRC4$^{-/-}$ BMMs presented robust caspase-1 activation and IL-1β secretion. However, ASC$^{-/-}$ BMMs are not able to activate caspase-1. To study the signaling pathways mechanisms involved in inflammasome activation we investigated the influence of the potassium efflux and oxygen radicals reactive (ROS), which are described to be required for activation of caspase-1 via ASC. Interesting, in the absence of ROS and potassium did not occur activation caspase-1 in WT BMMs. To test the susceptibility of these animals *in vivo*, WT, caspase-1$^{-/-}$, ASC$^{-/-}$ and NLRC4$^{-/-}$ mice were infected with 1000 forms of *T. cruzi* Y strain. Parasites in bloodstream and heart inflammation were measured. Further, the mortality was daily evaluated. Our results show that ASC and Caspase-1-/- mice present higher mortality whereas WT and NLRC4-/- did not die. Beyond the Asc-/- and caspase-1 mice presented diminished inflammation in the heart after 14 days of infection. However 17 days post infection, ASC$^{-/-}$ and caspase-1$^{-/-}$ mice showed increased inflammatory score compared to WT. Together, these results suggest that ASC inflammasome is crucial in the host innate immune response against *T. cruzi* infection.
Financial Support: FAPESP

IM.74 – 5171 THE ROLE OF ARYL HYDROCARBON RECEPTOR (AHR) IN THE REGULATION OF IMUNE RESPONSE DURING *TRYPANOSOMA CRUZI* EXPERIMENTAL INFECTION

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Chagas' disease is caused by the protozoan *Trypanosoma cruzi* and is a major cause of heart disease in endemic areas. The *T.cruzi* infection is an appropriate and interesting model for study/understanding the balance between effective immune response and immune-pathology. Lipoxin(LX)A_4 production by the activation of the enzyme 5-lypoxigenase(5-LO) is important to the regulation of pro-inflammatory cytokine production during *T.cruzi* infection. Therefore, the role of aryl hydrocarbon receptor(AhR), the nuclear receptor for LXA, during Chagas' disease is not know. Herein, we infected WT and AhR$^{-/-}$ mice with *T. cruzi* (Y strain) and parasitemia and imune response were assessed. We found significantly reduced parasitemia in AhR$^{-/-}$ mice when compared with WT. AhR deficiency resulted in the increased levels of IL-12p70 and IFN-γ production during the early and late acute phase of *T. cruzi* infection, as detected by FACs and ELISA. Next, to investigated which was the mainly factor responsible for the dramatically increase of parasite clearance in the AhR$^{-/-}$ mice, we test whether parasite killing was mediated by nitric oxide (NO). For this, peritoneal macrophages were isolated from WT and AhR$^{-/-}$ mice, infected with *T.cruzi* and/or followed by IFN-γ stimulation and evaluated for nitrite production (Griess method) and trypanocidal activity. Our in vitro results demonstrated no diference in the uptake of parasite by AhR$^{-/-}$ and WT cells, however, we found an increased trypanocidal activity by *T. cruzi*-infected AhR$^{-/-}$ macrophages when compared with WT. Nevertheless, no significant difference in NO levels was detected in the supernatants harvested from *T. cruzi*-infected-AhR$^{-/-}$ and -WT macrophages cultures. By contrast, an increased NO production by AhR$^{-/-}$ macrophages was detected when IFN-γ was added in the uninfected cultures when compared with WT counterparts. In summary, our data indicate the role of AhR in the regulation of immune response during *T. cruzi* experimental infection. Supported by CNPq and PRPq-UFMG.

IM.75 – TREATMENT WITH A PARTIAL CCR1/CCR5 ANTAGONIST (Met-RANTES) ameliorates *Trypanosoma cruzi*-ELICITED CARDIOMYOPATHY: POTENTIAL DELETERIOUS ROLE OF CCR1

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Chagas' heart disease, caused by Trypanosoma cruzi, is mainly characterized by chronic fibrogenic inflammation and loss of heart function. In affected cardiac tissue, the presence of pro-inflammatory cytokines and chemokines might drive leukocyte migration contributing to CCC formation. Chemokines, a group of mediators of the inflammatory process play an essential role in the recruitment and activation of leukocytes in various models of inflammatory diseases. Chemokines type CC (CCL2, CCL3, CCL4 and CCL5) are produced by cardiomyocytes and macrophages in response to T. cruzi infection, preferentially attracting mononuclear cells to inflammation sites. These are the predominant cell types in heart lesions of patients and infected animals. Therefore, CC-chemokines are proposed to crucially define the leukocyte subtypes composing the inflammatory infiltrates in the heart tissue of T. cruzi-infected individuals, being, consequently, involved in the immunopathogenesis of T. cruzi-triggered cardiomyopathy. Independent studies showed that CCR5 and LFA-1 are expressed in PBMCs enabling their migration to the heart. Further, most of the heart invading inflammatory cells are CCR5+ and LFA1+. Importantly, treatment with the selective partial antagonist CCR1/CCR5 during the chronic phase of infection resulted in 20-30% reduction in CD4+ cell numbers as well as IL-10, IL-13 and TNF expression in the cardiac tissue. Furthermore, Met-RANTES treatment led to reduction in parasite load, fibronectin deposition and cardiomyocyte lesion. Mice CCR5-/- infected with T. cruzi had lower survival when compared to C57BL/6 counterparts. Our preliminary data suggest that treatment of CCR5-/- mice with CCR1/CCR5 partial antagonist increases the survival of these animals, indicating a role for CCR1 in pathogenesis during T. cruzi infection. Thus, therapeutic strategies based on the modulation of CCR1/CCR5-mediated cell migration and/or effector function may contribute to cardiac tissue damage limitation during chronic Chagas disease.

IM.76 – EXPERIMENTAL INFECTION WITH *Plasmodium berghei* COMPROMISES THE THYMUS IN MULTIPLE WAYS

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Maturation of T cell occurs within the thymus and pathologies such as malaria cause thymus atrophy, which alters thymic microenvironment and impairs T cell development in multiple ways. Leptin is an important hormone linked to obesity and immunological activities whose decrease can also lead to thymic atrophy. Cytokines are essential for proper T cell maturation and besides them other factors such as the extracellular matrix and galectins control cell migration into the thymus. Galectin3, for instance, arouses cell de-adhesion smoothing cell migration. Here, we used 8-week old male mice infected with *Plasmodium berghei* and non-infected control mice to analyze serum levels of leptin (by ELISA), expression of thymic cytokines (by real time PCR) and the expression of thymic gal-3 (by IHQ) as well as the role of exogenous gal-3 in thymic-nurse cell complex. Thymuses from infected and control mice were collected, washed and submitted to Real time PCR to detect expression of IFN-γ, TNF-α, IL-17, IL-7, IL-10 and TGF-β; they were also embeded in paraffin for IHQ analysis and enzymatically digested for TNC isolation. Thymic expression of IFN-γ, TNF-α and IL-10 was higher in infected mice (twenty-fold, eight-fold and thirty-fold higher than in control, respectively). Expression of IL-7 and TGF-β was lower in infected mice (three-fold higher than in control for both cytokines). IL-17 expression did not alter in infected mice whereas these mice had lower levels of leptin when compared to control. Gal-3 increased in infected thymic tissue while TNC levels were smaller in infected mice. Further, the treatment of gal-3, ex vivo, altered thymocyte migration. We conclude that mice infected by *Plasmodium berghei* present clear alterations in thymic function arising from different pathways, and compromising the profile of mature peripheral T cells.
Supported by FAPESP #05/59774-9, #04/03599-1

IM.77 – ANTIBODIES PRODUCTION AND CIRCULATING LEUCOCYTES IN BALB/c MICE REINFECTED WITH RECOMBINANT *TOXOPLASMA GONDII* STRAINS AFTER IMMUNOSUPPRESSION WITH CYCLOPHOSPHAMIDE

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It still is not established if reinfection by *Toxoplasma gondii* is genotype-dependent and no work is available on reinfection of immunosuppressed mice. To verify the interference of differences among genotypes of *T. gondii* strains and immunosuppression with cyclophosphamide (Cy) in reinfection process, groups of BALB/c mice were prime-infected with the non-virulent strains: D8 (recombinant I/III) or ME49 (type II). Mice were challenged 45 days after primary infection with CH3 or EGS strains (recombinant I/III and virulent). Other groups of mice were inoculated following the same protocol, however, they were treated weekly with Cy, beginning 5 days before challenge. To evaluate the kinetic of anti-*T. gondii* antibodies (IgG, IgG1, IgG2a, IgM and IgA) by ELISA, blood was collected from each mouse immediately before challenge and at 30 days after challenge. Thirty days after challenge, the brain from surviving mice was submitted to PCR-RFLP analysis (genetic markers *L363* and *cS10-A6*) to confirm reinfection. Besides, circulating leucocytes of mice was evaluated 45, 55 65 e 75 days after primary infection with D8 or ME49. PCR-RFLP demonstrated that mice prime-infected with ME49 strain were reinfected with CH3 and EGS strains, while mice prime-infected with D8 strain were reinfected only with EGS strain. The immunosuppression increased reinfection and mortality after challenge. Reinfection in non-immunosuppressed mice was associated with increase of IgM and IgA levels after challenge. Otherwise, 30 days after challenge, immunosuppressed mice presented IgM and IgA levels significantly smaller than non-immunosuppressed mice, suggesting that those immunoglobulins participated in the process of protection against reinfection. Cy treatment caused significant leukopenia in prime-infected mice, with reduction of lymphocytes and neutrophils which probably favored reinfection. In conclusion, reinfection is related to genetic differences among *T. gondii* strains and to alteration of immune integrity of the host, which probably favor reinfection.
Supported by CNPq and FAPEMIG

IM.78 – 2751 QUANTIFICATION OF SPECIFIC AND LOW AVIDITY IgG IN THE DIAGNOSIS OF TOXOPLASMOSIS

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Toxoplasmosis is usually benign and asymptomatic, but affects humans by visual loss or severe diseases, often lethal in fetuses and immunocompromised patients. The transmission is related to acute infection allowing adequate treatment. The avidity of IgG antibodies has been used for diagnosis of acute infection, but conventional indirect avidity tests allow only the proportion of low avidity antibodies. Markers of disease activity, low avidity antibodies (LAA), are highly prevalent in acute infection. We devised an enzyme immunoassay (ELISA), with pH as chaotrope, which allows LAA elution, with subsequent neutralization and direct determination. We tested 150 serum samples from L.Protozoology IMTSP. ELISAs were performed in microtiter plates (Costar ®), adsorbed with 10 µg/mL of *T. gondii* soluble extract antigen and reacted with 1/200 sample dilution. Low avidity immune complexes were dissociated with citrate phosphate buffer at different pHs for 10 minutes, transferred to a new well, neutralized to neutral pH and incubated by 1 hour and 12 hours with the absorbed antigen. Bound IgG was revealed with 1/20.000 of the anti-human IgG conjugate (Sigma ®). Washing with pH 3.5 present similar results to usual 6M urea (r^2=0,7348), allowing recovery of the antibodies of low avidity. Neutralization allows reaction to the antigen, with similar results between 1 hour and 12 hours incubation. Antigen specificity of LAA were tested by western blotting against *T. gondii* antigens, compared to total or high avidity antibodies from our samples and also from samples from rabbits experimentally infected with *T. gondii*. LAA showed a clear difference in antigen specificity as compared to whole serum or high avidity antibodies. Direct quantification of low avidity antibodies open a new perspective for future studies both on diagnosis and pathogenesis of toxoplasmosis related to disease severity, especially in congenital infection and in immunocompromised individuals. This work was partially supported by CNPq and LIM49HCFMUSP.

IM.79 – DETECTION OF TH17 AND T REGULATORY CELLS DURING THE COURSE OF EXPERIMENTAL TOXOPLASMOSIS IN SUSCEPTIBLE AND RESISTANT MICE

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It has been described that peroral infection with *Toxoplasma gondii* in mice leads to the development of an intense intestinal inflammation dependent on Th1 cytokines. C57BL/6 susceptible mice succumb to this exacerbated inflammation, while resistant BALB/c mice are able to control this inflammatory response and develop a chronic disease. Previous studies have implicated IL-17 production in resistance to *T. gondii* and in development of immune-mediated pathology during infection. In parallel the regulatory T cells (Tregs) function is suggested to be corrupted during toxoplasmosis. In this work, we compared the Th17 and Treg responses in susceptible and resistant mice during course of *T. gondii* infection. C57BL/6 and BALB/c mice were orally infected with 40 cysts of ME49 *T. gondii* strain and, 4, 8 and 11 days post-infection the immune response was evaluated. During the course of infection, the number of T $CD4^+$ lymphocytes increased in Lamina Propria and decreased in Peyer´s patches from C57BL/6 mice. In BALB/c mice T $CD4^+$ cell numbers in Lamina Propria were significantly reduced when compared with C57BL/6 mice, but in Peyer´s patches, the number of these cells increased in the 8^{th} day. The frequency of Tregs ($CD4^+CD25^+Foxp3^+$) decreased and the Th17 ($CD4^+ROR\gamma^+IL\text{-}17^+$) cells increased during the toxoplasmosis progression in C57BL/6 mice while BALB/c mice sustained increased frequency of Tregs. In addition, the frequency of Th17 cells in BALB/c mice also increased during the course of infection, but it was lower than in C57BL/6 mice. The transfer of $Foxp3^+(GFP^+)$ cells sorted from non-infected mice, but not from infected mice, increased the survival of C57BL/6 infected mice. In addition IL-17 knockout mice were even more susceptible than C57BL/6 mice to oral infection with *T. gondii*. These results suggest that the balance between Tregs and Th17 cells might be essential to resistance of *T. gondii* infection in BALB/c mice.
Supported by: FAPESP and CNPq

IM.80 – ASSOCIATION BETWEEN IMMUNOGLOBULIN LEVELS AND DIFFERENT TYPES OF SCARRED OCULAR LESIONS DUE TO *TOXOPLASMA GONDII* INFECTION

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Toxoplasma gondii infects almost one third of human population worldwide. Congenital and acquired toxoplasmosis may cause acute scarring ocular lesions. It has been shown that the size of scarred lesions is related to the specific anti-*T. gondii* IgA levels in human sera. This study aimed to characterize the anti-*T. gondii* specific humoral immune response in 1714 individuals of three villages, located in Vale do Jequitinhonha, Brazil and to associate the specific antibody levels against *T. gondii* with number and size of lesions. The presence of specific anti-*T.gondii* IgG was measured by the ELISA assay. The number, type, size and location of retinochoroidal lesions where documented by retinographs. The lesions were classified into three types: A, B and C. Lesions of type A and B fulfill the morphological criteria of being caused by *T. gondii*, while type C lesions are considered to be of uncertain etiology. Our findings show a seroprevalence of 43.4% (744/1714) in the population studied. The prevalence increased with age, revealing the cumulative effect of age in toxoplasmosis seropositivity and was gender independent. Lesions were present in 9.95% of individuals studied. We observed that 87% of type A lesions (27/31) and 73% of type B (47/64) were present in seropositive subjects. The chance of being seropositive once the individual has developed a type A lesion is 10.3 higher as compared to individuals without a lesion. In comparison with those without lesions, the chance of being seropositive is of 5.3 for the type B lesions, (p<0.0001/99% IC). The levels of anti-*T. gondii* specific IgG and IgG1 did not correlate with the numbers of lesion per individual. The association between lesion size and immunoglobulin levels is still under investigation. In conclusion we found a strong relationship between the seropositivity to *T. gondii* antigens and the presence of ocular lesions type A and B.
Financial support: CNPq, CAPES, NIH.

IM.81 – IgG AND IgM WESTERN BLOT ASSAY FOR DIAGNOSIS OF CONGENITAL TOXOPLASMOSIS

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The aim of this study was to evaluate the use of Western blot (WB) in 215 newborn infants under suspicion of congenital toxoplasmosis. The children were submitted to clinical examinations to assess macular, neurological and hearing signals. The WB results obtained were compared to the persistence of IgG antibodies at the end of 12 months, regarded as the 'gold standard' diagnosis of congenital toxoplasmosis. It was also verified whether there was any association between the WB results and the clinical signals presented by the infants. Of the 215 children, 177 had confirmed congenital toxoplasmosis diagnosis, and 38 did not have the infection. IgG-WB showed sensitivity of 73.5% and specificity of 97.4%. IgM-WB showed sensitivity of 54.8% and specificity of 94.7%.The IgG-WB and IgM-WB combination increased the sensitivity to 86.5%. The IgM-WB positive children were found to have 1.4 times more risk to present active macular lesion than those with negative testing of IgM-WB. This study showed that WB is a useful tool in the confirmation of congenital toxoplasmosis diagnosis, and that the IgM-WB positive results can be an indication of active macular lesion in newborn infants. Financial support was provided by Secretaria do Estado de Saúde de Minas Gerais, Brazil; FAPEMIG (CBB-APQ-00129-09) and CNPq (301110/2009-3).

IM.82 – ANALYSIS OF ACUTE IMMUNE RESPONSE IN CD1$^{-/-}$ AND WILD TYPE C57BL/6 MICE INFECTED WITH *ENTAMOEBA HISTOLYTICA*

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Recent studies have shown that Natural Killer T (NKT) cells may represent a barrier against hepatic amebic abscess formation at acute phase of infection. The aim of the study was to characterize the immune components involved in the development and control of lesions caused acute infection with *E. histolytica* and its relationship with NKT cell activity. Frequencies of spleen and mesenteric lymph node (MLN) NKT, TCD4$^+$, TCD8$^+$, and B cells were analyzed in CD1-deficient C57BL/6 mice infected with *E. histolytica*. Four CD1 $^{-/-}$ C57BL/6 mice (Eh-CD1$^{-/-}$ group) and four wild type mice (Eh-WT) were inoculated via intracecal with 10.000.000 *E. histolytica* trophozoites of EGG strain. Control groups (CTRL-CD1$^{-/-}$ [N=4] and CTRL-WT [N=4]) were inoculated with sterile culture medium YI-S-32. 48hr after infection were collected spleen, MLN and caecum of the mice. Spleen and MLN cells were isolated for cell culture and analyzed by flow citometry. Caecum was analyzed by histopathology. The frequency of spleen NKT (CD3$^+$panNK$^+$) cells was reduced in CTRL-CD1$^{-/-}$ when compared to CTRL-WT groups (3.86 ± 0.39 to 2.61± 0.18) as expected since CD1 is a restriction element for NKT cells. Activated T cells were also reduced in CD1$^{-/-}$ mice but activated B cells were elevated. Cecal lesions have occurred more frequently and with higher magnitude in the animals of Eh-CD1$^{-/-}$ group. Thus, our results suggest that NKT cells may function as regulatory cells for the inflammatory effects of Eh infection.
Financial support: FAPEMIG

IM.83 – LOCAL IMMUNE RESPONSE TO TROPHOZOITES IN LIVER OF HAMSTERS INFECTED WITH *ENTAMOEBA HISTOLYTICA* AND *ENTAMOEBA DISPAR*

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Entamoeba histolytica is a protozoan parasite capable to penetrate and destroy the intestinal mucosa leading to the amoebic dysentery. Since then, trophozoites may reach the liver through blood circulation. Amoebic liver abscesses are the most frequent extraintestinal form of amoebiasis. The mechanisms involved in generating lesions by *E. histolytica* are not still completely understood, as well as the role of immune responses raised against trophozoites. In this study we evaluated the *in situ* binding of antibodies, C3 and C9 complement components on trophozoites, in livers of hamsters infected with *E. histolytica* and *E. dispar*. These parameters were correlated with the extension of the hepatic lesions observed in these animals and with trophofozoites survivor. Hamsters were inoculated intra-hepatically with 100,000 trophozoites of *E. histolytica* or *E. dispar* strain. Different groups of animals were necropsied 12, 24, 48, 72, 144 and 192h after inoculation. Antibodies, C3 and C9 binding to trophozoites were detected by immunohistochemistry. Although, binding of antibodies on trophozoites was searched for both strains, it was weaker in *E. histolytica* ($p<0.05$). Trophozoites of *E. dispar* were also more frequently vacuolated and high labeled cellular debris observed in the lesions. $C3^+$ and $C9^+$ trophozoites debris immunostaining was higher in livers of *E. dispar* than in livers of *E. histolytica*. Overall, these findings indicate that *E. histolytica* strain possesses an enhanced ability to evade the immune responses compared to *E. dispar,* although it also causes experimental hepatic lesions. This might be related to the larger amount and types of cysteine proteinases and expression of CD59-like lectin produced by *E. histolytica* trophozoites. The binding of antibodies and activation of the complement system were not sufficient to impair the progression of the amebian abscess, on the other hand, their action on trophozoites could promote selection of resistant subpopulations.
Financial support: FAPEMIG

IM.84 – CELL MIGRATION INDUZED BY DIFFERENT VACCINE ADJUVANTS IN MICE

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The use of the adjuvant for generation of effective cell-mediated immune response is of fundamental importance in vaccine against visceral leishmaniasis. In the context of innate immunity, inflammatory response deserves special attention, since it is rapidly initiated and independent of antigen, and precedes the onset of antigen-specific response. In these study was evaluated the inflammatory reaction and the activity of the enzymes myeloperoxidase (MPO) and N-acetyl-β-D-glucosaminidase (NAG) in the inoculation local by adjuvants: aluminum hydroxide ($AlOH_3$), saponin (SAP), Bacille Calmette-Guerin (BCG), monophosphoryl lipid A (MPL) and Freund Incomplete Adjuvant (FIA) at times (1, 12, 24, 48, 96 hours and 7, 14 days) in *Swiss* mice. The results demonstrated an increase in the cell recruitment in 1 hour in SAP and BCG groups until 14 days after the inoculum. In MPL group, this increase was observed after 12 hours. In FIA group this increase was visualized in 7 and 14 days. Any alteration in relation to number of cells circulation was observed in the $AlOH_3$ group. In concerning to the MPO activity enzyme, increase levels of this enzyme in BCG and AIF groups during 1 hour when compared with MPL. The increase of MPO was maintained in the BCG group rather than MPL group in 12 hours. Also, we analyzed the activity NAG enzyme and were observed an increase NAG levels in $ALOH_3$ group (1 hour) when compared to the groups (CS, SAP, BCG, MPL and AIF). In 96 hours, an increase of NAG levels occurred in SAP group when compared to MPL. In 14 days, were observed increased NAG levels in AIF group when compared to the CS group. These results will help in the understanding of the mechanism involved in the cell recruitment induced by adjuvants and thus may contribute in future vaccine formulations with the *Leishmania* antigens. Supported by PRONEX - FAPEMIG/CNPq and UFOP.

IM.85 – N VITRO BINDING AND SURVIVAL ASSAYS OF *LEISHMANIA* PARASITES TO MONOCYTES AND MONOCYTE-DERIVED MACROPHAGES ISOLATED FROM DOGS WITH VISCERAL LEISHMANIASIS AND TREATED WITH MEGLUMINE ANTIMONIATE-CONTAINING NANOMETRIC LIPOSOMES

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Canine visceral leishmaniasis (CLV), in America, is a zoonotic disease caused by *Leishmania (Leishmania) chagasi* (*syn* = *infantum*). It is endemic in several Latin American countries, where the parasite is transmitted to man and animals by infected blood-sucking sandflies of the genus *Lutzomyia* (Grimaldi et al. 1989). Our group, following the treatment protocol with meglumine antimoniate-containing lipossomes (LMA), has recently reported of both long-term parasite suppression and reduction of infectivity to sand flies in naturally-infected dogs (Ribeiro et al. 2008). The aim of this study was to evaluate the influence of LMA treatment in the in vitro binding and survival assays among amastigotes forms of *L. chagasi* and monocytes and macrophages derived from peripheral blood monocytes of dogs with CVL. Twenty four dogs was obtained from City Hall Zoonosis Department of Ribeirão das Neves (Metropolitan area of Belo Horizonte, MG) and divided in three groups of eight animals. Monocytes were obtained from the peripheral blood and resuspended to 3.0×10^6 cells/mL. Then, these cells were distributed in plates of 24 wells. The binding and the survival assays were carried out during 1 and 48 hours, respectively. The same protocol was applied to monocyte derived macrophages obtained after ten days cultures. The numbers of monocytes and monocyte derived macrophages bound to *Leishmania* promastigotes were significantly increased when C5D serum was used during the interaction with the canine cells. Moreover, parasite survival, characterized by the presence of amastigote forms of *Leishmania* in macrophages, was also significantly increased in the presence of serum ($p < 0.01$) in despite of the treatment with LMA.
Supported by CNPq Process 504252/2008-9