IM001 - **SYNTHETIC PEPTIDES ARE RELIABLE TOOLS TO DIAGNOSE CANINE VISCERAL LEISHMANIASIS BUT NOT FOR HUMAN VISCERAL LEISHMANIASIS**

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In attempt to contribute to the control measures for Visceral Leishmaniasis, we have been working to identify antigens that can be used to improve the diagnosis of this disease. In a previous study, using proteomic approach (bidimensional electrophoresis and Western Blot), we identified 47 immunodominant proteins from Leishmania infantum (= L. chagasi). These proteins were analyzed using bioinformatic tools to predict B cell epitopes, providing 360 peptides, which were synthesized in cellulose membranes. After immunoassays, the best peptides were selected to be synthesized by solid phase technique, in order to be used as antigens in ELISA. These peptides showed great performances when used to diagnose canine serum samples, with sensitivity between 70,9 and 88,7%, and specificity between 55,0 and 95,0%, according to the peptide. In order to evaluate these peptides in human visceral leishmaniasis (HVL) diagnosis, we tested serum samples from 80 patients with parasitological diagnosis of HVL and serum samples from 20 healthy patients in immunoenzimatic assays. Peptides were used in solution prepared with PBS (20μg/μL). The sensitivity, according to the different peptides, varied from 53,7 to 75,0% and the specificity varied from 52,6 to 63,1%. The tests showed low accuracy, with AUC ranging from 0,54 to 0,69. These results suggest that synthetic peptides in solution are not reliable tools to diagnose HVL in immunoassays and indicate that some optimizations must be done, such as the use of carriers to improve peptides immunogenicity.
Supported by:CAPES, CNPq, FAPEMIG

IM002 - **MILTEFOSINE EXERTS ITS LEISHMANICIDAL ACTION VIA PAF RECEPTOR**

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Miltefosine, first used as treatment in patients with cancer, has been used as an effective oral drug in visceral leishmaniasis. There is a structural similarity between miltefosine and platelet activating factor (PAF). PAF acts by binding to the PAF receptor (PAFR) present in target cells. The mechanism by which the drug works is not well established. Our hypothesis is that miltefosine uses the PAF receptor to bind to the cell. To test this hypothesis, we used wild-type mice (wt) and mice deficient in the PAFR (PAFR-KO). In vitro infections with L. donovani revealed that macrophages (MØ) from wt mice treated with miltefosine were more effective in controlling the growth of the parasite than MØ from PAFR-KO mice. However, the treatment did not induce production of significant amounts of nitric oxide (NO), which may indicate a cytotoxic mechanism independent of NO. Our data also showed that treatment with the drug alone did not alter the activity of the enzyme arginase, but in combination with IFN-γ and LPS was capable of downregulating its activity. In vivo infection with L. donovani showed no differences in susceptibility between wt mice and PAFR-KO. Treatment with different doses of miltefosine led to a reduction in parasite load in the liver and spleen of wt mice. Interestingly, PAFR-KO mice showed a 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 supernatant of splenocyte cultures and their effect on the expression of activation markers in MØ. Our results indicate that part of the antileishmanial activity of miltefosine is mediated by PAFR and that, in addition to a direct cytotoxic effect on Leishmania, miltefosine has an immunomodulatory effect.
Supported by:CNPQ, CAPES, FAPEMIG

IM003 - **EVALUATION OF THE INTERACTION BETWEEN NEUTROPHILS AND LEISHMANIA BRAZILIENSIS**

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Experimental cutaneous leishmaniasis caused by *L. braziliensis* (Lb) is characterized by a necrotic lesion in the skin that heals spontaneously after ten weeks. In parallel to lesion development and cicatrization, an intense influx of polimorphonuclear neutrophils (PMN) is observed at the infection site. We have previously shown that PMN depletion leads to increased lesion size and parasite load and, based on these observations, our objective was to evaluate the interaction between PMNs and *L. braziliensis*. PMNs, obtained by thyoglicolate stimulation, were magnetically separated and infected with serum-opsonized *L. braziliensis* (1:2). Infected PMNs were seen with 1h of infection and infection rate increased with time, similarly with the number of amastigotes per cell. PMNs exposed to *L. braziliensis* showed increased expression of CD18, CD49d and CD62L as well as enhanced production of myeloperoxidase, superoxide and neutrophil elastase. However, Lb-exposed PMNs did not show up-regulation in TLR2 or TLR4 expression. Moreover, both serum-opsonized and stationary *L. braziliensis* induced PMN apoptosis, differently from *L. major*. *In vivo*, we observed that *L. braziliensis* inoculation into the ear dermis of BALB/c mice promotes rapid PMN recruitment to the infection site and, at later time points, PMNs were observed within draining lymph nodes, indicating migration to secondary lymphoid organs, as seen with expression of surface molecules. Importantly, supernatants from PMNs exposed to *L. braziliensis* promoted migration of dendritic cells (DC), suggesting a modulation in DC function towards the development of the adaptive immune response. We can conclude that PMNs have mechanisms to control *L. braziliensis* infection and, in parallel, interaction of PMNs and DCs may favor the development of the adaptive immune response towards disease cure.
Supported by:CNPq

IM004 - **RECOGNITION OF NEOSPORA CANINUM BY DECTIN-1 INDUCES DOWN-MODULATION OF IMMUNE RESPONSES IN MICE**

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The parasite Neospora caninum has been associated to abortions in cattle since the early 1990's. In order to unravel the initial host-parasite interactions, our group has recently demonstrated the importance of Toll-like receptors in innate recognition of N. caninum. TLR2 is required for IL-12/IL-23p40 production and MHCII/B7 expression by dendritic cells exposed to parasitic antigens, which in its turn is required for an appropriate Th1 induction after infection by the protozoan. In that sense, this work aimed to evaluate the role of the C-type lectin receptor Dectin-1 during the infection by N. caninum. In order to observe the role of Dectin-1 in cytokine production after exposure to live parasites, Bone marrow-derived macrophages (BMMs) were pretreated with Laminarin (LAM; competitive inhibitor of Dectin-1) and co-cultured with different concentrations of N. caninum for 24 hours. After this period the supernatants were collected for cytokine (IL-12/IL-23p40, IL-6 and IL-10) determination by commercial ELISA kits. The experiments revealed that pretreatment with LAM resulted in an increment of proinflammatory cytokine production by BMMs infected with N. caninum, in a dose-dependent manner, independently of induction of anti-inflammatory cytokines as IL-10. To observe the role of dectin-1 in vivo, groups of mice were pretreated or not with LAM and infected with N. caninum for determination of acute ROS and NO production, chronic phase parasitism and inflammation in the central nervous system, antibody production, and survival. Mortality was inhibited in 50% of LAM-treated mice upon lethal parasite challenge. In association, it was observed a higher percentage of ROS+ peritoneal cells and lower parasite burden in acute infection. In a chronic model of infection, LAM-treated mice presented similar specific IgG production, however with lower IgG1 levels, as well as reduced parasitism and brain inflammation. The gathered data indicate that Dectin-1 is involved in parasite evasion of pro-inflammatory responses by the host and, in that sense, the results gathered here indicate that this signaling pathway is a promising target for future development of prophylactic and therapeutic protocols against neosporosis.
Supported by:CNPq, CAPES, Fapemig

IM005 - **IDENTIFICATION OF CONSERVED AND POLYMORPHIC EPITOPES IN DISTINCT TRYPANOSOMA CRUZI STRAINS WITH POTENTIAL APPLICATION FOR SEROTYPING**

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The factors influencing the variation in the clinical manifestation of Chagas disease have not been elucidated, but it is likely that genetic of the host and parasite is involved. Several studies trying to correlate the parasite strain and clinical manifestation have used hemoculture or PCR-based genotyping. Hemoculture requires parasite isolation from patient blood and growth in animals or in vitro cultures and offers opportunity for subpopulation selection. Further, the parasitemia in the chronic phase is very low. Parasite genotyping directly from infected tissues is a very invasive and hampers studies with large numbers of samples. The goal of this work is to identify *T. cruzi* conserved and polymorphic B-cell linear epitopes that could be used for serodiagnostic and serotyping using ELISA. We have performed B-cell epitope prediction on proteins derived from single copy genes represented by pair of alleles in the CL Brener genome. The rationale behind this strategy is that because CL Brener is a recent hybrid between TcII and TcIII lineages, it is likely that polymorphic epitopes in its pair of alleles could also be polymorphic in the parental genotypes. We have excluded epitopes also presented in *L. major*, *L. infantum* and *L. braziliensis* to minimize the chance of cross-reactivity. The reactivity of 150 peptides linked on a cellulose membrane has been tested using sera from C57BL/6 mice chronically infected with Colombiana (TcI) and CL Brener (TcVI) clones and Y (TcII) strain. A total of 36 peptides were reactive and the cross-reactivity between the strains is in agreement with the evolutionary origin of the different T. cruzi lineages. Five peptides were tested by ELISA against a panel of chagasic patients. We obtained 84% of sensibility and 93% of specificity with a conserved peptide. A polymorphic peptide recognized 70% of patients infected with TcII isolates and patients with chagasic cardiopathy had higher reactivity levels than undetermined clinical form.
Supported by:INCTV, FAPEMIG, CNPq, CAPES

IM006 - **TOLL RECEPTORS TYPE-2 IN PERIPHERAL BLOOD MONOCYTES AND ITS CORRELATION WITH CD11B/CD18 EXPRESSION AND XENODIAGNOSIS IN CANINE VISCERAL LEISHMANIASIS**

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The aim of the present study was to investigate TLR2 expression in peripheral blood monocytes cells (PBMC) from dogs naturally infected with *Leishmania infantum* to determine whether it correlates with CD11b/CD18 expression, and to evaluate the potential of dogs as sources of infection using phlebotomine xenodiagnosis (XENO). Forty eight dogs were serologically diagnosed with *L. infantum* infection by indirect immunofluorescence and enzyme linked immunosorbent assay. All infected dogs were defined as symptomatic under physical clinical exams. Parasitological exams from bone-marrow aspirates were positive by PCR analysis. Ear skin tissue samples were obtained for immunohistochemistry (IHQ) analysis. The potential of these dogs as a source of infection using phlebotomine XENO was evaluated. Density gradient separation was used to enrich for PBMC and flow cytometry was carried out on PBMC cells using mouse anti-human monoclonal antibodies anti-CD14, anti-TLR2, anti-MHCII and mouse anti-canine anti-CD11b. IHQ ear skin tissue parasite load and XENO were done where we found a strict correlation (r=0.4787). Infected dogs with higher expression of MFI of CD11b inside CD14 monocytes were represented by dogs without parasite ear tissue load that were unable to infect phlebotomines ($IHQ^-/XENO^-$) (p=0,0032). In contrast, infected dogs with lower expression of MFI of CD11b inside CD14 monocytes were represented by dogs with parasite ear tissue load and able to infect phlebotomines ($IHQ^+/XENO^+$). Comparable results were obtained for MFI of MHCII (p=0.0054). In addition, considering the population frequency of $CD11b^+TLR2^+$ and $CD11b^+MHCII^+$, higher values were obtained from dogs with $IHQ^-/XENO^-$ than dogs with $IHQ^+/XENO^+$ (p=0.01; p=0.0048, respectively). In addition, NO assays results showed higher levels for dogs with $IHQ^-/XENO^-$ than dogs with $IHQ^+/XENO^+$. These results led to the conclusion that $IHQ^-/XENO^-$ dogs are more resistant or could modulate the cellular immune response essential for *Leishmania* tissue clearance.
Supported by:CNPq, FAPEMIG

IM007 - **RETINOIC ACID ENCAPSULATED IN SOLID LIPID NANOPARTICLES IS AN ADJUVANT TO THE ANTILEISHMANIAL LAAG INTRANASAL VACCINE**

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Vaccination with *Leishmania amazonensis* promastigotes total antigen (LaAg) induces protection in mice against *L. amazonensis* infection when given by oral and intranasal routes, contrasting with the disease-promoting effect when given parenterally (Pinto *et al*., 2003; 2004). Retinoic acid (RA), a metabolite from dietary retinol required for the efficient differentiation of naïve T cells to $FoxP3^+$ regulatory T cells in the intestinal mucosa, has been associated with oral tolerance (Sun *et al*., 2006). Little is known about tolerance induction in nasal mucosa. In this study, the role of RA as an intranasal adjuvant to the LaAg vaccine was investigated. Since RA may be irritating to the nasal mucosa, RA encapsulated in solid lipid nanoparticles (SLN) at 0.1 % (RA-SLN) was employed. Thus, BALB/c mice were intranasally immunized with two doses of LaAg (10 µg of protein) with a 7 day interval in the presence of 15 µL of RA-SLN. Empty SLN were used as a control. Seven days after the last dose, mice were infected subcutaneously in the footpad with $2x10^5$ *L. amazonensis* promastigotes and lesion development was monitored periodically. On day 60 post infection, the parasite burden and the cytokine profile were evaluated in the infection site. The lower lesion development and the decreased parasite burden indicated that RA-SLN improves the LaAg intranasal vaccine efficacy. Moreover, animals vaccinated with LaAg in the presence of RA-NLS produced higher levels of IFN-γ, IL-4, IL-10 and TGF-β in the infection site. These results suggest that RA-mediated tolerance improved the LaAg vaccine efficacy possibly by modulating the peripheral immune response to parasite antigens.
Supported by:CAPES

IM008 - **CASPASE-1 IS REQUIRED TO IL-33 PRODUCTION AND MURINE PROTECTION IN THE INFECTION BY T. CRUZI.**

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The Toll-like receptors (TLRs), 2, 4 and 9 are important to immune protection against *Trypanosoma cruzi*, the etiologic agent of Chagas disease. A new family of pattern recognition receptors, Nod-like receptors (NLRs) was described, including NLRP3, which associates to ASC forming a complex that actives caspase-1, called inflammasome. This molecular complex is necessary for cleavage of the active forms of the IL-1β, IL-18 and IL-33. Here, we investigated the role of caspase-1 in the immune response against *T. cruzi*. First we asked if *T. cruzi* triggers caspase-1 activation in murine bone marrow-derived macrophages (BMMs). We found high caspase-1 activation in BMMs from WT mice, but not from $ASC^{-/-}$ mice. The blockage of potassium efflux, oxygen radicals reactive (ROS) and B catahepsin (activators of caspase-1 via ASC) resulted in inhibition of caspase-1 activation in BMMs from WT mice. To understand the role of caspase-1, WT, $caspase\text{-}1^{-/-}$ and $ASC^{-/-}$ mice were infected with Y strain of *T.cruzi*. Our results showed that $ASC^{-/-}$ and $caspase\text{-}1^{-/-}$ mice presented higher mortality, whereas WT are resistant to infection. In addition, $caspase\text{-}1^{-/-}$ mice presented higher myocarditis, increased cardiac damages and more parasites in the heart tissue on day 17 p.i. Curiously, the IL-33 production, quantified by ELISA, on the heart was reduced in $ASC^{-/-}$ and $caspase\text{-}1^{-/-}$ when compared with WT infected mice. The role of IL-33 was observed in ST2 deficient mice (receptor of IL-33) infected by *T. cruzi*. We observed higher mortality of $ST2^{-/-}$ as compared with WT mice. Together, these results suggest that caspase-1 dependent of ASC is important to cleavage of IL-33, which is required to protection in the experimental infection against *T. cruzi*. These results reveal new mechanisms involved in the innate immunity against this parasite, which could contribute to new approaches in the control of the Chagas disease.
Supported by:FAPESP

IM009 - **IMPACT OF AMASTIGOTES OF TRYPANOSOMA CRUZI ON CARDIOMYOCYTE APOPTOSIS AND THE IMMUNOLOGICAL ROLE OF THEIR GLYCOLIPID**

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The haemoflagellate Trypanosoma cruzi is the causative agent of the protozoan zoonotic Chagas' disease, or American trypanosomiasis that affects approximately eight million people in Latin America. The parasite is transmitted by blood-sucking insects of the triatomine species, blood transfusion, organ transplantation or congenitally. Recent incidence rates show an increase in Chagas outbreaks and vector propagation in South America after the vector has been successfully eradicated in the nineties.
The acute phase of disease triggers an immune response that restricts the dissemination and proliferation of parasites. However, parasites are able to persist in different tissues for decades causing the characteristics of Chagas' disease, cardiomyopathy and megasyndromes of the gastrointestinal tract and can even lead to death. Infection with T. cruzi, elicits macrophages to produce high levels of pro- inflammatory cytokines. Even though parts of the host-parasite interactions have been elucidated, many interactions of the parasite with the host cell remain in many aspects undefined. In that context, glycosylphosphatidylinositols (GPIs), components of a dense surface coat of glycolipids expressed by T. cruzi play possible roles. GPIs serve ubiquitously as anchors for proteins, complex carbohydrates or mucins and are known to induce an inflammatory response in macrophages. My diploma thesis describes a new method of purifying amastigote forms of T. cruzi and deals with the potential capability of GPIs of amastigotes to induce an pro- inflammatory response in macrophages in vitro. Secretion of TNF and IL-12, as well as nitric oxide (NO), incubated with purified GPIs has been observed. Furthermore, the induction of apoptosis in rat cardiomyocytes was determined after incubation with amastigote parasites. These results suggest that cardiomyopathy observed in Chagas' disease might be in part due to local inflammation in response to the GPIs and to apoptosis of cardiomyocytes induced by T. cruzi.

IM010 - **LEISHMANIA MAJOR INHIBITOR OF SERINE PEPTIDASES, ISP2, PREVENTS THE ACTIVATION OF MAMMALIAN PKR, PROMOTING PARASITE SURVIVAL IN MACROPHAGES**

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Ecotins are bacterial proteins that inhibit S1A serine peptidases such as neutrophil elastase (NE) and cathepsin G. *Leishmania major* has three genes similar to ecotin, *ISP*1, *ISP*2, *ISP*3. We showed that ISP2 prevents the activation of NE at the surface of murine macrophages during parasite phagocytosis. *L. major* mutants deficient in *ISP*2 and *ISP*3 (Δ*isp*2/3) are internalised more efficiently by macrophages but have diminished capacity to develop inside those cells. The increased phagocytosis of Δ*isp*2/3 requires CD11b,TLR4 and NE. Recently, it was described that *L. amazonensis* leads to the activation of the macrophage's serine/threonine kinase, PKR. PKR activation modulates the production of IL-10, influencing parasite survival and growth within macrophages. PKR can be activated by a variety of stimuli, and ligands to either TLR2 or TLR4 induce PKR phosphorylation. We asked if Δ*isp*2/3 activates PKR as a result of the triggering of the TLR4-NE pathway. Δ*isp*2/3 are internalised more efficiently than wild type parasites by RAW macrophages, but display reduced survival and intracellular growth. The phagocytosis of Δ*isp*2/3 returned to the levels of WT in the RAW line expressing dominant-negative PKR (RAW-DN-PKR), and the mutant parasites survived and proliferated normally in those cells. Blockade of TLR4, TLR2 or CD11b, or the inactivation of NE diminished the phagocytosis of Δ*isp*2/3 by RAW, while those treatments had no effect on parasite uptake by RAW-DN-PKR. The neutralisation of TLR4 or TLR2, but not of CD11b, before parasite phagocytosis, prevented the subsequent death of Δ*isp*2/3 in RAW within 24h, but did not influence parasite survival in RAW-DN-PKR. Furthermore, antibodies to IL-10, but not to TNF-a, restored the growth and survival of Δ*isp*2/3 in RAW. We propose that, in the absence of ISP2, the unregulated activity of NE coupled to the mobilisation of TLR4 and TLR2 by *L. major* results in the activation of PKR, influencing parasite growth and survival in macrophages.
Supported by:The Wellcome Trust/CNPq

IM011 - **CHARACTERIZATION OF REGULATORY T CELLS (TREGS) FUNCTION IN PATIENTS WITH CUTANEOUS LEISHMANIASIS DUE TO LEISHMANIA BRAZILIENSIS INFECTION**

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Patients with cutaneous leishmaniasis (CL) due to L. braziliensis develop strong Th1 responses, but do not necessarily heal the lesions. Sub-clinical patients (SC), however, present milder Th1 responses, and are able to control parasite replication, suggesting that excessive Th1 responses may be related to tissue damage. Tregs are important modulators of Th1 responses, and impaired Treg function results in excessive inflammation. Regarding this, our objective was to characterize the role of Tregs in human infection with L. braziliensis. PBMC from CL patients, non-infected donors (ND) and SC patients were phenotyped by flow cytometry and the suppressive functions of CD4+CD25+ and CD4+CD25- populations evaluated. Also, IFN-γ, TNF-α, IL-27, IL-10, TGF-β, FoxP3 and IL-10R mRNA expression in the lesions was measured. The frequency of Tregs in ND was higher than in CL and SC patients (ND>CL>SC). CD4+CD25+ cells from CL patients suppressed PBMC proliferation similarly to CD4+CD25+ cells from ND or SC patients. Also, CD4+CD25+ cells from CL patients suppressed IL-17, IFN-γ and TNF-α production similarly to ND or SC patients CD4+CD25+ cells. Interestingly, we observed that increased amount of CD4+CD25- cells from ND and CL patients in the cultures, resulted in decreased proliferation of PBMC. Also, mainly in CL patients, IL-10 and TGF-β production was higher inside CD4+CD25- cells, that were the main producers of pro-inflammatory cytokines. In the lesions, the pro-inflammatory cytokines expressions were positively correlated with anti-inflammatory cytokines. We conclude that the excessive Th1 response in CL patients is not related to impaired Treg function. However, these results suggest that in CL patients, L. braziliensis may trigger production of anti-inflammatory cytokines by effector or non classical Treg cells (CD4+CD25-), which may interfere in parasite replication control, and strategies that interfere with this escape system may improve leishmaniasis treatment.
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IM012 - **IFN-GAMMA PLAYS A PIVOTAL ROLE IN CONTROLLING INFECTION BY LOW VIRULENT TRYPANOSOMA CRUZI STRAIN**

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Trypanosoma cruzi is the etiologic agent of Chaga´s disease. *T. cruzi* strains have been divided into six discrete typing units (DTUs) according to their genetic background. These groups are designed *T. cruzi* I to VI. In this context, extracellular amastigotes from G strain (*T. cruzi* I) are highly infective *in vitro* and show no parasitemia *in vivo*, while amastigotes from CL strain (*T. cruzi* VI) are low infective *in vitro* and highly infective *in vivo*. Here we aimed to understand why amastigotes from G strain are highly infective *in vitro* and does not contribute for a patent *in vivo* infection. Our *in vitro* studies demonstrated the first evidence that IFN-γ would be associated to the low virulence of G strain *in vivo*. After, infection of wild-type and knockout mice for TNF-α, iNOS, IL-12, IL-18 and IFN-γ we found that the latter is crucial for controlling infection by G strain amastigotes. Our results showed that amastigotes from G strain are highly infective *in vitro* but did not contribute for a patent infection in vivo due to its susceptibility to IFN-γ production by host immune cells. These data could be useful to understand the *T. cruzi* multiple behavior in *in vivo* infection by different strains.
Supported by:FAPEMIG/CAPES/CNPQ/FAPESP

IM013 - **REGULATORY EFFECTS OF ARYL HYDROCARBON RECEPTOR (AHR) ON CYTOKINE PROFILES AND DEVELOPMENT OF PATHOLOGY DURING TRYPANOSOMA CRUZI INFECTION**

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Trypanosoma cruzi infection is an appropriate and interesting model to understand the balance between effective immune response and immune-pathology. Lipoxins$(LXA)_4$, an anti-inflammatory eicosanoid, is produced by the activation of the enzyme 5-lypoxigenase and it is important in the regulation of inflammatory cytokines during *T.cruzi* infection. The role of aryl hydrocarbon receptor(AhR), the nuclear receptor for LXA, during *T.cruzi* infection is not known. Herein, we infected wild type(WT) and AhR-/- mice with *T.cruzi* (Y strain) and the parasitemia was periodically assessed. The spleens, livers and hearts were harvested at different days post-infection(dpi) for histology or cytokines analyses by RT-PCR and/or flow cytometry. We found reduced parasitemia in AhR-/- mice when compared with WT. AhR deficiency resulted in the high levels of IL12 and IFN-γ production, associated with increased number of dendritic and T cells at 10dpi. The inhibition of pro-inflammatory cytokine production was observed in WT, but not in AhR-/- mice, 15dpi. The AhR deficiency also resulted in increased inflammation in the heart and liver 10dpi when compared with WT counterparts. However, 21dpi hearts, but not livers, from infected AhR-/- mice exhibited less intense inflammation when compared with infected WT. In vitro, we investigated which was the mainly factor responsible for the increased efficiency to control the parasite grown in infected AhR-/- mice. We found an increased trypanocidal activity by *T.cruzi*-infected AhR-/- macrophages (M) when compared with WT. Nevertheless, no significant difference in NO levels was detected in the supernatants harvested from *T.cruzi*-infected-AhR-/- and WT M cultures. By contrast, an increased NO production by AhR-/- M was detected when IFN-γ was added in the uninfected cultures when compared with WT. Our data suggests a critical role of AhR in the modulation of immune response and development of pathology during experimental T.cruzi infection.
Supported by:CNPq e FAPEMIG

IM014 - **LEISHMANIA (VIANNIA) SHAWI MODULATES CYTOKINE PRODUCTION OF DENDRITIC CELLS**

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Dendritic cells (DC) are powerful antigen-present cells (APC) which can be considered as initiator and modulator of the immune responses against pathogens. Some works demonstrated that *L. (L.) amazonensis* and *L. (V.) braziliensis* parasites can inactivate and activate DC functions, respectively. However, there is no information regarding the modulation of dendritic cells by *L. (V.) shawi*. In this way, bone marrow-derived DCs (10^5/well) were infected with amastigotes or promastigotes of *L. (V.) shawi* parasites (1 or 10 forms)/DC during 24h. Then, 5 $CD4^+$ or $CD8^+$ T lymphocytes/DC were added to the culture, and 24h later, the supernatants were collected to quantify the levels of IL-2, IL-12, IL-4, IL-10 and TNF-a by ELISA. The parasitism of DCs also has been analyzed. After infection, IL-10 was detected in all group of infected DC, and it was high after infection with 10 amastigotes/DC; IL-12 production was induced by 10 promastigotes/DC; and TNF-a showed increased level after infection with 10 amastigote or promastigote/DC. The interaction of $CD4^+$T lymphocytes with amastigote-infected DCs leads to increasing of IL-10 and decreasing of both IL-12 and TNF-a; however, interaction of $CD8^+$T lymphocytes with 10 amastigote-infected DCs leads to mild production of IL-12 and TNF-a, but the maintenance of high amounts of IL-10 was verified. The interaction of both T lymphocytes populations with promastigote-infected DCs inhibited the production of inflammatory cytokines, but low level of IL-10 was maintained. The parasitism of DC infected with 1 amastigote or promastigote/DC was similar, however it was lower compared to infection using 10 parasites/DC. IL-2 and IL-4 have been not detected. The results indicate that amastigotes and promastigotes can induce different immunological response by DCs through up-regulation of IL-10 and down-regulation of IL-12 and TNF-a after infection with amastigotes and promastigotes, respectively. Supported by FAPESP and LIM50-HCFMUSP.

IM015 - **SOCS2 MODULATES IMMUNE RESPONSE AND CARDIAC FUNCTION DURING EXPERIMENTAL TRYPANOSOMA CRUZI INFECTION**

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Background. Infection with Trypanosoma cruzi results in a robust inflammatory reaction in many organs that limits parasite proliferation and results in tissue damage. The regulation of inflammation during this infection is multifactorial involving many complex pathways including the enzyme 5-Lipoxygenase (5-LO) pathway. 5-LO induces suppressor of cytokine signaling (SOCS)2 expression which in turn regulates cytokine production. Methods and results. In the present study, first we investigated the induction of SOCS2 during T. cruzi infection and its effects in the development of the immune response and cardiac pathology. We infected WT and SOCS2 Knockout (KO) mice with the Y strain. When compared with WT mice, the parasitemia was significantly reduced in SOCS2 KO mice. The expression of IFN-γ, TNF-α, IL-6, IL-10, SOCS1 and SOCS3 was reduced in the spleen of SOCS2 KO infected mice. The expression of IFN-γ, TNF, SOCS1 and SOCS3 was also reduced in the hearts of infected KO mice. Moreover, the modulation of the generation/expansion of T regulatory and memory cells during the T. cruzi-infection is dependent of SOCS2. In addition, our electrophysiological and echocardiogram analyses suggested that SOCS2 deficiency during T. cruzi infection results in altered cardiac remodeling by increasing cardiac ventricular mass and reducing calcium and potassium levels in ventricular myocytes. Conclusions. Taken together, the observations indicate a role for SOCS2 in the regulation of the inflammatory response and development of cardiac pathology during experimental T. cruzi infection.
Supported by:CNPq e FAPEMIG

IM016 - **FACTORS THAT MODULATE INFECTIVITY OF AN AVIRULENT TRYPANOSOMA CRUZI STRAIN**

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T. cruzi is constituted by genetically heterogeneous populations with diverse characteristics.
The parasite is widespread and may be harbored by numerous wildlife mammalian species including opossum, which is an important reservoir of T.cruzi. Depending on the parasite genotype, it is possible that the course of infection in different host species is differentially modulated. Here we aimed at investigating the factors that modulate T. cruzi infectivity in mice, by using G strain, isolated from an opossum (Didelphis marsupialis), and Y strain as a highly virulent control. Mice infected orally or intraperitoneally (i.p.) with 106 metacyclic trypomastigotes (MT) or tissue culture trypomastigotes (TCT) of G strain failed to produce patent parasitemia or mortality, in sharp contrast to Y strain. Histological sections of diverse organs, collected from G strain-infected mice three weeks later, displayed inflammatory cellular infiltrate and very few parasite nests, in the heart only. By contrast, Y strain-infected mice displayed tissue damage and high number of parasite nests in the heart, spleen and liver. G strain-infected mice challenged i.p. with 5x104 Y strain TCT were protected against acute infection and mortality. Adoptive transfer of total T cells (CD3+) from G strain-infected mice to nude mice, which were challenged with 5x104 TCT 24 hs later, resulted in reduced parasitemia and no mortality up to 10 months post-challenge. We are currently investigating the immune response profile induced by G strain in mice and whether surface molecules play a role in this protective response. It has previously been shown that mice immunized with MT surface glycoprotein gp90, which negatively regulates host cell invasion, can be protected against acute infection by virulent, highly invasive T. cruzi strain MT.
Supported by:CNPq, CAPES e FAPESP

IM017 - **PHOSPHATIDYLSERINE EXPOSURE ON INTRACELLULAR AMASTIGOTES OF LEISHMANIA AMAZONENSIS IS A SENSOR OF MACROPHAGE ACTIVATION AND MODULATES IN VIVO INFECTIONS AND DENDRITIC CELL FUNCTIONS.**

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L. amazonensis parasites cause diverse forms of leishmaniasis in humans and persistent lesions in most inbred strains of mice. In both cases, the infection is characterized by a marked immunossupression of the host. We previously showed that amastigote forms make use of exposed phosphatidylserine (PS) molecules to infect and to promote alternative activation of MΦs, leading to uncontrolled intracellular proliferation of the parasites. Moreover, the amount of PS molecules exposed on the surface of amastigotes correlates with the susceptibility of the host. Now we aimed to understand the immunological mechanisms that control PS exposure on intracellular amastigotes and to determine whether is possible to revert the immunossupression of the infected host by blocking the recognition of PS molecules on the parasite's surface. PS exposure is a feature of intracellular amastigotes that is modulated by MΦ immune activation. L. amazonensis infection generates an unpolarized CD4+ T cell activation providing the optimal MΦs stimulation that consistently up-regulates PS exposure on intracellular amastigotes. Stimulation of low levels of iNOS expression is mandatory to up-regulate PS exposure on the parasite whereas concomitant activation of Arginase I prevents parasite death. Anti-PS antibody treatment decreases parasite tissue loads and induces increased DCs activation and T cell proliferative responses upon exogenous or parasite antigen stimulation. However, there is no induction of protective Th1 or inflammatory responses. Our data clarify the role of pathogenic T cells for disease progression and point to PS as a critical parasite strategy to subvert host immune responses. Moreover, we observed that anti-PS antibody treatment ameliorates the disease by leading to decreased parasite loads, but is not sufficient to induce protective T cell responses, probably due to endosomal recognition of PS on intracellular amastigotes, which cannot be blocked by antibody treatment.
Supported by:NIH, CAPES, CNPq

IM018 - **C57BL/6, BALB/C AND BALB/C NUDE IMMUNE MODULATION ON LEISHMANIA AMAZONENSIS AMASTIGOTES EXPRESSION OF POTENTIAL SURVIVAL RELATED PROTEINS**

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Leishmaniasis is a zoonosis caused by *Leishmania* parasites, which may produce different clinical forms according to the parasite species and the host immune status. Studies of molecules involved in immune evasion have already been performed, but no analysis of the effect of host immune system on the expression of these molecules in amastigotes has ever been described. The present study investigates the modulation of the immune system of three mice strains- BALB/c, BALB/c nude and C57BL/6 on the expression of proteins (Metacaspase, PDI, STI and LACK) potentially involved in *L. amazonensis* (LV79 strain) survival. We also aim to evaluate the pattern of immune response developed by each animal during infection. Western Blottings performed with protein extracts of promastigotes and amastigotes isolated from the three mouse strains indicated that the four proteins were expressed in both forms. When we used sera from infected animals BALB/c showed recognition of more proteins than C57BL/6. These data were confirmed by ELISA assays using extract of promastigotes, showing higher levels of IgG in BALB/c, followed by C57BL/6 and BALB/c nude. Analyses of IgG subtypes (IgG1, IgG2a and IgG2c) are under way to verify the pattern of immune response in each strain. Real Time RT-PCR was used to compare the expression of Mgl2, INF-γ, CD68, IL-1β, TGF-β, IL-4, CD3e, iNOS and TNF-α genes in spleens and lymph nodes from the three types of infected mice. In lymph nodes of C57BL/6 Mgl2 and CD68 were significantly increased compared to BALB/c and BALB/c nude, and IL-4 and CD3e were less expressed in BALB/c nude, as expected. In spleens a decrease in IL-1β and CD3e in BALB/c nude and IL-4 in C57BL/6 and BALB/c nude were observed, while INF-γ was not differentially expressed in C57BL/6 and BALB/c, showing no clear dichotomy of Th1 x Th2 responses. This gene was equally expressed in BALB/c nude, possibly due to the activity of NK cells.
Supported by:FAPESP E CNPq

IM019 - **LEISHMANIA BRAZILIENSIS ISOLATES DIFFERING IN ECTO-NUCLEOTIDASIC ACTIVITIES DISPLAY DISTINCTIVE BIOLOGICAL FEATURES**

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Leishmania braziliensis has been associated with a broad range of clinical manifestations ranging from simple cutaneous ulcers to destructive mucosal involvement. Factors or mechanisms leading to this diversity of clinical presentations are not well known, but parasite factors have lately been recognized as important. Since ecto-nucleotidases have a crucial role in metabolism of extracellular nucleotides, which can be correlated to parasitism and the development of infection, we focused our study on the activity of these enzymes in L. braziliensis isolates as a possible parasite-related factor that could influence the clinical presentation of disease. Our results show that isolates obtained from patients with mucosal leishmaniasis (ML) hydrolyze higher amounts of adenine nucleotides than isolates obtained from patients with cutaneous leishmaniasis (CL). We evaluated the biological features of two isolates with high (PPS6) and low (SSF) enzymatic activity and observed the correlation between ecto-nucleotidasic activity and virulence. The lesions caused by PPS6 (ML isolate) were larger and persisted longer than lesions caused by SSF (CL isolate), and lesion development was accompanied by increased parasite load in footpad, evaluated by limiting dilution assay. Furthermore, macrophages infected with PPS6 were not able to control the infection after 48 hours and produce less nitric oxide than SSF-infected macrophages. Interestingly, PPS6 infects a higher proportion of DC than SSF. In addition, dendritic cells infected with promastigotes of PPS6 and SSF showed decreased expression of MHCII and CD86, however this decrease was more evident in PPS6 infected cells. Thus, these data suggest that the ecto-nucleotidases may interfere with the establishment of the immune response with consequent impaired ability to control the infection and this may be an important factor for clinical signs of leishmaniasis.

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IM020 - **SIGNALING PATHWAYS ASSOCIATED WITH NEUTROPHIL EXTRACELLULAR TRAPS INDUCTION BY LEISHMANIA, PMA AND FMLP.**

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Leishmania is inoculated in a pool of blood in the vertebrate host and neutrophils are the major leukocytes in blood, and are recruited to the site as well. Thus, neutrophils are one of the first cells to interact with the parasite playing an important role in the infection. Neutrophils can die by apoptosis, necrosis or by NETosis. Upon NETosis, neutrophils release fibrous traps (NETs) of DNA, histones and granule proteins, which can kill bacteria, fungi and also Leishmania as demonstrated by our group. NETs are released upon neutrophil activation by several molecules like PMA, microorganisms or their components. Our group reported that L. amazonensis (La) stimulates neutrophils to release NETs and the parasite is trapped and killed by these fibers. Here we investigate the molecular mechanisms behind NET release by human neutrophils stimulated with La, PMA and the bacteria formilated peptide, fMLP. Thus, neutrophils, isolated from healthy human blood, were incubated with inhibitors of G-protein (Pertussis Toxin), protein kinase C (PKC; Bisindolilmaleimide - BIS I), phospholipase C (PLC; U73122) and an inhibitor of phosphoinositide 3-kinase (PI3K; LY-294002). After 30 min, neutrophils were activated by PMA, fMLP and La for 40 min and NETs were quantified by measuring DNA in the supernatant with PicoGreen kit. Our results demonstrate that inhibition of G protein and PLC decreases NETs release by neutrophils stimulated with fMLP and La, but not with PMA. Besides, addition of BIS I decreases NET release stimulated by La, fMLP and PMA. It was demonstrated that PMA induce NET release via PI3K signaling. Our results confirm this event and, in addition, we demonstrate that La seems to induce NETosis in the same way. Although, much progress has been done on the investigation of the cellular signaling associated with NETs release, the full process remains unclear.

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IM021 - **CELL THERAPY FOR CHRONIC CUTANEOUS LESIONS IN MICE (TNFRP55-/-) 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 cells from bone marrow. We showed that when infected with L. major, TNFRp55-/- mice develop chronic lesions and 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 as a treatment for chronic lesions in TNFRp55-/- mice infected with L. major. After 15 weeks of infection groups of mice were treated intravenously with purified monocytes from bone marrow and analyses were performed 4 weeks after the treatment. After the treatment with purified cells the lesions were reduced and the histological analysis showed evidence of healing in the treated mice in comparison with animals injected with PBS. Purified GFP+ monocytes were transferred to infected mice and after 24 hours or 7 daysthe GFP+ cells were located at the site of infection. 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:CNPq FAPEMIG

IM022 - **ROLE OF REACTIVE OXYGEN SPECIES (ROS) ON NEUTROPHIL EXTRACELLULAR TRAPS (NETS)INDUCTION BY PMA,FMLP AND LEISHMANIA**

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Neutrophils are one of the first lines of defense against invading microorganisms, which can be eliminated by phagocytosis, degranulation or by a newly described mechanism named NETosis. In this mechanism, neutrophils upon activation by different molecules, such as phorbol myristate acetate (PMA), LPS and microorganisms, release their DNA, associated to histones and granule proteins to the extracellular medium. This structure was called neutrophil extracellular traps (NETs) and it was demonstrated that its release was dependent on reactive oxygen species (ROS) production by NADPH oxidase. Recently, our group has demonstrated that the parasite Leishmania amazonensis (La) not only induces NETs but also is trapped and killed by these structures. The aim of this work is to dissect the role of ROS on NETs induction by PMA, formyl-methionyl-leucyl-phenylalanine (fMLP) and La. For that, neutrophils, isolated from healthy human blood, were incubated with inhibitors of ROS production, such as diphenylene iodonium (DPI), N-acetylcystein (NAC), apocynin (APO), and rotenone (ROT) for 30 min and then activated with PMA, fMLP and La for 1h. NETs were quantified by measuring DNA in the supernatants from neutrophil stimulated cultures. Our results show that DPI, an inhibitor of NADPH oxidase, inhibits the release of NETs when neutrophils were activated by PMA, fMLP and La. To ensure the participation of ROS on NETs release, NAC (a non-selective inhibitor) and APO (a selective NADPH oxidase inhibitor) were used. NAC and APO seems to reduce the formation of NETs by PMA and fMLP, and inhibit NETs production when La was used as activator. We wondered whether the production of other ROS pathway could also play a role on NETs formation. Rotenone, an inhibitor of mitochondrial complex 1, does not seem to reduce NET release by any of the activators. We thank the Hemotherapy Service of Clementino Fraga Filho Hospital, UFRJ.
Supported by:FAPERJ, CAPES, CNPQ, PIBIC-UFRJ

IM023 - **CLONING AND EXPRESSION OF THE PROTEIN MSP-142 OF PLASMODIUM VIVAX FUSED WITH THE SEQUENCE OF THE ANTI-DEC205 MONOCLONAL ANTIBODY.**

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Malaria kills approximately one million people per year, the majority being children under 5 years of age. The development of a vaccine against this disease would have a great impact on mortality and morbidity rates. In the last decade, a strategy aimed at targeting antigens to dendritic cells (DCs) in vivo is being developed successfully in animal models. DCs are antigen-presenting cells essential for the activation of immune responses. The antigen targeting is accomplished by the administration of antibodies directed to a DC endocytic receptor genetically linked to the antigen of interest. We fused a C-terminal 42kDa protein derived from the P. vivax merozoite surface protein-1 (MSP-142) to the sequence of a monoclonal antibody directed to the DEC-205 receptor, present at the surface of the mouse CD11c+CD8+ DC subpopulation. The anti-DEC-MSP-142 chimeric monoclonal antibody was successfully produced in vitro by transient transfection of HEK293T cells and purified using protein G beads. The chimeric antibody purity and integrity was analysed by SDS-PAGE and western blotting while its binding capacity was evaluated using CHO cells transfected with the murine DEC205 receptor and splenocytes and lymph node cells derived from normal mice.

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IM024 - **PRIME AND BOOST IMMUNIZATION PROTOCOL EMPLOYING NOVEL VACCINE CANDIDATES FOR CHAGAS' DISEASE.**

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We have previously screened an epimastigote-subtracted trypomastigote cDNA expression library of Trypanosoma cruzi by genetic immunization and challenge. This approach allowed us to identify 22 novel vaccine candidates. Sequence analysis showed that most of them are fragments of hypothetical proteins or unannotated T. cruzi open reading frames (ORFs). As the candidates identified were gene fragments that produce peptides ranging 4-21 kDa, we expanded the original sequences to include a greater number of T and B epitopes and to increase their immunogenicity. For this study, we selected the T. cruzi annotated proteins (n=4) and the ORFs whose products have high density of T and B epitopes and/or trans-membrane domains (n=5). They were amplified from CL Brener DNA, cloned and expressed into pQE or pGEX expression vectors and purified by affinity chromatography. As most of the recombinant proteins were insoluble in all the assayed protocols, they were purified from inclusion bodies. Mice (n=6) were immunized with 2 doses of mammalian-expression plasmid DNA carrying the gene fragments of T. cruzi genes/ORFs, administered with a plasmid coding for murine GM-CSF as adjuvant (control mice received empty plasmids plus GM-CSF; n=6). Animals were boosted with a 3rd dose of recombinant proteins coupled to aluminum hydroxide (control mice: GST + his-tagged protein + aluminum hydroxide). Sera were collected to analyze the humoral response. Animals were challenged with a lethal dose of trypomastigotes of the RA strain. Levels of bloodstream trypomastigotes were lower in the vaccinated mice than in control group. This data suggests that the immunization protocol employed allows partial control and/or delay of parasitemia.
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IM025 - **EPITOPE AND SUBCELLULAR LOCALIZATION PREDICTION IN LEISHMANIA INFANTUM GENOME: IMMUNOINFORMATICS FOR THE DEVELOPMENT OF A VACCINE AGAINST CANINE VISCERAL LEISHMANIASIS**

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Canine visceral leishmaniasis (CVL) is a zoonose in Latin America, and dogs have a central role in the urban cycle of *Leishmania infantum*. Immunoinformatics revolutionized vaccine design, allowing reduced cost and time. In this work, we developed a structural database approach to identify epitopes that could be tested for vaccine development. Predictions from nine algorithms including MHCI, MHCII and B–cell epitope predictors jointly with protein subcellular localization from *L. infantum* genome were integrated in a database. Initially, predicted proteome, with 8,214 proteins, was used in all analysis, and copies of each algorithm were installed in local servers. We analyzed 12 human MHC-I alleles and six mouse MHC-I alleles and, in the context of MHC-II, we analyzed 14 human alleles and three mouse alleles. NetMHC predicted 21,696 strong binders and 195,127 weak binders, with a total of 216,823 predictions. Yet regarding MHC-I prediction, NetCTL totaled 1,511,866 predicted epitopes. NetMHCII predicted 210,849 strong epitopes and 1,243,357 weak epitopes. Concerning B-cell epitopes, BepiPred did 47,195 predictions and AAP12 did 2,233,999 predictions, total of 2,281,194 predicted B-cell epitopes. For protein subcellular localization, Sigcleave and TargetP identified, respectively, 1,909 and 1,722 secreted proteins and WoLF PSORT identified 1,920 proteins, either secreted or located on plasmatic membrane. Specific parsers, developed in PERL language, were used to analyse data. The use of the specific conceptual schema developed will be important in establishing an environment that will make possible to accommodate the data from diverse approaches predictions, manipulate and extract the relationships between entity classes in order to define vaccine targets in the *L. infantum* predicted proteome. The resource developed represents an important tool that can be used to drive vaccine development against CVL.
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IM026 - **THE TNFRP55 MODULATE THE INFLAMMATORY RESPONSE IN LEISHMANIA AMAZONENSIS INFECTION**

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

IM027 - **STUDY OF INITIAL RESPONSE AGAINST LEISHMANIA BRAZILIENSIS: IS IT POSSIBLE TO PREDICT DISEASE OUTCOME BASED ON THE GENE EXPRESSION PROFILE?**

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The host immune response is essential to determine resistance or susceptibility to leishmaniasis. Cells from naïve volunteers exposed to Leishmania parasites present two profiles of interferon gamma (IFN-γ) production following in vitro stimulation with Leishmania: high production of interferon gamma (IFN-γ), characterizing the individual as a high responder (HR); or low production, characterizing the individual as a low responder (LR). There is still no consensus about the mechanism underlying these different responses. Therefore, we aimed at identifying differences at the molecular level by comparing the initial gene expression in cells obtained from polarized individuals, either HR or LR. For this purpose, we stimulated PBMC from healthy volunteers with L. braziliensis. Based on IFN-y production, we identified 2 HR individuals (IFN-y > 1.500 pg/ml) and 2 LR individuals (IFN-y< 700 pg/ml). Next, we analyzed gene expression of these same individuals using real time PCR arrays for IFN-y and receptors, chemokines and Th1/Th2/Th3 responses Following analysis with Ingenuity® software, we selected a group of candidate genes that are differentially expressed in HR and LR individuals: IFN-y, IL-6, IL-10, IL-8, IL-9, CD28, CXCL10, CXCL1, CXCL13, CXCL5 and CCL11. We are presently validating the expression of these genes by flow cytometry or Real Time PCR in a new group of HR and LR individuals. Next, we will verify the expression of these genes in patients with localized cutaneous leishmaniasis and in individuals from endemic areas, without history of disease, but with positive Leishmania skin test. In this manner, we will be able to correlate the pattern of gene expression and IFN-y production with clinical outcome of the disease, aiming at understanding the initial events which may lead to development cutaneous leishmaniasis or not.
Supported by:CNPQ

IM028 - **THE INFLAMMATORY ROLE OF REACTIVE OXYGEN SPECIES (ROS) IN INFECTIONS CAUSED BY L. AMAZONENSIS**

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The leishmaniasis are a wide spectrum of diseases caused by parasites of Leishmania genus. Because it is an intracellular obligate pathogen, infecting mainly macrophages and neutrophils, the oxygen and nitrogen radicals production could be an important factor for parasite killing. Several data show that nitric oxide has an important role in parasite killing, but specifically in L. amazonensis, the superoxide and hydrogen peroxide do not appear to be associated with parasite killing in vivo. Moreover, we found that mice deficient in NADPH oxidase of phagocytes (Phox-/-), enzyme responsible for superoxide production in these cells, develop larger lesions in the first weeks of infection than wild type animals, even with same parasite load. So, we evaluated the role of reactive oxygen species in control of L. amazonensis lesions in mice. We infected mice with 1 x 106 metacyclic promastigote forms of L. amazonensis in the hind paw and analyzed cellularity of spleen, draining lymph node and paw at 4, 8 and 12 weeks post infection. Our results show an increased influx of neutrophils at 6h as well as 72h post infection, compared with wild type animals. The neutrophil numbers in Phox-/- mice persisted higher until 8 weeks post infection when the inflammation is reverted. At 12 weeks, neutrophil numbers decreased with the lesion size. Following the same tendency, there was an increase in CD4+CD25+Foxp3+ T regulatory cells at 12 weeks post infection in Phox-/- mice. Despite of these data, both groups show the same parasite charge in all times studied. The experiments suggest that oxygen radicals have an important role in early stages in L. amazonensis infection, controlling the neutrophil influx to the lesion and consequently the inflammation of infected tissue. This highly inflammatory environment could contribute to strong regulation at later stages of infection, leading to the faster resolution seen in Phox-/- mice.
Supported by:CNPq, FAPEMIG e INCT de processos REDOX em biomedicina

IM029 - **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 able to activate macrophages to kill intracellular parasites, by the induction of NO production. The susceptibility 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*, which susceptibility is not associated with a polarized Th2 response. In addition, surprisingly, IFN-γ can induce the replication of these parasites in vitro. Also, IFN-γ-/- mice present higher susceptibility only 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 IFN-γ-/- mice present lesser parasites in the footpad 8 weeks after infection, and the same size of lesion 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 as wild-type controls, suggesting an IFN-γ-independent mechanism of induction of this enzyme. In this time of the infection they also showed a lower expression of IL-1β and a higher expression of IFN-α4 and IFN-β, which are cytokines that can induce iNOS expression in other models of infection. 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. In conclusion, our results indicate that it is possible to induce iNOS in the absence of IFN-γ and maybe this induction is important for the initial containment of the lesion progress.
Supported by:CAPES, CNPq, FAPEMIG and INCT/Redoxoma

IM030 - **CHARACTERIZATION OF A MURINE MODEL (TNFRP55-/-) FOR STUDY OF CHRONIC CUTANEOUS LESIONS BY LEISHMANIA MAJOR INFECTION**

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A chronic skin manifestation of leishmaniasis is caused by an exaggerated cellular immune response. Lesions in nasal mucosa and cartilage, months or years after an initial skin lesion, cause mutilation and morbidity in affected individuals. There is no murine model for the study of mucocutaneous leishmaniasis. However, data from our group showed that TNFRp55-/- mice, when infected with L. major, develop chronic lesions, which are not progressive as in the BALB/c classic susceptible strain. Based on this information the aim of this study is the characterization of chronic infection by L. major in TNFRp55-/- mice. Wild-type (C57BL/6) and TNFRp55-/- mice were inoculated in the foot-pad with L. major (1x106 parasites) and lesions were followed for 20 weeks. In the chronic phase of infection (15 weeks) samples from the site of infection were collected and processed for assessment of pro-inflammatory cytokines, histopathological analysis, quantification of parasites and analysis of the inflammatory infiltrate. Our results show that lesions in TNFRp55-/- mice were chronic non-progressive lesions, did not ulcerate, were hardened and fibrotic from the eighth week, and persisted for more than 20 weeks of infection. Interestingly, these animals control parasitism similarly to wild-type mice at 15 weeks of infection. Persistence of lesions was due to intense inflammatory infiltrate at the site of infection with increase percentage of Ly6G+ and TCD8+ cells. Cytokine analyses of the site of infection showed that TNFRp55-/- mice produced high levels of pro-inflammatory cytokines (IFN-γ and TNF-α) when compared with wild-type animals ($p<0.05$). The characterization of TNFRp55-/- shows the potential of developing a murine model for studies of pathogenesis for chronic lesions caused by infection with parasites of the genus Leishmania, mucosal leishmaniasis for example, and enables the experimental search for treatment of these lesions.
Supported by:CNPQ e FAPEMIG

IM031 - **DIFFERENT APPROACHES OF IMMUNIZATION USING THE ACIDIC RIBOSOMAL PROTEIN FROM LEISHMANIA INFANTUM (LIP0) AGAINST INFECTION WITH LEISHMANIA CHAGASI IN HAMSTERS**

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The acid ribosomal protein of *Leishmania infantum* (*L. infantum*) - LiP0 is a structural component of the ribosomal subunit and it was described as an immunodominant antigen recognized either by serum of patients and dogs infected by *Leishmania chagasi* (*L. chagasi*) and cooperates in the synthesis of other proteins. *Mycobacterium smegmatis* (*M. smegmatis*) is a opportunistic bacteria, which presents rapid growth, is a potent adjuvant and has been used as carrier of antigens in several different experimental models of immunoprotection. In this work we evaluate the immunoprotective capacity of recombinant *M. smegmatis* (r*M. smegmatis*) carrying the gene of LiP0 and the DNA or protein of LiP0 using homologous strategy (composed of plasmid DNA) and heterologous (consisting of plasmid DNA and recombinant protein more CpG) to immunize hamsters against infection by *L. chagasi*. The immunized animals produced anti-*M.smegmatis* antibodies but they did not produce antibody against LiP0, detected only in animals who received the heterologous strategy of vaccination. In the analysis of cytokines, we observed that animals immunized with r*M.smegmatis* LiP0 had higher concentration of IFN-γ and lower amounts of TGF-ß and IL-10 compared to control groups, suggesting a Th1 response. At different times after challenge, the degree of protection, evaluated by parasite load in the target organs, was estimated by limiting dilution assay. No difference was observed in the parasite load in the spleen, liver and lymph node between immunized hamsters and controls at all points of evaluation. There was no protection in animals immunized with r*M. smegmatis* expressing the acidic ribosomal protein gene, suggesting that LiP0 did not protect hamsters immunized with either *Mycobacterium* expressing the gene encoding the protein or DNA as a vaccine strategy using homologous or heterologous against infection *L.chagasi*.
Supported by:CNPq

IM032 - **EX VIVO KINETICAL STUDY OF IMMUNE RESPONSE IN MURINE INFECTION BY LEISHMANIA (LEISHMANIA) AMAZONENSIS**

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Leishmania (Leishmania) amazonensis is the main causative agent of cutaneous leishmaniasis in South America. Clinical manifestations can be experimentally partially reproduced in inbred strains of mice, which differs in resistance or susceptibility to Leishmania infection. The outcome of *L. (L.) amazonensis* infection depends largely on host immune responses to the parasites, such as T-cell responses: a Th1 profile usually leads to host protection, whereas a Th2 profile may lead to disease exacerbation. The aim of this study is to perform a kinetic ex vivo monitoring of host immune responses induced during murine infection by *L. (L.) amazonensis*. Experimental infections were performed with female BALB/c mice, which were injected subcutaneously in the left hind footpad with 10^6 promastigotes from stationary phase. The progress of disease was monitored by measuring lesion area and flow citometry analysis of cells from popliteal lymph nodes draining the lesions, for 20 weeks. The citometry analysis was performed using cell markers anti-CD3, CD4, CD8 and CD19 and non-infected Balb/c mice were included as control assay. Results indicate that immune response to infection is defined in the first week post injection of promastigotes. This immunity profile is characterized by high levels of B cells and by a decrease of T cells; the B cells level were three times higher when compared to control non-infected mice and T cells were close to 1.5 times lower. These values remain constant throughout infection until the last time point analyzed and the maintenance of high levels of B cells, which do not protect the host against Leishmania, is in accordance with the chronicity of experimental infection, with lesion development. Our perspective is to further evaluate the cytokines profile produced during the infection and, also, the presence of T cell clones able to recognize complexes of recombinant H-2 L^d molecules/synthetic peptides derived from infection-related parasite's proteins
Supported by:CAPES, FAPERJ, PAPES V (CNPq/Fiocruz)

IM033 - **EFFECTS OF EXPRESSION OF ECTONUCLEOTIDASES IN LEISHMANIA INFECTED MACROPHAGES**

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Macrophages act as the major reservoirs of Leishmania parasites and are the principal effector cells that determine the fate of the host parasite interaction. Studies have shown that cellular damage or injury in the sites of inflammation usually promote the release of elevated levels of extracellular nucleotides which act on a variety of immune cells. These nucleotides, such as ATPs are quickly hydrolyzed into extracellular adenosine in a coordinated two-step enzymatic manner via nucleotide triphosphate disphosphohydrolase-1 (CD39) and the ecto-5'-nucleotidase (CD73) expressed on activated immune cells. Adenosine has been implicated as a potent anti-inflammatory molecule. In this study, we looked at the expression of CD39 and CD73, by flow cytometry, on murine macrophages infected with metacyclic forms of Leishmania parasites and the percentage expression of these molecules was analyzed. Our preliminary results showed that uninfected macrophages, cultured at 37C in complete media, down regulate the expression of CD39 and CD73 after 24hrs. Interestingly, Leishmania prevented infected macrophages from down modulating these molecules in 24hrs. In an experiment conducted in vivo, inflammatory monocytes (GR1+) compared among drug treated or untreated BALB/c, and C57BL/6 mice, also demonstrated changes in CD73 expression. CD73 was down regulated during infection in both strains after 2 weeks. In susceptible BALB/c mice the expression was however recovered after 6 weeks of infection in contrary to resistant C57BL/6 and treated mice. CD73 is low in undifferentiated and inactivated monocytes. In summary, we observed that expression of these ectonucleotidases changes after Leishmania infection. The altered expression of these enzymes in Leishmania-infected macrophages is possibly a parasite-induced - mechanism that may lead to reduced activation of anti-leishmanial immune response against the parasite within the macrophages.
Supported by:CNPq

IM034 - **LEUKOCYTE PROFILE AND HISTOPATHOLOGICAL CHANGES INVOLVED IN THE RECRUDESCENCE OF PREGNANCY-ASSOCIATED MALARIA**

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Pregnancy-Associated Malaria (PAM) is characterized by sequestration of parasites in the placenta and concomitant maternal anemia, intrauterine growth retardation and decreased fetal viability. In areas of high transmission, women exposed to malaria before pregnancy and thus with a high premunition, may have increased susceptibility to malaria during pregnancy, which represents a high risk of death for the mother and for the developing fetus. Due to the ethics and logistics issues that restrict studies in human populations, experimental models of PAM have been used to investigate the mechanism related with this infection. In this work, we use a murine model of pregnancy-induced malaria recrudescence with chronic Plasmodium berghei ANKAGFP infection in BALB/C mice previously developed by our group (PLoS ONE. 2009;4(5):e5630) to evaluate the histological changes and analyze immune cell profiles that occur in recrudescence malaria. Morphometric analysis of placenta sections shows that primiparous females with recrudescent malaria presented a reduction in placental vascular space when compared with both the uninfected primiparous and no recrudescent primiparous groups (37.35±7.16, 56.39±5.26, 51.31±4.31 % of vascular space, respectively). The analysis of spleen cells populations by flow cytometry shows an increase in the percentage of CD4+, CD19+, TCRgamma-delta+, GR1+ cells on the infected animals when compared with the uninfected controls. No differences were observed in the percentage of cells from placenta. Together, these data suggest that the recrudescence is directly linked to tissue damage in the placenta, and promote a change in the profile of leukocytes in response to infection. The comprehension of the mechanisms that lead to parasite recrudescence in pregnancy-associated malaria is important to support public policies of prevention and care.
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IM035 - **LEISHMANIA'S E-NTPDASE IS ABLE TO ALTERS THE PATTERN OF IMMUNE RESPONSE IN MACROPHAGES**

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NTPDase are enzymes that have the ability to hydrolyze nucleotide di and triphosphates under stimulation of bivalent cations (Ca2+ or Mg2+). This enzyme is important in modulating the immune response by affecting the concentration of extracellular ATP. We previously found that incubation of L. amazonensis with anti-NTPDase is able to reduce adhesion of the parasite in macrophages and that modulation of the expression of the enzyme alters the profile of adhesion and internalization. We also found that pre-incubation of macrophages with recombinant NTPDase reduces the adhesion and internalization of parasites. Thus, the e-NTPDase seems to be an important molecule involved in parasite adhesion to host cells. The aim of this study was to evaluate the role of Leishmania's e-NTPDase in the modulation of the response of macrophages. Peritoneal macrophages (pMo) from C57BL/6 mice were previously stimulated with IFN-γ and LPS and then incubated with 1 or 5 µg of NTPDase recombinant (rNTPDase) without activity for 48 hours and nitric oxide production assessed by Greiss method. Our data demonstrate a reduced of 50 percent in production of nitric oxide (NO) by stimulated cells treated with rNTPDase. Moreover, a reduction of IL-10 (37.1 pg/mL to 17.5 pg/mL) and IL-12 production (29,2 pg/mL to 9,2 pg/mL) and a significant increase in the production of TGF-β (24,1 pg/mL to 70,2 pg/mL) by cells treated with rNTPDase was observed, demonstrating that the binding of Leishmania's E-NTPDase is able to modulate machophage activity. Finally, pMo were treated for 30 minutes with rNTPDase and then incubated with FITC-labeled latex beads to evaluate the phagocytic ability of these cells. We observed that cells treated with rNTPDase lost, after 30 minutes, phagocytic capacity. We conclude that binding of the enzyme is able to alter the response pattern of these cells, which could indicate that the E-NTPDase is another surface molecule of Leishmania involved in subverting the immune response against the parasite thus, facilitating infection.
Supported by:CNPq, CAPES, FAPEMIG

IM036 - **HISTOPATHOLOGIC MODIFICATIONS IN P. VIVAX INFECTED PLACENTAS FROM WOMEN LIVING IN THE BRAZILIAN AMAZON**

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Pregnancy-associated malaria (PAM) is characterized by marked accumulation of parasites in the intervillous space of the placenta, together with maternal anemia as well as low birthweight, prematurity and increased infant mortality. The objective of this work is to study placental histopathological changes associated to Plasmodium vivax infections in pregnant women living in Alto Juruá, Acre, Brazil. In this study we observed 136 placentas from term pregnancies, without other complications, of pregnant women infected during pregnancy with P. vivax (n=64) or P. falciparum (n=21), and also of uninfected (n=51), collected during the year 2009. To acess the number of knots syncytial (KS) and fibrinoid necrosis (FN) in histological sections H&E stain of placentas from pregnant women, we count the number of affected villi per 100 villi on microscopic fields. Our results shows an increase in the number of KS and FN in the infected compared with the non-infected groups (KS: 20.3±1.4 and 12.0±1.4; FN: 8.5±0.6 and 4.3±0.7, respectively). These values, however, were lower than those observed in P. falciparum infected group (KS: 25.3±1.6 and FN: 10.4±1.0). The weight of newborns from both infected groups did not present differences. However, we found a higher percentage of low birth weight (<2500g) in neonates of group infected with P. vivax compared with both group infected by P. falciparum as well as with non-infected group (15.6%, 10.0% and 9.5%, respectively). Together, these results suggest an association between changes in placental villous syncytiotrophoblast and fetal development. Changes that compromise the exchange of oxygen and nutrients between mother and fetus may explain the deleterious effect of placental malaria, such as fetal growth restriction or low birth weight. For a better understanding of the P. vivax damage to the fetal development, further studies allowing a more detailed description of placental lesions are needed.
Supported by:FAPESP/CNPq

IM037 - **L. INFANTUM-CHAGASI KMP11-LOADED PLGA NANOPARTICLES AS A TOOL FOR ANTIGEN DELIVERY AGAINST LEISHMANIASIS**

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The development of alternative prevention strategies such as vaccination has been a major goal in the field of leishmaniasis, a group of diseases caused by infection with unicellular protozoan parasites of the genus Leishmania. With this in mind, we analyzed the immunostimulatory effects of PLGA nanoparticles (NPs) loaded with L. infantum-chagasi KMP11, either in DNA or recombinant protein form. In vitro treatment of BALB/c macrophages with DNA-loaded NPs (rDNA NP) or rKMP11-loaded NPs (rProtein NP) followed by infection with L. braziliensis led to a significant reduction in parasite burden, when compared to empty NPs. This effect was associated with an increase in cytokine (TNF-α, IL-6 and IL-1β) and chemokine (CCL2/MCP-1 and CXCL1/KC) production and, in parallel, with enhanced macrophage and neutrophil chemotaxis. Moreover, caspase-1 activity and mature IL-1ß secretion were also observed upon macrophage stimulation with KMP11-loaded NPs and LPS. We then analyzed the in vivo protective capacity of recombinant NPs against infection with L. braziliensis, employing an experimental model of cutaneous leishmaniasis. BALB/c mice were immunized with rDNA NP (homologous strategy) or with rDNA NP followed by rProtein NP+CpG (heterologous strategy). Following infection with L. braziliensis, mice immunized with rDNA NP followed by rProtein NP+CpG presented a significant reduction in parasite load. In parallel, this immunization strategy also led to the development of a powerful inflammatory reaction at the inoculation site. We conclude that KMP11-loaded PLGA NPs are able to strongly stimulate innate responses and, doing so, promote L. braziliensis killing. These results point to the possibility of employing nanotechnology to the field of vaccine design or immunotherapy against cutaneous Leishmaniasis.
Supported by:CNPq, CAPES and Fundación CAN

IM038 - **CORRELATION BETWEEN INOS EXPRESSION AND PARASITISM IN LYMPH NODES OF DOGS NATURALLY INFECTED WITH LEISHMANIA (LEISHMANIA) INFANTUM CHAGASI**

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Introduction and Aim: Nitric oxide (NO) production by the inducible NO synthase (iNOS or NOS2) represents one of the main microbicidal mechanisms of murine macrophages, but its role in other animal models is poorly studied. Recent reports suggest that iNOS expression by macrophages plays an important role during the control of *Leishmania* infection in dogs. Therefore, the aim of this work was to evaluate NOS2 expression in lymph node of dogs naturally infected with *Leishmania (L.) i. chagasi*.
Material and Methods: Twenty one *Leishmania* positive dogs, 11 symptomatic and 10 asymptomatic from the Center of Zoonosis Control of Araçatuba city were submitted to euthanasia and fragments of lymph nodes were collected and processed by immunohistochemistry using as primary antibodies mouse anti-*Leishmania* and rabbit polyclonal anti-human NOS2 (Santa Cruz) polyclonal antibody, and LSAB (DakoCytomation) and NovoLink (Novocastra), respectively to develop the reaction. Quantitative analysis was performed using the image analysis system (Zeiss - Germany) in order to quantify the number of amastigotes and the number of $NOS2^+$ cells. Correlation between the number of parasites and the number of cells expressing NOS2 was assessed using Spearman test.
Results: Higher tissue parasitism was observed in symptomatic than in asymptomatic dogs (p=0.0084). In spite of no significant difference in NOS2 expression between both clinical groups, there was negative correlation between tissue parasitism and $NOS2^+$ cells in symptomatic (rs= -0.8273, p= 0.0017) and asymptomatic (rs= -0.6565, p= 0.0391) animals. In other words, high number of $NOS2^+$ cells was related with low number of amastigotes in the lymph nodes of symptomatic and asymptomatic dogs.
Conclusion: The results suggest that NOS2 expression could be responsible for the control of *Leishmania* parasites in the canine lymph nodes tissue.
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IM039 - **MYD88 SIGNALING IS IMPORTANT TO PREGNANCY-ASSOCIATED MALARIA PATHOGENESIS**

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Pregnancy-associated malaria (PAM) is characterized by a range of complications to the mother and to the fetus, such as anemia, intrauterine growth retardation, increase of the abortions and low birth weight of the newborns. It has been described that members of the Toll Like Receptors (TLR) family, like TLR2, TLR4 and TLR9, recognize components of Plasmodium sp. and influenced the host immune response. In common these receptors signal through MyD88, which leads to induction of NF-kB-dependent inflammatory cytokine productions. To study the influence of MyD88 in the pregnancy-associated malaria, we start by infecting pregnant females MyD88 knockout (MyD88KO) and C57BL/6 with P. berghei NK65GFP to evaluate the parasitemia course and survival rates. Our results did not show differences in parasitemia between the strains. However, when the same experiment was conduct in absence of pregnancy, parasitemia of MyD88KO was higher than the observed in C57BL/6 (76% vs. 54% of parasitemia) mice. Surprisingly, malaria disease in MyD88KO (pregnant or not) lead to high survival rates, since they presented a diminished number of deaths when compared with the controls. Histopathological analysis of the placentas shows a decrease of vascular spaces in C57BL/6 infected mice when compared with placentas from non-infected mice (38 vs. 48% of vascular space). On the other hand, placentas from infected and non-infected MyD88 did not present differences. In addition, we evaluate the occurrence of abortion during pregnancy period. C57BL/6 infected mice present an increased number of abortions when compared with non-infected animals (1.8 vs. 0.2 abortions/female) whereas we not observed abortions in MyD88KO mice. Taken together, ours results indicated that signaling via MyD88 contributed to PAM pathogenesis, probably due to induction of a severe inflammatory process during the infection. To confirm this hypothesis, others experiments are been conducted.
Supported by:Fapesp

IM040 - **A COMPARATIVE ANALYSIS OF DPP TEST WITH ELISA-BIO-MANGUINHOS AND L.CHAGASI-ELISA FOR THE DIAGNOSIS OF CANINE VISCERAL LEISHMANIASIS**

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The diagnostic screening test for canine visceral leishmaniasis (canVL) recommended by the Brazilian Ministry of Health is the ELISA-Bio-Manguinhos (ELISA-Bio), however the effectiveness of the method has been questioned over the years.Our aim was investigate the performance of the DPP test, a new quick test recently developed by Bio-Manguinhos and to compare with ELISA-Bio and *L.chagasi*-ELISA standardized in our laboratory that uses crude lysate of *Leishmania (L.) i. chagasi* promastigotes as antigen. In this way sera of dogs with proven infection (n=66), confirmed by immunohistochemistry (IHC), and negative controls, including sera of healthy animals living outside endemic area (n=18) and those without proven parasitism from endemic area (n=44) were tested. To detect cross-reactions, sera of dogs with ehrlichiosis (n=17), toxoplasmosis (n=9), neosporosis (n=6), neosporosis/toxoplasmosis co-infection (n=4) and Chagas disease (n=6) were also investigated. To compare the performance of the tests, the sensitivity and specificity of each one were determined, taking the IHC as the gold standard method. Besides, the agreement index among them was calculated by the kappa test. The sensitivity of the DPP test (93.9%) was equal to ELISA-Bio (93.9%) and slightly inferior to *L.chagasi*-ELISA (96.9%). The specificity was higher in both DPP test (87.5%) and *L.chagasi*-ELISA (87.5%) compared with ELISA-Bio (65.3%). Also, cross- reactivity was only observed by using ELISA-Bio, especially with sera from dogs with Chagas disease (50%) and ehrlichiosis (35%). Excellent agreement (0.93) was found between the quick test and *L.chagasi*-ELISA, but only moderate (0.67) when both tests were compared with ELISA-Bio. In conclusion, besides the advantage of being a quick test, the DPP showed better performance than ELISA-Bio, especially concerning cross-reactivity, and provided very similar results to the *L.chagasi*-ELISA that used the specific causing pathogen of canVL, as antigen.
Supported by:LIM-50 HCFMUSP and FAPESP

IM041 - **THE AVIRULENT PROTOZOAN PARASITE *TRYPANOSOMA RANGELI* INFECTION ELICITS PROINFLAMMATORY RESPONSES IN VITRO AND IN VIVO**

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Trypanosoma rangeli has been documented to be a non-virulent protozoan parasite in mammalian hosts. However, information regarding *T. rangeli*-host interactions during infection remains scarce. In this study, parasitemia as well as immune responses of BALB/c and C57BL/6 mice i.p. injected with culture-derived trypomastigotes of either *T. rangeli* (Choachí strain) or *T. cruzi* (Y strain) were comparatively assessed. In contrast with *T. cruzi* infection, which presented a peak of parasitemia on day 9 p.i., *T. rangeli* trypomastigotes were found in the blood of infected mice as early as 1 day p.i. in both C57Bl/6 and BALB/c mice, reaching a peak of parasitemia at 2 and 4 days p.i., respectively. In BALB/c, parasite clearance occurred on the 11th day p.i., whereas in C57Bl/6, *T. rangeli* clearance was observed until day 8 p.i. Moreover, *T. rangeli*-infected mice displayed increased levels of serum cytokines such as IL-12, MCP-1, IFN-γ and increased IL-6 and TNF as well. Histological analysis of the liver revealed that this parasite induced a macrophages-rich inflammatory infiltrate observed up to 30 days p.i., suggesting these cells may play a role in *T. rangeli*-host interactions. To further investigate the parasite-cell interactions, CFSE-labeled *T. rangeli* were incubated with murine macrophages at either 4°C or 37°C. The frequency of CFSE-positive cells increased with time and temperature, thus indicating that macrophages and *T. rangeli* trypomastigotes indeed interact. However, light-microscopy analysis of macrophages incubated with *T. rangeli* trypomastigotes revealed the majority of parasites are not inside macrophages. Nevertheless, macrophages exposed to *T. rangeli* produced TNF-α and IL-6. Taken together, these results suggest *T. rangeli* infection may modulate its entrance in macrophages and induce persistent inflammatory responses in vivo.

Supported by:CNPq, CAPES, FINEP, UFSC

IM042 - **CYTOTOXIC CD8+ T CELLS CONTRIBUTE TO INFLAMMATION IN LESIONS FROM HUMAN LOCALIZED CUTANEOUS LEISHMANIASIS**

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Introdution: The role of CD8+ T cells in human cutaneous leishmaniasis (CL) is not established. In the present study, we explore the participation of CD8+ T cells from lesions of CL patients caused by *L. braziliensis* in the inflammatory process and parasite killing. Methods: Expression of homing markers (CLA, CCR7), cytotoxic marker (CD107a), cytokines and granzyme B in PBMC and in lesion cells were analyzed by flow cytometry. Cells from CL lesions were also compared to those obtained from normal skin. Confocal analysis was used to identify and localize CD8 lymphocytes producing granzyme B in the lesions of patients. Data were analyzed by Mann-Whitney test, Paired T test and Image Pro Plus. Results: The frequency of CD3+CD8+ T cells and CD8+CLA+ T cells was higher in CL lesions than in normal skin. Granzyme B+ CD8+ T cells from lesions were more frequent than in PBMC with few of them expressing CLA. A significant expression of cytotoxic marker CD107a was observed in the lesions cells after stimulation with *L. braziliensis*. We also detected that co-culture of infected macrophages and effector lymphocytes resulted in release of granzyme B, but there was not change in parasite load in the presence of granzyme B inhibitors, whereas anti-IFNγ significantly increased parasite load. Conclusion: Taken together our results suggest CD8+ T cells, in LCL patients act as cytotoxic cells which may contribute to local inflammatory response.

Supported by:CNPq, FAPESB (PRONEX)

IM043 - **ENTERIC NERVOUS SYSTEM IS DIFFERENTIALLY AFFECTED BY INFECTION WITH DISTINCT TRYPANOSOMA CRUZI STRAINS IN THE BEAGLE DOGS**

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Megaesophagus and megacolon, the most common manifestations of the Chagas disease digestive form, are characterized by achalasia and partial/total colon obstruction, respectively, and their development are related to geographic distribution of distinct *Trypanosoma cruzi* subpopulations. The motor abnormalities observed in these visceromegalies have been related to lesions of the enteric nervous system, however, few studies have addressed the denervation limit required for the motility impairment. In this study, *Beagle* dogs were infected with Y or Berenice-78 *T. cruzi* strains and necropsied during the acute (30 days post infection-DPI) or chronic phases (730DPI) of the experimental disease for esophagus and colon *post-mortem* histopathological evaluation. Both strains infected the esophagus and colon and cause an inflammatory response during the acute phase. In the chronic phase, the conventional PCR detected only tissue parasitism in the Be-78 infected group, which may be related to persistent inflammatory process observed exclusively in this group. Only in animals infected with the Y strain was observed fibrosis in chronic phase. Denervation of myenteric ganglia was observed in infection with both strains during the acute phase, but in chronic phase, denervation was persistent only in animals infected with Be-78 strain. The glial cells involvement occurred earlier in animals infected with Y strain while animals infected with Be-78 strain showed a reduction in enteric glial cells GFAP-IR only in the chronic phase. These results suggest that although the two strains causing lesions in the digestive tract, infection by Y strain leads to early control of the lesion, while infection with Be-78 strain results in progressive lesions of the gut during the Chagas disease experimental in the dog model.
Supported by:: FAPEMIG (PPM, Redes TOXIFAR e BIOTERISMO), CNPq and UFOP

IM044 - **DIFFERENT CHANGES IN HISTOLOGICAL ANALYSIS OF HEART AND SPLEEN OF MICE INFECTED WITH BLOOD OR METACYCLIC TRYPOMASTIGOTES OF TRYPANOSOMA CRUZI**

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We previously demonstrated that infection by different infective forms of *T. cruzi* causes different immune responses in peripheral blood and in the cytokines produced by NK and T cells in the spleen. Herein we have further focus in the evaluation of the histopathological changes in heart and spleen following infection with metacyclic (MT) or blood (BT) trypomastigotes of Be-78 strain. The morphometric analysis showed an early increase in the number of inflammatory cells at day 7 after infection in the BT group. In the 28th and 42nd day after infection (DAI), both groups showed an increase in the number of inflammatory cells, however in the MT group it was observed a reduction in the 28th to 42nd DAI. The analysis of the spleen white pulp demonstrated that both experimental groups showed no change in this region in the 7th DAI. However, in the 14th DAI, animals of the MT group showed moderate hyperplasia in the white pulp which remained at 80% and 100% of animals in 28th and 42nd day after infection, respectively. The BT group showed an intense white pulp hyperplasia in 40% and 100% of the animals in the 28th and 42nd DAI, respectively. The analysis of inflammatory cells in the trabecula demonstrated that the MT group showed no change in that region until the 28th DAI. However, in the BT group, 60%, 20% and 80% of the animals presented trabecular inflammation in the 7th, 28th and 42nd DAI, respectively. On the 14th DAI, as observed in the trabecular area, no alteration in the spleen capsule was observed in the BT group, but it was observed a progressive increase in the thickening and inflammation of this region in the 28th and 42nd DAI. The initial interaction between metacyclic trypomastigotes and the vertebrate host induces a histophatological profile different from that observed in infection with blood trypomastigotes, the latter featuring an intense and persistent cardiac inflammatory process and severe alterations in the spleen. Supported by FAPEMIG (PPM, Redes Toxifar e Bioterismo), CNPq and UFOP.
Supported by:FAPEMIG (PPM, Redes Toxifar e Bioterismo), CNPq and UFOP

IM045 - **ZVAD-PRE-TREATED NEUTROPHILS KILL LEISHMANIA CHAGASI BY INDUCING AN INFLAMMATORY RESPONSE**

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Introduction and Objectives: Neutrophils are considered the host's first line of defense against infections and have been implicated in the immunopathogenesis of Leishmaniasis. Previously, we have demonstrated that *Leishmania chagasi,* the etiological agent of Visceral Leishmaniasis, take advantage by induction of murine neutrophil apoptosis. Because different cell death pathways have been implicated in the pathogen killing or survival by host cells, we tested whether inhibition of neutrophil apoptosis by pre-treatment with zVAD-fmk, a pan caspase inhibitor, is effective on *L. chagasi* killing, since it has been demonstrated that zVAD-fmk induces a TNF-α-dependent necroptosis.
Methods and Results: C57BL/6 mice peritoneal neutrophils obtained by thioglycolate solution injection were pre-treated with zVAD-fmk, medium, DMSO and zFA-fmk and infected with *L. chagasi*. Only zVAD-pre-treated neutrophils presented a significant decrease on viable parasite (4.68x104 ± 1.59) compared with controls (medium: 13.06x104 ± 2.08; DMSO: 13.81 x104 ± 1.28; zFA-fmk: 16.13 x104 ± 2.01). In addition, we also evaluate the inflammatory mediators in the supernatant of infected neutrophils. Interestingly, when infection was performed in neutrophils pre-treated with zVAD-fmk, it was observed higher levels of TNF-α (-zVAD 231.8pg/ml ± 88.3; +zVAD 474.5pg/ml ± 198.5) and ROS (MFI: -zVAD 44.95 ± 2.05; +zVAD 64.8 ± 1.27). In another hand, TGF-β release was reduced (-zVAD 107ng/ml ± 23.52; +zVAD 57.15ng/ml ± 12.15).
Conclusion: Taken together, our data suggest that molecular mechanisms underlying zVAD-induced inflammatory response on neutrophils have biological significance in the control of *Leishmania* infection.
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IM046 - **EVALUATION REACTIVE OXYGEN AND NITRIC OXIDE PRODUCTION BY NEUTROPHILS INFECTED WITH LEISHMANIA AMAZONENSIS AND LEISHMANIA MAJOR OF C57BL/6 MICE**

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Leishmania amazonensis and Leishmania major are primary etiologic agents of cutaneous leishmaniasis. Neutrophils are important first line defense cells which destroy invading parasites by phagocytosis through the action of various proteolytic enzymes and reactive oxygen substances. In this study, we investigated the pathogenic mechanisms of these host cells against Leishmania parasites. The reactive oxygen species (ROS) and nitric oxide (NO) triggered by neutrophils against L. amazonensis and L. major were measured. Bone marrow neutrophils were isolated from C57BL/6 mice of 4-8 weeks old and were purified by percoll gradient centrifugation. The cell suspension generated was incubated in presence of L. amazonensis and L. major (1:5). To determine ROS levels, infected neutrophils were analysed in the luminometer for fourty minutes. After that, zymozan was added in all groups and was analysed again for fourty minutes. Culture supernatants of neutrophils incubated for 1 and 3 hours were used in the presence/absence of L. amazonensis and L. major to measure NO levels. Our preliminary results demonstrated that ROS production by neutrophils in the presence of L.amazonensis and L.major was lower when compared to neutrophils in absence of parasites. However, in the presence of zymosan and parasites, ROS levels were elevated. Moreover, the results support that there is a possible tendency in a decreased NO production by neutrophils in the presence of parasites when compared to neutrophils in the absence of parasites. Thus our data suggest that L.amazonensis and L.major are decreasing ROS and NO production in infected neutrophils. The results of reactive oxygen species production may help to understand the early death/survival of parasites inside neutrophils.
Supported by:FAPEMIG/CAPES/CNPq/UFOP

IM047 - **THE HUMORAL IMMUNE RESPONSES IN HAMSTERS INFECTED WITH L. CHAGASI USING DIFFERENT INFECTION ROUTES**

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Golden hamster has been evaluated as animal model to investigate different immunopatological parameters during visceral leishmaniasis. In fact, this model reproduces several clinical signs, such as hepatosplenomegaly, anemia, pancytopenia, cachexia and hypergammaglobulinemia. In this work, different infection routes (intradermal, intracardiac and intraperitoneal) and strains from L. chagasi (wild and reference WHO MHOM/BR/74/PP75 strains) were tested to evaluate the disease progression. The quantification of specific total-IgG and IgG2 was carried out by ELISA during 1, 3, and 6 months post infection with 10^7 L. chagasi promastigotes. Our majors results demonstrated that the levels of anti-Leishmania IgG were increased 1 month after infection using intraperitoneal inoculum compared to control group, regardless of the strain inoculated. After 3 months was observed increased IgG levels in all groups experimentally infected with wild strain when compared with the control. Animals inoculated with reference strain by intracardiac route showed high IgG levels when compared with others groups. The IgG2 profile analysis revealed increase of this isotype during the first month using wild strain in all groups when compared to the control group. The increased IgG2 levels were maintained after 3 and 6 months in the animals inoculated by intracardiac and intraperitoneal routes when compared to control and intradermal groups. Interestingly, animals inoculated with the reference strain showed IgG2 profile similar to IgG, with increased IgG2 levels in the first month of infection in animals inoculated by intracardiac route, resulting higher levels at 6 month after infection. In this context, our data revealed that evaluation of humoral immune responses in hamster model can be useful tool for estimating the activity of VL infection using IgG and IgG2 immunoglobulins as biomarkers of disease progression.
Supported by:CAPES, CNPq, FAPEMIG and UFOP

IM048 - **EXPRESSION OF TYPE-2 AND TYPE-9 TOLL-LIKE RECEPTORS IN JEJUNE AND COLON IN CANINE VISCERAL LEISHMANIASIS**

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Toll-like receptors (TLRs) function as pathogen recognition receptors (PRRs) that recruit active signaling molecules involved in innate immunity and it is one of the first defensive systems against pathogen like Leishmania. TLRs recognize “pathogen-associated molecular patterns” (PAMPs) such as glycolipids, peptidoglycans and lipopeptides, which are produced only by microorganisms and not by host cells. After recognition of PAMPs by leukocytes, trigger NF-kB, which then proceeds to the nucleus and promotes the transcription and further synthesis of pro-inflammatory cytokines. A total of 11 human and 13 mouse TLRs have been identified and each responds to distinct PAMPs, leading to the activation of specific signaling pathways Experimental studies reveled that TLR2 and TLR9 contribute to the recognition of Leishmania major and to the subsequent immune response. The aim of this work was to investigate TLR2 and TLR9 expression by flow cytometry of cells obtained from lamina propria of the gastrointestinal tract (TGI) segments: jejune (LPJ) and colon (LPC). Twenty-four dogs naturally infected with L. chagasi were divided into symptomatic and asymptomatic groups. In general, all the segments showed an exudate of mononuclear cells of the lamina propria mainly characterized by macrophage, plasma cells and lymphocytes. However, the parasite load was higher in the LPC, but without statistical difference between symptomatic and asymptomatic dogs. A positive correlation between the parasite load and cellular exudate in the lamina propria was found in both TGI segments: LPJ (r2=0.3852) and LPC (r2=0.2544). Under flow cytometry analysis, the mean fluorescence intensity (MFI) showed higher TLR2 expression in LPC than LPJ (p=0,0001). Moreover, in LPC higher TLR2 expression were higher in symptomatic than asymptomatic dogs. In the other hand, the MFI of TLR9 showed be higher in LPJ than (p=0,0364) in the LPC, but without statistical difference between symptomatic and asymptomatic animals. Thus, these preliminary results might indicate that TLR9 has a potential anti-parasite effect role in leishmaniasis.
Supported by:CAPES

IM049 - **ROLE OF NLR2 IN TH1 INDUCTION DURING NEOSPORA CANINUM INFECTION**

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N. caninum is an apicomplexan parasite first described as the cause of encephalomyelitis in dogs serologically negative to T. gondii. Infection by this protozoan is the major identifiable cause of reproductive failure in cattle worldwide, causing considerable economic losses. NLR are a specialized group of intracellular proteins that play a critical role in the regulation of the host innate immune responses. However, the role of NLR in sensing intracellular parasites is unclear. In that sense, we aimed to observe the role of NLRs in host response to N. caninum infection. For that purpose, we infected NLR1-/-, NLR2-/- and Rip2-/- mice, along with WT littermates, with N. caninum tachyzoites, and evaluated acute phase parasitism, inflammatory cell migration and cytokine pattern. During acute phase, NLR2-/- mice demonstrated to be the most susceptible lineage to the infection, since higher parasite burden were detected at the peritoneal exudate and lungs. Inflammatory cell migration was impaired in both compartments, as NLR2-/- mice presented decreased migration of dendritic cells (DCs), B and T lymphocytes to the peritoneal cavity. Mononuclear cell infiltrates were also significantly reduced in the lungs of NLR2-/- mice, if compared to WT. In parallel, we observed that DCs and macrophages presented lower MHCII expression in NLR2-/- mice, fact that was associated to lower IFN-g production during spleen cell antigenic recall, and detection in lung and brain homogenates, which was IL-10-independent. Corroborating with those results, parasite specific IgG2a/IgG1 ratio was also altered in NLR2-/-, indicating a lack of Th1 induction in these animals. Our group has previously described that TLR2 is required for an appropriate Th1 induction after infection by the protozoan and, based on the results herein presented, NLR2 has a complimentary role in Th1 programming initial immune responses to N. caninum.
Supported by:CNPq, CAPES, Fapemig

IM050 - **ANALYSIS OF MONOCYTE SUBPOPULATIONS FREQUENCY IN THE BLOOD DURING LEISHMANIA MAJOR INFECTION IN MICE AS A PREDICTOR OF SEVERITY OF DISEASE.**

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Monocytes are important cells from innate immunity that give rise to macrophages and dendritic cells. Recently, two subpopulations of monocytes were described based on the expression of the surface marker, GR1 (Ly6C). GR1+ monocytes are inflammatory cells that migrate to the site of inflamed tissues, producing cytokines and controlling some diseases such as toxoplasmosis and brucelosis. We have shown that these cells can migrate very quickly to the site of L. major infection where they are able to kill the parasites. The Health Ministry of Brazil in 2006 shows that in the last two decades the number of deaths in patients with visceral leishmaniasis has increased. There is no good indicator of prognosis for this disease and the characterization of clinical or immunological markers that could be used as such are important to reduce morbidity and mortality and to guide possible prophylactic and therapeutic strategies. Although L. major causes a typical cutaneous disease in humans, infection of BALB/c mice with L. major induces a cutaneous and visceral disease, similar to that observed in humans with visceral leishmaniasis. Thus, the aim of this study was to analyze the frequency of GR1+ inflammatory monocytes, in the blood, during L. major infection in BALB/c susceptible and C57BL/6 resistant mice. In addition, we have analyzed monocytes in mice treated with Amphotericin B for 4 weeks. Our results show that GR1+ monocytes increase in frequency in the blood during L. major infection and this increase is related to lesion size. C57BL/6 mice present decreased GR1+ monocyte numbers relative to BALB/c mice, that correlate with decreased lesion size . Similar results were observed in the BALB/c mice treated with Amphotericin B for 4 weeks. Thus, in mice, the analysis of monocyte frequency in the blood can be used as a predictor of disease progression in treated animals, where the decrease in the number of blood monocytes during treatment, represents an improvement in the animal's clinical status.
Supported by:PIP/UFOP

IM051 - **AVALIATION OF REACTIVE OXYGEN SPECIES IN NEUTROPHILS INFECTED BY LEISHMANIA MAJOR OF C57BL/6 MICE**

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Neutrophils appear to play a protective and/or immunoregulatory role in the early response to infection with *Leishmania major*. Oxygen-dependent cytotoxic responses mediated by phagocytes play an important role in immune response against infections agents. Reactive oxygen species (ROS) and nitric oxide (NO) are production by neutrophils and to act as an early proinflammatory mediator. The metabolites of ATP such as adenosine and drug suramin are considered as anti-inflammatory molecules that can inhibit respiratory burst. In our work, we evaluated the levels of ROS and NO production in neutrophils treated with adenosine or suramin in presence or absence of *L.major*. Neutrophils from bone marrow of 4-8 weeks old C57BL/6 mice were purified by percoll gradient centrifugation. To determine ROS levels, we pretreated neutrophils with suramin or adenosine for thirty minutes and then infected with *L.major*. Moreover, neutrophils were treated and infected at the same time. Neutrophils were analyzed in the luminometer for fourty minutes. Later zymozan was added in all groups and analyzed again for fourty more minutes. To analyze the production of NO, we treated uninfected and infected neutrophils with suramin or adenosine for 3 hours. Our data showed that non infected neutrophils treated with adenosine or suramin reduce the production of ROS. This reduction was more evident later with the addition of zymozan in neutrophils treated thirty minutes before measure ROS. There was a similar reduction in ROS production in neutrophils treated and infected with *L.major*. Similarly, this reduction was higher when zymozan was added in neutrophils treated thirty minutes before the time of *L.major* infection with adenosine or suramin. NO production by neutrophils was decreased in both treatments after 3 hours of infection. Thus, our preliminary results suggest that *L. major* inhibits neutrophils for the production of ROS and NO.

Supported by:FAPEMIG/CAPES/CNPq/UFOP

IM052 - **NITRIC OXIDE AND CYTOKINES PROFILE IN DOGS IMMUNIZED WITH LBSAP VACCINE AND CHALLENGED WITH LEISHMANIA CHAGASI AND SAND FLY SALIVA**

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A vaccine against visceral leishmaniasis (VL) would be an important tool in the control of canine visceral leishmaniasis (CVL), and would also dramatically decrease the infection pressure of L. chagasi for humans. In this context, we analyzed a vaccine composed by *L. braziliensis* promastigotes proteins plus saponin as adjuvant (LBSap) as a pre-requisite to understanding the mechanisms of relationship with the immunogenicity. Cytokines (IL-4, TGF-ß, IL-12, IFN-γ, and nitric oxide (NO) in the *in vitro* context from supernatants of peripheral blood mononuclear cells (PBMC) cultures and the bone marrow parasite load were evaluated after third vaccine dose (T3) and at times 90, 435 and 885 days after experimental challenge (dac). Our major results demonstrated that LBSap group displayed a significant decrease of TGF-ß at 90 dac when the PBMCs were stimulated with soluble *Leishmania* antigen. In contrast, the PBMC cultures subjected to *Leishmania*-stimulation *in vitro* displayed increased levels of IL-12 and IFN-γ in both T3 and at 90 dac. Also, the higher levels of NO after experimental challenge were observed. The parasitological analysis in bone marrow do not displayed amastigotes of *L. chagasi* in this tissue until 885 dac. In conclusion, the major findings in the present study point to a strong immunogenicity elicited when dogs were vaccinated with LBSap against CVL indicating a compatible action of this immunobiological with the effective control of the parasitism replication in dogs.

Supported by:FAPEMIG, CNPq, PAPES-V, CAPES and UFOP

IM053 - **THE SIGNALING PATHWAY NOD1/RIP2/MAPK IN THE RESPONSE TO TRYPANOSSOMA CRUZI INFECTION**

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Chaga's disease is caused by Trypanosoma cruzi and affects approximately 7,7 million people in Latin America. It was described that the immune response against T. cruzi is dependent on recognition of parasite by pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs). However, the role of non-TLR receptors remains largely obscure. We have demonstrated that Nod1, a member of the family, accounts for host response against T. cruzi infection. Here, we aimed to investigate the signaling pathway downstream to Nod1 in response to T. cruzi infection. It is well established that in response to bacterial products, Nod1 signals via the Rip2 kinase to trigger mitogen-activated protein kinase (MAPK) and NF-kB. Thus, we used bone marrow macrophages from C57BL/6 (WT), nod1-/- or rip2-/- mice to investigate the role of Rip2 in response against the infection. Macrophages were pre-treated with IFN-γ and infected with T. cruzi. The number of intracellular amastigotes was analyzed 48 hours after infection by Giemsa staining and the release of trypomastigotes in supernatant from cells at 3, 4 and 5 days after infection. We measured activation of the MAPK Erk1/2, p38 and JNK in macrophages infected with T. cruzi. Parasite multiplication was evaluated by Giemsa in macrophages pre-treated with MAPKs inhibitor. We found similar numbers of intracellular amastigotes and released trypomastigotes in all unstimulated-macrophages tested. However, IFN-γ-stimulated Nod1-/- macrophages presented higher number of intracellular amastigotes and released trypomastigotes as compared to IFN-γ-stimulated-WT and Rip2-/-. Thus suggesting that Nod1-dependent responses do not require Rip2. Our data suggest that the activity of JNK, but not Erk1/2 and p38, is required for Nod1-dependent response against T. cruzi. Together, these results indicate that Nod1 is essential to control intracellular replication of T. cruzi in by a novel pathway independent of Rip2 kinase and dependent on JNK kinase.
Supported by:CNPq

IM054 - **RELATIONSHIP BETWEEN THE INOS AND CD3+T CELLS EXPRESSION IN THE CUTANEOUS LESIONS DEVELOPED IN CEBUS APELLA MONKEY BY LEISHMANIA (L.) AMAZONENSIS AND LEISHMANIA (V.) BRAZILIENSIS INFECTION.**

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Cebus apella monkey has been shown to be susceptible to experimental infection by different species of New World dermotropic *Leishmania sp*. and is considered a suitable animal model for studying cutaneous leishmaniasis. The aim of this study was to correlate the parasite burden with the $CD3^+$T cells and $iNOS^+$ expression during the evolution of cutaneous lesions in *C.apella* developed by *L.(L.)amazonensis* and *L.(L.)braziliensis* inoculation. Ten specimens of monkey were intradermally inoculated with $3x10^6$ promastigotes in six different spots on the tail, two groups of 5 animals inoculated with each *Leishmania* species. Skin biopsies were collected at 30, 60, 90, 120, 150 and 180 days post-infection (PI) for immunohistochemical staining using as primary antibodies anti-Leishmania, anti-CD3 and anti-iNOS. A quantitative analysis of the immune-stained cells was done in each section using an image analysis system. A gradual increase on the parasitism was observed in the cutaneous lesion until the 60^{th} day PI. After this period the parasitism decrease was so pronounced that in the 180^{th} day PI there were not found parasites in the healed lesions. The amount of parasites was smaller in *L.(V.)braziliensis* than in *L.(L.)amazonensis* infection. High densities of stained $CD3^+$T and $iNOS^+$ cells were observed at 30^{th} and 60^{th} days PI in *L.(L.)amazonensis* infection followed by a progressive decrease. Nevertheless, the $CD3^+$T and $iNOS^+$ cellular densities were higher in *L.(V.)braziliensis* infection since 90^{th} day PI, reflecting a more lasting and efficient cellular immune response, which was related to decreased parasitism from the 90^{th} day PI. Our results suggest an efficient activation of the cellular immune response of *C.apella* monkey, with the subsequent activation of dermal macrophages and NO production, which is directly related to the reduction of parasite burden and the infection healing in the skin of *C.apella* infected by both *Leishmania* species.
Supported by:FAPESP, LIM-50/HC-FMUSP, Evandro Chagas Institute, Federal University of Pará

IM055 - **ALTERNATIVE ASSAYS FOR DETECTION OF IGG ANTI-T. GONDII IN SAMPLES OF HUMAN SALIVA**

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Toxoplasmosis is high prevalent zoonotic disease that affects about one billion people in the World. Antibody detection is the primary tool in the diagnosis of toxoplasmosis mainly in serum samples. Obtained without invasive procedures and acceptable for children, saliva contains sera from mucosal and gingival crevicular fluid and may be an alternative material as a source of IgG for use in the diagnosis of toxoplasmosis. The low and variable amount of IgG in this material implies in high sensitivity assays and some internal controls, for avoiding false negative results. We devised to use an specific IgG ligand, staphylococcal protein A in two phase solid assays for capture saliva IgG in a controlled way. Microplate protein A binding was used in a capture ELISA and a membrane bound protein A was used in a dot-ELISA assay. Biotinilated T.gondii extract was reacted and avidin peroxidase conjugate used for identification of formed complex. In microplate assay o-phenilenediamine was used as soluble peroxidase color reactive and diaminobenzidine used for membrane assays. Both assays were tested with saliva for 20 volunteers who also provided sera for conventional ELISA. IgG in saliva was also concentrated by etanol precipitation and used 10 x concentrated in conventional anti T.gondii ELISA anti human Fc assays. Capture ELISA was highly discriminative between seropositive or seronegative saliva,.with higher signal than conventional ELISA. Sensitivity and specificity was 100%. Capture dot-ELISA presented the same efficiency with those samples, but presented more difficult visual qualitative analyses. Protein A capture assays using saliva provided a controlled IgG amount which could be used for several tests, as here we presented for toxoplasmosis, but also for vacinal control without invasive procedures in children.
Supported by : LIMHCFMUSP and CAPES.

IM056 - **CHARACTERIZATION OF HEART MUSCLE INFLAMMATORY ANGIOGENESIS DURING THE ACUTE PHASE OF CHAGAS DISEASE IN MICE INFECTED WITH THE BERENICE-78 STRAIN OF T. CRUZI**

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Compelling evidence indicates that angiogenesis and inflammation are key components to the maintenance of a variety of pathological conditions. Chagas disease is known for its intense cardiac inflammation in acute phase. Herein we focus on characterizing the inflammatory angiogenesis in heart muscle during the acute phase of Chagas disease in mice infected with the Berenice-78 strain of T. cruzi.
The effects on various components of inflammatory angiogenesis (cell recruitment, blood vessel formation) were evaluated at 7, 14, 28 and 42 days post-infection. Blood vessel formation was assessed by hemoglobin content and morphometric analysis. The evaluation of the inflammatory process showed that the infected animals presented an increased number of inflammatory cells in the 7th and 28th day after infection (DAI) when compared to control group. Interestingly, the peak of parasitemia occurred on the 15th day after infection in which was not found inflammation in these animals. It also conducted an analysis of the amount of hemoglobin present in heart tissue in both experimental groups to evaluate the extent of vascularization in this organ during infection. Despite the levels of hemoglobin did not differ, morphometric analysis shows a smaller number of vessels at all times evaluated. The infiltration of mononuclear cells into the implants was quantified by measuring the levels of the lysosomal enzyme N-acetyl-β-d-glucosaminidase (NAG) which is present in high levels in activated macrophages. However, macrophage accumulation (as NAG activity) showed no change over the 42 days evaluated. Our preliminary results suggest that infection with Be-78 strain of T. cruzi does not induce inflammatory angiogenesis in heart muscle. More experiments are being conducted to confirm the antiangiogenic activity induced by T. cruzi.
Supported by:FAPEMIG (PPM, Redes Toxifar e Bioterismo), CNPq and UFOP

IM057 - **INTRANASAL IMMUNIZATION WITH LACK DNA INDUCES LONG-TERM PROTECTION AGAINST L. AMAZONENSIS INFECTION IN BALB/C MICE**

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We have previously shown the effectiveness of intranasal vaccination using LACK DNA against cutaneous leishmaniasis (Pinto, E.F. et al., 2004) and visceral leishmaniasis (Gomes, D.C.O. et al., 2007) in mice challenged one week after boost vaccination. In this work, we proposed to evaluate whether the intranasal vaccination with LACK DNA induces a long-term protection against cutaneous leishmaniasis. For this, BALB/c mice were immunized twice by the intranasal route with 30 ug of LACK DNA. Nine, six or three months after boost vaccination BALB/c mice were challenged with fluorescent *L. amazonensis*-GFP promastigotes and the lesion growth was followed for 120 days. Animals immunized 3 and 6 months before challenged presented slower lesion growth (p≤0,001 and p≤0,01) and reduced parasite burden quantified by fluorimetry (p≤0,05 and p≤0,001) and limiting dilution assay (p≤0,05 and p≤0,05) on footpad as compared to PBS. Immunization of animals 9 months before challenge was not able to control lesion growth, however, was also observed a reduced parasite load when compared to control PBS. Quantification of transcripts in the draining lymph node by real time PCR showed higher amounts of INF-g and no difference in the levels of IL-4 and TGF-b as compared to control PBS in 3 and 6 months of immunization indicating a Th1 profile induction. These data show that intranasal vaccination with LACK DNA was able to induce immunological memory, demonstrating to be a potential long-term vaccine against leishmaniasis. Supported by CNPq.
Supported by:CNPq

IM058 - **PARASITEMIA AND SPLEEN STRUCTURE DURING P. CHABAUDI MALARIA IN INNATE RESPONSE SELECTED MICE FOR HIGH (AIRMAX)) AND LOW (AIRMIN) ACUTE INFLAMMATORY RESPONSE**

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Malaria is usually controlled by the host by using all segments of the immune response. Looking for innate immune response, we studied the evolution of a non lethal rodent malaria, Plasmodium chabaudi strain CR, in two mouse strains genetically selected for high (AIRmax) or low (AIRmin)acute inflammatory reaction. Obtained by bidirectional genetic selection, these strains differ in susceptibility to Salmonella enterica serotype Typhimurium and Listeria monocitogenes experimental infections. Groups of BALB/C (control background), AIRmax and AIRmin mice were infected with 106 parasitized erythrocytes obtained during ascending parasitemia of strain CR Plasmodium chabaudi chabaudi infected mouse. Infection was monitored by daily parasitemia, and groups of mice were killed at 4th, 6th, 8th, 10th e 12nd days after infection, for organ collection. Parasitemia increased more rapidly in BALB/C mice, as compared to both AIRmax and AIRmin, which presented similar evolution. Spleens were diversely affected in these models, with AIRmin mice presenting higher spleen weight as compared to other groups, with a huge infiltration of red pulp by blood precursors cells in the 4th days after infection. This fact leads to the disorganization of spleen structure more clearly seen from 8th and 10th days after infection, with high infiltrate of hematopoietic cells in red pulp. AIRmax and BALB/C presented a less intense infiltration of the red pulp with proliferating cells, but subsequently also evolves to huge activation of the red pulp. The infiltration of proliferative cells is important for adequate hematopoietic and immune response to malaria in the spleen, affected by acute inflammatory response. Our findings of high proliferative cells infiltration in the spleen suggest that the low acute inflammatory response results in high cell spleen infiltration and proliferation, resulting in higher spleen weight in those animals, but without affecting the parasitemia control.
Supported by:CAPES

IM059 - **AVALIATION OF REACTIVE OXYGEN SPECIES AND NITRIC OXIDE LEVELS BY NEUTROPHILS INFECTED WITH LEISHMANIA AMAZONENSIS OF C57/BL6 MICE**

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Leishmania amazonensis is an etiological agent of cutaneous leishmaniasis. Neutrophils are one of those primary immune cells that can interact with *Leishmania* and can destroy parasites via formation of respiratory burst. The metabolites of ATP such as adenosine production *in vivo* and commercial drug suramin are considered as anti-inflammatory molecules that can inhibit respiratory burst bi-products. In our work, we evaluated the levels of reactive oxygen species (ROS) and nitric oxide (NO) production in neutrophils treated with adenosine or suramin in presence or absence of *L. amazonensis*. Neutrophils harvested from bone marrow of 4-8 weeks old C57BL/6 mice were purified by percoll gradient centrifugation. To determine ROS levels, we pretreated neutrophils with suramin or adenosine for thirty minutes and then infected with *L. amazonensis* and compared with the group of neutrophils treated and infected at the same time. These neutrophils were analysed in the luminometer for fourty minutes. Later zymozan was added in all groups and analyzed again for fourty more minutes. To analyze the production of NO, we treated uninfected and infected neutrophils with suramin or adenosine for 3 hours. Our data showed that non infected neutrophils treated with adenosine or suramin reduce the production of ROS. This reduction was more evident later with the addition of zymozan in neutrophils treated thirty minutes before measure ROS. There was a similar reduction in ROS production in neutrophils treated and infected with *L. amazonensis*. However, this reduction was higher when zymozan was added in neutrophils treated with adenosine at the moment of infection, as well as neutrophils treated thirty minutes before the time of *L. amazonensis* infection with suramin. NO production by neutrophils was decreased in both treatments after 3 hours of infection. Hence, our preliminary results suggest that *L. amazonensis* inhibits neutrophils for the production of ROS and NO in early infection.
Supported by:CAPES/CNPQ/FAPEMIG/UFOP

IM060 - **NETOSIS IN HEALTHY AND LEISHMANIA-NATURALLY INFECTED DOGS**

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Visceral leishmaniasis is an important zoonosis and dogs are the main reservoir in the urban zone. Neutrophils are among the first cell to interact with the parasite when they are inoculated in the mammalian host. Neutrophils can die releasing traps of DNA, histones and proteins, the neutrophil extracellular traps (NETs), in a mechanism named netosis. Here, we investigate netosis of neutrophils from healthy and naturally Leishmania chagasi-infected dogs, sub-grouped as symptomatic and asymptomatic dogs, stimulated with L. chagasi promastigotes in vitro. Release of NET was measured in neutrophils culture supernatants by its content of DNA through the picogreen assay. Our results showed that neutrophils from healthy dogs released 3 times more NETs after parasite stimulation, and that from asymptomatic dogs, released 2 times more NETs, compared to unstimulated controls. However, neutrophils from symptomatic dogs were unable to increase NET release upon parasite stimulation [5.7 ± 5.2 ng/mL vs 7.8 ± 4.6ng/mL] or upon an unspecific E. coli stimulus. We also evaluated netosis by myeloperoxidase (MPO) activity, as this enzyme is associated with NETs. Thus, MPO activity was quantified in supernatants of cells stimulated with Leishmania, or non-stimulated control cultures. No difference in the MPO activity was observed comparing spontaneous NET release by neutrophils from healthy and symptomatic dogs. However, in symptomatic dogs, MPO activity was accompanied by the NET release pattern, displaying increased MPO activity after Leishmania-stimulation in contrast to symptomatic dogs. Our results suggest that NET mechanism seems to be exhausted in symptomatic animals, preventing neutrophil response to other stimuli. The increased NET release and MPO activity in symptomatic unstimulated neutrophils suggest spontaneous neutrophil activation during ongoing canine visceral leishmaniasis.
Supported by:FAPERJ, CNPq, FAPEMIG.

IM061 - **EXPRESSION OF CD4+/CD25+ TREG CELLS, TGF-B AND IL-10 IN AMERICAN CUTANEOUS LEISHMANIOSIS**

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The immune response of american cutaneous leishmaniasis (ACL) is complex and characterized by Th1 or Th2 cells, which are responsible for resistance or susceptibility to the disease. Several studies suggest that T reg cells, whose phenotype is CD4+CD25+Foxp3+, are responsible for immunologic tolerance, by inducing IL-10 and TGF-b production and modulating the intensity of effector cells and apoptosis of these cells, favoring the parasite survival and increasing the susceptibility of the host. Recents experimental infections with *Leishmania major* have evaluated the role of T CD4+CD25+Foxp3+ in ACL.
The aim of this study was to determine the CD4+CD25+FoxP3+T reg cells, TGF-b and IL-10 expression in the cellular infiltrate of skin lesions of twenty-two cases of localized cutaneous leishmaniasis (LCL) from a typical *L. (V.) braziliensis* endemic area, Buriticupu municipality, pre-Amazonian region of Maranhão State, Brazil.
The paraffin-embedded biopsies were submitted to immunohistochemistry using monoclonal antibodies for FoxP3 (1/600, Sc-28705), TGF-b (1/1000, Sc-146) and IL-10 (1/800, MAB 217-R&D). 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 density of IL-10 was 784,7 mm2, TGF-b was 405 mm², and CD4+CD25+FoxP3+T cells was 301,3mm² in the lesions of these patients.
Our findings confirm the presence of CD4+CD25+FoxP3+T cells, IL-10 and TGF-b in the skin lesions of these patients from Maranhão. Although the role of Treg in the pathogenesis of *L braziliensis* is not completely enlightened, these results suggest that Treg contribute to the control of the T effector cells mediated immunological response.
Supported by:LIM50/HC FMUSP; FAPESP, CAPES.

IM062 - **CLONING OF THE C-TERMINAL DOMAIN PEPTIDES OF LEISHMANIA DONOVANI NUCLEOSIDE HYDROLASE (NH36) AIMING THE IDENTIFICATION OF ANTIBODY EPITOPES**

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The C-terminal domain of NH36, called F3 (amino-acids 199–314) induced a main CD4+ T cell driven response. Immunization with F3 exceeds in 36.73 ±12.33% the protective response induced by NH36. Increases in IgM, IgG2a, IgG1 and IgG2b antibodies, CD4+ T cell proportions, IFN-γ secretion, ratios of IFN-γ/IL-10 producing CD4+ and CD8+ T cells and percents of antibody binding inhibition by synthetic predicted epitopes and a 90.5–88.23% decrease in parasite load were detected in F3 vaccinated mice [Nico et al., 2010 Plos NTD]. In order to map the domain which is the target of the adaptive immunity in F3 protein, three recombinant peptides: F31(amino acids 199–241), F32 (a.a. 242–274) and F33 (275–314) were cloned in pET28b system. The Protean Pad algorithm program predicted two epitopes for antibodies in F31, none in F32 and only one in F33. The peptide sequences were amplified by PCR and oligonucleotides containing the NcoI and XhoI restriction sites were cloned into the pMOS vector for sequencing confirmation and further cloned into pET28b. The recombinant peptides were expressed in E. coli BL21 DE3 cells and purified in a Ni-NTA column. Female Balb/c mice, were vaccinated at weekly intervals, by the sc route, with 3 doses of 100 µg of F3 recombinant protein and 100 µg of Quillaja saponaria (SIGMA) saponin. Seven days after immunization sera were collected and anti-F3 antibodies were measured by an ELISA assay using 2 µg of F31, F32 or F33 recombinant peptides. An absorbance increase was found against the F31 peptide (mean=0,960) and the F33 peptide (mean=0,814) that both fell outside the CI95% of the F32 peptide (mean=0,622; CI95% 0,433-0,811). The One way ANOVA analysis for correlated samples disclosed that the reactivity to F3-1 was significantly higher than the one of F3-2 ($p<0.018$). These results agree with the prediction of algorithm program and confirm the presence of the important epitopes for antibodies in F31 and F33.
Supported by:CAPES; FAPERJ; CNPQ

IM063 - **ORAL VACCINATION WITH TRANSGENIC TOBACCO (NICOTIANA TABACUM L.) EXPRESSING THE LEISHMANIA SPP. ANTIGEN LACK PROTECTS MICE AGAINST LEISHMANIA AMAZONENSIS INFECTION**

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LACK (Leishmania homolog of receptors for activated C kinase) is a well conserved Leishmania ssp. protein. Our group has previously shown that oral and intranasal vaccination with Leishmania amazonensis promastigote antigens (LaAg) and intranasal vaccination with LACK in DNA form conferred protection against visceral and cutaneous leishmaniasis. Based on these findings, in this study we investigated the potential vaccine effect of the transgenic tobacco expressing LACK antigen (Tobacco-LACK+/+). Thus, fresh leaves of Tobacco-LACK+/+ were crushed to powder and lyophilized. Afterwards, BALB/c mice were orally vaccinated by intragastric gavage with 4 doses of Tobacco-LACK +/+ (10 mg) or with 2 doses of LaAg (100 ug), both with a one week interval; or received Tobacco-LACK +/+ (80 mg) mixed with pelleted food ad libitum for 4 weeks. Wild type tobacco (Tobacco-WT) was used as a control. One week after the last dose, mice were subcutaneously infected with 2x106 L.amazonensis-GFP promastigotes and lesion development was monitored periodically. On day 90 post infection, the parasite load in the infection site was evaluated. A lower lesion development and a decreased parasite load indicated that the oral Tobacco-LACK+/+ vaccine conferred more protection against infection than the oral LaAg vaccine. Tobacco-WT conferred no protection against infection. Interestingly, Tobacco-LACK+/+ administered with pelleted food ad libitum showed a stronger protection than the oral Tobacco-LACK+/+ vaccine (administered by intragastric gavage). These results suggest a protective effect of oral Tobacco-LACK+/+ vaccine against L. amazonensis infection and indicate its potential use as an edible vaccine.
Supported by:CNPq

IM064 - **SAPONIN AS ADJUVANT CHANGES CELL INFILTRATE PROFILE IN HYPERSENSITIVITY RESPONSE AND INDUCES STRONG PROTECTION AGAINST MURINE LEISHMANIASIS WHEN ASSOCIATED TO LAAG AND LASP-I VACCCINES**

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Leishmania amazonensis is the main agent of diffuse cutaneous leishmaniasis. Our previous studies demonstrated that intramuscular (i.m.) immunization with whole Leishmania amazonensis antigen (LaAg) in the absence of adjuvant enhances mouse susceptibility to cutaneous leishmaniasis. Using a single-step aprotinin-agarose chromatography, serine proteases were partially purified from membrane fraction of LaAg (LaSP-I). In this study, we investigated the effectiveness of i.m. vaccination with LaAg and LaSP-I associated or not with the adjuvant saponin against leishmaniasis. BALB/c mice were twice vaccinated in the thigh with 25µg of LaAg or LaSP-I in the presence or absence of 100ug of saponin (Riedel-de Haën), prior to footpad infection with *L. amazonensis*-GFP. We found that i.m. vaccination with LaAg alone promotes enhanced susceptibility to infection, but its association with saponin reverts this disease-promoting effect, partially reducing lesion sizes and parasite burdens. Analysis by histology of cells infiltrate 18h after infection demonstrated that mice vaccinated with LaAg plus saponin showed an increase of lymphocytes and eosinophils and decrease of mast cells, neutrophils and macrophages infiltration in comparison to PBS vaccinated mice;, indicating an immune modulation at the beginning of infection that can be associated with the protection observed. On the other hand, vaccination with LaSP-I alone induces a partial protection that is strongly enhanced when associated with saponin, as seen by the significantly smaller lesion sizes and parasite burdens. The LaSP-I plus saponin protection was associated with increased production of IFN-γ in the infected footpads. These findings indicate that the use of saponin leads to immune deviation in the case of LaAg, and an enhanced effect in the case of LaSP-I vaccination. We propose that the use of saponin should be further explored as an adjuvant in potentialy defined vaccine against cutaneous leishmaniasis.
Supported by:CNPq, FPERJ

IM065 - **PARTIAL CONTRIBUTION OF THE B2 RECEPTOR OF THE KININ INFLAMMATION PATHWAY IN MICE VACCINATED WITH THE C-TERMINAL DOMAIN OF NUCLEOSIDE HYDROLASE AND SAPONIN AGAINST VISCERAL LEISHMANIASIS**

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The F3 peptide is the C-terminal domain of the Nucleoside Hydrolase (NH36) of L. donovani. NH36 is the main component of the FML antigen of the Leishmune® vaccine. F3 conferred the best protection against murine visceral leishmaniasis (VL). Here we investigated the potential contribution of G-protein coupled kinin receptors (B2R) in the protective immunity induced by F3. B2R-/- and wild-type C57BL/6 controls (B2R+/+) (n=5) were vaccinated with F3 (100 µg) + saponin (100 µg), challenged with amastigotes of Leishmania chagasi and euthanized 30 days later. The intradermal response (IDR) to leishmanial antigen before and after infection increased in all vaccinated animals over the saline control. IDR from B2R-/- vaccinated mice was lower than controls before infection and higher after infection ($p<0.05$). The increase of the relative weight of liver and spleen are important signs of the advanced disease. A 44.7% ($p<0.05$) of significant decrease of spleen relative weight was noted in vaccinated wild type but not in vaccinated B2R-/- mice. A 26.9% ($p<0.05$) of significant decrease of liver relative weight was noted in vaccinated wild type and 32.5% ($p<0.05$) in vaccinated B2R-/- mice. Our results indicate that the B2R of kinin has a partial contribution to protection against VL splenomegaly. Despite this difference, both vaccinated groups diminished their liver parasite load (96.7% in B2R-/- and 98.5% in B2R+/+; $p>0.05$). In previous work, we observed that B2R-/- mice vaccinated with the FML-saponin vaccine completely lost the protection detected in wild type mice. This was thought to be related to the pro-inflammatory component of saponin treatment. Our preliminary results suggest hence that vaccination with the recombinant peptide F3 conferred stronger protection against VL than the FML-saponin vaccine and has a lower contribution of the B2 receptor of the kinin inflammation pathway, despite the common use of saponin in both vaccines.
Supported by:CAPES; FAPERJ; CNPQ

IM066 - **A COMPLEMENT INHIBITORY ACTIVITY FROM SECRETED PRODUCTS FROM METACYCLIC TRYPOMASTIGOTES FORMS OF TRYPANOSOMA CRUZI**

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During infection of mammals, the insect-derived trypomastigote metacyclic forms of *Trypanosoma cruzi* have to avoid the lysis by the complement system and invade host cells. Our previous data support that resistance to complement-mediated killing is not a strict characteristic of the metacyclic trypomastigote of *T. cruzi*; rather, there are strains sensitive and resistant to complement killing by human serum. The features that make some strains resistant and others sensitive are still unknown. We have hypothesized that the metacyclic tripomastigotes could be releasing serine protease inhibitors (SERPINS) at the first contact with mammalian host. We have tested complement inhibitory activity in secreted products from epimastigotes and metacyclic tripomastigotes forms. Briefly, we have incubated cultured epimastigotes and metacyclic trypomastigotes Y strain at 28, 37 and 42 ºC for 1 h in a serum free DMEM and the supernatant was collected after parasites removal .We made a complement-lysis assay using log growth epimastigotes parasites in presence of 50 % Normal Human Serum (NHS) diluted in DMEM (a condition that lysed 100 % of the parasites at 37 C in 30 min) or in the supernatant obtained at 28, 37 and 42ºC from epimastigotes and metacyclic cultures. We have found that supernatant derived from metacyclic culture at 28 and 37 ºC showed an inhibitory effect ranging from 50-80 % of complement-mediated lysis of epimastigotes forms .However the supernatant derived from epimastigotes at 28, 37 and 42 ºC and 42º C metacyclic supernatant did not show any inhibitory effect. In complement-lysis assay using NHS and NHS treated with EGTA (an inhibitor of classical and Lectin pathway) we have seen no differences in lysis inhibition in different complement pathways. Moreover we have detected that the inhibitory effect is devoid of parasite burden and is not strain dependent. We have searched at the *Trypanosoma cruzi* genome and we have detected the presence of ecotin-like genes, termed inhibitor of serine protease. Ecotin is an 18 kDa protein, it forms dimers and inhibits a wide range of serine proteases from the S1A protease family (trypsin fold) of clan PA, which includes trypsin, chymotrypsin, neutrophil elastase (NE) and cathepsin G. We have designed oligonucleotide primers to those genes and we have cloned and overexpress in epimastigotes forms using Ptex vector. The ability of the parasite to resist to complement lysi and the specificity of the enzymatic inhibition and the expression level of these genes at the different strains is under investigation.
Supported by:cnpq

IM067 - **CHARACTERIZATION OF COSTIMULATORY MOLECULES AND INHIBITORY RECEPTORS DURING PLASMODIUM VIVAX MALARIA**

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In Brazil, malaria is still a significant public health problem. Although the numbers of malaria cases are decreasing, the incidence was higher than 300,000 cases in the past two years. Of these cases, Plasmodium vivax was found to be the causative agent in 90% of these cases. The adaptive immune response, along with the mechanisms of innate immunity, has the task of overcoming the strategies imposed by infectious agents, leading to the control of the disease. It is know that T cell-mediated responses are essential for parasite control, however the mechanisms behind this response are not clear. The goal of this study is to assess the phenotype of T cells from P. vivax-infected patient focusing on the expression of molecules such as inducible T cell stimulator (ICOS), cytotoxic T lymphocyte attenuator (CTLA-4) and programmed death 1 (PD-1). Peripheral blood mononuclear cells from P. vivax-infected patients were obtained in Porto Velho, RO, where lymphocytes were analyzed by flow cytometry. Our data show increased levels of inflammatory cytokines and decreased absolute numbers of lymphocytes during acute infection. Interestingly, the expression of costimulatory and inhibitory molecules has been associated with impairment of T cell function during a variety of infectious diseases. Our data show that the above-mentioned costimulatory and regulatory molecules are also upregulated on T cells from malaria patients. Importantly, the increased expression of PD-1 correlates with liver damage during acute P. vivax infection. Identification of mechanisms that regulate T cell responses during malaria will provide important information on the development of immunological protection against Plasmodium.
Supported by:CNPq, FAPEMIG, REDE MALARIA, NIH

IM068 - **REGULATORY PROFILE IS ASSOCIATED TO ANTI-INFLAMMATORY MEDIATORS IN PATIENTS SERA WITH ACTIVE DIFFUSE CUTANEOUS LEISHMANIASIS (DCL)**

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Diffuse Cutaneous Leishmaniasis (DCL) is a rare clinical manifestation of tegumentary leishmaniasis caused by Leishmania amazonensis. It is characterized by an inefficient parasite-specific cellular response and heavily parasited macrophages. It has been demonstrated “in vitro” that murine macrophage infection by L. amazonensis increases arginase I, TGF-β and PGE2 contributing to parasite proliferation enhancement. However, the relevance of these mediators for DCL pathogenesis remains unknown. Here, we evaluate systemic release of inflammatory mediators in DCL patients.
Sera from 12 active DCL patients and 35 endemic health controls (HC) from Maranhão were obtained between 1980 and 1990. All patients had clinical and laboratory diagnosis of DCL, whereas 15 HC had positive skin response to leishmanin (DTH+) and 20 had DTH-. Serum samples were evaluated for arginase I, TGF-β, PGE2, LTB4 and MCP-1 levels by ELISA. The levels of arginase, TGF-β1 and PGE2 , mediators involved with macrophage deactivation, were increased in the active DCL sera (3,4; 603; 379 pg/ml, respectively) compared with DTH+ (0,16; 294; 43 pg/ml) and DTH- (0,12; 262; 36 pg/ml), indicating a involvement of these anti-inflammatory mediators in DCL clinical state. Additionally, MCP-1 amount, a chemokine that stimulates the oxidative burst in macrophages, was decreased in DCL patients (23 pg/ml) compared with DTH+ (524 pg/ml) and DTH- HC (185 pg/ml). In another hand LTB4, an eicosanoid known by opposite PGE2 effects, did not show difference between DCL (283 pg/ml) and DTH+ (378 pg/ml) or DTH- HC (386 pg/ml). However, PGE2/LTB4 ratios were higher in DCL patients compared with DTH+ or DTH- HC, supporting an anti-inflammatory immune profile in DCL patients. Interestingly, arginase and TGF-β were also higher in DCL patients (603; 4,4 pg/ml, respectively) when compared with sera from patients which present the responsive poles of Leishmaniasis, Localized (126; 0,7 pg/ml) and Mucocutaneous Leishmaniasis (164; 0,4 pg/ml).
Taken together, our data suggest that a regulatory profile might be implicated in the inability of DCL patients to mount an efficient immune response against L. amazonensis. Investigating the involvement of arginase and eicosanoids in DCL pathogenesis can favor the development of new strategies for parasite proliferation controlling.
Supported by:CNPq/FAPESB

IM069 - **THE C-TERMINAL AND N-TERMINAL DOMAIN PEPTIDES OF LEISHMANI DONOVANI NUCLEOSIDE HYDROLASE (NH36) IN MICE CROSS-PROTECTION AGAINST LEISHMANIA AMAZONENSIS INFECTION**

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The C-terminal domain of NH36, called F3 (amino-acids 199–314) induced a main CD4+ T cell driven response against murine visceral leishmaniasis by Leishmania chagasi. Immunization with F3 exceeds in 36.73 ±12.33% the protective response induced by NH36. Increases in IgM, IgG2a, IgG1 and IgG2b antibodies, CD4+ T cell proportions, IFN-γ secretion, ratios of IFN-γ/IL-10 producing CD4+ and CD8+ T cells and percents of antibody binding inhibition by synthetic predicted epitopes and a 90.5–88.23% decrease in parasite load were detected in F3 vaccinated mice [Nico et al., 2010 Plos NTD]. In order to map the domain which is the target of the adaptive immunity in NH36 protein against tegumentar leishmaniasis the recombinant peptides were expressed in E. coli BL21 DE3 cells and purified in a Ni-NTA column. Female Balb/c mice, were vaccinated at weekly intervals, by the sc route, with 3 doses of 100 µg of NH36 protein (aa 1-314), F1 (aa. 1-103), F2 (aa 104-198) or F3 (aa 199-314) recombinant peptides and 100 µg of Quillaja saponaria (SIGMA) saponin. Seven days after immunization sera were collected the intradermal reaction performed and mice were challenged with 106 infective promastigotes of Leishmania amazonensis PH8. The IDR response at 24h and 48 h after injection were significantly increased by the NH36 and the F3 vaccines only. The increase of the size of the footpad lesions was monitored with a Mitutoyo apparatus weekly and significant differences ($p<0.001$) appear on week 6. The F1, NH36 and F3 saponin vaccines reduced the footpad lesions in comparison to saline controls and the F2 vaccine ($p<0.05$). The RTPCR method discloses that only the F1 and F3 vaccines induced a pronounced reduction of parasites in footpad lesions. Our preliminary results suggest that the C-terminal and N-terminal domain of the Nucleoside hydrolase of Leishmania donovani contain relevant epitopes in cross-protection against Leishmania amazonensis infection.
Supported by:CAPES; FAPERJ; CNPQ

IM070 - **INCREASE OF THE ADJUVANT CAPABILITY OF CHIOCOCCA ALBA SAPONIN BY THE INCREASE OF ONE SUGAR RESIDUE IN THE C-28 TRITERPENE ATTACHED SUGAR CHAIN. EFFECT ON PROTECTION AGAINST VISCERAL LEISHMANIASIS**

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We recently isolated two typical Glucuronide Oleanane-type Triterpene Carboxylic Acid 3,28-O-Bisdesmoside (GOTCAB) coded as CA3 and CA4 from the plant Chiococca alba. QS21 saponin from Quillaja saponaria and CA3 and CA4 from C. alba are triterpene saponins containing glycidic moieties attached to the C3 and C28 carbon of their aglycone. In this investigation we compared the adjuvant potential of CA3 and CA4 from C. alba in murine vaccination against visceral leishmaniasis with the FML antigen. We used a QS21 containing saponin as positive control. CA3 and CA4 saponin share a common triterpene nucleus, a Glucuronic acid on C3 and Arabinose, Rhamnose and Apiose on C-28. CA4 shows an additional Apiose residue attached to the Rhamnose unit. We investigated the effect of 100µg of each saponin and 150µg of FML on Balb/c mice challenged with 107 amastigotes of Leishmania chagasi. After vaccination, both CA3 and CA4 saponins increased the IgM, IgG1 and IgG3 antibodies with a mild dominance of CA4 for the IgG2a antibodies. After challenge significant increase of IgG, IgG2a and IgG2b antibodies were only noted in CA4 vaccinated mice. The CA4 saponin vaccine was the only one that sustained the intradermal response to leishmanial antigen at 48h after injection and that increased both the CD4+ and CD8+ Leishmania–specific T cell proportions and induced a higher production of IFN-γ by CD8+ T cells and of TNF-α by CD4+ T cells in spleens. The CA4 also determined the highest parasite load reduction (78%, $p<0.0001$) followed by the CA3 saponin with 57% ($p<0.0001$). The difference between CA4 and CA3 was significant ($p<0.0125$) hence confirming the superiority of the CA4 saponin in protection against visceral leishmaniasis that might be related to the longer and more hydrophilic carbohydrate chain [Oda K et al., Vaccine 2003].
Supported by:CAPES, FAPERJ, CNPQ

IM071 - **IL-17 PROMOTES PROTECTION AGAINST LEISHMANIA INFATUM CHAGASI INFECTION**

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Although Th17 cells are involved in host defense against several pathogens, its function to the resistance to *Leishmania infantum/chagasi*-(Lic) infection, the etiologic agent of visceral leishmaniasis-(VL) in Brazil, is unknown. Thus, our aim was to evaluate whether Th17 participates in the host immune response against Lic and such mechanisms involved. Our results showed that Lic induces high amounts of TGF-β, IL-1β, IL-6, and IL-23 in bone marrow-derived dendritic cells (BMDC), cytokines involved in Th17 differentiation. Accordingly, co-cultivated naïve-C57BL/6 spleenocytes with Lic-infected BMDC produced IL-17 by TCD4+ and TCD8+ cells. Interestingly, IL-17 was produced in high amounts in liver and spleen of C57BL/6WT infected mice, being peaked at the 4th week of infection. The IL-17 is critical for protective immunity against Lic, since that IL-17R-/- infected mice showed a high amount of parasite associated with enhancement of IL-10 production by CD4+T compared to WT mice. Strictly, in the absence of IL-17, a smaller inflammatory infiltrate was observed in the liver of infected mice. Furthermore, IL-23p19-/- and IL-6-/- mice were more susceptible to infection, supporting our hypothesis that Th17 profile development is important for the parasite control. Curiously, IL-17 added into infected-macrophages culture induces significant amounts of NO, and such effect was potentiated when suboptimal dose of IFN-γ was administered together, suggesting that IL-17 acts in synergism with IFN-γ in the control of parasite growth. Taken together, our results show that Lic trigger Th17 response that promotes the host protection during infection. The determination of the mechanisms by which Lic interacts with host defense may open new perspectives for treatment and prophylaxis to VL.Supported by:FAPESP

IM072 - **ROS-DEPENDENT NLRP3-INFLAMMASOME ACTIVATION ACCOUNTS FOR RESTRICTION OF LEISHMANIA AMAZONENSIS INFECTION IN MACROPHAGES AND IN VIVO**

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Nod like receptor Nlrp3 is among the important members of the inflammasomes, which activates caspase-1 through Asc adapter protein. It can be activated by reactive oxygen species-(ROS) generation or after sensing intracellular pathogens. Nlrp3 participates in the detection/control of several bacterial infections. However, Nlrp3-inflammasome role during trypanosomatids parasite infection is unknown. Herein, we investigated Nlrp3-inflammasome role in the host resistance against L.amazonensis infection. Using Nlrp3, Asc and caspase-1 deficient macrophage, L.amazonensis infection induced Nlrp3-dependent caspase-1 activation. Besides, in the absence of Nlrp3 inflammasome, macrophages failed to process IL-1β. Strikingly, ROS inhibitors-treated macrophages failed in the IL-1β production after infection. Nlrp3, Asc, caspase-1 and inducible nitric oxide synthase (NOS2)-deficient macrophages fail to reduce intracellular parasite multiplication. Moreover, ROS scavengers treatment induced a diminished leishmanicidal activity in macrophages. Addition of exogenous IL-1β, in combination or not with IFN-g and TNF-α, contributed to L. amazonensis restriction in macrophage, regardless inflammasome activation triggering NO production. In vivo infection demonstrated that inflammasome activation was crucial for efficient control parasite replication and resolve cutaneous lesions after infection with L.amazonensis and L.braziliensis. Moreover, the inflammasome activation was crucial to parasitism control in the liver and spleen during the L.chagasi infection. Controversially, no difference was observed after L.major infection. Altogether, Leishmania sp trigger the Nlrp3 inflammassome pathway contributing to control of infection, providing a novel function for inflammasome in parasite-host interactions. Elucidating Nlrp3 inflammasome-dependent activation in response to Leishmania infection may help our understanding how NLRs proteins trigger host resistance against infectious diseases.
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IM073 - **FOXP3 EXPRESSION IN CUTANEOUS LESIONS OF DIFFERENT FORMS OF THE AMERICAN CUTANEOUS LEISHMANIASIS CAUSED BY LEISHMANIA (LEISHMANIA) AMAZONENSIS AND L. (VIANNIA) BRAZILIENSIS**

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T cell CD4+ CD25+ Foxp3+ (Treg) is a subpopulation of CD4+ T cells with important role on the induction and control of inflammatory response. Recent reports have shown a major role of Treg Foxp3+ cells in the control of immune response against Leishmania. This study aimed to evaluate the Treg cell profiles through the Foxp3 expression in skin lesions of different clinical forms of American cutaneous leishmaniasis (ACL) caused by L. (V.) braziliensis and L. (L.) amazonensis, addressing a better understanding on the role of Treg cells in the immunopathogenesis of ACL in Brazil. Thirty-one patients were examined: anergic diffuse cutaneous leishmaniasis (ADCL): 6; borderline disseminated cutaneous leishmaniasis (BDCL): 6, both by L. (L.) amazonensis (DTH–); localized cutaneous leishmaniasis (LCL) also due to L. (L.) amazonensis with DTH– (8) and DTH+ (5) and, LCL due to L. (V.) braziliensis with DTH+ (6). Paraffin-embedded biopsies were submitted to immunohistochemistry using the primary antibody anti-Foxp3 (SC-28705), 1:500 dilution. For amplification and visualization of the reaction Novolink max polymer was used. The immunostained cells were counted in 5–10 fields (400x) in section by using an image analysis system (Zeiss). The comparison of Treg cellular density in the clinical-immmunological spectrum of ACL showed a progressive increase in Foxp3+ cells from the central LCL (DTH+) caused by L. (V.) braziliensis to the polar forms, ADCL and BDCL (DTH–) caused by L. (L.) amazonensis, as follows: (ADCLDHT-[636]>BDCLDHT-[487]>LCL/LaDTH-[321]>LCL/LaDTH+[278]>LCL/LbDTH+[354]). In conclusion, the differences observed in Foxp3 expression in the wide ACL spectrum suggest an important role of Treg cells in the genesis of these different clinical forms, possibly by the control of the immune response mediated by effector T cells.
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IM074 - **CYTOKINE PROFILE AND DEVELOPMENT OF CEREBRAL MALARIA DURING PLASMODIUM BERGHEI ANKA INFECTION: ROLE OF SOCS2**

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Introduction: Human cerebral malaria (CM) is the most important complication of infection with Plasmodium falciparum. Mice infected with P. berghei ANKA (PbA) faithfully recapitulate many of the characteristics of human CM. Although the pathogenesis of CM is not completely understood, it likely involves a vasculopathy and an inflammatory response. The suppressor of cytokine signaling (SOCS) 2 is an intracellular protein induced by eicosanoids and hormones, and is important to modulate the inflammatory response. However, the involvement of SOCS2 in CM is not known. Objective: Investigate the role of SOCS2 in the regulation of immune response and development of CM. Method: C57Bl/6(WT) and SOCS2-/- mice were infected with PbA and the parasitemia, survival and body weight were monitored periodically. The production of cytokines (TNF-α, IL-1β, TGF- β, IL-6, IFN-γ, IL-12 and IL-10) in the brain and spleen was assessed by ELISA and flow cytometry. Leukocyte recruitment in the brain was evaluated by intravital microscopy. Histopathological analysis was performed in cerebral cortex, brainstem and hippocampus. Results: The parasitemia was significantly lower in SOCS2-/- compared with WT mice and no difference in lost weight was detected among the groups. In the brain and spleen of PbA-infected SOCS2-/- mice there was a significant increased expression of TGF-β and decreased expression of IFN-γ and IL-10 when compared with infected WT mice. Additionally, there was an increased expression of IL-6 and decreased expression of IL-1β, TNF-α and IL-12 in the brain of infected SOCS2-/- mice when compared with WT counterparts. Moreover, a significant increase of leukocyte rolling in the brain microvasculature and microvascular obstruction of the hippocampus was found in the PbA-infected SOCS2-/- mice when compared with WT. Conclusions: These findings indicate, for the first time, a role for SOCS2 in the immunopathogenesis of PbA-associated CM.
Supported by:CAPES, CNPq, FAPEMIG

IM075 - **INTRANSAL VACCINATION WITH ANTIGENS OF LEISHMANIA AMAZONENSIS BUT NOT OF L. MAJOR, L. BRAZILIENSIS, L. CHAGASI AND L. DONOVANI ARE PROTECTIVE AGAINST CUTANEOUS LEISHMANIASIS**

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We have previously shown the effectiveness of intranasal vaccination using whole antigen from *L. amazonensis* (LaAg) against *L. amazonensis* infection (Pinto, E.F. et al., 2004).The species of Leishmania present a genetic homology between from 70% to 90%. In this work, we proposed to evaluate wheter intranasal efficacy is extensive to antiegns of L. braziliensis (LbAg), L. chagasi (LcAg), L. donovani (LdAg) and *L. major* (LmAg). For this, BALB/c mice (n=8) were immunized twice by the intranasal route with 10 mg of each antigen. One week after booster vaccination the animals were challenged with $5x10^5$ *L. amazonensis* promastigotes in the footpad. Three days after challenge the total number cells of cervical and popliteal lymph nodes was assessed. The lesion growth was followed for 80 days and then animals were sacrificed to assess the parasite load by limiting dilution assay. The results showed that on day 3 of infection all vaccinated groups, especially the one with LcAg, had a higher number of cells in cervical lymph nodes as compared to control PBS. On the other hand, only the LaAg group had lower cells in popliteal lymph nodes than PBS. The protective effect of LaAg was confirmed both by lesion growth and parasite load measurements. Interestingly, LbAg immunization had no effect whereas LcAg, LdAg, and more pronouncedly LmAg increased the susceptibility to infection. In conclusion, despite the great antigen homology between different Leishmania species, LaAg contains an especific component responsible for protection.
Supported by:CAPES

IM076 - **LACK OF VITAMIN A INCREASES SUSCEPTIBILITY TO INFECTION WITH LEISHMANIA AMAZONENSIS AND REDUCES THE EFFICACY OF AN ORAL VACCINE**

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LaAg oral immunization (Leishmania amazonensis promastigote lysate) protected BALB/c mice against L. amazonensis infection in a manner related to peripheral tolerization to TH2 responses. Retinoic acid (Vitamin A - VitA) is powerful inducer of FoxP3 expression and enhances commitment to Treg cell lineage. Then, we evaluated infection outcome with L. amazonensis in VitA deficient C57BL/6 and BALB/c mice, oral LaAg vaccine ability in CD4+Foxp3+ Treg cells expansion and their efficacy in VitA deficient BALB/c mice. Gravid females received lacked VitA (VitA-) diet or normal (VitA+) diet. Pups were maintained on the same diet. Serum retinol levels were analyzed by HPLC. VitA+ and VitA- C57BL/6 and BALB/c mice were infected in footpad with L. amazonensis. We observed that VitA- were more susceptible to infection than VitA+, presenting higher parasite load and diminished IFN-γ and TGF-β production in peripheral lymph nodes and infection site. After, VitA+, VitA- and CT BALB/c mice (treatment with Citral, 14 days before vaccination until the day of second dose) received 2 doses of LaAg (100 µg) or PBS once a week, by gavage. Mesenteric lymph nodes were collected 2 days after second dose. LaAg increased CD4+FoxP3+ expansion in VitA+ but not in VitA- or CT. After, VitA+, VitA- and CT were immunized, as previously describe, and 7 days after second dose, were infected in footpad. LaAg did not impair capacity of infected VitA- and CT to mount a disease-associated hypersensitivity response. VitA- were more susceptible to infection than VitA+ (higher parasite load and lesion). VitA+ producing higher IFN-g and TGF-β and decreased IL-4 and IL-10 production in infected footpads and peripheral lymph nodes. Dietary Vitamin A is required for an effective response against cutaneous leishmaniasis caused by L. amazonensis, and for effectiveness of oral LaAg vaccine in BALB/c mice. The requirement of Vitamin A may be associated with CD4+Foxp3+ Treg cells expansion in the gut-associated mucosa.
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IM077 - **BRADYKININ B2 RECEPTORS ARE CRITICAL FOR THE DEVELOPMENT OF PROTECTIVE TYPE-1 RESPONSES IN VISCERAL LEISHMANIASIS**

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Visceral leishmaniasis is characterized by complexity of clinical manifestations ranging from asymptomatic infection to life threatening illness. The pathology of VL is associated with immunosupression and immunological dysfunctions. Experimental evidences and clinical studies indicate a development of organ-specific immunity in the two main target tissues of infection: the spleen and the liver. Kinins, the vasoactive peptides proteolytically liberated from kininogens, were recently recognized as signals alerting the innate immune system. Our main purpose was to evaluate the role of bradykinin B2 receptors (B2R) during visceral leishmaniasis. B2R-/- and wild-type C57BL/6 controls (B2R+/+) (n=5) were infected with with 3 x 107 amastigotes of L. chagasi. After 30 days post-infection (30 d.p.i), mice were sacrificed to evaluate the parasite load in the liver and T cell mediated-response from spleen. B2R-/- mice infected display a 6-fold increase in parasite load of liver as compared to B2R+/+ mice. Analysis of the clinical parameters indicate that the infection on B2R-/- mice resulted in loss of corporal weight and a relative increase in both liver and spleen weight as compared to B2R+/+. Moreover, analysis of recall responses (30 d p.i.) showed production of IFN-gamma from B2R-/- splenocytes was significantly reduced as compared with wild-type mice. Our results demonstrate that activation of B2R is critically required for development of acquired resistance to Leishmania infection.
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IM078 - **MYCOBACTERIUM BOVIS AND TRYPANOSOMA CRUZI CO-INFECTION DOWN REGULATES PARASITE REPLICATION IN HUMAN MACROPHAGES**

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Chagas' disease is an endemic disease caused by *Trypanosoma cruzi* that affects about 18 million people in Central and South America with more than 10 million at risk of infection.
Almost 100% of Brazilian population has been immunized with BCG Moreau RDJ to protect against tuberculosis. BCG is also the only immunomodulator used as treatment for a cancer disease (superficial bladder cancer). Aiming to understand the immunomodulator effect of BCG in Chagas' disease we have studied the effect of BCG in the in vitro model of *Trypanosoma cruzi* infection in human macrophages. The human macrophages were infected with BCG and after 24 hours were infected with trypomastigotes forms of *Trypanosoma cruzi* Y strain. When evaluating the number of intracellular parasites through the endocytic index at 72 hours after infection, we found that both macrophages pretreated with conditioned medium of BCG and macrophages previously infected with BCG had a reduced endocytic index of 71.6% and 56% amastigotes respectively compared to untreated controls. Our preliminary results have shown that amongst cytokines detected by ELISA in culture supernatants we observed increased levels of TNF-α, IL1-ß and IL-10. Further studies are in progress to investigate the participation of IL-1β andTNF-α proinflammatory cytokines and IL-10 cytokine in the control of the replication and immune response to *Trypanosoma cruzi.*
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IM079 - **INTERACTIONS BETWEEN MOUSE SIGLEC-E (SIALIC ACID-BINDING IG-LIKE LECTIN-E) AND T.CRUZI LEAD TO IMPAIRED IMMUNE RESPONSE AGAINST THE PARASITE**

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Trypanosoma cruzi is an obligate intracellular protozoan parasite. The trypanosomal trans-sialidase (TS) is described to be a major virulence factor. This enzyme allows the parasite to acquire sialic acids from its environment by cleaving sialic acids from host glycoconjugates and to transfer these directly to GPI-anchored mucin-like molecules on its own cell surface. In the current investigation we provide evidence that members of the Siglec family, which are sialic acid-specific lectins, directly interact with *T. cruzi* parasites. Siglecs have been shown to act as inhibitory receptors, ascribed to the presence of conserved immunorecepor tyrosine-based inhibition motifs (ITIMs) in the cytoplasmic regions. This suggests that the natural role of Siglecs is the modulation of immune function.
Using Siglec-E transfected CHO-cells and a Siglec-E-Fc fusionmolecule we demonstrate that Siglec-E, which is expressed on mouse phagocytic cells, neutrophils as well as NK cells, binds with a high affinity to trypomastigotic parasites of the pathogenic *T. cruzi* Tulahuen strain, but not to the non-pathogenic *T. cruzi* Tehuantepec. To further investigate if Siglec-binding correlates with virulence and persistence, we analyzed in collaboration with the INP (Buenos Aires, Argentina) 12 additional *T. cruzi* strains. Moreover we observed that the microsomal fraction of *T. cruzi* lysates binds with high affinity to Siglec-E. The amount of sialic acids was determined with the lectin Mal II and correlates with Siglec-binding.
These results further support the notion that the interaction of Siglec-E with sialylated mucins modulates the immune response of the host, which favours parasitemia and persistence of *T. cruzi*.
Supported by:

IM080 - **HUMAN NEUTROPHILS ACTIVATED BY FIBRONECTIN CONTROL LEISHMANIA AMAZONENSIS INFECTION IN HOST MACROPHAGES**

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Introduction and objective: Neutrophils are involved in the initial responses against pathogens and they are activated by their passage through the endothelium in direction to the inflammatory site. In the present study, we activated the human neutrophils using proteins of the extracellular matrix and evaluated the effect of viable activated human neutrophils on macrophage infection by Leishmania amazonensis.
Results: Fibronectin interaction with neutrophils induced a higher level of activation than collagen, laminin or even an artificial extracellular matrix . This neutrophil activation was evidenced by an increased in the levels of matrix metalloprotease (MMP)-9 and neutrophilic elastase (NE) released from neutrophilic granules. Interaction between viable fibronectin-activated neutrophils and Leishmania-infected macrophages decreased the parasite burden in a mechanism independent on contact and mediated by action of neutrophilic granule proteases. We also observed an increase in the levels of TNF-alpha, leukotriene B4 (LTB4) and superoxide production, suggesting the contribution of these factors in the intracellular killing of the parasites. Moreover, we observed a major population of neutrophils -Annexin V and PI - double labeled after activation with fibronectin, indicating that they were in secondary necrosis. Treatment of the neutrophils with proteases inhibitor reversed this phenomenon.
Conclusion: These results indicate that macrophage interactions with fibronectin-activated neutrophils can control parasite in the initial events after L. amozenensis infection, but also may contribute to the inflammation observed in lesions caused by Leishmania spp.
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