Imunologia- Immunology

IM01 - Complement Lectin Pathway activation for trophozoites forms of *Giardia intestinalis*

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Giardia intestinalis is a ubiquitous intestinal protozoan parasite causing disease in humans and animals worldwide. Molecular and evolutionary studies localize this parasite as one of the earliest divergent eukaryotic lineages. In spite of recent advances in biochemistry and molecular biology of *G. intestinalis*, little is known about the role of innate immunity in host defence.
The complement system is comprised of three different cascades: the classical, the alternative, and the recently described lectin complement pathway (LCP). The LCP is an antibody-independent cascade initiated by binding of mannose-binding lectin (MBL) and Ficolins to cell surface carbohydrates. The aim of this work is to investigate the ability of axenically cultured trophozoites **of** *Giardia intestinalis* to activate the lectin Pathway.
First, The Complement activation by *Giardia intestinalis* was showed by measuring Normal Human Serum (NHS) deposition factor on the parasite membrane by FACS.The relative FITC fluorescence using anti C3 , anti MBL, anti H and anti L ficolin antibodies showed 55 %, 30 %, 41 % and 61 % of deposition in 5 minutes with NHS.
Secondly we detected an inhibition from 20 % to 80% of complement-mediated Lyses when NHS were incubated with 20 to 80 mM of Mannose and Nacetil glucosamine and lower inhibition (5 to 20%) was obtained with ficose , Galactose and glicose (20- 80 mM). These results suggest the involvement of MBL and ficolins at the complement activation.
Finally Kinetics of complement- mediated lyses with NHS (C , L and A pathways) and EGTA-treated NHS (AP) and a pre NHS activated Parasites/ EGTA treated NHS (LP) measure from 0 to 30 Min have being done showing *Giardia intestinalis* activates the three pathways (under studies).Taken together, we are showing for the first time a efficient an rapid activation of Complement Lectin Pathway by trophozoites of *Giardia intestinalis*.
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IM02 - Adhesion of *Toxoplasma gondii* infected maternal cells on trophoblast in the placenta

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【Background】*Toxoplasma gondii* is an intracellular parasite. It has been thought that *T. gondii* disseminate to the peripheral organs, including placenta, by circulation of tachyzoite-infected leukocytes in the blood flow. In vitro studies show that tachyzoite-infected maternal cells leukocytes bound to trophoblast. However, it has not yet been known whether tachyzoite-infected maternal leukocyte bound to chorion/villus of the placenta. The aim of this study is to determine whether tachyzoite-infected maternal leukocytes bound to chorion/villus using mouse model.
【Material and methods】Heterozygous GFP transgenic female mice (GFP+/-) were crossed to wild type male mice (GFP-/-). Genotype of fetus were GFP+/- or GFP-/-. When GFP+/- mice conceive GFP-/- fetuses, maternal and fetal tissues can be clearly distinguished by green fluorescence (GFP positive and negative, respectively). At 14 days gestation, the pregnant model mice were infected with transgenic red fluorescent *T. gondii* tachyzoites which express DsRed Express. The placentas were observed at 4d.p.i.

【Results】Accumulation of green fluorescent maternal leukocytes on the surface of non-fluorescent the fetal tissue of the placenta. Approximately 50 % of the green fluorescent maternal leukocytes were infected with red fluorescent tachyzoites.

【Conclusions】

It was confirmed that tachyzoite-infected maternal leukocytes bound to the fetal tissue of the placenta. Considering the frequency of infected rate of maternal leukocyte in general circulation was less that 0.1 %, the much higher infection rate of the green fluorescent maternal cells on the surface of fetal tissue suggests that tachyzoite-infected maternal leukocytes selectively attach to the fetal tissue of the placenta.

IM03 - SALIVARY GLAND HOMOGENATES ENHANCES PLASMA LEAKAGE INDUCED BY *L.chagasi* PROMASTIGOTES

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Leishmaniasis is a vector-borne disease transmitted by phlebotomine sand flies such as *Lutzomyia longipalpis*. Saliva of arthropods could enhance infectivity of pathogens like *Leishmania chagasi* promastigotes. Using intravital microscopy, we have recently showed that *L. chagasi* promastigotes induce plasma leakage in postcapillary venules of the hamster cheek pouch (HCP) by mechanisms involving activation of the kinin pathway (Svensjö et al., Microbes and Infection, 2006) while homogenates of salivary glands evoke leakage responses via the PAC-1-receptor pathway, simulating the proinflammatory effects of purified maxadilan (Svensjö et al., J. Vasc. Res, 2009). Here we studied the vascular leakage responses (FITC-dextran) and arteriolar diameter changes in the HCP induced by topical applications of *L. chagasi* promastigotes (LCP), alone or combined with salivary gland homogenates (SGH) of *Lutzomyia longipalpis*. Plasma leakage (extravasation of FITC-dextran) and arteriolar diameter was measured in images using computer software (AxioVision 4.4). Animals (n = 22) were divided into 4 groups, saline control, salivary gland homogenates (one salivary gland = SGH), *L. chagasi* promastigotes $3 \cdot 10^7$/ml (LCP) and SGH + LCP. Applications were made topically to the HCPs and lasted for 9 minutes after which images were recorded for 60 minutes. There was no plasma leakage increase in the saline control group (100%) but there was a lasting increase in the SGH-group with a maximum of 90 % above the saline control at 20 min, 74% in the LCP-group at 50 min and 273% in the LCP+SGH-group at 30 min. The plasma leakage response in the LCP+SGH-group was larger than the arithmetic sum of the LCP and SGH effects until 60 min when it still was 23% above the arithmetic sum. **Conclusion**: Our data suggest that PAC1 receptor activation by maxadilan and/or by other mediators present in salivary gland homogenates from *L. longipalpis* may potentiate the intrinsic inflammatory effects of *L. chagasi* promastigotes (LCP).

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IM04 - GENERATION AND ANALYSIS OF THE IMMUNOGENICITY OF A RECOMBINANT PROTEIN BASED ON THE APICAL MEMBRANE ANTIGEN 1 (AMA-1) OF *PLASMODIUM VIVAX* EXPRESSED IN *PICHIA PASTORIS*

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The apical membrane antigen 1 (AMA-1) is one of the most promising vaccine candidates against the asexual stages of malaria. In the last years, we have studied several aspects of the immune responses against bacterial recombinant proteins based on *P. vivax* AMA-1 (bPvAMA-1) ectodomain in individuals from malaria endemic areas of Brazil. These studies demonstrated that a high frequency of individuals infected by *P. vivax* had IgG antibodies to this protein. In the present study, aiming at future immunizations in non-human primates, we designed a codon-optimized synthetic gene encoding PvAMA-1 ectodomain for large scale expression in the methylotrophic yeast *Pichia pastoris (*yPvAMA-1*)* using the pPIC9K vector. In addition, three potential N-glycosylation sites were mutated and the construct was made as C-terminal His_6 fusion proteins to enable purification of the protein. The protein yPvAMA-1 was expressed as a secreted, soluble protein and the yield was superior to that obtained previously in *E. coli.* This recombinant protein maintains its antigenicity, being recognized by a high percentage of sera from individuals infected by *P. vivax.* The immunogenicity of this protein was evaluated in BALB/c mice using protocols of individual immunization and prime/boost strategies with plasmid DNA (*pIgSP-Pvama-1*) and recombinant protein (yPvAMA-1). After 3 immunizing doses with 10 μg of the protein emulsified in Complete (CFA) or Incomplete (IFA) Freund′s adjuvant or 100 μg of the DNA plasmid, we observed that DNA/Protein (IFA), Protein/Protein (CFA/IFA) and Protein (CFA)/DNA protocols induced significantly higher IgG antibody response than DNA/DNA and Protein/Protein (without adjuvant) regimens. Pre-clinical studies in mice comparing other adjuvant formulations are under way. We concluded that the protein yPvAMA-1 is necessary in the formulation of the malaria vaccine and deserves further evaluation in pre-clinical immunizations against *P. vivax* in nonhuman primates. Supported by FAPESP and CNPq (IMTEV).

IM05 - C57BL/6 MICE WITH 200 DAYS OF INFECTION WITH A NON-LETHAL STRAIN OF *Plasmodium chabaudi* ARE CAPABLE OF INHIBITING HOMOLOGOUS REINVASION, BUT NOT HETEROLOGOUS REINVASION BY A LETHAL STRAIN

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Studies performed in our research group showed a time–correlated decrease in memory immunological responses to *P. chabaudi* AS (a non-lethal strain) in C57BL/6 mice, culminating with lack of challenge infection control when animals have 200 days post-infection (10^6 parasitized erythrocytes - PEs). To evaluate the relationship of this decrease with a decrease of controlling red blood cells (RBCs) reinvasion by merozoites, we compared the reinvasion ratio (RR) against *P. chabaudi* AS between non-infected and 200 days p.i. – infected mice (with patent and subpatent infection models), utilizing a flow cytometry-based assay, in which we analyzed parasitemia levels periodically in mice i.v. inoculated with $5x10^8$ PEs (only mature forms, isolated by Percoll gradient), and calculated RR values as a ratio between 8h and 30 min post-inoculation values. At the same time, we analyzed the RR of AS-infected mice against a lethal strain of *P. chabaudi* (AJ). Results showed lower RR values in patent and subpatent mice compared to non-infected animals, when AS strain was inoculated ($p<0,001$; n=5). However, when AJ strain was inoculated, there was not reinvasion inhibition in subpatent AS-infected mice, compared to non-infected mice ($p>0,05$; n=5). ELISA tests were performed to measure specific IgG1 and IgG2a in sera of AS-infected mice before, 24h and 7 days after i.v. inoculation. Results showed a decrease in antibody titers for all experimental groups 24h after inoculation, even in animals inoculated with AJ strain ($p<0,05$; n=5), making clear that antibodies present in sera of these mice are capable of recognizing AJ-strain merozoites, despite absence of reinvasion inhibition. This work also confirm that antibodies present in mice with 200 days p.i. are capable of controlling reinvasion by AS strain, thus showing that lack of challenge control are not correlated with lack of reinvasion inhibition, adding evidence for an antibody-independent component of responses against challenge in this model.
Supported by FAPESP and CNPq.

IM06 - CONTRIBUITION OF PERFORIN IN DEVELOPMENT OF CHRONIC CHAGASIC CARDIOMYOPATHY PATHOGENESIS

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Comprehension of the dual participation of the immune response controlling the invader and leadingto tissue damage might contribute to design effective vaccines and new therapies for Chagas disease. Perforin, a cytolytic protein employed by killer cells, is involved in resistance to acute *Trypanosoma cruzi* infection. However, the contribution of perforin in parasite control and chronic chagasic cardiomyopathy is unclear. Perforin+ cells were detected in the heart tissue during the acute and chronic infection of C57BL/6 mice inoculated with low dose (10^2 parasites) of the Colombian *T. cruzi* strain. This protocol led to acute phase survival in both wild-type and perforin null (pfp-/-) mice lineages. During the chronic infection, parasitism and inducible nitric oxide synthase as well as IL-4+ and, mainly, IFN- + cells were more elevated in the heart tissue of pfp-/-
mice. Higher levels of circulating nitric oxide and anti-parasite IgG2c and IgG3 paralleled by a prominent frequency of IFN-+ and IL-10+ splenocytes were evidenced in pfp-/- infected mice. Therefore, although the perforin-dependent pathway plays a role, it is not crucial for anti-*T. cruzi* immunity and acute phase survival of mice infected with a low inoculum. Further, perforin deficiency resulted in lower activity of creatin kinase-MB isoenzyme (CK-MB) in serum and a more restricted connexin 43 loss, markers of cardiomyocyte lesion. Moreover, perforin deficiency hampered the development of severe electrocardiographic abnormalities. Hence, our results corroborate that perforin-bearing cytotoxic cells might contribute to cardiomyocyte lesion and heart dysfunction during chronic *T. cruzi* infection, shedding light on immunopathogenesis of chronic chagasic cardiomyopathy.
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IM07 - Involvement of CD43-mediated T cell homing in pathogenesis of experimental chagasic cardiomiopathy: elucidation of a mechanism to exploit as a therapeutical target during *Trypanosoma cruzi* infection.

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One characteristic of infection by *Trypanosoma cruzi* is the pronounced inflammatory process in the myocardium due to parasite persistence and immune response at early stage of infection. Prevalence of myocarditis correlates with the severity of chronic heart cardiopathy. Thus, the intervention in leukocyte recruitment during acute phase of infection may be an important step to attenuate tissue damage and fibrosis that occur in chronic phase of Chagas' disease. Several works have demonstrated that CD43, a sialoglycoprotein expressed on leukocyte surface, functions as E-selectin ligand during inflammatory process. In this study, we infected CD43 deficient mice with *T. cruzi* (Y strain) aiming to shed light into the possible role of CD43 in leukocyte migration to heart during acute phase of murine infection. We observed that the CD43 deficient mice are more resistant to parasite infection, presenting lower heart parasitism and mortality. CD43KO infected mice showed a decrease number of inflammatory cells in cardiac tissue leading to attenuated myocarditis at day 15 post infection, although it did not change parasitemia. Treatment of wild type mice with an inactive form of *T. cruzi trans*-sialidase (iTS), which is a lectin that bind to CD43, led to a significant decrease in both subpopulations of T cells infiltrating the heart, decreasing tissue injury and animal mortality. iTS effects were not observed in infected CD43KO, suggesting that the decrease in inflammation and injury is mediated by binding of iTS to CD43 sialoglycoprotein. Corroborating with the data obtained during the acute phase, heart from chronically infected CD43KO mice, had lower tissue fibrosis. Together, our results suggest that CD43 plays an important role in the cardiomyopathy observed during *T. cruzi* infection, emerging as a potential target for therapeutic intervention in Chagas disease and that riTS can be an attractive tool to attenuate chagasic myocarditis.
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IM08 - ACE inhibitors harness the adjuvanticity of bradykinin in a vaccine formulation that promotes recruitment of immunoprotective CD8$^+$ effector T cells to peripheral sites of *Trypanosoma cruzi* infection in mice

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Bradykinin (BK), a nonapeptide that induces dendritic cell (DC) maturation via bradykinin B_2 receptors (B_2R), was recently characterized as a type-1 directing adjuvant of endogenous origin. Here we used the mouse model *Trypanosoma cruzi* infection to ascertain the efficacy of alum-based vaccine formulations supplemented with exogenous BK. Using soluble epimastigote extracts as the immunogen, we initially found that Balb/C mice immunized with [Alum/BK/Ag] succumbed (100%) to lethal challenge. Analysis of their immune responses showed evidence of B_2R-dependent IgG isotype switch (IgG1>IgG2a). In addition, mice immunized with the above formulation developed high-frequency of Ag-specific IFN-γ-producing CD4$^+$ and CD8$^+$ effector T cells in lymphoid-associated tissues, but these effectors were virtually absent in the heart. Considering that angiotensin converting enzyme (ACE) degrades bradykinin, we checked if BK adjuvanticity and vaccine efficacy could be harnessed by the ACE inhibitor captopril (Cap). Indeed, Balb/C mice receiving a single-dose of Cap shortly before immunization with [Alum/BK/Ag] developed resistance to lethal challenge (100% survival). Induction of protective immunity in vaccinated Cap-mice was linked to presence of high frequencies of intracardiac IFN-γ-producing CD4$^+$CD44$^+$CD69$^+$ and CD8$^+$CD44$^+$CD69$^+$ T cells. Adoptive transfer experiments showed that protective immunity in vaccinated Cap-mice was mediated by CD8$^+$ T cells, but not by CD4$^+$ T cells. Consistent with our working hypothesis, HOE-140 (B_2R antagonist) abolished vaccine-induced protection in Cap-mice. In addition, mice immunized with splenic CD11c$^+$ DCs that were previously pulsed *ex-vivo* with Ag in the presence of BK and Cap resisted lethal challenge, this effect being linked to presence of intracardiac type-1 effector T cells. Collectively, our studies suggest that drug-assisted maneuvers (such as ACE inhibition) that prolong the half-life of the BK adjuvant in the immunized mice translates into increased frequencies of IFN-γ-producing /effector function of CD8$^+$ T cells in peripheral tissues, thereby improving efficacy of vaccination against intracellular pathogens.
Supported by: CNPq.

IM09 - INDOLEAMINE 2,3-DIOXIGENASE (IDO) IS IMPORTANT FOR THE CONTROL OF *T. CRUZI* AMASTIGOTE GROWTH IN MACROPHAGES.

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Resistance to *T. cruzi* infection is reported to be dependent on the capacity to generate IFN-γ and TNF which can activate macrophages (Mo) to produce the microbicidal product nitric oxide (NO) generated by NO synthase (iNOS). In addition, these proinflammatory cytokines are able to induce IDO activity in Mo and dendritic cells. IDO is an intracellular enzyme that catalyses the initial rate-limiting step of tryptophan (Trp) catabolism leading to the production of immunoregulatory catabolites, collectivelly called "kynurenines". Depletion of Trp and the production of "kynurenines" are responsible for the activities observed after IDO induction including effects on intracellular pathogens replication and lymphocyte proliferation, survival and anergy. We have demonstrated that *in vivo* IDO blockade using 1-MT impairs the resistance to *T. cruzi* infection in mice. In order to study the effect of IDO activity on the regulation of intracellular *T cruzi* growth, we used murine bone marrow derived Mo. IDO blockade in Mo resulted in a strong stimulatory effect on parasite growth that was dependent of 1-MT dose. In addition, Trp supplementation could prevent IDO-mediated inhibition meanwhile the addition of L-kynurenine has no effect on parasite replication. In order to know the specific contribution of IFN-γ-inducible IDO and iNOS to control *T. cruzi* replication, Mo were cultured in medium alone or containing IFN-γ or IFN-γ plus LPS for 24 h and then infected. The activation with INF-γ or INF-γ plus LPS resulted in NO production, induction of IDO activity and a strong inhibitory effect of amastigote growth. Moreover, the IDO blockade in these cultures resulted in a reversion of the inhibitory effect of amastigotes growth induced by IFN-γ or IFN-γ plus LPS. IDO activity, through Trp starvation, is critical for the control of *T. cruzi* amastigote growth in Mo.
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IM10 - TUMOR NECROSIS FACTOR ALPHA (TNF-α) and TNF-RECEPTOR 1 (TNF-R1) ARE INVOLVED IN THYMOCYTE MIGRATION DURING *TRYPANOSOMA CRUZI* INFECTION

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Although TNF-α is protective during *Trypanosoma cruzi* infection, its overproduction could be detrimental to the host and might contribute to the pathophysiology of the disease. During murine acute infection, we detected an increased exit of immature $CD4^+CD8^+$ double positive (DP) T-cells linked to a massive loss of DP cells by apoptosis, in parallel to increased intrathymic contents of tumor necrosis factor alpha (TNF-α) and extracellular matrix proteins (ECM), including fibronectin (FN). DP cell death seems to be TNF-α-independent, but since normal thymocyte export is influenced by ECM and cytokines/chemokines-mediated interactions, we explored the TNF-α plus FN combined contribution to the migratory activity of thymocytes during infection. Briefly, 2,5x10^6 thymocytes from infected or non-infected mice were incubated in transwell devices and allowed to migrate during 3 hours through a membrane coated with FN (10 mg/ml) plus TNF-α (25 pg/ml) or BSA (PBS/BSA 0.5%). Migrating cells were counted and their phenotype analyzed by cytometry. In all cases, thymocytes from infected mice showed a higher migratory response than non-infected independently of membrane treatment. Strikingly, in infected animals, FN interactions with TNF-α enhanced motility of total thymocytes compared with FN/BSA ($p<0.05$). The analysis of thymocyte subset input showed similar results, especially in DP cells from infected mice ($p<0.05$). The blocking of TNF-α activity by anti-TNF-α antibody, diminished significantly DP migration. Thymocytes from infected TNF-R1-/- mice turns down in half the input on FN/TNF-α compared with their normal TNF-R1+/+ counterparts, whereas the addition of TNF-α blocking antibody deeply abrogated DP cell migration, suggesting a role for TNF-R2 in thymocyte migratory activity.
Our results put forward that *in vivo* thymic anomalous exportation of immature and potentially auto reactive DP T cells could be related with a high expression of molecules involved in migratory activity and co-stimulated by TNF-α-deposition.
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IM11 - THE INVOLVEMENT OF NEUTROPHILS IN IMMUNE RESPONSE TO *LEISHMANIA AMAZONENSIS*

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Neutrophils provide the first line of defense against infection and also contribute to the initiation of inflammation. However the role of neutrophils in response to *Leishmania* is not clear yet. Moreover, most of the studies were performed in *Leishmania major* model of infection, hence there is little information on the involvement of neutrophils in the immune response to *Leishmania amazonensis*. For this reason, the aim of this work was to investigate the role of neutrophils during the initiation of the immune response to *L. amazonensis*. In the present work we showed that neutrophils are the first cells to migrate to the site of infection in response to *L. amazonensis*, representing the predominant cellular type in the infiltrating area, and that the accumulation of these cells correlates with the production of CXCL1, CXCL2 and TNF-α in the infected tissue. Another goal was to determine the stimulus responsible for neutrophil migration to the site of infection with *L. amazonensis*, however the blockade of the major mediators of neutrophil recruitment was not able to prevent neutrophil migration in response to *L. amazonensis*, which indicates that there might be more than one stimulus acting simultaneously, or another possibility is that the parasite may produce neutrophils chemotactic factors. Furthermore, we found that the presence of neutrophils at the site of infection is essential for the formation of an intense inflammatory infiltration and for the expression of CCL2, CCL5, TNF-α and IL-1β in response to *L. amazonensis* during the first twenty four hours after infection. Finally, we showed that neutrophil depletion exacerbates disease in BALB/c mice. In conclusion our data suggests that neutrophils seem to be involved in immunomodulatory mechanisms that confer certain resistance to BALB/c mice in response to *L. amazonensis*.
Supported by CAPES, CNPq and FAPEMIG.

IM12 - NEUTROPHIL EXTRACELLULAR TRAPS-*Leishmania* INTERACTION: ROLE OF LIPOPHOSPHOGLYCAN AND HISTONES AND OCCURRENCE *IN VIVO.*

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Neutrophils die by apoptosis, necrosis or by NETosis. Upon NETosis, neutrophils release fibrous traps of DNA, histones and granule proteins, which can kill bacteria and fungi. Previous results of our group showed that *Leishmania amazonensis* promastigotes induce and are killed by neutrophil extracellular traps (NETs). Here, we investigated parasite molecules that could mediate NETs' release from naïve neutrophil as well as molecules associated with NETs that could promote parasites' killing. Human neutrophils were incubated with different concentrations of LPG and the DNA estimated by the picogreen assay. Our results showed that the amount of DNA released by 10 µg/ml of LPG was 2.5 times higher than that released by non-treated neutrophils. Additionally, we incubated neutrophils with promastigotes of *L.chagasi* and *L.major* for 1h at different parasite:neutrophil ratios. DNA content determined in supernatants showed that the amount of DNA released by the 1:1 *L.major*:neutrophil ratio was five times higher (6.6 ± 0.8 µg/ml) than the control (1.5± 0.3 µg/ml), while the amount released by 1:1 *L.chagasi*:neutrophil ratio was eleven times higher (15.9 ± 0.7 µg/ml) than the control. To find NETs' molecules that could mediate parasite killing, we added anti-histone antibody to neutrophil-*Leishmania* co-cultures. Immune neutralization of histone resulted in a 42% increase in parasite survival relative to non-treated controls. Next, we incubated promastigotes of *L.amazonensis* with supernatants from DNase-treated PMA-activated neutrophils showing that even disrupted NETs killed promastigotes. Addition of anti-histone antibody to these supernatants inhibited promastigote death. To confirm the cytotoxic properties of histones, we incubated purified H2A histone with promastigotes. A concentration of 20 µg/ml killed 62% of the promastigotes after 30 min incubation. NET and histone toxicity to promastigotes were confirmed by live/dead assays. Importantly, meshes composed of DNA, elastase and histone were evidenced in biopsies of human cutaneous leishmaniasis.
Supported by: Capes, CNPq, CNPq-PIBIC, Faperj.

IM13 - Nod1 AND Nod2 ARE PATTERN RECOGNITION RECEPTORS IMPORTANT TO DETECTION AND RESTRICTION OF *Leishmania major* INFECTION

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Pattern recognition receptors such as the toll-like receptors (TLRs) play a central role in the control of *Leishmania* infection. Mice lacking the TLRs adaptor molecule MyD88 are highly susceptible to *Leishmania major* infection. Recently, it was described a new family of cytosolic pattern recognition receptors, called Nod-like receptors (NLRs). Among NLRs are Nod1 and Nod2 proteins, which trigger NF-κB activation pathway similarly to membrane associated TLRs. Thus, we aimed to determine whether NLRs are also able to sense this parasite. To evaluate the NF-κB activation in response to *L. major* infection, we employed reporter cell lines expressing the luciferase gene under the control of NF-κB promoter. We showed that NF-κB activation in response to *Leishmania* is highly depended on the transient expression of Nod1 or Nod2 in the reporter cells. To determine the role of Nod1 and Nod2 for recognition of parasite in physiological conditions, we used macrophages from mice deficient for Nod1, Nod2 or Rip2, a downstream kinase in the NF-κB activation pathway. In macrophages, NF-kB activation leads to cytokine production and expression of co-stimulatory molecules. Accordingly, macrophages deficient for Nod1 or Nod2 fail to express co-stimulatory molecules, such as CD80 and CD86 and produce low levels of cytokines such as IFN-γ and IL-12. In addition, Nod1, Nod2 and Rip2 are required for NO production. Compared to wild type, Nod1-, Nod2- and Rip2-deficient macrophages fail to restrict intracellular infection *in vitro*. Preliminary results suggest that these receptors are important for restriction of the infection *in vivo* as well. Collectively, these data propose that Nod1 and Nod2 effectively contribute to trigger NF-kB activation in response to *Leishmania*, being important innate immune sensors against *L. major* infection. This seems to be the first report of the role of NLRs in the recognition and control of *Leishmania* infection.
Financial Support: FAPESP, WHO/TDR, CNPq and PEW Latin America.

IM14 - PROTECTION AGAINST CANINE VISCERAL LEISHMANIASIS CONFERRED BY A RECOMBINANT CYSTEINE PROTEINASE

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In a previous study we demonstrated that a recombinant cysteine proteinase from *Leishmania (L.) chagasi*, rLdccys1, is a suitable immunological marker for several stages of visceral leishmaniasis (VL) in humans and dogs, and appears to be useful for serodiagnosis of human visceral leishmaniasis. We have also shown with rLdccys1-based ELISA assays and DTH reactions an inverse correlation between humoral and cellular responses in the course of canine visceral leishmaniasis. In the present study, the rLdccys1 antigen was used for immunization of dogs in Teresina, Piauí, an important endemic area of VL in Brazil. Non-infected dogs were immunized with three subcutaneous doses of 150 μg per dose of rLdccys1 with 250 μg of *Propionibacterium acnes* as the adjuvant. Fifteen days after the last dose the animals were challenged by bite of *Lutzomyia longipalpis* previously infected by feeding dog blood containing *L. (L.) chagasi* promastigotes. The bloodfed *Lu. longipalpis* showed an average infectivity rate of 43% and 25 sandflies per dog were used for challenge. Immunization with rLdccys1 led to an increase in serum levels of IFN-γ in immunized dogs which peaked one week after challenge. In contrast, a very low concentration of IL-10 was detected in the serum of these animals. All dogs immunized with rLdccys1 plus *P. acnes* survived sixteen weeks after challenge, whereas control dogs injected with PBS or *P. acnes* alone died after seven and nine weeks, respectively. Control dogs displayed a significant number of *L. (L.) chagasi* amastigotes in liver and spleen, whereas no parasites were found in dogs immunized with rLdccys1. These results opened perspectives for immunization of dogs in the field to test the effect of the vaccine on the incidence of visceral leishmaniasis.
Supported by FAPESP, Faculdade NOVAFAPI and FACIME/UESPI.

IM15 - TGFβ expression in American tegumentary leishmaniasis is differently regulated by *Leishmania (L.) amazonensis* and *Leishmania (V.) braziliensis*.

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Transforming growth factor (TGF)-beta has several down-regulatory functions on the immune system. TGFβ blocks nitric oxide production and the ability of IFN-γ treated macrophages to kill *Leishmania*. This factor has also been recognized as an important immune-regulator in murine leishmaniasis, once it increases the susceptibility to disease.

This study was carried out to determine the TGFβ1 expression in the lesions of American cutaneous leishmaniasis (ACL) due to the major pathogenic leishmanial parasites found in Brazil: *L. (V.) braziliensis* and *L. (L.) amazonensis*.

A total of 26 patients distributed into the clinical-immunological spectrum of ACL were examined: anergic diffuse cutaneous leishmaniasis – ADCL (n=5) and borderline disseminated cutaneous leishmaniasis – BDCL (n=5), both caused by *L. (L.) amazonensis* with negative delayed-type hypersensitivity (DTH^-); localized cutaneous leishmaniasis (LCL) due to *L. (L.) amazonensis* with two groups, DTH^- (n=5) and DTH^+ (n=3) and, LCL caused by *L. (V.) braziliensis* with DTH^+ (n=8). Paraffin-embedded biopsies were submitted to immunohistochemistry using anti-TGFβ1 antibody (SC-146). Immunostained cells were counted in 5–10 fields (400x) in each section by using an image analysis system (Zeiss).

The comparison of TGFβ1+ cells density(mm^2) among these ACL forms has shown a progressive decrease in the TGFβ1 expression from the non-reactive$^{(DHT-)}$ ADCL and BDCL extremity forms caused by *L.(L.) amazonensis* to the reactive $LCL^{(DTH+)}$ central form due to *L.(V.) braziliensis* (ADCL[1534] > BDCL[944] > LCL/La^{DTH-} [600] > LCL/La^{DTH+} [562] > LCL/Lb^{DTH+} [492]).

These findings suggested a strong correlation between the TGFβ1 expression and the Th1/Th2 immune response dichotomy associated with these two *Leishmania* species; while *L.(L.) amazonensis* shows a clear tendency to increase the TGFβ1 expression toward non-reactive$^{(DTH-)}$ BDCL and ADCL, the more immune suppressed forms, with a Th2-type immune response activation, *L.(V.) braziliensis* shows the opposite, decreasing the TGFβ-1 expression toward the reactive $LCL^{(DTH+)}$ associated to a marked Th1 response.

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IM16 - *Leishmania* ENCODED INHIBITORS OF SERINE PEPTIDASES (ISPs) PREVENT TOLL 4-LIKE RECEPTOR ACTIVATION AND PROMOTE PARASITE SURVIVAL IN MACROPHAGES

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Bacterial ecotins are competitive inhibitors of Clan PA, family S1 serine peptidases such as neutrophil elastase (NE), enzyme released from neutrophil azurophilic granules in inflammatory exudates. Three ecotin-like genes, *ISP1*, *ISP2* and *ISP3*, were identified in the genome of *Leishmania major*. We generated *L. major ISP2*/*ISP3* deficient mutants (Δ*isp2/3)* and analysed their interaction with the mammalian host. Δ*isp2/3* mutants were internalised by mice peritoneal macrophages more efficiently than wild type, a phenotype that was reversed by the re-expression of genes in the mutant. The higher uptake of Δ*isp2/3* was reduced to WT levels upon addition of aprotinin, recombinant ISP2 or NE synthetic inhibitors (NEI), suggesting that the increased phagocytosis is promoted by NE in macrophages. After uptake by macrophages, approximately half of Δ*isp*2/3 promastigotes died, while the remaining parasites transformed to amastigotes, but failed to divide for 48 hours. The complement type-3 receptor (CR3) was found to be the predominant receptor used by Δ*isp2/*3 for internalization, but not by WT or the re-expressing parasites. Although blockade of CR3 during Δ*isp2/*3 interaction with macrophages reversed increased parasite uptake, it failed to prevent intracellular death and delayed multiplication of amastigotes. In contrast, NEI prevented death of Δ*isp2/*3 and restored intracellular growth, suggesting that the NE activates pathways leading to increased phagocytosis of *Leishmania*, with the subsequent impairement of amastigote growth. Neutralization of Toll-like receptor 4 (TLR4) during the *Leishmania*-macrophages interaction prevented both the elevated phagocytosis of Δ*isp2/*3 and the subsequent intracellular death, indicating that TLR-4 and NE act cooperatively to downregulate *Leishmania* growth in macrophages. Likewise, the uptake of Δ*isp2/3* by macrophages of TLR4-deficient mice was comparable to that of WT, and the intracellular parasites survived and grew normally. Our results indicated that ISPs act as virulence factors by preventing the triggering of a NE-TLR4-CR3 activation pathway in order to guarantee parasite survival and the establishment of infection.

IM17 - ABSENCE OF FAS-L AGGRAVATES RENAL INJURY IN ACUTE *TRYPANOSOMA CRUZI* INFECTION

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Trypanosoma cruzi infection induces an important myocarditis, congestive heart failure, and other systemic alterations. However, the physiological network of disturbs imposed by the infection are less addressed. Regarding to myocarditis induced by *T. cruzi* infection, we observed in a previous work that Fas-$L^{-/-}$ mice (*gld/gld*) have very mild inflammatory infiltration, when compared to BALB/c. However, all *gld/gld* died in the early acute phase. Therefore, in this work, we studied the development of cardiac insufficiency, possibly evolving renal/erythropoietic failure and cardiac dysfunction that could lead to death. Only *gld/gld* showed on day post infection (dpi) 15 an intense renal inflammatory response, with tubular damage and reduced filtration capacity that compromised renal function. We also observed on dpi 6 a peak of arterial hypotension in both groups of infected mice. Cardiac echocardiography and electrocardiography of BALB/c and *gld/gld* on dpi 7 showed the evolution of cardiac insufficiency, always worsened in *gld/gld*, and we believe that renal dysfunction and anemia contribute to cardiac failure. The mechanistic evaluation of renal damage/insufficiency and heart failure connection suggested that glomerular deposition of IgM and activation of renin-angiotensin system could lead to a cardio/anemic/renal syndrome. This could be found in human patients in indeterminate period and help to predict the development of chronic cardiomyopathy.
Suported by CNPq, Faperj and Fiocruz/IOC

IM18 - Differential extent of kinins released in inflamed tissues may account for the variable pro-inflammatory and TH-inducing phenotype of isolates representative of lineages *T. cruzi* I and *T. cruzi* II

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In the past years, we have studied the contribution of the kinin system in the pathogenesis of Chagas disease. The starting point of our research was the discovery that tissue-culture trypomastigotes (TCT, Dm28c) rely on the proteolytic activity of cruzipain to liberate kinin peptides (e.g. bradykinin) in inflamed extravascular tissues. Acting in the surrounding microvasculature, kinins amplify inflammation by activating the endothelium through their cognate GPCRs, i.e., bradykinin B_2 receptor (B_2R; constitutive) and the B_1 receptor subtype (inducible). Notably, bradykinin potently activates dendritic cells (DCs), driving their migration to draining lymph nodes. Once settled in T cell-rich areas, the matured DCs present *T. cruzi* antigens to naïve T cells, while coordinating their differentiation into type-1 effector T cells. Here we sought to determine if isolates belonging to the lineage *T. cruzi* II (Y strain, VL-10, 115) are able to activate the kinin pathway *in vivo*. As previously reported, Balb/C mice infected by TCT Dm28c developed an overt edema that was blocked by HOE-140 (B_2R antagonist). In contrast, TCT Y, VL-10 and 115 evoked a mild edema. Surprisingly, however, the reaction evoked by the *T. cruzi* II isolates was strongly enhanced by HOE-140. This implies that the endogenously released kinins exerted anti-inflammatory effects in mice infected by *T. cruzi* II isolates, while playing a pro-inflammatory role in mice infected by Dm28c. Preliminary analyzes of T cell recall responses in mice infected by TCT 115 suggest that Th1 responses are attenuated in comparison to the overt Th1 response observed in Dm28-infected mice. Knowledge about the mechanisms underlying differential *in vivo* activation of the kinin pathway by members of the major filogenetic lineages of *T. cruzi* may provide new clues to explain the variable pathogenic outcome of Chagas disease. Supported by: FAPERJ, CAPES, CNPq (Instituto Nacional de Pesquisa em Biologia Estrutural e Bioimagem).

IM19 - IMMUNOGENIC PROPERTIES OF A RECOMBINANT PROTEIN BASED ON DOMAIN II OF APICAL MEMBRANE ANTIGEN 1 OF *PLASMODIUM VIVAX*

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The apical membrane antigen 1 (AMA-1) has been considered a malaria vaccine candidate against asexual stages of *Plasmodium*. Recently, we identified the domain II (DII) of *Plasmodium vivax* AMA-1 (PvAMA-1) as a region highly recognized by IgG antibodies from the Brazilian individuals infected by *P. vivax*. In the present study we evaluated the immunogenic properties of a bacterial recombinant DII in mice in the presence of different adjuvant formulations: Complete/Incomplete Freund's adjuvant (CFA/IFA), MPL-TDM, TiterMax, Alum (Aluminum hydroxide), Quil A, QS-21, CpG-ODN 1826. Our goal is to select formulations for future non-human primate immunizations. Groups of 6 female BALB/c we immunized four times with 10 ug of the protein DII in the presence of each adjuvant separately or in combination (Alum + QS-21 or Alum + CpG-ODN 1826). The IgG antibody titers against PvAMA-1 ectodomain were determined by ELISA two weeks after each immunization. The presence of IgM and IgG subclass (IgG1, IgG2a, IgG2b and IgG3) were also evaluated after four immunizations. We found that the recombinant DII was highly immunogenic in BALB/c mice when administered in the presence of all adjuvants tested. High titers of IgG1, IgG2a and IgG2b were observed in all groups, suggesting a mixed Th1/Th2 response. Finally, we show that DII-specific antibodies recognized the native protein expressed on the merozoite surface of *P. vivax* parasites. Together, our data demonstrated that the recombinant DII of PvAMA-1 was immunogenic in mice when administered in different adjuvant formulations, suggesting that this protein can be used as part of a sub-unit vaccine against malaria *vivax*. Supported by: FAPESP and CNPq (IMTEV).

IM20 - MOTOR BEHAVIOR DYSFUNCTION, NEURONAL DEATH AND GLIAL ACTIVATION IN IL-12p40KO MICE INFECTED WITH *TRYPANOSOMA CRUZI*

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Chagas' disease is a protozoosis caused by *Trypanosoma cruzi* that is endemic in most Latin America countries. Neurological disorders due to *T. cruzi* infection have been described in children and in immunosuppressed hosts; nonetheless, little is known about this form of the disease. Here, we studied motor behavior and neuronal compromise as well as macrophage/microglia and astrocyte activations at the spinal cord in a murine model of the disease. IL-12p40Knockout (KO) and wild-type (WT) female mice infected with *T. cruzi* Sylvio X10/4 clone were evaluated in a scale of motor impairment, inclined plane test and computerized infrared motion sensor. Mice were euthanized when IL-12p40KO individuals presented a complete paralysis of the forelimbs. Spinal cord sections were immunolabeled for localization of proteins expressed by neurons (NF-200), macrophages/microglias (CD11b) and astrocytes (GFAP). Behavioral evaluation showed an ascending paralysis from the tail to the forelimbs. Unbiased stereological analysis revealed a decrement of 60% ($p<0.05$) on estimated neuronal density of IL-12p40KO mouse entire spinal cord, accompanied by an increment of 780.6% ($p<0.01$) on macrophage/microglia estimated density, where 36.3% ($p<0.01$) of these macrophages/microglias were infected by *T. cruzi*. Additionally, spinal cord morphometric/microdensitometric image analysis showed an increment of 317.0% ($p<0.01$) on GFAP immunoreactive area in IL-12p40KO compared to WT mice. In view of those data, we suggest that motor dysfunction displayed by *T. cruzi*-infected IL-12p40KO mice can be closely related to neuronal death and glial reaction.
Supported by FAPESP and CNPq.

IM21 - THE ADJUVANT EFFECT IN EXPERIMENTAL IMMUNIZATION WITH A *Trypanosoma cruzi*-like STRAIN ISOLATED FROM BAT

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In previous studies, we presented that prior inoculation of a *Trypanosoma cruzi*-like strain isolated from bats were able to reduce parasitaemia in animals challenged with *T. cruzi* strains, nevertheless not able to prevent histopathological lesions. The adjuvant presence in immunizations can induce tolerance to antigens, response exacerbation after subsequent inoculation or an appropriate immunomodulation. In this study, we aimed to evaluate the effect of adjuvants with different immunological patterns (Th1, Th2 and Th1/Th2) in association with a *T. cruzi*-like strain without ability to infect mice, in immunizations against experimental Chagas disease. Non-isogenic mice were inoculated with RM1 *T. cruzi*-like strain in association with aluminum hydroxide gel (Alum), incomplete (IFA) and complete (CFA) Freund adjuvant, Iscoms (immunostimulating complex) or inulin. Afterwards, they were challenged with ROM *T. cruzi* strain and monitored for quantification of parasitaemia, survival and total/differential counting of blood leukocytes. In animals immunized with Alum, IFA and non-immunized group the parasitaemia was significantly higher when compared to those immunized with CFA, inulin, Iscoms and in mice that received the RM1 strain without adjuvant. A higher mortality was observed in groups immunized with IFA, Alum and inulin. It was observed an increased number of leukocytes after immunization followed by a reduction in the period post-infection. There was no correlation between parasitaemia and leukogram. The immunization with RM1 strain was able to reduce parasitaemia, despite the unnecessary adjuvant association to accomplish that. Nevertheless, the adjuvant effect significantly influenced the response to *T. cruzi*, due to absence of protection in groups immunized with IFA and Alum (Th2 profile). Therefore, adjuvants which are able to induce a Th1 response are important in immunizations against *T. cruzi*. Prospective histopathological studies are required to confirm the adjuvant's ability to protect or their participation in the development of tissue lesions.
Supported by CNPq, FAPEMIG and FUNEPU-UFTM.

IM22 - THE IMPORTANCE OF IPAF, ASC AND CASPASE-1 IN THE CONTROL OF EXPERIMENTAL INFECTION BY *L. amazonensis*

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Active caspase1 is present in multiprotein complexes called Inflammasome. Important members of the inflammasome comprises the Nodlike receptor Ipaf, the adapter protein Asc and caspase1. Ipaf activates caspase1, after sensing intracellular pathogens. In this context, we investigated the participation of Ipaf Inflammasome during the experimental infection by *L. amazonensis*. Experiments in vitro were performed using BMMΦ from WT, Asc/, Ipaf/, caspase1-/- mice in a C57BL/6 background, infected with promastigotes of *L. amazonensis* stably expressing GFP, in order to evaluate killing, stimulatory and costimulatory activity, cytokines and NO production and expression of NOS2 and arginase1 enzymes. Moreover, macrophages were evaluated for its ability to kill parasites after activation with IFNγ or IFNγ+TNFα. Additionally, WT, Asc/, Ipaf/ and caspase1-/- mice were infected and evaluated lesion development weekly, burden parasite, production of cytokines and expression of NOS2 and arginase1 enzymes. Regarding the in vitro infection, BMMΦ from Asc/, Ipaf/ and caspase1-/- mice presented lower leishmanicidal activity and NO production comparing with controls. Similar results were observed in macrophages activated with IFNγ or IFNγ+TNFα. Moreover, BMMΦ from Asc/, Ipaf/ and caspase-1-/- mice displayed lower expression of CD80, CD86, MHCII and CD1d and higher expression of PDL1, than do macrophages from C57BL/6 mice. BMMΦ from Asc/, Ipaf/ and caspase1-/- mice produced significantly lower levels of IL12p40, IFNγ, TNFα and IL1β and expression of NOS2 and higher levels of TGFβ, IL10 and expression of arginase1 compared to BMMΦ from WT mice. Experiments in vivo showed that Asc/, Ipaf/ and caspase-1-/- animals, infected with *L. amazonensis* had higher lesion development and parasitism in the ear, lymph nodes and spleen, lower levels of production of IL12p40, IFNγ and IL1β and expression of NOS2 and higher levels of IL10 and expression of arginase1 when compared with respective controls. Our results suggest that Ipaf inflammasome complex contributes for the effector functions of macrophages in response to parasitism, and is necessary for the control of *L. amazonensis* infection in mouse.

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IM23 - INFLUENCE OF *Leishmania (Leishmania) infantum* INFECTED-KUPFFER CELLS ON HEPATOCYTE DAMAGE

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Viscerothropic *Leishmania* species such as *Leishmania infantum* (syn. *Leishmania chagasi*) are protozoan parasite which target resident macrophages in the liver, the Kupffer cells (KC). The control and resolution of the liver infection depends on the granuloma formation that are assembled around fused, parasitized resident macrophages surrounded by both cytokine secreting T cells and influxing leishmanicidal blood monocytes. As a collateral effect of the pathogen clearance, tissue damage is exacerbated. We developed experimental conditions for the isolation and co-cultivation of Balb/C mice KC and hepatocyte to mimic *in vivo* conditions for studding the infection with axenic amastigotes of *L. chagasi.* We found that KC are less infected than peritoneal macrophages (Mϕ) in the same conditions, and the potential for intracellular parasite elimination is limited. We measured the hepatic transaminases in the supernatant of infected KC-hepatocytes co-cultures, as damage markers, and the percentual of enzyme released observed was very little compared to co-cultures with infected Mϕ. In previous studies we showed the pivotal role of the nitric oxide in such damage and it was observed that KC was able to produce appreciated levels of this molecule. However, production of reactive oxygen intermediates is dramatically reduced in *Leishmania*-infected KC which point to a possible synergistic effect of these molecules or even production of nitrite peroxide as the mediator of such damaged. In fact, the addition of H_2O_2 to the culture medium leaded to transaminase release. In the other hand, infected KC secreted low amounts of TNF-α, and TGF-β when compared with Mϕ although they can secrete at least four times more IL-10 than Mϕ. These results suggest that KC may play an important role for the modulation of the immune response and also limiting disease by secreting IL-10.

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IM24 - EVALUATION OF THE EFFECT OF INTESTINAL MICROBIOTA IN MICROBICIDE ACTIVITY IN INFECTED MACROPAGHES *IN VITRO* BY *LEISHMANIA MAJOR*

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Animals are colonized by their indigenous microbiota from the early day of life. The estimated number of associated bacterial cells is around of 10^{14} per individual, most of them in the gut. Several studies have investigated the microbiota-host relationship and the use of germ-free animals has been an important tool in these studies. These animals, when infected with a pathogen, have shown to be sometimes more resistant and other times more susceptible than conventional animals, as during infection by *Leishmania major*. Previous studies showed that in the infection by this parasite, Swiss/NIH germ-free animals developed a typical Th1 response, but failed to heal lesions, while conventional mice developed the same response and controlled the infection. Th1 response is clearly related in the literature with healing and parasite clearance in this infection. Thus, our aim in this study is to evaluate a possible adjuvant efect of the microbiota in the microbicidal activity of the macrophages, the major cell that kills the parasite. Therefore we infected resident peritoneal macrophages and bone marrow-derived macrophages from germ-free and conventional Swiss/NIH animals *in vitro* with *L .major* amastigotes to measured NO, TNF-α (pro-inflammatory profile), IL-10 and arginase activity (anti-inflammatory profile). Our results showed that macrophages from germ-free animals surprisingly produced more NO and showed more arginase activity. However, no significant difference was found in TNF-α and IL-10 production. When we analysed the quantification of parasites, we found the same parasite loads in macrophages from of conventional and germ-free animals, in presence of IFN-γ. These data suggest that macrophages from both groups are able to kill *L. major* amastigotes similarly.

Support: CNPq, CAPES, FAPEMIG

IM25 - EVALUATION OF DIFFERENT IMMUNIZATION PROTOCOLS IN ATTEMPT TO INDUCE A TH1 IMMUNE RESPONSE TOWARDS A NEW *Leishmania chagasi* RECOMBINANT ANTIGEN

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Aiming at modifying the immune response type, from a Th2 to Th1 one, towards a new *Leishmania chagasi* recombinant antigen (namely rLc9), naturally immunogenic for dogs, we described several different immunization protocols. Groups of BALB/c mice were injected 3 times at 3-week intervals with (I) rLc9 alone or associated with Freund's adjuvant, saponin, peanut oil, alum or a plasmid encoding single chain murine IL-12 (pcDNA3.1-scmIL-12); (II) naked plasmid DNA encoding Lc9 (pBK-CMV-Lc9) alone or pBK-CMV-Lc9 twice followed by a rLc9 protein booster (pBK-CMV-Lc9/rLc9), (III) rLc9-saponin associated, in the first dose, with different amounts of pcDNA3.1-scmIL-12 or (IV) rLc9 in combination, in the first dose, with different amounts of pcDNA3.1-scmIL-12 or with CpG-ODN 1826. The mice immunized with rLc9-saponin or rLc9 associated with any amount of pcDNA3.1-scmIL-12 tested, in combination with saponin or not, or CpG-ODN 1826 produced specific IgG1 and IgG2a, while the animals immunized with rLc9 alone or rLc9 associated with any of the other adjuvants, generated IgG1 but fail to produce IgG2a specific antibodies. Mice injected with pBK-CMV-Lc9 or pBK-CMV-Lc9/rLc9 generated, exclusively or almost exclusively IgG2a specific antibodies, although at lower amounts in comparison with the groups mentioned above. The mice immunized with rLc9-saponin or rLc9 associated with any amount of pcDNA3.1-scmIL-12 tested in combination with saponin showed IFN-γ and IL-5 production, whereas the groups injected with pBK-CMV-Lc9, pBK-CMV-Lc9/rLc9 synthesised IFN-γ but no IL-5, after *in vitro* stimulation with rLc9. In conclusion, Th2 response, following immunization with rLc9 alone in mice, can be slightly modified towards a Th1 response by the association of rLc9/pcDNA3.1-scmIL-12 or rLc9/CpG-ODN 1826 and the combination of rLc9/saponina, but never reached an exclusively Th1 immune response. On the other hand, murine immunization by the administration of naked plasmid DNA pBK-CMV-Lc9 alone or followed by rLc9 protein booster induced a predominantly Th1 immune response.
Supported by CNPq, RENORBIO-Ministério da Ciência e Tecnologia, INOVABIO-Ministério da Saúde, Programa Instituto do Milênio, CNPq.

IM26 - COMPARISON OF THE IMMUNOGENICITY IN MICE OF DIFFERENT RECOMBINANT PROTEINS REPRESENTING THE *PLASMODIUM VIVAX* MEROZOITE SURFACE PROTEIN-3

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Plasmodium vivax Merozoite Surface Protein-3α (PvMSP-3α) and 3β (PvMSP-3β) are members of a family of related merozoite surface proteins that contain a central alanine-rich domain with heptad repeats. Recently, we found that a high frequency of individuals had IgG antibodies to recombinant proteins based on PvMSP-3α and PvMSP-3β. The present study was designed to evaluate comparatively the immunogenicity of the different fragments of these proteins after immunization of mice. Recombinant proteins representing the C-terminal region of MSP-3α (FP-1) and different regions of MSP-3β [N-terminal (FP-1), C-terminal (FP-2) and full-length protein (FP-3)] were expressed in *Escherichia coli* from histidine-tagged vectors. Previously, the secondary structure of each one recombinant protein representing PvMSP-3α and PvMSP-3β was analyzed by dichroism circular experiments. Groups of six to eight-week-old female BALB/c mice were immunized s.c. three times with 10 μg of each recombinant protein in the presence of Complete/Incomplete Freund's Adjuvant (CFA/IFA). The IgG antibody titers against the homologous protein on the sera of immunized mice were determined by ELISA and were expressed as logarithm. Immunization with PvMSP-3α (FP-1) was potent (5.37 ± 0.13). Similarly, both fragments of PvMSP-3β induced high antibody titers (FP-1= 5.01 ± 0.21, FP-2 = 5.07 ± 0.50) as well as the full-length protein (FP-3 =5.25 ± 0.13). Overall, IgG1, IgG2a and IgG2b were produced predominately in all mice groups. We concluded that after three doses all these recombinant proteins were highly immunogenic in BALB/c mice. Currently we are evaluating the immunogenicity of these proteins in the presence of the different adjuvant formulations aiming future immunizations studies in non-human primates.
Supported by FAPESP and CNPq (INCTV).

IM27 - EVALUATION OF DIFFERENT ROUTES AND LOW DOSES OF SOLUBLE *LEISHMANIA* PROTEINS TO INDUCE PROTECTION IN MICE AGAINST *LEISHMANIA* INFECTION

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Leishmaniasis is currently one of the most common infectious in tropical countries. The pathology of the infection is determined by the parasite species, genetics background and immune factors of the host. In this work, we investigated the effect of immunization using different routes (intranasal, oral and subcutaneous routes) with low doses of soluble Leishmania antigen (SLA) of *L. amazonensis*, *L. major* and *L. chagasi* in the course of murine infection with this parasite. Groups of BALB/c mice (n=8, per group) received three doses of SLA L. *amazonensis*, L. *chagasi* or L. *major* (1 μg per mouse), extracts plus alum (1 μg of SLA plus 0.5 μg of alum, per mouse) or PBS, as control. Thirty days after the last dose, mice were challenged subcutaneously with $1x10^5$ or phase stationary promastigotes of *L. amazonensis*. Measures of the footpad swelling, quantification of the parasite load and cytokine levels were performed. Significant reductions in the footpad swelling and parasite load of the BALB/c mice immunized with different SLA were observed, as compared to PBS group. The best immunization schedule was using oral route with SLA *L. amazonensis,* without alum. In this case, a high level of IFN-γ and low levels of IL-4 and IL-10 by spleen cells in response to SLA *L. amazonensis* was observed. In conclusion, oral immunization of BALB/c mice with low doses of SLA was able to induce protective immunity against *L. amazonensis* infection.
Supported by: FAPEMIG and CNPq.

IM28 - CELL POPULATION AND CITOKYNE PRODUCTION IN SPLEEN OF DOGS WITH VISCERAL LEISHMANIASIS

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Visceral leishmaniasis is associated with atrophy and disorganization of splenic lymphoid tissue in mice and in dogs. In this work we study the changes in the distribution of cell populations and cytokines in splenic lymphoid tissue of dogs with naturally acquired visceral leishmaniasis. Spleens of 20 dogs, 10 with and 10 without visceral leishmaniasis were subjected to immunohistochemistry for identification of positive cells for CD79-alpha (B cells), CD3 (T cells), protein S100 (dendritic cells), IH1 (marginal zone macrophages), CD45 (pan-leukocyte marker), KI-67 (proliferating cells). Spleen fragments were also collected for measuring TNF, IFN-gamma, IL10, CCL19, CXCL13 and CCL21 expression using real time RT-PCR. A preliminary analysis showed a decrease in the density of B cells, KI-67- and CD45-expressing cells in the white pulp and marginal zone, and of KI-67-expressing cells in the red pulp (student t-test*,* P=0.0076) of the animals with lymphoid disorganization of the spleen. So far, CCL19, IL10 and TNF expression has been examined in the spleens of 18 dogs. Although the expression of IL10 was slightly higher in the animals with disorganized lymphoid tissue, such difference was not statistically different. Our data show that cell populations in the spleen are differentially affected in the disorganization process induced by visceral leishmaniasis. Now we are using morphometry to quantify the cell populations present in the different compartments of the spleen and also expanding the data on the cytokine expression in the spleen of the animals from the different groups.
Supported by CNPQ and FAPESB.

IM29 - TARGETING RECOMBINANT *Plasmodium vivax* AND *Trypanosoma cruzi* ANTIGENS TO DENDRITIC CELLS *IN VIVO* BY GENETIC FUSION WITH AN ANTIBODY TO DEC-205: A NEW STRATEGY FOR THE DEVELOPMENT OF VACCINES.

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Dendritic cells (DCs) are the most efficient antigen-presenting cells for the activation of immune responses. Hawiger *et al.* (2001) showed that antigens fused to an anti-DEC-205 antibody could be delivered to DCs *in vivo* via receptor mediated adsorptive endocytosis. Boscardin *et al.* (2006) described that the *in vivo* administration of the anti-CD40 antibody and the toll-like receptor agonist poly I:C simultaneously with chimeric antibodies induced immune responses measured by specific serum antibodies and antigen-specific cytokine secretion by CD4 Th1 and CD8 Tc1 T cells. These results led me to test the hypothesis that anti-DEC-205 recombinant antibodies containing parasite antigens could be a feasible strategy to elicit protective immunity against these pathogens. Here, I present the generation of chimeric anti-DEC antibodies in fusion with two proteins of *Trypanosoma cruzi* (amastigote surface protein-2 and *trans*-sialidase) and with a protein of *Plasmodium vivax* (merozoite surface protein-1_{19}). The recombinant genes expressing each of the three proteins in fusion with the heavy chain of the anti-DEC-205 or an unrelated control antibody were expressed in transfected 293T cells. Chimeric antibodies were purified from culture supernatants and their integrity/purity confirmed by SDS-PAGE. The binding capacity and specificity of the chimeric antibodies to DEC-205 was also confirmed using DEC-205 receptor transfected cells. Currently, were are injecting these chimeric antibodies containing the parasite antigens in naïve BALB/c and C57BL/6 mice to evaluate their ability to generate immune responses measured by serum antibodies and T cell-mediated cytokine secretion, as well as protective immunity against a challenge infection.

References:
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IM30 - BONE MARROW CELLS TREATMENT INTERFERE IN INFECTION COURSE OF SUSCEPTIBLE MICE INFECTED BY *LEISHMANIA MAJOR*

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Leishmaniasis is a medical problem causing mortality and morbidity in tropical and subtropical areas. Three clinical forms of leishmaniasis are well characterized and in many cases these clinical forms are difficult to treat with the drugs available, for example, the cutaneous injuries, that in some cases are mutilating and debilitating in the affected individual. The BALBc mouse is a susceptible model for *L. major* infection with non-curative and ulcerative cutaneous lesions. In this study, our interest is to assess the potential treatment for these lesions with total bone marrow cells. The results show that the intravenously transference of syngeneic total bone marrow cells is effective in reducing foot-pad *L. major*- induced lesions by approximately 30% and 70% ($p<0.05$), respectively in animals treated with one or three consecutive doses of cells [$6x10^6$ of total BMC], for a limited time [4-5 weeks]. The treatment reduced the number of parasites at the site of infection, draining lymph node and spleen ($p<0.05$). However, in histological analysis, no significant alteration in tissue organization was detected in association with treatment. In order to monitor the engraftment of bone marrow cells in the recipient's tissues, cells were labeled with PKH26, transferred and after 24 hours the animals were sacrificed and their organs [heart, spleen, lung, liver, lymph and foot-pat] were analyzed. The marked cells were found only in the spleen and draining lymph nodes, but were not detected neither at the site of infection nor in the other examined organs. In conclusion, we observed that treatment with total bone marrow cells in susceptible animals infected with *L. major* can induce temporary reduction of lesion size and the number of parasites in this experimental model of cutaneous leishmaniasis. Supported by CNPq and FAPEMIG.

IM31 - IMMUNOBLOT ASSAY WITH EXCRETED-SECRETED ANTIGENS OF *L. (L.) CHAGASI, L. (L.) AMAZONENSIS* AND *L. (V.) BRAZILIENSIS* (IB-*LEISH)* TO HELP THE DIAGNOSIS OF CANINE LEISHMANIASIS

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In Brazil, domestic dogs are an important reservoir of *Leishmania (Leishmania) chagasi*. The elimination of serological positive cases is an important part of the control program of canine leishmaniasis. Since the incidence of zoonotic canine leishmaniasis is rising in Brazil, several research groups are working on specific and sensitive screening techniques for identification of infected hosts. Here, we present an evaluation of diagnostic performance of Immunoblot assay (IB), for detection of canine anti-leishmania IgG. The IB-*leish* was performed with excreted-secreted (exoantigens) antigens of promastigote forms of 3 species of Leishmania: *L. (L.) chagasi*, *L. (L.) amazonensis* and *L. (V.) braziliensis* obtained in a protein-free medium and used without purification. The study was performed with 82 dogs living in leishmaniasis endemic area of Araçatuba - São Paulo, Brazil. Fifty dogs were diagnosed as parasite-positive by the immunohistochemical method (viscera and/or skin) and divided in two groups. The symptomatic group (34 dogs) and the asymptomatic group (16 dogs) recognized 3 immunodominant bands with MW ranging from 27 to 31kDa, in IB-*leish* performed with *L. (L.) chagasi* exoantigen. Any positive bands were showed by twenty dogs, living in non-endemic area and 21 with other infections (*T. cruzi, T. evansi, Babesia* sp*, Erlichia* sp*, Neospora* sp, *Toxoplasma* sp). The evaluation of IB-*leish* with *L (L.) chagasi* resulted in 100% sensitivity and 100% specificity. In the next evaluation we included thirty two dogs parasite-negative, but with positive ELISA (Pinedo-Cancino, 2008) for leishmaniasis. The results of IB-*leish* showed positivity of 50% (10/20) among symptomatic and 83.3% (10/12) among asymptomatic dogs. These data led us to think that IB-*leish* could be a reliable method for diagnostic and epidemiological assays, specially to help the diagnosis of canine leishmaniasis.
Supported by CNPq, LIM 49-IMTSP

IM32 - SOLUBLE ANTIGEN FROM *LEISHMANIA (VIANNIA) SHAWI* PROMASTIGOTES INDUCES PARTIAL PROTECTION ASSOCIATED WITH MIXED TH1/TH2 IMMUNE RESPONSE.

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Some reports indicate that *L. shawi* antigens showed high immunogenicity to antibody detection in patients infected with New World *Leishmania* sp. in North of Brazil as well as in experimental immunization using released proteins from promastigote forms. However, data about the levels of protection induced by soluble antigen (SA) in experimental immunization are not yet evaluable. The aim of this work was evaluate the level of protection generated by SA from *L. shawi* promastigotes in experimental immunization. Promastigote forms of *L. shawi* in stationary phase of growth were disrupted by 3 cycles of frozen in liquid nitrogen and thawed at room temperature, followed by centrifugation at 10,000g, 30min, 4°C. The supernatant was collected and protein concentration was quantified. BALB/c mice were immunized subcutaneously in the dorsal skin with 25□g of SA or PBS twice, at 0 and 7 day. At 14th day, 10^6promastigote forms or PBS were injected into the mice footpad. After 6 week pi mice were sacrificed and the parasite load was quantified through limiting dilution assay in skin; and cytokines and isotypes were quantified by ELISA. Immunized BALB/c mice showed significant decreasing in the lesion size (70%) and in parasite load (68%) compared to non-immunized, associated with increased levels of IL-2, IL-4 and IL-12 in supernatant of lymph nodes cells culture, as well as IFN-□ in the sera. Decreased levels of IgG1 isotype were recorded in immunized mice. SA induced partial protection associated to elevated levels of IL-2, IL-12, IFN-□ and IL-4 characterizing a mixed Th1/Th2 immune response. Further research of purified molecules from SA will performed to improve experimental immunization. Support: FAPESP and HCFMUSP-LIM-50.

IM33 - PREDOMINANT TH-1 IMMUNE RESPONSE ASSOCIATED TO PROTECTION BY LOW FRACTION SECRETED BY *LEISHMANIA (VIANNIA) SHAWI*

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L.shawi parasites can be an important source of antigens for the development of vaccines against American Tegumentar Leishmanisis (ATL), as some reports indicate that their antigens show strong immunogenicity and cross reactions with other Leishmania sp causing ATL. Therefore the aim of this work is to evaluate the protective effect of low protein fraction isolated from supernatant of *L. shawi* promastigotes. Supernatants from virulent cultures of *L. shawi* were collected after 24h in RPMI 1640 protein-free. The proteic fraction of low molecular weight was separated by eletroelution and used to immunize 30 BALB/c mice (25□g/weekly, two times). At day 21, mice were injected with promastigote forms or with PBS into the footpad. After 5 weeks pi the parasite load was quantified and CD4+, CD8+ and non-CD4/CD8+ T cells were purified from popliteal lymph nodes using monoclonal antibodies. RNA was extracted from separate Tcells subpopulations and reverse transcribed into cDNA. The expression of IFN-□□ TNF-□□□IL-4, IL-10 and TGF-□ were quantified by real time PCR. Low fraction induced 92% of protection, associated to high expression of IFN-□ by CD4+ and non-CD4/CD8+Tcells, TNF-□ by CD8+ and non-CD4/CD8+Tcells and TGF-□ by CD8+Tcells. Reduced expression of IL-4 by CD8+ and non-CD4/CD8+Tcells and IL-10 by CD4+ and CD8+Tcells were also observed. In immunized BALB/c mice, high expression of IFN-□, TNF-□ and TGF-□ were recorded by CD4+ and CD8+Tcells, IL-4 byCD8+ and non-CD4/CD8+Tcells and IL-10 by non-CD4/CD8+Tcells. The low fraction gave partial protection against *L.shawi* infection, associated with a predominant Th1 immune response and macrophage activation. Possibly, higher expression of IL-10 and TGF-□ in immunized mice could be associated to generation of cell memory. This study demonstrates that proteins release by *L.shawi* may bring important insights for develop of an efficient vaccine able to induce protection against ATL. Supported by: FAPESP/LIM50-HCFMUSP/Portuguese FCT and by the PTDC/FEDER (PTDC/CVT/70275/2006).

IM34 - Assessment of immune response in dogs immunized with a new recombinant Leishmania chagasi antigen (Lc9)

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Zoonotic visceral leishmaniasis (VL) is an infectious disease caused by *Leishmania infantum/chagasi* that affects humans and dogs. Domestic dogs are the major reservoir of the causal agent. An effective vaccine against canine VL may contribute to infection/disease control. We describe the canine immune response following immunization with a new recombinant *L. chagasi* antigen (rLc9). Groups of dogs were injected four times at 3 to 6-week intervals. These animals were injected with: (i) naked DNA pBK-CMV-empty plasmid twice (pBK-CMV, negative control group), followed by saline/oligodeoxynucleotide (ODN-CpG)/Montanide 720 (Mont720) and saline/ODN-CpG; (ii) pBK-CMV twice, followed by rLc9/ODN-CpG/Mont720 and rLc9/ODN-CpG; (iii) plasmid encoding Lc9 (pBK-CMV-Lc9) twice, followed by rLc9/ODN-CpG/Mont720 and rLc9/ODN-CpG. Naked plasmid DNA was administered intramuscularly with electroporation. After immunization, anti-rLc9 IgG antibodies were assessed by ELISA. Four months after last immunization, the three groups were challenged with an injection of 1×10^8 *L. chagasi* (IV). After three months, infected dogs had their spleen punched and cultivated in a biphasic medium. After immunizations and challenge the cellular immune response was assessed by lymphoproliferative assays and interferon gamma (IFN-γ) measurement, after *in vitro* stimulation with rLc9.

Comparing with the negative control group, dogs immunized with rLc9/adjuvants or pBK-CMV-Lc9/rLc9/adjuvants produced low and high levels of anti-Lc9 IgG antibodies after the third injection series and both groups generated high levels of specific antibodies after the fourth injections. Dogs immunized with pBK-CMV-Lc9/rLc9/adjuvants showed a specific lymphoproliferative response and preliminary data suggested that these animals produced IFN-γ□after the fourth immunization. However, three months after challenge, dogs' spleen cultures were positive for *L. chagasi*. The results described suggest that dogs immunized with rLc9/adjutants developed only a humoral response while the ones that received pBK-CMV-Lc9/rLc9/adjuvants generated both humoral and cellular immune response which wasn´t sufficient to protect them against L.chagasi infection. Assessment of IL-10 production is underway.

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IM35 - IDENTIFICATION OF IMMUNOSTIMULATORY SEQUENCES DERIVED FROM PROTOZOAN PARASITE GENOMES

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Toll-like receptors (TLRs) are part of the innate immune system responsible for the recognition of Pathogen-Associated Molecular Patterns (PAMPs). TLR9 was initially identified as the mammalian component of innate immune system responsible for the recognition of unmethylated CpG motifs derived from bacterial DNA. We and others have demonstrated the role of TLR9 in parasite infections. DNA preparations from protozoa parasites such as *Trypanosoma cruzi*, *T.brucei, Plasmodium falciparum* and *Babesia bovis* are able to activate macrophages to produce proinflammatory cytokines and nitric oxide in a TLR9-dependent manner. B-Class ODNs, a class of CpG, are strong stimulators of proinflammatory cytokine production. Here we have performed *in silico* analysis to identify the presence of sequences containing human- and mouse-like B-class CpG motifs in the genome of the protozoa parasites *T.cruzi*, *Leishmania major, T.brucei, P.falciparum and Toxoplasma gondii. T.cruzi*, *T.gondii* and *L.major* have the higher contents of both human- and mouse- B-Class-like CpG motifs, followed by *T.brucei* and finally by *P. falciparum*. Although, we have identified some CpG motifs in the VAR genes of *P.falciparum*, it was expected a smaller content of these motifs in this genome, since it is 70-80% AT-rich. In contrast, the genomes of the other protozoan are approximately 50% CG-rich, and therefore expected to have a higher number of CpG motifs. We have also investigated the immunostimulatory properties of synthetic oligonucleotides containing CpG motifs, derived from *L.major* genome. To this end, dendritic cells and IFN-gamma-primed macrophages from C57BL/6, TLR9-/- and TLR4-/- mice were incubated in the presence of different concentrations of *L.major* CpG ODNs. Some, but not all, *L.major* CpG ODNs were able to stimulate the immune system via TLR9. We speculate that the content of CpG in the genome of different parasite species may be an important factor affecting virulence, pathogenesis and favoring parasite adaptation to the vertebrate host.
Supported by CNPq, FAPEMIG and WHO.

IM36 - A NOVEL MOLECULE IMPLICATED IN THE PATHOGENESIS OF *LEISHMANIA SPP*

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Leishmania spp are intracellular protozoan parasites that cause serious human infections throughout the world. These parasites cause disease by infecting host macrophages, where they replicate within parasitophorous vacuoles with properties of lysosomes. There are significant gaps in our knowledge of the strategies used by Leishmania to survive within the hostile environment of macrophage phagolysosomes. In particular, amastigotes of *Leishmania amazonensis* have been reported to be more resistant to macrophage-mediated killing and the molecular mechanisms underlying this phenomenon are still unknown. Important differences are also observed in vivo: C57bl/6 mice, which heal from *L. major* infection, develop chronic disease when infected with *L. amazonensis*. Human infections with *L. amazonensis* also show a more complex pattern, with cutaneous, diffuse cutaneous and monocutaneous forms of the disease.
We identified expression of a molecule in macrophages infected by amastigotes of *L. amazonensis* that inhibit macrophage activation, allowing the intracellular growth of the parasite in vitro and in vivo. Real time RT-PCR of macrophages infected with amastigotes of *L. amazonensis* at different time-points confirmed upregulation. Also, infection of KO mice with *L. amazonensis* revealed decreased lesion formation when compared to control C57BL/6 mice. In vitro infection of Bone Marrow macrophages from KO mice with *L. amazonensis* also showed reduced intracellular replication. Apparently, the mechanism implicated in the downregulation of macrophage responses is the reduced expression of iNOs, a key factor controlling Leishmania growth. Thus, induction of expression of this molecule could explain how *L. amazonensis* can survive and proliferate during an inflammatory process. Support by NIH and PEW fellowship

IM37 - the Immunogenecity of LBSap Vaccine in dogs after challenge by intradermal inoculum using promastigotes of *Leishmania chagasi* and SAND FLY saliva

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A vaccine against canine visceral leishmaniasis (CVL) would be important tool in the control of the disease due to the dogs are the major domestic reservoir of the *L. chagasi*/*L. infantum*. Recently our group developed and evaluated the immunogenicity of a vaccine against CVL composed by *L. braziliensis* antigens plus saponin (LBSap). Dogs vaccinated with LBSap elicited strong immunogenicity related to high levels of T $CD8^+$ and *L. chagasi* antigen-specific T-$CD8^+$ after immunization with LBSap. Herein, we proposed to analyze the *ex-vivo* immune-response using flow cytometry including T-($CD5^+$, $CD4^+$, $CD8^+$), B-($CD21^+$) lymphocytes and circulating ($CD14^+$) monocytes after challenge by intradermal inoculum using $1x10^7$ metacyclic promastigotes of *L. chagasi* and saliva of *Lutzomyia longipalpis*. Vaccinated dogs were following to 885 days after challenge. The increase of T-$CD5^+$ lymphocytes after vaccination maintaining high during long time after challenge (541 days) were observed. Similar comportment in T-$CD8^+$ was observed indicating that LBSap vaccine induces an effective memory including the recruitment and activation even after challenge. Also, the increase of T-$CD4^+$ after vaccination was sustained high during 435 days with a peak elevation in 90 days after experimentally infection. The increase of B-$CD21^+$ cells after challenge until high by 541 days. Also, the increase of $CD14^+$ monocytes after vaccination was observed with a subsequent decrease immediately after challenge and a posterior restoration and increase during final phase of the monitoring. Our findings support a potential protective ability of LBSap mediated by T-$CD8^+$ activation associated with the high levels of B-$CD21^+$ and the recuperation levels of $CD14^+$ monocytes after challenge which induce immunity related to infection-induced resistance, may provide best protection against CVL. Further investigation focusing the parasitological and molecular analysis to evaluate the LBSap efficacy has been assessed in our lab.
Supported by: FAPEMIG, UFOP, FIOCRUZ, CNPq and CAPES.

IM38 - Chemokines and cytokines expression in the dermis of dogs immunized with antigenic compounds of *Leishmania braziliensis* plus saponin (LBSAP VACCINE)

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The complex connection among cytokines and chemokines is important in the arrangement of innate and adaptive immune-response against pathogens. Cytokine and chemokine expression drives the recruitment and activation of immune effector cells to sites of tissue infection. Thus, the aim of the present study was to evaluate the kinetics of chemokines (CCL2, CCL4, CCL5, CCL21 and CXCL8) and cytokines (IL-12, IFN-γ, TNF-α, IL-4, IL-13, TGF-β and IL-10) expression in dermis of dogs immunized with antigenic compounds of *L. braziliensis* plus saponin (LBSap vaccine) at different times (1, 12, 24, 48, 96 hours) by *Real Time*-PCR. The results demonstrated that there was a tendency to increase pro-inflamatory cytokines (IL-12, IFN-γ and TNF-α) and anti-inflamatory cytokines (IL-4 and IL-13) in the first 24 hours in LBSap group and a reduction of these cytokines levels in subsequent times of the kinetics. Furthermore, it was detected a significant increase of IL-10 in the LBSap group in the first 24 hours, suggesting the role of this cytokine on the control of the inflammatory process. Also we evaluated the chemokines expression and our major results showed a significant increase in the CXCL8 expression in 24 hours after immunization in the LBSap group in cooperation with other groups. In addition, the LBSap vaccine was able to induce increase of CCL4 in 24 hours after immunization. An increased expression of the CCL5/RANTES in the LB group in 48 hours after immunization was observed. In this context, we conclude that the immune response observed during the kinetics of the potential vaccinated antigens and adjuvant using dog model elicited the mechanisms and factors involved in a protective response against *Leishmania*. Also, it will help to characterize and to establish a rational approach for the development of vaccines against canine visceral leishmaniasis. Supported by CNPq, PRONEX - FAPEMIG and UFOP.

IM39 - Apoptotic cell uptake induces heme-oxygenase-1 production: impact on Trypanosoma cruzi infection

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Apoptotic cells are rapidly recognized and engulfed by professional phagocytes such as macrophages to avoid secondary necrosis and thus inflammation. Recognition of apoptotic cells polarizes macrophages toward an anti-inflammatory phenotype. However, mechanistic details provoking these phenotype alterations are incompletely understood. Previously our group has shown that there is intense lymphocyte apoptosis in an experimental model of Chagas' disease, a debilitating cardiac illness caused by the protozoan Trypanosoma cruzi. Here, we demonstrated a biphasic up-regulation of heme oxygenase-1 (HO-1), a protein that bears an anti-inflammatory potential, in infected murine macrophages, which were exposed to the apoptotic cells. The induction of HO-1 by apoptotic cell uptake or with single treatment with of the HO-1 inducer cobalt protoporphyrin (CoPPIX) correlated with increased number of infected macrophages, intracellular amastigote load and number of viable trypomastigote released. Also, induction of HO-1 correlated with increased production of anti-inflammatory factors (TGF-beta and PGE-2), and decreased production of TNF-□ and nitric oxide (NO). Infected macrophages cocultured with apoptotic cells in the presence of the HO-1 inhibitor tin-protoporphyrin IX (SnPPIX) drastically reduced the numbers of infected cells, intracellular parasites and trypomastigotes released. These results suggested that induction of HO-1 by uptake of apoptotic cells is a critical modulator of T. cruzi infection.
Financial Support: CNPq and FAPERJ

IM40 - CONTROL OF *Trypanosoma cruzi* GROWTH BY THE TIMING OF CD8$^+$ T CELL ACTIVATION

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After infection with the human protozoan parasite *Trypanosoma cruzi*, the parasitemia observed in C57BL/6 or CD8-deficient mice increases at the same rate until reach the peak of parasitemia. At that time, strong CD8$^+$ T cell immune response is triggered in C57BL/6 concomitant to a rapid reduction in the number of parasites. In contrast, CD8 deficient mice are unable to control the parasitemia, become severely ill, and die. We observed that this kinetics was controlled by the parasite load and suggested that the unusual delay in the CD8$^+$ T cell response might be critical for the establishment of a productive infection. If this assumption was correct, we hypothesized that the anticipation of the development of specific CD8$^+$ effector T cells would control the parasitemia and the pathogenesis after challenge. Initially, we tested whether the delay of activation of CD8$^+$ effector T cells could be due to active mechanisms of immune-suppression caused by the infection. For that purpose, mice immunized with recombinant adenoviruses were infected or not with *T. cruzi*. We found no evidence for active immuno-suppression as immunized mice, infected or not, developed until the peak of infection anti-viruses or parasite specific effector CD8$^+$ T cells equally well. Subsequently, we demonstrated that the induction of specific CD8$^+$ T cells for a parasite epitope by a single immunization with recombinant adenoviruses 7 days prior to infection was sufficient to reduce the pathogen load (parasitemia) and the pathogenesis (mortality). The delay of activation of specific CD8$^+$ effector T cells is not caused by immune suppression and the anticipation of the activation of these cells reduces the parasite load and pathogenesis.
Financial Support: FAPESP and INCTV (CNPq).

IM41 - CANINE LEISHMANIASIS: EVALUATION OF THE CELLULAR IMMUNITY

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A broad spectrum of clinical manifestations could be observed in dogs from endemic areas of canine leishmaniasis (CanL). The immune mechanisms underlying the clinical presentation of CanL have not been fully investigated. To characterize the cellular immune response in dogs naturally infected with *L.(L.)i.chagasi* in a region of high incidence of CanL in the São Paulo state, the expression of $CD3^+$, $CD4^+$ and $CD8^+$ T cells in lymph nodes as well as of serum cytokines were determined in symptomatic and asymptomatic dogs. Twenty *Leishmania* positive dogs, 10 symptomatic and 10 asymptomatic from the Center of Zoonosis Control of Araçatuba city were submitted to euthanasia and biopsies of lymph nodes were collected and processed by immunohistochemistry (avidin-biotin method) using mouse anti-human CD3 (DAKO), rat anti-canine CD4 and CD8 (SEROTEC) monoclonal antibodies. Quantitative analysis was performed using the image analysis system. Sera were processed by Capture-ELISA for TFN-α; and TGF-β; determination (R&D). Biopsies and serum of dogs from non-endemic area of visceral leishmaniasis were used as control. *Leishmania* infection promoted an increase on the number of T cells in the lymph nodes. There was predominance on the $CD3^+$, $CD4^+$ and $CD8^+$ cells expression in asymptomatic compared to symptomatic dogs that not reflected on the levels of TFN- α and TGF- β as they were found in equal amount in both groups of animals. However, increased TGF- β that is associated with susceptibility, and decreased TFN-α that is related with resistance were observed in the sera of dogs from endemic area compared to dogs from non-endemic area ($p<0.05$). In summary, the symptomatic and asymptomatic dogs showed similar immune response that was associated with a non-protective pattern, indicating susceptibility of this reservoir to *L.(L.)i.chagasi* infection despite the clinical status.

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IM42 - SPECIFIC SERODIAGNOSIS OF CANINE VISCERAL LEISHMANIASIS USING *LEISHMANIA* RIBOSOMAL PROTEIN EXTRACTS.

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Canine visceral leishmaniasis (CVL) is an important disease in various countries around the world. Due to their genotypic relationships, *L. chagasi* and *L. infantum* can be considered identical. The outcome of CVL is variable and infected dogs can develop different clinical forms of the disease: asymptomatic, oligosymptomatic or symptomatic. To reduce the transmission of *Leishmania* from dogs to humans, it is necessary to diagnose canine leishmaniasis as early as possible. The presence of anti-*Leishmania* antibodies in infected dogs has allowed the development of serologic tests. Diagnosis of CVL using ELISA based on soluble *Leishmania* antigens (SLA) have shown a high value of sensitivity but low specificity because of antigenic relatedness between *Leishmania* and other protozoa. As a strategy to develop specific serodiagnostic test for CVL, different parasite antigens have been analyzed. Some of the parasite ribosomal constituents like the parasite acidic P proteins induce strong humoral response in dogs suffering visceral leishmaniasis (VL). In addition, *Leishmania* ribosomal proteins (LRP) seem to be immunologically relevant molecules during murine experimental cutaneous leishmaniasis (CL), because high titres of antibodies recognizing the parasite ribosomal proteins were detected in the sera form BALB/c mice infected with *L. major*. In the present work, we have analyzed the antigenic properties of LRP in dogs infected with *L. infantum* and *L. chagasi*. For that reason, the diagnostic potential of LRP extracts was evaluated in comparison with SLA. We report that the ELISA tests using LRP have a similar sensitivity but higher specificity that SLA-based ELISA assays.

Supported by: FAPEMIG and CNPq.

IM43 - CD11c CELLS PROFILES EXPRESSED IN THE SKIN LESIONS OF BALB/c MICE EXPERIMENTALLY INFECTED WITH L. (L.) amazonensis AND L. (V.) braziliensis

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American cutaneous leishmaniasis (ACL) is an infectious disease caused by several Leishmania species. L.(L.)amazonensis and L.(V.)braziliensis are the two species with most medical interest in Brazil. In the BALB/c mouse model, L.(L.) amazonensis shows a progressive disease associated with a frank Th2-type immune response, while L.(V.)braziliensis presents, in contrast, a self-limited disease linked to a Th1-type immune response. In order to study the role of the dermal dendritic cells (dDCs) in the experimental L.(L.)amazonensis and L.(V.)braziliensis-infection, BALB/c mice were skin-inoculated into the hind footpads with 106 stationary-phase cultured promastigotes of both parasites. Control group was inoculated with PBS. The hind footpad swelling was evaluated weekly till 60th day post-infection (PI). At 30 and 60 days PI, biopsies of skin-inoculation sites were collected and processed by immunohistochemistry for CD11c cells staining; cells of draining lymph nodes were cultured under ConA and Leishmania-antigen stimulation and the culture-supernatant were used for IFN-γ, IL-4 and IL-10 determination by ELISA. A serum sample was also processed for IgG1, IgG2a and IgG2b by ELISA. The lesion size increased with the time of L.(L.)amazonesis-infection and was higher than that in L.(V.)braziliensis-infection, which developed a self-healing lesion with small pick at 4th week PI. At 30th day PI, no stained CD11c cells and no IgGs levels were detected in any experimental group, but increased levels of IL-4 were observed in L. (L.) amazonensis-infection. At 60th day PI, the CD11c cells expression was only noted in L.(V.)braziliensis-infection associated with an increased level of IFN-γ. Increased of IgGs levels were only demonstrated in L.(L.)amazonensis-infection, especially IgG1. These preliminary results suggest that the CD11c cells expression combined with the increased levels of IFN-γ□in L.(V.)braziliensis-infection might be associated to the development of the protective immune response in these animals.
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IM44 - Induction of humoral immune response following immunization with three different plasmid DNA vaccines against African trypanosomiasis

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African Trypanosomiaisis (AT), known as Sleeping Sickness, is an orphan and extremely debilitating disease in human, cattle and domestic animals. AT is caused by the protozoan *Trypanosoma brucei* and at the present, there's no safe or efficient pharmacology intervention. The DNA vaccines could be the answer for this disease by being able to induce production of IgG antibodies and induce of Th1/Th2 cytokines mediated by $CD8^+$ T cells and activating $CD4^+$ T helper cells. In this study, we shows that Balb-C mice immunized intramuscularly with a single dose of plasmids encoding three antigenic candidate genes from *Trypanosoma brucei,* named *Invariant Surface Glycoprotein* (ISG), *trans*-sialidase (TSA), and fosfolipase C (PLC) are able to produce IgG antibodies anti-trypanosoma. This immunization process was able to control the mortality level when mice were submitted to challenger assay with *Trypanosoma brucei brucei* parasites. After the challenger, 40% of mice immunized with ISGpVAX1 survive such as 60% of immunized with nTSApVAX1 and 20% of PLCpVAX1. The animal immunized with all plasmids intramuscularly and subcutaneous shows a protective rate of 40% and 75%, respectively. These results open up the possibility of the use of new attractive targets for vaccine development against AT.

KEYWORDS: African Trypanosomiasis, Sleeping Sickness, *Trypanosoma brucei*, recombinant antigens, DNA vaccine

IM45 - Meningoencephalitis in experimental infection of *Trypanosoma cruzi*: astrocyte role in parasitism control

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The experimental *T. cruzi* infection leads to intense inflammation in the central nervous system (CNS) in C3H/He mice restrict to the acute phase, characterized by the prevalence of F4/80+ and CD8+ and the presence of *T. cruzi* antigens in macrophages/microglia and astrocytes. These data suggest that glial cells are able to control the parasite in the chronic phase. Herein, we studied the role of the astrocyte in the genesis/resolution of the *T. cruzi*-elicited encephalitis, adopting astrocyte-enriched cell culture of C3H/He mice (susceptible) and C57BL6 mice (resistant) infected or not by the *T. cruzi* Colombian strain of, by measuring nitric oxide (NO) production by Griess reaction and TNF by ELISA. Our results show that astrocyte cultures of C57BL6 had higher production of NO. However, in the presence of IFN-γ NO production was diminished in both strains. In contrast with C57BL/6 mice, there is an increased TNF mRNA expression restricted to the acute infection in the CNS of the susceptible C3H/He mice, whereas high levels of circulating TNF were detected during acute and chronic phases of infection. Also, non-infected astrocytes did not produce significant amounts of TNFα, even in the presence of IFN. However, in IFN-γ-treated *T. cruzi* infected-astrocytes TNFα production was enhanced. In conclusion, NO production by astrocytes of resistant mice might be one of the factors involved in parasitism control. Additionally, TNF-α production by infected astrocytes is up-regulated by IFN-γ. Our findings suggest that TNF might be produced in the CNS as result of the infiltrating inflammatory cell production of IFN-γ, although the source of TNF in vivo remains unclear. Moreover, our data show that CNS inflammation formation/resolution is independent of peripheral TNF levels. Altogether our data show that astrocytes are target for *T. cruzi* infection and might contribute to resolution of the *T. cruzi*-elicited meningoencephalitis.
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IM46 - Cell migration in Chagas disease: the CC-chemokine receptors CCR1/CCR5 as targets for immunoregulation in *Trypanosoma cruzi*-elicited cardiomyopathy

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Chagas disease, caused by Trypanosoma cruzi, affects 16-18 million people in Latin American, leading to chronic chagasic cardiomyopathy (CCC) in about 30-40% of the patients. CCC is characterized by inflammation associated with prominent fibrosis and electrical dysfunction. In the affected cardiac tissue, the presence of pro-inflammatory cytokines and chemokines might drive leukocyte migration contributing to CCC formation. T. cruzi-infected cardiomyocytes and macrophages produce the CC-chemokines CCL3, CCL4 and CCL5 that stimulate infected cells to control T. cruzi growth in a nitric oxide-dependent manner. Conversely, elevated plasma concentrations of chemokines have been associated with heart failure severity and CCC. Enhanced expression of CCR5 (receptor for CCL3, CCL4 and CCL5) was detected in peripheral blood mononuclear cells (PBMC) of CCC patients and T. cruzi-infected mice. These led us to consider that CC-chemokines and the CC chemokine receptor CCR5 might be involved in the immunopathogenesis of T. cruzi-triggered cardiomyopathy. Independent studies showed that the CCR5 and the cell adhesion molecule LFA-1 are expressed in PBMC enabling them to migrate to the heart tissue. Further, most of the heart invading inflammatory cells are CCR5+ and LFA1+ (dos Santos et al., 2001; Marino et al., 2004; Michailowsky et al., 2004). Importantly, treatment with the selective partial antagonist CCR1/CCR5 during the chronic phase of infection resulted in 20-30% reduction in CD4+ cell numbers as well as IL-10, IL-13 and TNF expression in the cardiac tissue. Furthermore, Met-RANTES treatment led to reduction in parasite load, fibronectin deposition and cardiomyocyte lesion. Thus, therapeutic strategies based on the modulation of CCR1/CCR5-mediated cell migration and/or effector function may contribute to cardiac tissue damage limitation during chronic Chagas disease.
Medeiros et al. Treatment of chronically *Trypanosoma cruzi*-infected mice with a CCR1/CCR5 antagonist (Met-RANTES) results in amelioration of cardiac tissue damage. Microbes and Infection, 11:264-273, 2009. Support: CNPq, FAPERJ.

IM47 - *Leishmania major* ENCODED INHIBITORS OF SERINE PEPTIDASES (ISPs): INFLUENCE IN THE PARASITE-NEUTROPHIL INTERACTION.

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Establishment of *Leishmania* infection is associated with survival of the parasite within macrophages that fail to be properly activated and eliminate the parasite. It was recently shown that neutrophils are the first cells to be recruited to the site of the infection and they can also be parasitized. *Leishmania* delays neutrophil apoptosis, protecting the infected cells from premature death. *Leishmania major* has three genes sharing similarity with bacterial ecotins, which are potent inhibitors of family S1A trypsin-fold serine peptidases, termed ISPs (Inhibitors of Serine Peptidases). Recent work showed that recombinant ISP2 inhibits human neutrophil elastase, trypsin and chymotrypsin with high affinity. Moreover, it was shown that parasites lacking *ISP2* and *ISP3* (Δ*isp2/isp3*) are more efficiently phagocytosed by peritoneal macrophages than wild-type parasites, in a mechanism dependent on neutrophil elastase activity. Since protease based-mechanisms contribute to the microbicidal activity of neutrophils, we began to evaluate the possible role of ISPs in the interaction of neutrophils with *L. major* by using *isp2/isp3* null mutants and tool. Our results shows that Δ*isp2/isp3* are internalized more efficiently by C57B6 neutrophils when compared to wild type parasites, and this was reversed by addition of r-ISP2 or a synthetic inhibitor to neutrophil elastase. Evaluation of the apoptosis of murine elicited neutrophils by annexin-V labeling after in vitro culture showed that neutrophils co-cultured with Δ*isp2/isp3* were rescued from apoptosis more efficiently than those co-cultured with WT parasites. Moreover, peritoneal macrophages infected in vitro with Δ*isp2/isp3* were able to recruit neutrophils more efficiently than those infected with WT or with parasites re-expressing ISP2 and ISP3. Our results suggest that the regulation of host SP activity by *L. major* ISPs influences the parasite interaction with professional phagocytes, possibly contributing to the establishment of the infection.

IM48 - PENTOXIFYLLINE ACTION IN CHRONIC EXPERIMENTAL CHAGAS DISEASE

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In *Trypanosoma cruzi* infection the heart is the main damage organ. In the acute infection, inflammation in the cardiac tissue can result in regain of homeostasis. However, the inflammatory process can progress resulting in chronic myocarditis in about 30% of patients. Pro-inflammatory cytokines, such as TNF-alpha, play an important role in recruitment of inflammatory cells to target tissues, controlling the expression of cell adhesion molecules (CAM) and up-regulating chemotactic cytokines such as CCL5/RANTES and CCL3/MIP-alpha. Our previous data support that CD8-enriched chagasic myocarditis formation involves CCR1/CCR5-mediated cell migration. Furthermore, it has been shown that the plasma levels of TNF-alpha are correlated with the degree of heart dysfunction in chronically *T. cruzi*-infected patients, suggesting a role for this cytokine in the pathogenesis of chagasic myocarditis. In the present study, the action of Pentoxifylline (PTX), a drug known to inhibit the synthesis of TNF, upon myocarditis and heart dysfunction was investigated adopting a model of chronic chagasic cardiomyopathy. PTX therapy during the chronic *T. cruzi* infection did not alter the survival, parasitemia and heart parasitism, however had a beneficial effect, improving the cardiac function characterized by reduction of arrhythmias and atrio-ventricular block grade 1 and 2. In summary, treatment with PTX in the chronic phase of T. cruzi infection appears to be a promising therapeutic strategy. Further studies are required to support PTX as a potential adjunct therapy to treat chronic chagasic cardiomyopathy.

IM49 - IMMUNOGLOBULIN G SUBCLASS-SPECIFIC ANTILEISHMANIAL ANTIBODY RESPONSES IN AMERICAN TEGUMENTARY LEISHMANIASIS PATIENTS BEFORE AND AFTER TREATAMENT

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American Tegumentary Leishmaniasis (ATL) is pathology of infectious and chronic character that affects the man and several species of wild and domestic animals, caused by protozoa of the gender *Leishmania*. The clinical spectrum of ATL comprises cutaneous (CL), mucosal (ML) and diffuse cutaneous (DCL) forms. The diagnosis of ATL includes aspects epidemics, clinical and serological and frequently the association of some of those elements is necessary to obtain a definitive diagnostic. The analysis of antibodies anti-*Leishmania* allows to evaluate the evolutionary course of the infection, as well as to provide evidence of the characteristics of immune response. Among the immunological exams, the serologic tests, as Indirect Immunofluorescence (IIF) and Montenegro Skin Test (MST) are the most frequently used. The present work evaluated Immunoglobulin G profile (IgG) and their subclasses in samples of serum of 47 patients with confirmed clinical diagnosis attended the Hospital Anuar Auad (HDT) in Goiânia, Goiás. 74.2% and 87.5% of serums reagents were found for specific IgG in patients with the cutaneous and mucosal form of the leishmaniasis, respectively. The evaluation revealed that in the collected samples before treatment the patients with CL presented equivalent levels of the subclasses of IgG, while in the patients with ML the levels of IgG1 and IgG3 were higher. Six months after the beginning of the treatment, the patients with CL altered significantly the levels of IgG2 that increased and the levels of IgG3 that decreased, whereas in the patients with ML, the levels of IgG1, IgG2 and IgG3 subclasses increased and the levels of IgG4 decreased significantly. Twelve months after the beginning of the treatment all patients with CL and ML were cured, the levels of all subclasses decreased. A significant reduction of the levels of IgG1 and IgG3 in patients with CL twelve months after the beginning of treatment was observed. The prevalence of some IgG subclasses is associated with the type and the complexity of the immune response developed against *Leishmania*.
Supported by CNPq, FAPEG/GO.

IM50 - ISOTYPES PATTERNS OF IMMUNOGLOBULINS IN DISTINCT ASYMPTOMATIC CLINICAL FORMS OF CANINE VISCERAL LEISHMANIASIS CAN BE INDICATE THE RESISTANCE DURING THE NATURAL INFECTION

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During canine visceral leishmaniasis the immunological mechanism underlying the susceptibility or resistance to severe disease remains for less defined. Polyclonal activation of B-cells leading to high titers of circulating antibodies is finding of the course of *L. chagasi* infection. In the present study were evaluated 34 dogs naturally infected by *L. chagasi*. The asymptomatic clinical forms (AD) was subdivided into two distinct categories AD-I (n = 8, seronegative, PCR positive and without clinical signs) and AD-II (n = 10, seropositive , PCR positive and without clinical signs) and symptomatic dogs - SD (n = 16, seropositive, PCR positive and showed that more than three clinical signs of infection. Seven dogs with negative parasitological, serological and molecular diagnosis were used as control group (CD). These animals were submitted to a detailed analysis of serological parameters by ELISA using a specific anti-canine isotype antibodies (IgG, IgG1, IgG2, IgM, IgA and IgE) employing a soluble *L. chagasi* antigen. The result shows higher levels of IgG, IgG2 and IgM in AD-II and SD in comparison with CD and AD-I. In group AD-I, it was observed decreased levels of IgG1 in relation to CD group. Our results emphasize that the CVL disease progression is characterized by appearance of specific immunoglobulins isotypes such as IgA with which may contribute to the aggravation of the clinical status during ongoing CLV infection. Furthermore, we observed that the AD-I presented a similar profile of the Igs isotypes showed in CD group indicating that the B-cell polyclonal activation in this group is still lower. Also, the higher levels of total IgG, IgG2, IgM and IgA observed in AD-II and SD might suggest that a active disease was established in AD-II dogs probably because a failed cell immune response in this animals. Supported by FAPEMIG, CNPq, DECIT, PRONEX.

IM51 - EXPLORING THE ASYMPTOMATIC CLINICAL FORM IN CANINE VISCERAL LEISHMANIASIS (CVL) TO UNDERSTAND THE RESISTANCE MECHANISMS OF THE INFECTION THROUGHOUT CELLULAR IMMUNOLOGICAL BIOMARKERS INVESTIGATIONS

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To evaluate the immunological biomarkers of susceptibility and resistance in the natural infection by *L. chagasi* we assessed a total of 41 dogs naturally infected. The asymptomatic clinical forms - AD (n=18) was subdivided into two distinct categories AD-I (n=8, seronegative, PCR positive and without clinical signs) and AD-II (n=10, seropositive, PCR positive and without clinical signs) and symptomatic dogs - SD (n = 16, seropositive, PCR positive and showed that more than three clinical signs). Seven dogs from endemic area with negative parasitological, serological and molecular diagnosis were used as control group (CD). It was investigated, by flow cytometry, absolute number/mm^3 of the T lymphocytes (CD5$^+$ and Thy-1$^+$), T-subpopulations (CD4$^+$, CD8$^+$ and CD4$^+$/CD8$^+$), monocytes (CD14$^+$) and B (CD21$^+$) cells. Our results showed that dogs of the AD-I group presented an increased of circulating T lymphocytes (CD5$^+$ and Thy-1$^+$) that maintained high in the AD-II group. Meanwhile symptomatic dogs (SD) presented a decline in the number of T circulating (CD5$^+$ and Thy-1$^+$) lymphocytes in relation to AD-I and AD-II groups. The increase of T lymphocytes in asymptomatic (AD-I and AD-II) groups is closely related to the increase in subpopulations of T (CD4$^+$ and CD8$^+$) lymphocytes, mainly of CD4$^+$ in AD-I and CD8$^+$ in the AD-II group. There is also a decrease in the CD4$^+$/CD8$^+$ ratio according to the clinical progression. Furthermore, we observed that asymptomatic dogs presented distinct profiles of cellular phenotype according to serological and molecular diagnosis results. Such evidence suggests the existence of two distinct groups of dogs in asymptomatic being AD-I are more related to the resistance mechanisms. Our results suggest a new perspective in the analysis of immunological biomarkers in the asymptomatic CVL which may contribute to future clinical evaluations and in the understanding the mechanisms of resistance to infection.
Supported by FAPEMIG, CNPq, DECIT, PRONEX.

IM52 - IMMUNOGENICITY OF A NEW VACCINE AGAINST CANINE VISCERAL LEISHMANIASIS COMPOUND BY *LEISHMANIA BRAZILIENSIS* ANTIGENS, SALIVA OF *LUTZOMYIA LONGIPALPIS* AND SAPONIN ADJUVANT (LBSAPSAL VACCINE)

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Dogs represent the most important domestic reservoirs of *L. chagasi*/*L. infantum*, and a vaccine against canine visceral leishmaniasis would be an important tool in the control of visceral leishmaniasis. Furthermore, the sand fly saliva proteins were considerate as potential antigens to constitute a new vaccine candidate against leishmaniasis. For this reason, we performed a detailed analysis of the antigenicity/immunogenicity in dogs using a new canine visceral leishmaniasis vaccine composed of *Leishmania braziliensis* antigen, sand fly gland extract and saponin adjuvant (LBSapSal vaccine). No general adverse reactions occurred following immunization with LBSapSal. The specific anti-saliva and anti-*Leishmania* humoral responses showed significant increases in the serum levels of total IgG, IgG1 and IgG2 observed in dogs of the LBSapSal group. Western blot analysis revealed three different anti-saliva proteins (34, 45 and 71 Kda) after LBSapSal immunization. The immunophenotypic profiles of the peripheral blood revealed after first dose a significant increase in the number of circulating CD5+, CD4+ and CD8+ T-lymphocytes and CD21+ B-lymphocytes in LBSapSal group. Following LBSapSal vaccination, increased antigen-presenting cell (CD14 monocytes) counts gave rise to a lymphocyte activation profile associated with an up-regulation of both CD80 and MHC-I expression. A marked increase in *in vitro* cell reactivity in the presence of *Leishmania braziliensis* and *Leishmania chagasi* stimuli was the major characteristic following vaccination with LBSapSal. Moreover, the frequencies of CD8+ T-cells in antigen-stimulated *in vitro* cell proliferation cultures were increased by vaccination with LBSapSal and represented the major T-cell subset presenting a positive association with lymphoproliferation reaction. Futhermore, progressively higher levels of the reactive NO radical were recorded in sera from the LBSapSal. Our data suggested that the potential resistance profile elicited by the LBSapSal vaccine was compatible with effective control of the etiological agent of canine visceral leishmaniasis. *Supported by:* FAPEMIG, CNPQ, PAPES V/FIOCRUZ, CAPES, UFOP.

IM53 - IMMUNOGENICITY IN DOGS AFTER ADMINISTRATION OF A VACCINE COMPOSED BY ANTIGENS OF LEISHMANIA BRAZILIENSIS, SALIVA OF LUTZOMYIA LONGIPALPIS PLUS SAPONIN (LBSAPSAL) BEFORE AND AFTER CHALLENGE WITH LEISHMANIA CHAGASI

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Since no treatment method for canine visceral leishmaniasis (CVL) is acceptable by the government and current dog therapy is costly and not always effective, the development of new vaccines constitutes the priority in the control of this disease. In previous studies we showed that LBSapSal vaccine displays strong immunogenicity in dogs after vaccine protocol showing increased $CD21^+$ B-cells and $CD5^+$ T-cells, reflected by higher counts of $CD4^+$ and $CD8^+$ T-cells. The immunogenicity of LBSapSal a killed *Leishmania* vaccine with sand fly saliva extract and saponin adjuvant was investigated in dogs after challenge. Herein we studied the immunophenotypic profile of these cell populations during long follow up (885 days) after experimentally infection using *L. chagasi*-promastigotes plus *Lutzomyia longipalpis* saliva by intradermal rote. The preliminary results showed in LBSapSal group disclosure sustained high counts of $CD5^+$ T-cells later than 435 days after challenge. Similar results were observed in the T ($CD4^+$ and $CD8^+$) cells indicating that both subpopulations are involved with the immunogenicity of the LBSapSal vaccine. Further analysis suggest that the experimentally infection with the *L. chagasi* promastigotes induced increased levels of $CD21^+$ B-cells during long time of the follow up in LBSapSal group. Otherwise, the analysis of monocytes ($CD14^+$) showed high counts in the saliva immunized dogs and low numbers in LBSal and LBSapSal groups. Our data disclosures insights about the CVL vaccines and may contribute in the establishment of additional tools for analyze vaccine candidates to identify potential antigens against *Leishmania*-infection using dog model.
Supported by: FAPEMIG, UFOP, PAPES V-FIOCRUZ, CNPq and CAPES.

IM54 - GENOTYPIC CHARACTERIZATION OF *TOXOPLASMA GONDII* IN EXPERIMENTAL MOUSE REINFECTION WITH GENETIC DISTINCT STRAINS

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Toxoplasmosis, caused by the coccidian *Toxoplasma gondii,* is transmitted by ingestion of food and water contaminated with oocysts of the feces of felines. Although asymptomatic in most cases, the agent causes ocular disease or severe disease in immune compromised patients or fetus. Several strains of *T.gondii* have been described in recent years, with variable virulence that could be associated with severity of human disease, including the reinfection of a previous immune host. We study experimental double infections in mice with two strains of *T.gondii*, non virulent ME-49 type II strain and intermediate virulent VEG type III strain, looking for cerebral histology after infection, immune humoral response after challenge and genotypic characterization of strains in brain. In this study, groups of 8 mice were infected orally with cysts of each strain, alone, together or challenged after 1 month of first infection. Specific IgG were determined by ELISA. Brain involvement was determined in histology by cysts counts 56 days after infection in each group. The genotypic characterization was made by PCR-RFLP. ME-49 induces a higher cyst numbers than VEG strain in all animals' brains. Challenge with heterologous strain protects from the disease observed in each strain alone, without altering antibody profiles, with VEG infection protecting from ME-49 cyst burden. Mice challenged with a second strain presented brain cysts only from the first strain. Concomitant double infected mice with genetic distinct strains, the predominance of cysts varied with the time of infection, with more ME 49 cysts on 28th and more VEG cysts on 56th. The immune response and the brain disease is dependent of infecting strain and the immune response induced during acute infection do not abolishes reinfection but alters disease evolution. Strain determination would be a powerful tool in the prediction of the severity of toxoplasmosis.
Supported by CNPq and LIM49.

IM55 - QUICK COLLOIDAL GOLD IMMUNOCHROMATOGRAPHIC TEST FOR IN OFFICE DETECTION OF *TOXOPLASMA GONDII IgG* ANTIBODIES

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Toxoplasma gondii induce severe fetal disease, during pregnancy of acutely infected mother and their surveillance is mandatory during prenatal care, usually by serology. Uninfected women at risk of contracting the disease must be followed by monthly serology, for therapy of seroconverted diminishing fetal damage. We develop a new immunoassay based in immunochromatographic strips for in office detection of specific IgG serology for toxoplasmosis, for a rapid diagnosis of seroconversion. The test was based on colloidal gold particles (6 nm) adsorbed with *Toxoplasma gondii* antigen obtained from tachyzoites purified from peritoneal exudates of infected mice. This colored antigen was mounted in strips of high flow nitrocellulose membranes with dots of Staphilococcal Protein A (test dot) or anti-*T.gondi* IgG (control dot) (TIC-Toxo). Serum or blood samples was applied and flow through the particles with *Toxoplasma gondii* antigen to the membrane and if specific IgG was present in sample, the IgG covered particles stains both dots, while negative samples stained only control dot, allowing immediate detection of specific IgG. We evaluate TIC-Toxo test with 70 predefined sera samples from pregnant women in prenatal care of Cascavel – Paraná count public health system, according their anti *T.gondii* IgG serology. The results of TIC-Toxo was compared with two other techniques: enzyme-linked immunosorbent assay (ELISA) and indirect immunofluorescence assay (IFA), with determination of their comparative indexes. The test presented sensitivity of 88,6% (CI95% 72,3–96,3%), specificity of 80% (CI95% 62,5-90,9%), predictive value positive of 81,6% (CI95% 65,1-91,7%) and predictive value negative of 87,5 % (IC 70,1-95,9%), with a agreement of 0,857 and Kappa coefficient 0,712.The test lasts less than 10 minutes with visual detection. Our test are useful as a rapid, easy and simple screening serology method for anti-*Toxoplasma gondii* IgG in office prenatal care, especially for follow up of seronegative mothers at risk of infection.

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IM56 - PRELIMINARY STUDIES ON PARASITEMIA, ANTIBODY LEVELS AND ΡIFN PRODUCTION IN MICE INFECTED WITH *TRYPANOSOMA CRUZI* COLOMBIAN STRAIN.

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The treatment of Chagas disease is based on the administration of a single drug, either Benznidazol or Nifurtimox, but does not focus on the host's immune response. Treatment failure has very seldom been connected with failure of immune response, and with the escape mechanisms of *Trypanosoma cruzi*. IFN is a co-adjuvant molecule that modulates the host's immune response, and its production is induced by associated cytokines such as IL2. Our hypothesis is that the strains of the parasite which are resistant to current treatment with Benznidazole (BZL), such as Colombiana strain, do not activate an immune response strong enough in the host that co-adjuvates the chemotherapy. In a first attempt to test this hypothesis, we intraperitoneally infected 20 mice with 250 trypomastigotes of Colombiana strain. Mice were killed at days 10, 25, 35, and 95 post-infection, and their circulating parasites were measured by optical microscopy twice a week. Serum was extracted to measure levels of IFN and IgG in response to the infection. This inoculum with few parasites induced a low but continuous parasitemia that was still detectable after 3 months of infection, in contrast with other strains whose parasites are undetectable after a month. IFN and IgG levels were negative at day 10 post-infection, but progressively increased during days 25 and 35, where IFN reached its peak. In contrast, antibody levels continued increasing and at day 95 were at their highest point, whereas IFN became negative. These results show that although a high antibody response was mounted it was not enough to control the infection. In future experiments we will explore the mice response to a higher inoculum, and we will also compare it to the one induced by the susceptible strain Tulahuén.

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IM57 - TOXOPLASMOSIS IN MAPUTO, MOZAMBIQUE – PRELIMINARY RESULTS IN PREGNANT WOMEN AND AIDS PATIENTS WITH ENCEPHALITIS

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Toxoplasmosis, a protozoan disease, causes severe disease in fetus of pregnant women during acute infection and deadly encephalitis in HIV patients. There are several studies on its seroprevalence around the World, but for African countries only few and anecdotally reports. We studied by ELISA two groups of samples from Maputo Mozambique, using 150 pregnant women serum samples and CSF samples of 06 AIDS patients with encephalitis. HIV status was confirmed and CD4 blood counts were obtained of HIV positive pregnant women. In the whole group, IgG seroprevalence was 18.7% (28/150), more intense in HIV positive (31.3%, 18/58) than in HIV negative (10.9%, 10/92) patients. This data could be biased according to cumulative effect of exposition that affects similarly its prevalence, and if corrected, could show an interaction of HIV and *T.gondii*. Prevalence of both diseases increases with age, but this is more clearly seen in toxoplasmosis ($p<0.005$) than in HIV infection ($p<0.05$), explained by higher transmission of HIV after childhood. In encephalitis of HIV patients, CSF serology showed a 33% prevalence of specific IgG of high avidity in CSF, which is according to the prevalence in this group, based on our pregnant women data. Lower prevalence of both infections in older groups could be explained by more deaths from any cause in infected groups, resulting in lower prevalence. Toxoplasmosis prevalence is higher in HIV+ groups, that could be ascribe to an HIV and *T.gondii* associated risk factor. The low incidence of toxoplasmosis in younger age groups shows that the transmission could related to better access to cyst containing meat in adult life, as environmental transmission due to oocysts is usually associated to higher incidence in children. All these data supports the urgent need of research in toxoplasmosis in Africa, especially in the presence of HIV epidemics.
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IM58 - USE OF MEAT EXUDATE IN THE DETECTION OF IGG ANTI-*TOXOPLASMA GONDII*

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Toxoplasma gondii is a cosmopolitan obligate intracellular parasite that infects warm-blooded animals, causing serious problems in immunosuppressed individuals or congenital infection during acute infection in pregnancy. This agent causes also economic losses in animal production, due to abortion. The main human transmission is the ingestion of raw or undercooked meat containing cysts, being one of the main meat-transmitted zoonotic diseases. Currently, meat inspection in Brazil is devised to gross macroscopic lesions or parasites, as tuberculosis or teniasis, without *Toxoplasma* search. Several laboratory methods are used to detect this infection, mainly by serology, but detection in slaughter meat needs PCR or cumbersome biological tests in mice. We devised to study exsudates of frozen meat, composed mainly by blood retained in capillary vessels, as a source from "serum" of the animal for *Toxoplasma* serology. We standardize an Enzyme-Linked Immunosorbent Assay to detect IgG anti-*T. gondii* in meat exsudates from experimentally infected rabbits, normalizing the amount of blood in those samples by hemoglobin determination, using frozen blood from those animals as standard of hemoglobin, by a nitrite solution for 540nm OD stabilization. Clear discrimination of infection was obtained in those exsudates, allowing the assumption of infected or *T.gondii* free animals, with adequate cut-offs and serological indexes when we assayed the exsudates in a standizerd OD 540nm, before the ELISA assay. Moreover, we observed that the exsudate do not vary its antibody composition between its first and second thawing and also do not depend of freezing period, at least for 120 days. This exsudate is easily obtained after thawing, without affecting its commercial value. This diagnostic approach is very promising and important for meat safety quality, allowing control at end distributors or users in meat prepared for marketing, contributing directly to prevention of human infection.
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IM59 - Neutrophils and macrophages cooperate in host resistance against *Leishmania braziliensis* infection

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Neutrophils play an active role in the control of infections caused by intracellular pathogens such as *Leishmania*. In the present study, we investigated the interaction between neutrophils and *L. braziliensis*–infected macrophages in addition to the outcome of this interaction in terms of parasite survival. The in vivo depletion of neutrophils was shown to lead to a significant increase in parasite load in an experimental model of *L. braziliensis* infection. Consistent with these results, BALB/c mice co-inoculated with both parasites and live neutrophils displayed lower parasite burdens at the site of infection and in the draining lymph nodes. In the process of determining the mechanism associated with parasite killing, we observed that live neutrophils significantly reduced the parasite load in *L. braziliensis* infected murine macrophages in vitro. This reduction was dependent on the interaction between neutrophils and macrophages and was associated with an increase in the production of TNF-α and superoxide. Furthermore, the contribution of live neutrophils towards parasite elimination was also observed during the co-culture of human neutrophils and macrophages infected with *L. braziliensis* and with two other New World *Leishmania* species. These results suggest that neutrophils play an important and previously unappreciated role in *L. braziliensis* infection and may impact the induction of a protective immune response.
Supported by CNPq and FIOCRUZ

IM60 - THE ROLE OF TH17 IN THE PATHOGENESIS OF HUMAN MUCOSAL LEISHMANIASIS

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Mucosal leishmaniasis (ML) is characterized by disfiguring facial lesions partially attributed to an exacerbated Th1 inflammatory response. It has been described that intense Th17 response promotes collateral tissue damage in several chronic inflammatory disorders. In order to evaluate the role of Th17 cells in ML pathogenesis, we analyzed biopsy specimens from 16 ML patients. Local expressions of interleukin-17 (IL-17) as well as of IL-17-inducing cytokines (interleukin-1, interleukin-23, Iinterleukin-6 and transforming growth factor-) were detected by immunohistochemistry in ML biopsy specimens. IL-17+ cells exhibited CD4 or CD8 phenotypes, and numerous IL-17+ cells co-expressed the chemokine receptor 6 (CCR6), identified by confocal microscopy. Neutrophils, a hallmark of Th17- mediated inflammation, were regularly detected in necrotic and perinecrotic areas and stained positively for neutrophil elastase, myeloperoxidase and matrix metalloproteinase 9. Moreover, stimulation of cells obtained from mucosal lesions by *Leishmania braziliensis* lead to an increased frequency of Th17 cells. These observations show the existence of Th17 cells in ML lesions associated to neutrophil-mediated tissue damage suggesting an important role of IL-17 in ML pathogenesis.

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IM61 - The role of CD8$^+$ T lymphocytes in the pathogenesis of American Tegumentary Leishmaniasis

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American tegumentary leishmaniasis (ATL) is caused by protozoans of the genus *Leishmania*. In Brazil the disease is endemic all over the country, in which about 35,000 new cases are notified each year. CD8 T cell are important in the defense against virus, yet little is known of their participation in the defense against parasites. During the acute phase of infection, large numbers of CD8$^+$ T cells have been observed in the lesions as well as in peripheral blood. In our laboratory were verified that CD8$^+$ T cells have also been implicated in the chronicity of Leishmania infections by exacerbating the tissue lesions caused by *L. braziliensis*. The aim of this study is evaluated the role of CD8$^+$ T lymphocytes in peripheral blood and in biopsy of patients with ATL in early stages of infection. Markers of activation (CD25, CD71), address (CD62L, CCR7 e CLA), production of cytokines and Granzyme B were evaluated by FACS analysis in the ex-vivo cells, in peripheral blood mononuclear cell (PBMC) 24 hours after *L. braziliensis* stimulation and in cells obtained by biopsy from patients. Increase in the expression of activation markers (CD25 and CD71) and address (CLA) was observed in CD8$^+$ T cells of patients, both in the analysis of ex vivo and after stimulation with leishamnia. Higher frequency of CD8$^+$CLA$^+$ T cells was also found in the biopsies, suggesting that these cells would be migrating from peripheral blood to the tissue of the lesion. The expression of IL-10 and granzyme B were increase in the CD8$^+$ T cells after stimulation with leishmania, suggesting that these cells may participate in mechanisms of regulation and cytotoxicity. These results suggest that CD8$^+$ T cells in patients with ATL in early stages of infection, have a characteristic profile of effectors cells and that such cells would migrated to the tissue of the lesion, where exert a cytotoxic and regulatory response.

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