IM001 - **NETOSIS TRIGGERED BY *LEISHMANIA AMAZONENSIS* INVOLVES NADPH OXIDASE- AND MITOCHONDRIA-DERIVED ROS**

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Neutrophil extracellular traps (NETs) are composed by a DNA scaffold decorated with histones, cytosolic and granules proteins, which ensnare and kill microorganisms when extruded from neutrophils upon activation by microorganisms, such as *Leishmania*. This new microbicidal mechanism named netosis has been shown to be dependent on reactive oxygen species (ROS) generation by NADPH oxidase activity and also of the enzymes peptidyl arginine deaminase 4 (PAD4) and elastase (NE), both involved in chromatin decondensation. Here we analyze the role of ROS, NE and PAD4 in the netosis stimulated by *Leishmania amazonensis* (La) promastigotes, using human neutrophils isolated from healthy blood donors. NET release was quantified by measuring DNA in supernatants of neutrophils activated with La and ROS production was inhibited by several inhibitors. Our results show that diphenylene iodonium, a flavoprotein inhibitor, significantly decreased NET release induced by La. Similarly, the NADPH oxidase inhibitor apocynin reduced 34%, NET induction by La. To address the role of ROS produced by the mitochondria on NET formation we treated neutrophils with Rotenone, an inhibitor of mitochondrial complex 1, which inhibited 40% NETs release by La. Confirming the mitochondrial ROS participation, treatment with FCCP, an uncoupler of mitochondrial oxidative phosphorylation, and antimycin A, a mitochondrial complex III inhibitor, reduced 67% and 50%, respectively, NETs formation by La. ROS inhibition by the different treatments was ensured by ROS measurements with specific probes. The PAD4 inhibitor Cl-Amidine and elastase inhibitor III reduced 54% and 64%, respectively, the extrusion of NETs by La. Together, our results ensure that ROS, elastase and histones citrulination are involved in *La*-induced NET formation by human neutrophils, and suggest the involvement of mitochondrial-generated ROS in this process. We thank the Hemotherapy Service of HUCFF, UFRJ. **Supported by:**CAPES, FAPERJ e CNPq

IM002 - **THE NATURAL CYSTEINE PEPTIDASE INHIBITOR (ICP) OF THE AFRICAN TRYPANOSOME MODULATES INFLAMMATORY RESPONSES IN EXPERIMENTAL INFECTIONS**

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In Human African Trypanosomiasis, *T. brucei rhodesiense* penetrates the central nervous system by unknown mechanisms, leading to meningoencephalitis. The parasite cathepsin L-like cysteine peptidase was implicated in the penetration of the blood brain barrier. Its activity is modulated by the endogenous inhibitor of cysteine peptidases (ICP) that belongs to the chagasin family. To investigate the role of ICP in *T. b rhodesiense* we generated ICP null mutants (Δ*icp*). Δ*icp* has higher CP activity and traverse brain microvascular endothelial cell monolayers *in vitro* more efficiently than WT. BALB/c mice infected with Δ*icp* display lower blood parasitemia and delayed death, as compared to those infected with WT parasites. At day 5 of infection, immunohistochemistry of brain cryosections showed a dense cellular infiltrate and sparse microglia in the subarachnoid space of mice infected with WT, while this was not evident in mice infected with Δ*icp*. All infected mice exhibited splenomegaly and higher cellularity as compared to uninfected controls. Cell phenotyping by FACS revealed similar numbers of $CD4^+$ or $CD8^+$ cells but increased numbers of $CD11c^+$, $F4/80^+$ or NK 1.1^+. To obtain further insight into the immune response of the infected animals, we measured cytokine levels of splenocytes cultured for 24 hours. We found significantly higher levels of IFN-γ and IL-6 at the culture supernatants from animals infected with Δ*icp* as compared to WT or ICP-reexpressors. In contrast, TNF-α levels were more pronounced in supernatants from splenocytes of mice infected with WT. To study late-stage disease, infected mice were treated with Berenil at day 3 and parasitemia receded for one week. At day 20, immunohistochemistry of brain cryosections showed significantly increased numbers of Iba-1 stained cells in the cortex of mice infected with parasites Δ*icp*. We propose that ICP downmodulates early inflammatory responses with consequences to brain inflammation. **Supported by:**FAPERJ

IM003 - ***NEOSPORA CANINUM*** **INDUCES A P38 MAPK PATHWAY- DEPENDENT IN MURINE BONE MARROW-DERIVED MACROPHAGES**

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Introduction: Due to the high prevalence and economic importance of neosporosis, the development of safe and effective vaccines against this parasite is required in order to prevent abortions and vertical transmission in cattle, as well as to control the spread of oocysts by the definitive host. Such research is a priority in this field and is crucial to limit infection in its hosts. The major aim of the present study was to assess the mitogen-activated protein kinases (MAPK) signaling pathways in bone marrow-derived macrophages (BMDMs) under *N. caninum* infection Methods: In order to accomplish our goals, BMDMs derived from C57BL/6 WT mice and genetically deficient in PI3K and MyD88 were infected with *N. caninum* and stimulated with its antigens (NLA). The activation of the different MAPKs was observed by Cytometric Bead Arrays (CBA) and the cytokine profile (IL-12 and IL-10) was determined by ELISA. BMDMs were also phenotyped for B7 and MHC expression. Results: WT BMDMs treated with p38 or PI3k inhibitor or PI3k-/- BMDMs upregulated IL-12p40 production and B7 expression, but the treatment did not alter MHC expression, while IL-10 production was downregulated after exposure to live tachyzoites and parasite' soluble antigens. However, the production of IL-12p40 and IL-10 was completely abolished in the absence of MyD88. In order to check if this phenomenon could be associated with protection *in vivo*, C57Bl/6 mice were immunized with PBS, NLA, NLA plus p38 inhibitor and challenged with *N. caninum*. The group immunized with p38 inhibitor presented improved infection control. Conclusion: These results demonstrate that *N. caninum* manipulates p38 and PI3k pathways to downregulate the host innate responses, which aids the establishment of persistent infections. In that sense, this piece of information can be useful to develop a cellular vaccine against neosporosis. **Supported by:**CAPES, CNPq, FAPEMIG,

IM004 - **REPEATED AMINO ACID SEQUENCES FROM TRYPANOSOMA CRUZI ANTIGENS AS VIRULENCE FACTORS DURING PARASITE INFECTION**

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Using an immunoscreening approach, several antigens derived from a *Trypanosoma cruzi* amastigote cDNA library that react with sera from chronic chagasic patients were isolated. One such antigen, homologous to the eukaryotic L7a ribosomal protein and containing an Ala-Lys-Pro rich repetitive domain at its N-terminus, was characterized. To evaluate the role of amino acid repeats present in *T. cruzi* antigens, we generated recombinant versions of the complete antigen (TcRpL7a) as well as truncated versions containing only its repetitive (Rep) or the non-repetitive domain (∆R) and used them to immunize mice. Whereas mice immunized with TcRpL7a produced IgG antibodies against the complete protein as well as against the repetitive domain, they produced very low levels of antibodies against the non-repetitive domain. On the other hand, mice immunized with Rep did not generate antibodies against any of these antigens. Regarding cellular immune responses, whereas mice immunized with TcRpL7a produced high levels of IFN-γ, only very low levels of IFN-γ were detected in mice immunized with Rep. After challenging immunized mice with trypomatigotes, we observed a partial protection in mice immunized with the complete protein whereas immunization with ∆R did not alter parasitemia levels compared to controls. In contrast, immunization with Rep resulted in an exacerbation of the parasitemia compared to the other groups. Our results thus suggest that repetitive domains present in *T. cruzi* antigens may be used by the parasite to modulate host immune response, most likely by inducing B cell tolerance. To further investigate this hypothesis, we inserted the repetitive domain in fusion with GST and repeated the immunization protocol using GST only and GST::Rep, followed by challenge with virulent parasites. To verify the role of these repeats as a factor responsible for inducing immune tolerance, we also immunize animals with Rep before a boost immunization with the full length TcRPL7a.â€‹. **Supported by:**CAPES, CNPq and INCTV

IM005 - **THE MIRNA PROFILE OF MACROPHAGES IS ALTERED BY *LEISHMANIA AMAZONENSIS* INFECTION**

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The microRNAs (miRNAs) are non-coding RNAs (21-24 nt), that can modulate gene expression by the complementary binding of the initial 6 to 9 nucleotides of its 5' region to the 3'UTR of target mRNA, inducing the mRNA cleavage or causing the inhibition of the translation. The miRNAs are modulators of inflammatory mechanisms during immune response by post-transcriptional regulation of genes involved in these pathways. The miRNAs profile of macrophages is altered during infections by bacteria and virus, as well as in cancerous cells. In this work, we determine whether the infection by *Leishmania (L.) amazonensis* can subvert the miRNAs profile of mouse macrophages and the implications in regulating the infection establishment (entrance) and maintenance (parasite replication). We analyzed the miRNA prolife of total RNA from Bone Marrow-Derived macrophage (BMDM) of BALB/c mice infected by *L. (L.) amazonensis* wild-type (WT) and *L. (L.) amazonensis* arginase knockout ($arg^{-/-}$) using miScript miRNA PCR Array (Qiagen). We detected the modulation of mir721, mir294-3p, and miR9-5p of the BMDM infected by *L. (L.) amazonensis* WT during entrance (4 and 12 hours post-infection) and parasite replication (24, 48 and 72 hours post-infection). *In silico*, the mRNA targets for these miRNAs include pathways involved in phagolysosome maturation (LAMP-1) and regulation of iNOS, cationic amino acidic transporter (CAT) and Tumor-necrose factor receptor (TNFR). As expected, infection with *L. (L.) amazonensis* $arg^{-/-}$ did not induce these miRNAs from BMDM, corroborating the reduced infectivity of that mutant. We concluded that *L. amazonensis* infection alters the miRNA profile of macrophages to subvert the host immune responses. **Supported by:**FAPESP and CNPQ

IM006 - **THE PARADOXICAL ROLE OF ROS IN *TRYPANOSOMA CRUZI* INFECTION**

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Introduction: *In vitro*, *T. cruzi* is readily uptaken by macrophages and triggers respiratory burst. The main consequence of oxidative stress is the formation of DNA lesions, which can result in genomic instability and lead to cell death. Guanine is the base that is most susceptible to oxidation and 8-oxoguanine (8-oxoG) is the most common lesion. The GO-system is an 8-oxoG repair pathway, constituted by MutT, MutY and MutM in bacteria. Objective: The aim of this study was to investigate the importance of 8-oxoG during parasite infection of mammalian cells and to investigate the parasite burden in macrophages from C57BL6 mice and from mice deficient in NADPH phagocyte oxidase (phox KO). Methods and results: Macrophages isolated from murine peritoneal cavity of C57BL/6 and phox KO were infected with culture trypomastigotes of wild-type and recombinant parasites (overexpressing the TcMTH enzyme (homologous to MutT of bacteria) and heterologously expressing *E. coli* MutT) and the parasite burden was analyzed by optical microscopy. Our results demonstrate that both macrophages uptook parasites similarly. Both wild-type and recombinant parasites had the same capacity of infecting macrophages. The modified parasites presented enhanced replication inside murine inflammatory macrophages from C57BL6 mice when compared with wild type. Interestingly, when phoxKO macrophages were infected with these parasites, we observed a decreased number of all parasites when compared with macrophages from C57BL6. Conclusions: Our results indicate a paradoxical role for ROS. In large quantities, ROS can cause damage to parasites and our results highlight the importance of the 8-oxoG repair system for cell viability, since recombinant parasites were more successful in C57BL6 macrophages. On the other hand, ROS also can function as an important signal to induce the proliferation of parasites, since the multiplication of the parasites becomes reduced in macrophages devoid of ROS produced by phox. **Supported by:**INCT Redoxoma, FAPEMIG, CAPES, CNPq

IM007 - **GENOME-WIDE SCREENING AND IDENTIFICATION OF NEW *TRYPANOSOMA CRUZI* ANTIGENS WITH POTENTIAL APPLICATION IN CHRONIC CHAGAS DISEASE DIAGNOSIS**

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The diagnosis of the chronic Chagas disease is based mainly in serological tests, due to the low *Trypanosoma cruzi* parasitemia and high anti-*T. cruzi* IgG titers. The antigens used in these assays may present low specificity due to the occurrence of cross reactivity with other parasite infections, and low sensibility mainly caused by the high polymorphism of *T. cruzi* strains. Therefore the identification of new *T. cruzi* specific antigens conserved in the different parasite strains is still required. In this work, we have performed B-cell epitope prediction on all proteins derived from single copy genes represented by pair of alleles in the CL Brener genome. We assumed that, because CL Brener is a recent hybrid between TcII and TcIII lineages, it is likely that conserved epitopes in its pair of alleles could also be conserved in the parental genotypes. We have excluded proteins conserved in *Leishmania major*, *L. infantum* and *L. braziliensis* to minimize the chance of cross-reactivity. A peptide array containing the putative epitopes with the highest epitope prediction scores was synthesized and the reactivity of the peptides were tested by immunoblot using pool of sera of C57BL/6 mice chronically infected with *T. cruzi* strains that belongs to the TcI, TcII or TcVI lineages. The *T. cruzi* proteins that contain the reactive peptides against the 3 pools of sera were recombinantly expressed and submitted to ELISA experiments with sera from chagasic patients with distinct clinical manifestations, sera from patients known to be chronically infected with *T. cruzi* from TcII and TcVI lineages, and patients with cutaneous or visceral leishmaniasis. Two proteins, named Tc3 and Tc4, presented respectively 94.8 and 89.9% sensibility, 98.2 and 94.6% specificity, and a pool of these 2 proteins presented 96.6% sensibility and 98.2% specificity. This work led to the identification of two new antigens with high potential application in the diagnosis of chronic Chagas disease.
Supported by:FAPEMIG, CNPq, INCTV, CAPES

IM008 - **CROSS-PROTECTIVE IMMUNITY TO *LEISHMANIA AMAZONENSIS* IS MEDIATED BY CD4+ AND CD8+-EPITOPES OF *LEISHMANIA DONOVANI* NUCLEOSIDE HYDROLASE TERMINAL**

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Visceral (VL) and cutaneous leishmaniasis (CL) are severe anthroponotic and zoonotic protozoan diseases of increasing and overlapping worldwide incidence. In mice and dogs NH36 induces, a CD4+ T cell-driven protective response against *L. chagasi* infection that is directed against its C-terminal domain (F3). Preliminary results indicated that the C-terminal and N-terminal domain vaccines decreased the footpad lesion caused by *L. amazonensis*. We studied now the basis of this crossed immune response using recombinant generated peptides covering the whole NH36 sequence and saponin. Results: Both peptide vaccines (F3:199-314 and F1: a.a. 199-314) enhanced the IgG and IgG2a anti-NH36 antibodies to similar levels. The F3 vaccine induced the strongest DTH response, the highest proportions of NH36-specific CD4+ and CD8+ T cells after challenge, the highest ratios of IFN-γ/IL-10 and TNF-α/IL-10 CD4+ and CD8+ producing T cells and highest expression of IFN-γ and TNF-α. The F1 vaccine, on the other hand, induced a weaker but significant DTH response, a mild enhancement of IFN-γ and TNF-α levels and mild enhancement ratios of IFN-γ/IL10 producing CD4+ T cells. Accordingly, the F3sap vaccine was the most protective, reducing the footpad lesion in 75-79%, the parasite load by 99.93% and exceeding in 40.40% the protection due to the cognate NH36 protein, while the F1 vaccine promoted a 57-69% reduction of footpad lesion and 99.93% decrease of parasite burden. The *in vivo* depletion with anti-CD4 or CD8 monoclonal antibodies disclosed that protection against *L. amazonensis* was related to epitopes for CD4+ T cells of the C-terminal domain and epitopes for CD8+ T cells of the N-terminal domain. Conclusions: The identification of the C-terminal and N-terminal domains as the targets of the immune response to NH36 in the model of *L. amazonesis* infection represents a basis for the rationale development of a bivalent vaccine against leishmaniasis. **Supported by:**CNPQ, FAPERJ, CAPES

IM009 - **AHR IS A &LDQUO;TIME MACHINE&RDQUO; REGULATOR OF IMMUNITY AND PATHOLOGIES DURING *TRYPANOSOMA CRUZI* INFECTION**

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Introduction: Chagas' disease is caused by *Trypanosoma cruzi* (Tc) and requires immune response balance to control parasite growth and refrain pathology. Activity of 5-lipoxygenase enzyme results in lipoxin $(LXA)_4$ release which is important to regulate inflammatory cytokines (CK) production. However, the role of aryl hydrocarbon receptor (AhR), LXA nuclear receptor, during this infection is still uncertain. Methods and Results: Herein, wild type (WT) and AhR KO mice were infected with Tc (Y strain) and AhR expression, parasitemia and immune response was assessed. Hearts and spleens were harvested at different days post-infection (dpi) for histology, CK analyses by PCR, ELISA and flow cytometry. We found that AhR expression is up-modulated in heart and spleen during infection. Deficiency of AhR resulted in higher resistance to infection, related with precocious increased numbers of macrophages and dendritic cells producing IL-12, and T CD4 and T CD8 cells releasing IFN-γ in spleen at 10dpi. Reduction of pro-inflammatory CK levels were observed in Tc-infected AhR KO mice at 15dpi, which was similar or lower to the levels found in infected WT counterparts. In AhR KO mice modulation of immune response in heart "mirror" the phenotype described in spleen, where a precocious inflammation was found at 10dpi and it was partially reversed at the time point when myocarditis is in an upper limit in WT mice. We found that, beside CK, an increased reactive oxygen species, but not nitric oxide and peroxynitrite, production could be also a factor responsible for the increased efficiency to control the parasite grown in infected AhR KO mice. Conclusion: Collectively, our data demonstred that AhR is responsible to modulate immune response and development of myocarditis during experimental Tc infection. Thus, AhR is an essential "time machine" tool capable to control "where and when" immune response must acts to avoid pathology development due to parasite or inflammatory undertakings. **Supported by:**CNPq and FAPEMIG

IM010 -**DENDRITIC CELLS FUNCTION DURING EXPERIMENTAL *TRYPANOSOMA CRUZI* INFECTION: THE ESSENTIAL ROLE OF SOCS2**

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Background. Diverse cell types are essential to control *Trypanosoma cruzi* (Tc) infection. Suppressor of cytokine signaling (SOCS)2 is critical to modulate the immune response (IR) and its expression is partially mediated by Lipoxin $(LXA)_4$ generation in dendritic cells (DCs). DCs are antigen presenting cells (APC) responsible for primary IR against infections. Diphtheria toxin (DT) injection causes transient DCs depletion in a transgenic mouse expressing Simian DT receptors under the control of the CD11c promoter, allowing us to investigate the effects of DCs depletion in the IR against Tc infection and the role of LXA/SOCS2 in the modulation of DCs function. *Methods/results*. CD11cDTR mice were injected with DT (1ug/mouse, DP mice) or PBS (control group, CT mice) and 6h later infected with Tc (Y strain). Another groups of depleted mice received transference of DCs purified from SOCS2 deficient ($^{-/-}$) or WT mice and then infected with Tc. Parasitemia, development of IR and LXA effects in the modulation of pro-inflammatory mediators production induced by Tc was examined. Our results demonstrated that DCs depletion resulted in higher parasitemia when compared with CT mice. There was an increased presence of innate immune cells $CD11b^+GR1^+IL12^+$ and $F4/80^+CD11b^+IL\text{-}12^+$ in the DP mice compared with CT mice. Also, we found decreased generation and expansion of $CD3^+CD4^+IFN\text{-}g^+$ and $CD3^+CD8^+IFN\text{-}g^+$ in DP mice during the infection. The transfer of WT DCs, but not of $SOCS2^{-/-}$ DCs, recovered the capacity of DP mice to control the infection. Our *in vitro* results, demonstrated that LXA inhibit the induction of IL-6, IL-12 and TNF-a induced by Tc infection in DCs. Interesting, $SOCS2^{-/-}$ mice infected with Tc produced higher levels of LXA compared with WT. *Conclusions*. Our results demonstrated that SOCS2 is essential for DCs response against Tc infection modulating innate and acquire IR, and suggest that LXA is modulator of SOCS2 expression in this cell during infection. **Supported by:**CNPq e FAPEMIG

IM011 - **SEVERE MALARIA DEVELOPMENT IS MODULATE BY SUPPRESSOR OF CYTOKINE SIGNALING 2**

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Introduction: The cerebral malaria (CM) is the most severe neurological complication occurring upon *Plasmodium falciparum* infection. Mice infected with *P.berghei ANKA* (PbA) faithfully recapitulate many characteristics of human CM and it has been an important tool to investigate the disease pathogenesis. Although CM pathogenesis is not completely understood, it likely involves inflammatory response (IR) deregulation and alterations in neurotransmitters. The suppressor of cytokine signaling (SOCS)2 is an intracellular protein induced by eicosanoids and hormones and participate in IR, macrophage polarization, modulation of neural development and neurogenesis. However, SOCS2 involvement in CM is not known. Method: C57Bl/6 (WT) and SOCS2 deficient ($^{-/-}$) mice were infected with PbA and parasitemia and survival were monitored periodically. Cytokines production (TNFα, IL1β, TGFβ, IL6, IFNγ, IL12, IL10) in the brain and spleen was assessed by ELISA and flow cytometry. The SOCS1 and SOCS3 expression was assessed by qPCR. Leukocyte recruitment in the brain was evaluated by intravital microscopy. Nitric oxide (NO) was assessed by the Griess method in the brain. Histopathological analysis was performed in brain. Results: The parasitemia was significantly lower in SOCS2$^{-/-}$ compared with WT mice. In the brain of infected SOCS2$^{-/-}$ mice there was a significant increased expression of TGFβ and IL17 and increased level of NO when compared with infected WT mice. Additionally, there was a decreased expression in the brain of PbA-infected SOCS2$^{-/-}$ mice, but not in spleen, of IL1β, TNFα, IL10 and IL12 when compared with WT. Moreover, a significant decreased expression of glial cell line-derived neurotrophic factor (GDNF) and leukocyte rolling, and increased microvascular obstruction was found in brain of PbA-infected SOCS2$^{-/-}$ mice when compared with WT counterparts. Conclusions: These findings demonstrated, for the first time, a role for SOCS2 in CM immunopathogenesis **Supported by:**CAPES, CNPq, FAPEMIG

IM012 - **PHOSPHATIDYLSERINE EXPOSURE ON INTRACELLULAR AMASTIGOTES OF LEISHMANIA AMAZONENSIS IS A SENSOR OF MACROPHAGE ACTIVATION AND MODULATES IN VIVO INFECTIONS AND DENDRITIC CELL FUNCTIONS**

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

IM013 - **PROTECTIVE EFFECT OF NATIVE PROTEINS PURIFIED FROM TACHYZOITES OF NEOSPORA CANINUM AGAINST EXPERIMENTAL INFECTION**

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Neospora caninum is an obligate intracellular parasite that has the dog as definitive host and other mammals, especially cattle as intermediate hosts. Economically, neosporosis is considered a disease of great importance in veterinary medicine due to the fact that the parasite *N. caninum* be able to cause potentially serious complications, like abortion in cattle and neuromuscular paralysis in dogs. The present study aims to purify protein targets of *N. caninum* by electrophoresis native of Davis (1964) for mortality tests of mices C57/BL6 immunized with native proteins and after challenged with tachyzoites of *N. caninum.* For this, after obtaining the soluble antigen of *Neospora caninum* (NLA) was made fractionation by native electrophoresis of proteins applying 200µL of antigen in each gel. After the electrophoretic run, the gels were macerated in liquid nitrogen, rehydrated in phosphate buffered saline (PBS), homogenized for 5 minutes and kept in agitation overnight. In the next day, the samples were centrifuged at 12.000g for 15 minutes and the supernatant containing the proteins of interest was collected and concentrated in Amicon system. Mices C57/BL6 were subjected to immunization with three doses of 25µg each of the native proteins or PBS (control group) and then were challenged with a lethal dose of 3x10 ^{7} tachyzoites of *N. caninum* to evaluation of survival rates. The mices immunized with the native proteins had a survival rate of 80%, and were able to maintain their body weight being sacrificed 30 days after the challenge. Otherwise, the mices that were inoculated with PBS had a significant weight loss and died in the first 10 days after the infection. In conclusion, the native proteins of *N. caninum* provided protection against neosporosis when compared to the control group, and may be considered interesting protein targets of study for the development of vaccines and the consequent control of neosporosis animal. **Supported by:**FAPEMIG

IM014 - **INTRANASAL IMMUNIZATION WITH KMP11 ASSOCIATED TO MPLA INDUCED PROTECTION AGAINST *LEISHMANIA AMAZONENSIS*.**

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In this project, we developed a new vaccine using recombinant antigens against *Leishmania amazonensis*. KMP11 is a protein present in all kinetoplastids protozoa and it is considered as a potential candidate for a vaccine against leishmaniasis. Several studies have employed using KMP11, however, using by intranasal route is innovative. Furthermore, we evaluated the association of KMP11 with LTA (ligand for TLR2), MPLA (ligand for TLR4) and the combination of the two associated with AddaVax. C57BL6 mice were vaccinated twice with 7 day intervals intranasally with 10 ug of recombinant KMP11 associated with 2 µg MPLA, 5µg LTA and 10 µl of AddaVax ™. Seven days after the second dose, the animals were infected with 2 x 10^{5} promastigotes in stationary phase of *Leishmania amazonensis* (Josefa strain). The development of lesion was assessed by paquimeter. Immunization with KMP11 free of adjuvant induces protection in the acute phase, but the association with MPLA increased protection in the acute and chronic phases rated for control of lesion growth. The combination of MPLA with AddaVax worsened the effect of vaccine. This data set presents a new candidate vaccine and in the next step we are going to evaluate the increased concentration of MPLA and the increase in the number of doses in order to increase vaccine efficacy.

IM015 - **EXPRESSION OF PD-L1 ON HEART AND LYMPHOCYTES FROM C3H/HEPAS MICE CHRONICALLY INFECTED WITH *TRYPANOSOMA CRUZI* SYLVIO X10/4**

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Introduction: C3H/HePAS mice infected with *T. cruzi* Sylvio X10/4 parasites develop a chronic cardiomyopathy similar to observed in humans. Both parasite and host elements contribute to the incomplete elimination of *T. cruzi*, and an inefficiency of the effector mechanisms of the adaptive immunity due to exhaustion induced by the persistent stimulation of T lymphocytes. Senescence of $CD4^+$ T cells, and loss of the effector ability of $CD8^+$ T cells can occur by interaction of inhibitory molecules PD-1 and PD-L1 expressed in these cells. We assessed the expression of PD-1 and PD-L1 in lymphocytes of Sylvio X10/4 chronically infected C3H/HePAS mice, and evaluated the importance of these molecules for the *in vitro* activation of lymphocytes.
Methods and Results: Heart pathology progression was observed in the course of infection. There was an increase in the total number of cells in the spleen of chronically infected mice, and an increase in number of $CD4^+$, $CD8^+$ and B(CD19). The spleen of chronic mice showed an increase of activated $CD4^+$ cells, and a significant increase in PD-L1 expression by $CD4^+$ T lymphocytes. There were no differences in the expression of PD-L1 in other lymphocyte populations. In the heart of chronically infected animals, significant augment was observed in the expression of PD-L1, as assessed via qPCR. To evaluate inhibitory effect of PD-L1, splenocytes from chronically infected animals and controls were stimulated *in vitro* with *T. cruzi* antigen in the presence or not of anti-PD-L1. After 72h of stimulation, *in vitro* proliferation of $CD4^+$ T cells was increased by PD-L1 blockade. There was no increase in IFNg secretion in the presence of anti-PD-L1.
Conclusion: The increased expression of PD-L1 seen in the heart and spleen of chronically infected animals raises the possibility that T lymphocyte activity might be inhibited via PD-1/PD-L1 molecules, a process that could hinder control of the parasite at the inflammatory site, allowing its persistence. **Supported by:**FAPESP, CAPES and CNPq

IM016 - **IFN-&GAMMA;-INDUCED PRIMING MAINTAINS ACQUIRED IMMUNITY TO EXPERIMENTAL MALARIA**

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Introduction: The mechanism by which protective immunity to *Plasmodium* is typically lost in the absence of continued exposure to this parasite has yet to be fully elucidated. It has been recently shown that IFN-γ produced during human and murine acute malaria primes the immune response to TLR agonists. Herein, we investigated whether IFN-γ-induced priming is important to maintain long-term protective immunity against *P. chabaudi* AS malaria.
Methods and Results: On day 60 postinfection, C57BL/6 mice still had chronic parasitemia and efficiently controlled homologous and heterologous (AJ strain) challenge. The spleens of the chronic mice showed augmented numbers of effector/effector memory $CD4^+$ (T_E/T_{EM}) cells, which is associated with increased levels of IFN-γ-induced priming, *i.e.*, high expression of IFN-inducible genes and TLR hyperresponsiveness. After parasite elimination, 200 days postinfection, IFN-γ-induced priming was no longer detected and protective immunity to heterologous challenge was mostly lost with >70% mortality. Spontaneously cured mice had high serum levels of parasite-specific IgG, but T_E/T_{EM} cell numbers, parasite-driven $CD4^+$ T cell proliferation and IFN-g production were similar to non-infected controls. Remarkably, the priming of cured mice with low doses of IFN-γ rescued TLR hyperresponsiveness and the capacity to control heterologous challenge, increasing the T_{EM} cell population and restoring the $CD4^+$ T cell responses to parasites. The contribution of TLR signaling to the $CD4^+$ T cell responses in chronic mice was supported by data obtained in mice lacking the MyD88 adaptor.
Conclusions: These results indicate that IFN-γ-induced priming is required to maintain protective immunity against *P. chabaudi* and aid in establishing the molecular basis of strain-transcending immunity in human malaria. **Supported by:**FAPESP, CNPq

IM017 - **INTRAVITAL IMAGING HIGHLIGHTS A FUNDAMENTAL ROLE FOR SPLENIC DENDRITIC CELLS IN THE PHAGOCYTOSIS OF INFECTED ERYTHROCYTES DURING EARLY *PLASMODIUM CHABAUDI* AS MALARIA**

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Introduction: The study of elimination of *Plasmodium* parasites inside spleen by phagocytosis by *in vitro* and *ex vivo* approaches, such as flow cytometry and immunofluorescence, allowed the phenotyping of phagocytic cells, but not a quantification fully compatible with the importance of spleen for parasite control. Splenic dendritic cells (DCs) are central activators of immune system in response to blood-borne pathogens; however, a role in direct elimination of those pathogens was not described for DCs.
Methods and Results: Here we showed that *in vivo* depletion of DCs led to increased susceptibility to *P. chabaudi* infection; by using confocal intravital microscopy (CIVM), we studied the phagocytosis of *P.chabaudi*-infected red blood cells (*Pc*-iRBCs) by subcapsular splenic dendritic cells (DCs) at different phases of blood-stage *Pc* infection. In non-infected mice, immature DCs could promptly recognize and internalize *Pc*-iRBCs. This capacity was maintained during pre-crisis (5 days after infection), when DCs were more activated. However, during infection crisis (8 days after infection), DCs cannot internalize newly injected *Pc*-iRBC, mainly due to spleen closure. CIVM allowed us to visualize phagocytosis by DCs with high details, in a time-lapse manner; importantly, this approach yielded higher percentages of colocalization if compared to *ex vivo* techniques such as flow cytometry or immunofluorescence.
Conclusions: In summary, this article describes for the first time the DC phagocytosis of *Plasmodium* parasites by CIVM inside the spleen, indicating a possible role for splenic subcapsular DCs in the elimination of parasites through phagocytosis. **Supported by:**FAPESP, CNPq, CAPES, FCT

IM018 - **CELLULAR AND HUMORAL IMMUNE RESPONSES AGAINST ORAL INFECTION BY *NEOSPORA CANINUM***

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Neospora caninum is an intracellular protozoan parasite with worldwide distribution, that causes abortions in livestock and over a billion dollars in economic losses per year. However, the mechanisms involved in this host-parasite interaction remains unclear. To date, experimental models that assess host responses against the parasite are usually based on parenteral inoculations, but it is important to understand the mechanisms involved in resistance/susceptibility to infection. Thus, we aimed to evaluate the profile of the cellular and humoral immune responses in mice orally infected with *N. caninum*. Thus, female C57BL/6 mice were orally infected by gavage with $3x10^7$ live tachyzoites, and were euthanized at 7, 14 and 21 days post-infection (p.i.) to collect serum and brains, lungs, livers and gut sections for histological analysis, cytokine quantification, parasite DNA quantification and specific IgG antibody detection. Histological analysis revealed a severe diffuse inflammation in livers, lungs and central nervous system (CNS) of mice at 7, 14 and 21 days p.i. The cytokine balance of the target tissues were expressed by the detection of IFN-g and IL-10. Although the gut was the primary site of infection, increased expression of IFN-g was observed after 14 days p.i. in the duodenum, proximal jejunum and distal jejunum, while increased IL-10 production was mainly detected in the duodenum and proximal jejunum at 21 days p.i. On the other hand, expression of IFN-g increased progressively in the CNS since 7 days p.i., while IL-10 expression was observed only at 21 days p.i. We observed crescent levels of specific IgG in the serum samples obtained during the infection, which were mainly composed of IgG2a antibodies, while IgG1 was almost undetected, suggesting a predominance of a Th1 immune response in these animals, differently of that usually observed in parenteral protocols. In that sense, we can infer that the experimental model proposed presents a promising tool for the study of the immunopathogenesis of neosporosis **Supported by:**CAPES, CNPq e FAPEMIG

IM019 - **IL-10 AND FOXP3 EXPRESSION IN PATIENTS WITH CHRONIC CHAGASIC CARDIOMYOPATHY IS CORRELATED WITH DEATH AND CEREBRAL VASCULAR ACCIDENT RISK**

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The aim of this study was to evaluate the association among cytokines expression and death risk cerebral vascular accident (CVA) in patients with different clinical forms of chronic Chagas disease. Cytokines (IL-10, IL-17, IFN-γ and TGF-β) and transcriptional factors (GATA-3, RORγt, T-Bet, FoxP3) expression of Th1, Th2, Th17 and regulatory T cells were determined by real time PCR in peripheral blood mononuclear cells of patients that display indeterminate (IND=21), cardiac (CARD=22), digestive (DIG=7) and cardiodigestive (CARD-DIG=9) clinical forms of the disease. Samples from uninfected health individuals (CONT=10) were used as control. Cytokines and transcriptional factors expression were correlated with death risk in CARD chagasic patients and CVA with IND, CARD, DIG, CARD-DIG clinical forms patients. Higher expression of IL-10 and FoxP3 was observed in IND than CARD patients. Chagasic patients with cardiac involvement were grouped in low, medium and high death risk over ten years and compared with cytokines and transcription factors expression. Higher expression of FoxP3 and IL-10 was demonstrated in patients with low death risk than those with high death risk. Patients presenting IND, CARD, DIG, CARD-DIG clinical forms were also grouped according to CVA risk in low, medium and high, and was observed that CVA low risk patients displayed higher GATA-3, T-Bet and FoxP3 expression than those with CVA high risk. These findings indicate that in chronic chagasic cardiomyopathy patients the increased production of IL-10 could be correlated with low death risk, inasmuch GATA-3, T-Bet and FoxP3 enhanced expression to suggest association with low CVA risk. **Supported by:**CNPq, FAPERN, CAPES, MCTI/CNPq/MS-SCTIE-Decit

IM020 - **E-NTPDASE-2 OF *LEISHMANIA AMAZONENSIS* PROMASTIGOTES IS IMPORTANT TO DOWNMODULATION OF NO PRODUCTION AND SURVIVAL OF PARASITES.**

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E-NTPDase is known to be expressed in *L. amazonensis* and participates in the hydrolysis of ATP to AMP. We investigated the role of E-NTPDase-2 in the infection of J774 cells by *L. amazonensis*. Ectonucleotidase activity was assayed by measurement of Pi released by incubation of parasites with ATP or ADP and western blotting of membrane parasites were used to detect the expression of the enzyme. Metacyclic promastigotes of L. amazonensis PH8 strain exhibit increased activity and expression of E-NTPDase-2 when compared to a parasite clone with low infectivity (1IIId clone). J774 cells were infected with metacyclic promastigotes and stimulated with LPS and IFN-γ for 48 hours. NO production was evaluated by Greiss method. LPS and IFN-γ stimulus was able to reduce in 50% the percentage of infection by 1IIId clone while no decrease was observed in cells infected by the PH8 strain. This correlates with decreased NO production. We also show that DIDS was able to reduce the survival capacity of the PH8 strain by restoring NO production by infected cells. On the other hand, NECA increased survival of clone 1IIId, in part, by reducing the production of NO by cells. Incubation with MRS1754 (A2B receptor antagonist) reduced by 30% the percentage of cells infected with the PH8 strain in 48 hours. NO production by these cells was twice that observed in control. In addition J774 cells incubated with recombinant NTPDase, with negligible activity, presented a greater than 50% reduction in NO production by stimulated cells. Given that DIDS and A2 antagonists did not prevent the reduction of NO, our data suggests an activity-independent action of the protein over NO production. Our data show that E-NTPDase-2 is important to the survival of *L. amazonensis* by participating in the reduction of NO production by infected cells, both by providing substrate (AMP) for adenosine production that will activate A2B receptors and also by direct contact to a currently unknown cell surface molecule. **Supported by:**CAPES, CNPq, FAPEMIG

IM021 - **PARTICIPATION OF TLR-2 AND TLR-6 IN THE SKIN FIBROBLASTS IMMUNE RESPONSE DURING EXPERIMENTAL INFECTION BY *LEISHMANIA (LEISHMANIA) AMAZONENSIS.***

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The initial moments of infection by protozoa of the genus Leishmania are crucial to the evolution of the disease and involve the host and parasite factors, such surface molecules, parasite species and genetic background. Several studies have shown the involvement of TLRs in response to Leishmania infection. We have demonstrated that the absence of TLR-2 induce a lower susceptibility to infection by *L. amazonensis*, which controlling the parasite load and cellular profile alterations of the inflammatory infiltrate at the site of infection, however, the immune response developed during infection is not known. To identify the profile of response and participation of resident cells in modulation and recruitment of inflammatory cells in the absence of TLR-2, we evaluated the production of inflammatory mediators by skin fibroblasts (SF) in the early stages of infection. TLR-2 and TLR-6 deficient mice were inoculated in the ear with 105 *L. amazonensis* promastigotes. After 1, 7, 15 and 30 days of infection, the analysis the cellular profile was performed by light and electron microscopy and the production of inflammatory mediators evaluated by flow cytometry. Our results showed that the absence of TLR-2 induced a distinct cellular response, effective in reducing the parasite load and infection control. On the other hand, the absence of TLR-6 did not affect the infection, demonstrating that this receptor is not directly involved in infection by *L. amazonensis.* Furthermore, it was observed that the SF on site of inoculation are capable of responding to infection by producing cytokines such as IL-4, IL-10, TGF-b, IL-12 and IFN - g, contributing to the initial response to infection. In TLR-2 deficient mice were found significant increase in production of IL-4 and IFN-g by SF on the first day of infection different from that observed in WT mice, which showed high production of IL-4 only after 30 days of infection. These results may suggest the likely participation of fibroblasts in the intense recruitment of eosinophils in the initial moments of infection in the absence of TLR-2. Modulation of the TLR-2 may be a crucial factor for the development of a more efficient immune response in controlling infection by *L. amazonensis*, and a pathway in the search for alternatives in the development of new therapies for the treatment of leishmaniasis. **Supported by:**IOC/FIOCRUZ, CNPq, FAPERJ

IM022 - **ROLE OF CD39 AND CD73 SURFACE EXPRESSION IN RESIDENT MACROPHAGES INFECTED WITH LEISHMANIA AMAZONESIS**

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Endogenous nucleotides produced by various group of cells under inflammatory conditions act as potential danger signal *in vivo*. Extracellular release of nucleotides such as ATP is brief and is rapidly cleaved to adenosine (ADO) by coordinated ectonucleotidasic activities of CD39 and CD73. *Leishmania* which are the obligate intracellular parasites of macrophages (MØ) are capable of modulating their host cells in order for them to survive and multiply. In this current study, we investigated the effects of *Leishmania amazonensis* on infected resident MØ by flow cytometry and also the role of purinergic receptors in an infection *in vitro*. Our findings demonstrated that the resident MØ possess both CD39 and CD73 excessively on their surfaces. In infected populations, however, MØ were characterized mainly by increased CD73 expressions. When we inhibited these extracellular enzymes *in vitro* by the use of inhibtors DIDS and α,β MAD respectively, we observed that both percentage of infection and amastigote number decreased sufficiently. Furthermore, we evaluated the effects of adenosine receptors in the function of parasitic survivability and infection and we found that inhibition of A2b receptors by MRS 1724 diminished both percentage of inhibition and amastigote number after infection. Moreover, we also analyzed the supernatant from the culture incubated for CCL5, TNF-α and IL-10 production by ELISA and nitric oxide by Griess method. Infection with *Leishmania* did not alter any of these soluble mediators in all conditions. Our results support that *Leishmania amazonensis* is capable of regulating CD39/CD73 pathway and adenosine receptors mainly A2b for their survivability inside MØ. **Supported by:**TWAS, CNPq and FAPEMIG

IM023 - **SOLUBLE PROTEINS FROM *NEOSPORA CANINUM* INDUCE TNF-A PRODUCTION IN MACROPHAGES**

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Neospora caninum is an obligate intracellular protozoan parasite with worldwide distribution and ability to infect a broad range of warm-blooded hosts as dogs and cattle, which may present clinical signs of the infection. Considering the inefficiency of current prophylactic protocols, there is an urgent need for a greater understanding of the effector mechanisms of the host immune response against this pathogen. In that sense, we aimed to study the innate immune responses induced by *N. caninum* antigens. For that, we used as an experimental model bone marrow derived macrophages (BMDMs) stimulated with *N. caninum* soluble antigens (NLA). The model readout was based on TNF-a production measured by ELISA after 24h of in vitro stimulation. We evaluated the protein profile of the fractions obtained by electrophoresis of the NLA, and performed a cytotoxicity test with treated antigens using [3 - (4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide] (MTT). In a first experiment, we observed that stimulation of BMDMs with NLA induced a reasonable production of TNF-a after 24h. We then investigated whether the protein content of NLA was responsible for cytokine production; for that, we submitted the parasites' soluble antigens to protein degradation by Proteinase K treatment. In order to observe the changes induced by Proteinase K in NLA profile, we observed the treated fractions in polyacrylamide gels, and it was possible to observe a significant reduction in the number of proteins present in each sample, in a dose dependent manner. Interestingly, only proteins with molecular weights ranging from 12 to 70kDa resisted enzymatic degradation. In the cytotoxicity assay, it was found that cells remained 100% viability after incubation with treated protein fractions. In that sense, we conclude that proteins synthetized by *N. caninum* induce TNF-a production on BMDMs, and further studies are required in order to unravel which component(s) are responsible for this phenomenon. **Supported by:**Capes and CNPq

IM024 - **LECTIN FROM *ECTATOMMA TUBERCULATUM* VENOM: THE EFFECT ON THE INVASIVE ACTIVITY OF *TOXOPLASMA GONDII.***

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Lectins are a class of non-immune glycoproteins that bind specifically and reversibly to carbohydrates. These molecules are found ubiquitously in nature and for their ability to bind specifically to carbohydrates, and various applications have been described for these molecules in biological systems, making the target of interest in characterizing them for biotechnological innovation. The present study aims at the isolation a lectin from *Ectatomma tuberculatum* venom and analysis the effect of this lectin in the invasion and infectivity of *Toxoplasma gondii in vitro.* Therefore, the crude venom of this ant (EbEt - Extract Gross *Ectatomma tuberculatum*) was subjected to test interaction with microspheres attached to different carbohydrates. After the test run and electrophoretic EbEt, proceeded to the analysis of the bands of the SDS-PAGE gel stained with comassie blue. There was evidence of bands of approximately 40 and 105 kDa reagents microspheres containing D-mannose monosaccharide. Thereafter, isolation was initiated protein component of this affinity mannose by affinity chromatography on a column of D-mannose (EpEt). Regarding the effect of this fraction invasion and infectivity of *Toxoplasma gondii* tachyzoites *in vitro*, it was observed qualitatively, the ability to decrease in the invasion of HeLa cells in a dose of 20 µg/mL (EpEt). The results of this study show the presence of an extract venom *Ectatomma tuberculatum* binding to mannose residues containing protein components of 40 and 105kDa, and the effect decreased invasive activity of tachyzoites of *Toxoplasma gondii in vitro* cellular model. Thus, this study opens new perspectives for bioprospecting venom ants, especially of protein components with interaction with carbohydrate and its applicability in biological systems. **Supported by:**CAPES, CNPq, FAPESB

IM025 - **THE ROLE OF TNF-&ALPHA; IN THE HOST-PARASITE INTERFACE DURING INFECTION WITH *NEOSPORA CANINUM***

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Introduction: The protozoan parasite *Neospora caninum* has been associated to abortions in cattle since the early 1990's, and the infection leads to major economic impact to the segment. TNF-a is rapidly elicited during the acute phase of the infections. Produced mainly by activated macrophages, its actions are required for the induction of systemic inflammation. Given the importance of this cytokine during acute infectious processes, its role becomes important target for understanding the pathologies arising from clinical neosporosis. Methods and Results: C57BL/6 wild type (WT) and genetically deficient mice in TNF-α receptor I (p55, $TNFRI^{-/-}$), and double knockout for receptors I and II (p55 and p65, $TNFRI/II^{-/-}$), were infected intraperitoneally with a sublethal ($1x10^6$) or lethal dose ($3x10^7$) of *N. caninum* tachyzoites, by the intraperitoneal route. The animals were monitored every two days for their body weights and survival, and bled every week for serological analysis, during four weeks. We found that $TNFRI^{-/-}$ and $TNFRI/II^{-/-}$ animals showed increased survival and reduced weight loss during the acute phase of infection, if compared to WT mice. We also found a role for TNF-a in the production of immunoglobulins during chronic infection by protozoa. We observed that, regardless of the presence of TNF-α signaling, mice showed production of specific IgM to soluble antigens of the parasite. However, the recognition of antigenic targets of the parasite by specific IgG, as well as its subclasses, were severely compromised TNF receptor deficient mice. Conclusion: These results demonstrate the role of TNF-α signaling in the induction of acute systemic inflammatory responses and B cell class switch during infection by the protozoan *N. caninum*. **Supported by:**CAPES, CNPq, FAPEMIG

IM026 - **VACCINATION WITH NUCLEOSIDE HYDROLASE (NH36) OF *L. (L.) DONOVANI* OR ITS C-TERMINAL PORTION (F3) IN FORMULATION WITH SAPONIN PREVENT THE DEFECTIVE MIGRATION OF DENDRITIC CELLS IN MURINE EXPERIMENTAL VISCERAL LEISHMANIASIS**

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Visceral leishmaniasis, the most severe form of leishmaniasis is associated with immune dysfunction and migration failure of spleen dendritic cells (DCs) (Ato et al, 2002). The goal of this study is to evaluate the effect of vaccination with NH36 or its C-terminal portion, F3, in formulation with saponin, on DCs migration in *L. (L.) chagasi* challenged mice. Methods: C57Bl6 mice were immunized with 100µg of NH36 or F3 and saponin and challenged with $3x10^7$ amastigotes. Infected or untreated remained as controls. The intradermal response (IDR) against lysate of *L. (L.) donovani* was assayed. DCs migration test (Ato et al., 2002) was assayed on day 31 after infection. $5x10^5$ purified spleen DCs were platted in the presence or absence of CCL19 and incubated for 2h. Parasite load was evaluated in Leishman Donovan Units (LDU). Results: IDR was stronger in NH36sap and F3sap vaccinated mice than in controls ($p<0.0001$) with no difference between them ($p>0.05$) and only sustained up to 48h, after infection, by the F3sap vaccine. Parasite loads of livers ($p = 0.0012$) and spleens ($p = 0.0012$) were significantly reduced by both NH36sap and F3sap vaccines ($p>0.05$). Migration of DCs was abolished in infected animals while higher numbers of positive DCs migrated normally were detected in mice vaccinated with either F3sap or NH36sap ($p <0.005$). While the F3sap vaccine sustained migration values obtained for normal uninfected mice, the NH36sap vaccine even determined a significant increase. Our results indicate that animals infected with *L. (L.) chagasi* exhibit a defect in migration of DCs, as previously described for *L.(L.) donovani* (Ato et al., 2002) and that the immunization with F3sap or NH36sap prevent from this defect, and even cause a significant improvement in DCs migration which is probably related with the decrease of parasite load. **Supported by:**CNPQ, FAPERJ, CAPES

IM027 - **ANALYZED OF CELL PROLIFERATION AND PRODUCTION OF IFN-&GAMMA; BY FLOW CYTOMETRY IN SPLEEN CELLS OF THE IMMUNIZED MICE BY INTRAPERITONEALLY AND ORALLY WITH IRRADIATED TACHYZOITES OF *TOXOPLASMA GONDII***

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The *Toxoplasma gondii* is an excellent immunogen that induces innate and adaptive immune response. Primary infection with this agent stimulates the production of high levels of IFN-γ and this initial response is critical for the resistance of *T. gondii,* by limiting the expansion of parasite survival and induces the host for a long time or even for life in the form of cysts. Our development of a vaccine for toxoplasmosis, using irradiated tachyzoites induces response pattern similar to natural infection, allowing dissection of immunity against disease and cysts formation. In this work we evaluated the cellular immune response in immunized BALB/c mice by intraperitoneally and orally with irradiated 255Gy (Cobalt-60) of *T. gondii* tachyzoites. Immunized BALB/c mice with 10^7 irradiated tachyzoites by intraperitoneal (i.p.) or orally, the animals received three doses biweekly. Cell populations of T and B lymphocytes, cytokine production (CBA) and splenocyte proliferation (CFSE) were analyzed by flow cytometry. For the CBA and CFSE assays the cells were maintained in culture in the presence of the antigen of *T. gondii*. Immunized BALB/c mice by i.p. or orally had higher populations of T lymphocytes, $CD3^+CD4^+CD44^+$ and $CD3^+CD4^+CD45RB^{lo}$, B lymphocytes $CD19^+B220^+CD23^+$ when compared with control mice. The immunized mice showed a Th1 type response, with high levels of IFN-g produced by spleen cells. BALB/c mice i.p. showed a higher production of IFN-γ (8000pg/mL). There was an increase in the proliferation 10% of splenocytes and 13% of B cells in BALB/ i.p. in comparison with the control group. Our preliminary data suggest that our model vaccine induces a response similar to natural infection. Immunized mice with irradiated tachyzoites present an important production of IFN-γ and increased cell proliferation. The elucidation of mechanisms of immune response can assist in the immunogen producing suitable and that can be used in future animal use. Supported by LIMHCFMUSP & CNPq. **Supported by:**CNPq

IM028 - **HUMORAL RESPONSE AND PROLIFERATION OF SPLENOCYTES FROM BALB/C MICE IMMUNIZED WITH 60CO IRRADIATED SOLUBLE EXTRACT OF TACHYZOITES OF *TOXOPLASMA GONDII*.**

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Toxoplasmosis is a zoonosis of worldwide distribution affecting both human and animal populations. In general, radiation is used to prevent the reproduction of agents but can also change the proteins by oxidation products of radiolysis of water, improving its recognition and presentation to macrophages inducing immunity different from native proteins. Irradiated sterilized viable tachyzoites induces immunity similar to disease, with low counts of tissue cysts in challenged animals. In this study we evaluated the humoral immune response and proliferation of splenocytes from BALB/c mice immunized with ^{60}Co irradiated soluble acellular extract of *T.gondii* tachyzoites, for comparison with irradiated viable agents. RH strain *T.gondii* tachyzoite extract was purified and aliquots subjected to 500Gy, 1000Gy and 2000Gy and 4000Gy ^{60}Co radiation. SDS-PAGE showed dose response aggregate formation, but without changing antigenic properties by immunoblot. BALB/c mice were immunized with 3 biweekly doses s.c. of each extract and IgG production and avidity evaluated by ELISA. Mice immunized by antigen irradiated with 1000Gy 2000Gy showed significant levels of IgG antibodies specific high avidity ($p < 0.05$). After complete immunization with 1500Gy extract, spleens were recovered and antigen driven proliferation of splenocytes was analyzed by flow cytometry, showing an antigen driven 10% cytotoxic T lymphocytes ($CD3^+CD8^+$) and 9.95% B-lymphocytes ($CD19^+$) production as compared with unimmunized control group. Non irradiated soluble antigen immunization provide only 4,8% of cytotoxic T lymphocytes ($CD3^+CD8^+$) and 4,2% of B-lymphocytes ($CD19^+$) production. Our preliminary results suggest that irradiation of extracts could induce a better immune response than naïve proteins and ionizing radiation can act as natural adjuvant in vaccines. The use of those potential immune enhancing protocols could be an interesting alternative for developing a future vaccine for toxoplasmosis. **Supported by:**CAPES & LIMHCFMUSP

IM029 - **MULTIPLEX FLUORESCENCE-LINKED IMMUNOSORBENT ASSAY FOR IGG AND IGM DETECTION IN HUMAN TOXOPLASMOSIS**

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Toxoplasmosis is a high prevalent parasitic infection with low morbidity, which results in significant affected people, mainly ocular disease, fetal infections and encephalitis in immune deficient patients. Diagnosis is performed mainly by specific antibody search, especially for acute infections, with different commercial tests and antigens, but the use of low thresholds and individual variation in patients lead to frequent inconsistencies. New fluorimetric immunoassays have been described using high performance fluorophores conjugates in microplates, allowing simultaneous detection with direct and linear quantification of specific antibody. Those techniques allow high throughput protocols, as necessary in screening for antenatal diagnosis of toxoplasmosis. We devised to standardize multiplex FLISA anti-*T.gondii* IgG/IgM and, after this, analyze the efficiency of those techniques in 120 serum samples previously screened by the ELISA IgG/IgM. Compared to ELISA IgG, multiplex FLISA IgG demonstrated agreement of 94.1% (n=113), with significant statistical correlation (r>0.8, p<0.0001) and kappa coefficients showing excellent concordance (K=0.8837; sensitivity=89.0%, CI=78.7%-95.4%; specificity=100%, CI=93.6%-100%). Analysis of serologic reactivity by the multiplex FLISA IgM provided results comparable to ELISA IgM with agreement of 99.1% (n=119), with significant statistical correlation (r>0,5, p<0,0001) and excellent concordance Kappa index (K=0,9187; sensitivity=85.7%, CI=42.1%-99.6%; specificity=100%, CI=96.7%-100%). These data suggests that the multiplex FLISA can be used effectively for screening toxoplasmosis in large populations, useful in high throughput applications as antenatal care. **Supported by:**CAPES

IM030 - **COMPARISON OF CONVENTIONAL AND DISSOCIATIVE SEROLOGICAL TESTS FOR THE DIAGNOSIS OF VISCERAL LEISHMANIASIS IN DOGS AND HUMAN SAMPLES IN ENDEMIC AREAS OF S&ATILDE;O PAULO AND TOCANTINS**

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Caused by protozoa of the genus *Leishmania*, leishmaniasis is present in tropical and subtropical areas of the world, with wide clinical manifestations, which generally include the involvement of skin, mucosa, organs such as the spleen and liver, thus characterizing cutaneous, mucosal or visceral leishmaniasis. Visceral leishmaniasis (VL) annually affects about 500,000 people in endemic areas, including Brazil, caused by *Leishmania (L.) infantum*, which if transmitted by female sand-flies with domestic dog as main reservoir. Several methods are used for diagnosis of VL, with invasive parasitology aspirates of infected organs as reference technique. IFI and ELISA can be used for the diagnosis of VL, but may differ in sensitivity and specificity, and also with interference due to circulating immune complexes (CIC). To clarify whether the presence of CIC interferes in serological diagnosis, we standardized an enzyme immunoassay with acid phase for dissociation of CIC. We tested 64 samples from dogs and 100 human samples from endemic areas in the states of São Paulo and Tocantins, respectively. The samples were tested by IFI (gold standard), conventional and dissociative ELISA. The results of the methods were compared and showed change in the profile of some samples. The results showed that 86% of samples of dogs and 12% human samples were negative by IFI and positive in conventional and dissociative ELISA. The dissociative ELISA showed 3.5% positivity in samples defined as negative by conventional ELISA and IFI. The difference between the results may be explained by the variation of parameters such as sensitivity and specificity, and also due to interference by CIC. Those data shows clearly a possible use of dissociative ELISA in the screening of serology for suspected dogs or patients for visceral leishmaniasis. **Supported by:**CNPQ

IM031 - **EXERCISE IMPROVES THE TH1 RESPONSE BY MODULATING CYTOKINE AND NO PRODUCTION IN BALB/C MICE: A MODEL OF *LEISHMANIA MAJOR* INFECTION**

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The genus *Leishmania* is an ethiological agent of leishmaniasis, an important neglected parasitosis that is endemic in Brazil.
Physical exercise can improve health and may lead to changes in the functionality of the immune system. Moderate intensity exercise can reduce the risk of infection by shifting the overall immune response towards a T helper type 1 pattern. This study investigates the effect of 12 weeks of swimming on the cytokine profile of lymph node cells and macrophages, of the nitric oxide production by these cells and the effect on the macrophage infection by *Leishmania major*. BALB/c mice were divided into two groups. The exercise group was subjected to swimming exercise. The cytokines were measured by ELISA methods. Lymph node cells culture showed that concentrations of interferon-γ and tumor necrosis factor-α were higher in the exercised group, while levels of interleukine-4 and interleukine-10 were significantly decreased in this group. The interleukine-10/interferon-γ ratio tended towards a T helper type 1 profile. Moreover, macrophages isolated from exercised mice produced more interleukine-12 and tumor necrosis factor-α following lipopolysaccharide stimulus. These macrophages when infected by *Leishmania major* showed a higher interleukine-12 production than was observed with macrophages from the control group. Nitric oxide production was increased in macrophages isolated from exercised group following lipopolysaccharide stimulus but not following infection with *Leishmania major*. The mechanisms of escape from the parasites could interfere with the exercise effect. These data suggest that exercise biases the immune system towards a T helper type I response profile that could be important to defense against parasites such as *Leishmania* sp. **Supported by:** FAPERJ, PIBIC-UERJ

IM032 - **DIAGNOSIS OF ACUTE TOXOPLASMOSIS IN HUMAN MILK**

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Toxoplasmosis is a zoonosis caused by an intracellular parasite, *Toxoplasma gondii,* that infects different hosts including up to one third of the world's human population and can cause severe damage to the fetus in congenital infection. Maternal breastfeeding is the most natural and safe way to feed a newborn, with ability to provide resistance to infections during the infant's first months of life. All immunoglobulin isotypes may be found in human breast milk, mainly in colostrums, from systemic origin or locally produced. Few efforts have been directed to identifying the presence of specific antibodies against parasitic infections, including *T. gondii*, in human milk. In that sense, this study was conducted in order to detect and evaluate the presence of specific *T. gondii* IgG, IgM and IgA antibodies in paired serum and colostrum samples. The study was carried out on 289 puerperal women that was attended in the Clinical Hospital of the Federal University of Uberlândia (mean age 24.8 years, range 14 – 43 years). ELISA immunoassays showed that 136 (47.0%), 20 (6.9%), and 8 (2.8%) serum samples, and 133 (46.0%), 23 (7.9%), and 8 (2.8%) colostrums samples were positive for specific IgG, IgM and IgA antibodies to *T. gondii*, respectively, with a good correlation between the different samples tested. Immunoblotting assays showed that it's possible to detect IgG, IgM and IgA antibodies specific to diverse antigens of *T. gondii* in human serum and colostrums. As expected, IgG antigen recognition was more intense in serum samples, if compared to specific IgM and IgA recognition. On the other hand, milk samples presented specific IgA antibodies recognizing a higher number of antigens than IgG and IgM. Our results showed that is possible to diagnose toxoplasmosis using human milk, a noninvasive way. More studies are needed to determine the role of these bioactive components in the protection against the clinical manifestation of congenital toxoplasmosis. **Supported by:**CAPES, CNPq e FAPEMIG

IM033 - **SEROLOGICAL DIAGNOSIS USING RECOMBINANT PROTEIN PEROXIDOXIN TO DETECT INFECTION OF *LEISHMANIA (LEISHMANIA) INFANTUM* IN NATURALLY INFECTED DOGS**

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The search toward the establishment of novel serological tests for an accurate differential diagnosis of canine visceral leishmaniasis (CVL) and the precise diagnosis may represent one of the most relevant challenges for the control and possible eradication of the disease. In the present work, we have investigated the potential use of recombinant Peroxidoxin (*r*Peroxidoxin), a highly conserved protein in *Leishmania* genus, as potential antigen for the immunodiagnostic of dogs naturally infected with *L. infantum*. The serological assay (ELISA) demonstrated that dogs infected by *L. infantum* showed high levels of antibodies against *r*Peroxidoxin, allowing identification of CVL with considerable sensitivity. The evaluation of animals infected with other relevant species of canine infection (*Trypanosoma cruzi*, *Babesia canis* and *Erhlichia canis*) by *r*Peroxidoxin ELISA showed greater ability to discriminate dogs suffering from visceral leishmaniasis of these infections, compared to *r*k39 antigen and the reference test for diagnosis of CVL in Brazil (EIE-LVC, Bio-manguinhos, FIOCRUZ). *r*Peroxidoxin ELISA also showed greater ability to discriminate between vaccinated and infected animals, an important requirement for a public campaign control of CVL. Our results demonstrated that *r*Peroxidoxin could be among the target molecules which could be used as a potential antigen for the immunodiagnostic of visceral leishmaniasis. **Supported by:**FAPEMIG/CAPES/CNPq

IM034 - **HSP90-*LIKE* IN SEROLOGICAL DIAGNOSIS OF CANINE VISCERAL LEISHMANIASIS**

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Heat shock proteins (HSP) are highly conserved molecules in prokaryotes and eukaryotes that play important roles in protein folding, assembly of protein complexes, and translocation of proteins across cellular compartments. The potential use of HSP proteins of families have been demonstrated in the serodiagnosis of leishmaniasis and obtained promising results for development diagnosis kits using these antigens. In this study, we present data on the development of enzyme-linked immunosorbent assay (ELISA)-based techniques for the detection of antibodies against the recombinant HSP90-*like* protein and a comparison of the results with EIE-LVC Kit, the gold standard test for CVL diagnosis in Brazil. Our data showed that HSP90-*like* ELISA have a greater ability to discriminate dogs with visceral leishmaniasis, offering an excellent discrimination of other canine infections and lower cross-reactivity with commercial vaccines anti-CVL compared to reference test for diagnosis of CVL in Brazil. **Supported by:**FAPEMIG/CAPES/CNPq

IM035 - **LAAG PLUS SAPONIN INDUCED PROTECTION ON BALB/C, BUT NOT ON C57BL/6**

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Leishmania amazonensis is the main agent of anergic diffuse cutaneous Leishmaniasis. Our previous studies demonstrated that LaAg vaccine plus Saponin induced a partial protection against *L. amazonensis* infection observed by partial reduction of lesion growth and parasite load. In this study, we investigated the initial immune response after challenge to characterize the protective response. Prior to footpad infection with *L. amazonensis*, BALB/c mice were twice vaccinated by the intramuscular route with 25 ug of LaAg containing 100 ug of Saponin. We found that vaccinated mice developed delayed hypersensitivity peaking at 15-18 hour similar to Jones-Mote reaction. Histological studies demonstrated a lymphocyte and eosinophil infiltration and reduction of neutrophil, macrophage and mast cells infiltration in comparison to nonvaccinated mice. Eosinophil and lymphocyte (Th1) cells are related to parasite control andNeutrophils and Mast Cells are associated to susceptibility of *L. amazonensis* infection, suggesting that vaccine induced a cellular infiltrate to corroborate to parasite control. Indeed,We observed by mRNA quantification in the peak of hypersensitivity an increase of T-bet and GATA-3, but not FOXP3 on popliteal lymph node cells and demonstrated in footpad an increase of IL-4, IL-10, IFN-γ and IL-12 production and TGF-β reduction demonstrating a Th1 and Th2 profile. However, we observed an increase of iNOS levels in the footpad of vaccinated mice. The increase of iNOS in infected footpad is related to Th1 profile and associate with parasite elimination. In C57BL6, LaAg plus saponin induced a smaller hypersensitivity, without eosinophils migration. LaAg plus saponin did not induce protection on C57BL6 Our result demonstrated a new-type of delayed hypersensitivity associated with Th1 response different of classical DTH reaction and suggests the involvement of this new-type of delayed hypersensitivity to parasite control in vaccinated mice Balb/c. **Supported by:**CNPq, FAPERJ and CAPES

IM036 - **ASSOCIATION OF LAAG PLUS ADDAVAX™ (SQUELENE-OIL-WATER) FAILED TO IMPROVE VACCINE EFFICACY AGAINST LEISHMANIA AMAZONENSIS INFECTION**

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Leishmania amazonensis is the ethiologic agente of diffuse cutaneous leishmaiasis. LaAg (whole antigens of leishmania amazonensis) is a contra –protective antigen when used by intramuscular route and it is protective by intranasal route for Balb/c. In the C57BL6, LaAg does not affect infection when used by intramuscular route (similar to control) and it is protective by intranasal route. In this study, we evaluated the use of AddaVax, a squelene-oil-water based adjuvant, as adjuvant for LaAg vaccine. This adjuvant is a nano-emulsion that induces a lot of effect as cells activation, citokines production, a balanced Th1/Th2 responses and increases production of antibody. Similar adjuvants have been used by intransal route to improve the vaccine efficacy against viral infections. Our hypothesis was to evalulate Adda Vax as adjuvant for intranasal vaccine in comparison with the effect on intramuscular immunization. We immunized C57BL6 mice twice with 10µg of LaAg by intramuscular and intranasal routes associated or not with AddaVax (50%). PBS associated or not with adjuvant was used as control. After seven days, mice (WT) were infected with 5 x 10^5 promastigotes in footpad. The lesion development was evaluated by paquimeter and the parasite load by limited-diluition. As expected for C57BL6, LaAg did not induce protection by intramuscular route. The association with AddaVax did not affect any parameter in intramuscular immunization. However, when used by intransal route the vaccine became worse than in comparison with LaAg free of adjuvant. We observed the increase of Th2 response in infected footpad of vaccinated mice with LaAg plus AddaVax. In this study, we demonstrated that using LaAg as antigen, AddaVax is not a candidate adjuvant to improve vaccine efficacy.

IM037 - **VITAMIN D AS ADJUVANT FOR NASAL AND ORAL VACCINES AGAISNT *LEISHMANIA AMAZONENSIS***

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Vitamin D (VitD) has several effects on immune system such as the participation in the resolution of infection with *M. tuberculosis* thought of induction antimicrobial peptides. Besides, Vitamin D is a potent inducer of cell migration to skin thought induction expression of CCR10 in T cells. *Leishmania amazonensis* is the main agent of anergic diffuse cutaneous Leishmaniasis, a skin disease. Immunization with LaAg (whole *Leishmania amazonensis* antigens) by nasal and oral routes induces protection against leishmaniasis. Our hypothesis was to increase cell migration to the skin of the cells generated from vaccination with LaAg in the mucosa through the association with vitamin D. In this work, we immunized C57BL6 mice twice by nasal route with LaAg (10 ug) associated or not with Vitamin D2 (40ug – Ethanol/PBS solution) or Vitamin D3 (40ug – Ethanol/PBS solution); by oral route with LaAg (100 ug) associated or not with Vitamin D2 (200ug – Ethanol/PBS or Ethanol/oil) or Vitamin D3 (200ug – Ethanol/PBS or thanol/oil); and PBS or Vitamin D2 or D3 as control. After seven day of second dose, mice were infected with 5 x 10^5 promastigotes in stationary phase of Leishmania amazonensis (josefa strain). The lesion development was evaluated by paquimeter and the parasite load by limited-diluition. We observed in nasal immunization that association of LaAg with Vitamin D2 or D3 reduced the lesion grown and the parasite load in comparison with controls and with mice immunized with LaAg. For oral immunization, the association with vitamin D in Ethanol/PBS did not work as adjuvant, however, using in Ethanol/oil, we observed an increase in vaccine efficacy, showed by reduction of the lesion grown and the parasite load in comparison with controls and with mice immunized with LaAg. Vitamin D2 and Vitamin D3 improved both oral and nasal vaccinations in C57BL6, with better results observed for Vitamin D3. In conclusion, our results indicate Vitamin D as a new mucosal vaccine adjuvant against *Leishmania amazonensis*. **Supported by:**FAPERJ, CAPES, CNPq.

IM038 - **OOCYST-SPECIFIC ANTI-*TOXOPLASMA GONDII* ANTIBODY DETECTION IN NATURALLY INFECTED CHICKENS IN COMPARISON WITH EXPERIMENTALLY IMMUNIZED ANIMALS.**

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Diagnosis and epidemiological surveys of toxoplasmosis in humans and animals has been focuses on immunoassays using whole parasite antigens. However, theses assays determine only if there was previous exposure to *T. gondii*. Thus, the possible sources and routs of infection (oocyst or cyst) remain indeterminate, which represents a considerable difficulty concerning the establishment of an epidemiological surveillance of the parasite. In the present study, we used a panel of 11 specific peptides designed from T. gondii oocyst (named as p1 to p11) and tested with serum samples from chickens naturally infected with *T. gondii* compared with chickens immunized experimentally with soluble taquizoite antigen (STAg). It was carried out indirect ELISA by using peptides or soluble toxoplasma antigen against serum samples from naturally infected chickens or samples from chickens immunized with STAg. Furthermore, *Western blotting* was performed to confirm the results. In serum samples from naturally infected chickens, it was observed reactivity for all peptides and STAg. In sera from experimentally immunized chickens it was observed reactivity with STAg and only peptide 3 (P3). These results were confirmed by Western blotting. Considering that chickens are infected by oocysts ingestion only, these data suggest that these peptides may be useful to detect exposure of chickens to sporozoites in *T. gondii* infection. **Supported by:**CNPq, FAPEMIG, CAPES

IM039 - **HETEROGENEITY OF DOG´S MONOCYTES AND ITS ROLE IN CANINE VISCERAL LEISHMANIASIS: PRELIMINARY RESULTS**

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In Brazil, the parasite *Leishmania (Leishmania) chagasi (L. infantum)* is the cause of canine visceral leishmaniasis. The dog represents the major reservoir of the parasite, having a central role in the transmission to humans. *Leishmania* spp. is obligate intracellular parasites affecting mainly the mononuclear phagocyte system. Monocytes are largest pool of circulating progenitor cells and play an important role in inflammation, tissue repair and immune response. The aim of this project is to study monocytes subpopulations and markers of susceptibility and resistance by the analysis of peripheral blood monocytes of naturally infected dogs and to describe the behavior of these cells during infection. Peripheral blood samples were collected from dogs from different groups: uninfected and infected (asymptomatic and symptomatic) with *L. infantum,* which the cells were subjected to labeling by anti-CD14, anti-CD16 and anti-MHCII and the samples obtained by flow cytometry. Our preliminary results show that dogs exhibit three distinct populations of peripheral blood monocytes. One of these populations corresponds to 90% of the total monocytes, presenting high expression of CD14 molecule. The second and third populations show different levels of CD14, CD16, MHCII and together, represent about 10% of the total blood monocytes. Infected dogs show increased frequency of CD14+ monocytes both in asymptomatic and symptomatic animals, when compared to uninfected. In monocytes subpopulations described above, the frequency of monocytes expressing MHCII is homogeneous in the three groups, however there was a reduction in MHCII expression in infected dogs monocytes when compared to the negative ones and this is more evident in the symptomatic group compared to the asymptomatic group. Our results show for the first time that monocytes in dogs are a heterogeneous population, and as it has been described in mice and humans, can play different roles in the immune response during infection. **Supported by:**CNPq e UFMG

IM040 - **SYSTEMIC AND LOCALIZED IMMUNE RESPONSE IN PATIENTS WITH CUTANEOUS LEISHMANIASIS IN THE XABRIABÁ INDIGENOUS COMMUNITY, SOUTHEASTERN BRAZIL**

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Leishmania are injected into the vertebrate host as a promastigote, which is phagocytosed by different phagocytic cells in the host. In this study, we evaluate the phenotypic features of phagocytes apart from cytokine signature of circulating T-cells from individuals presenting positive Montenegro skin test (MST+, n=09) without lesions and patients with cutaneous leishmaniasis (CL, n=18) as compared to control group (CT, n=09). These individuals are indigenous resident in Xakriabá community, northern of Minas Gerais State, Brazil, where the prevalence of the disease is around 9.0%. Our data demonstrated that monocytes from CL patients displayed an impaired ability to phagocytize *L. braziliensis*. This impaired phagocytic capability did not reflect on the expression of the MHC-I, CD23, CD80, TLR-2, TLR-4 and TNF-alpha molecules that are upregulated in CL as compared to MST+. The lymphocyte-derived cytokine signature demonstrated that a down-regulated synthesis of IL-10 or IL-4 is associated with lower *L. braziliensis* phagocytic index and lower nitric oxide levels produced by monocytes. The histopathological features of recent CL lesions (less than 90 days) showed an intense inflammatory reaction, characterized by mononuclear cells and polymorphonuclear leukocytes, whereas late CL lesions (more than 90 days) exhibit a predominance of mononuclear leukocytes. Moreover, the genic expression of cytokines and chemokines of skin biopsies from CL group evidenced in recent CL lesions, increased expression of IL-10, TGF-beta, IL-4, TNF-alpha, IL-12, CCL2, CCL3, CCL5 and CXCL10 as compared with late CL lesions. Our findings demonstrate that recent lesions presented a higher cytokine/chemokine expression than late lesions showing that time evolution of lesions is an important feature able to influence CL lesion outcome, and therefore it should be assessed in studies from immune response during CL infection. **Supported by:**CAPES

IM041 - **POTENCIAL EFFECTS OF *TITYUS SERRULATUS* VENOM IN *TOXOPLASMA GONDII* AND *TRYPANOSOMA CRUZI* CLEARANCE DURING EXPERIMENTAL INFECTION**

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Introduction: *Trypanosoma cruzi* (Tc) and *Toxoplasma gondii* (Tg), agents of Chagas Disease and Toxoplasmosis, respectively, are obligatory intracellular parasites that infect several hosts and subvert their initial immune response. Both cause ample morbidity and mortality mainly in immunosuppressed host, despite present rarely clinical signals/symptoms during acute phase. Nitric oxide (NO) is a critical agent, produced by macrophages (MO) through cytokines (CK) induction, that controls the parasites burden/dissemination in both infections. *Tityus serrulatus* venom (TsV) modulates inflammatory mediators production by MO, albeit its effect in MO microbicidal function against Tc and Tg infection is unknown. So, this study aimed to verify the potential action of TsV in modulates MO function against Tc or Tg infection. Methods and Results: Peritoneal MO harvested from C57Bl/6 mice, were plated, infected in a 2:1 (Tc:cell) or 5:1 (Tg:cell) proportion and incubated with different TsV concentrations (100|200|400mg/ml). Parasites uptake and replication were verified counting their number into the cells (amastigotes) and in the supernatant (tripomastigotes), wich was collected after 24 and 48h for NO (Griess) and CK (ELISA) analysis. We found TsV didn't interfere in parasite's uptake but increased MO microbicidal activity, which were associated with greater ability to induce NO. Moreover, despite Tc infection per se acted as a weak NO stimulator by MO, its presence resulted in partially subversion of NO production induced by TsV. NO production could be modulated by a difference in CK levels produced by Tc-infected MO stimulated with TsV, which demonstrated great ability to enhance IL-6 and TNF-α and inhibit IL-10 release when compared with unstimulated cells. Our data also demonstrated that TsV "mirror" its effects in Tg-infected MO. Conclusion: Briefly, our results suggest TsV as a "power tool" against intracellular infection as Tc and Tg. **Supported by:**CNPq; CAPES; FAPEMIG

IM042 - **DEFICIENT DIETS ON VITAMINS B6 OR B9 OR D INDUCED AN INCREASE RESISTANCE AGAINST LEISHMANIA AMAZONENSIS INFECTION IN BALB/C.**

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Vitamins have several effects on immune system as dendritic cells maturation and T cell differentiation. Leishmania amazonensis is the main agent of anergic diffuse cutaneous Leishmaniasis. The infection in mice, a mixed of Th1 and Th2 response is present in susceptible and resistant animal. Our aim is to investigate the role of Vitamin D (VitD), Vitamin B9 (VitB9) and Vitamin B6 (VitB6) on L. amazonensis infection. BALB/c mice with one month of life were submitted on a diet without Vit B6 or VitB9 or VitD or with normal diet ,as control, for 45 days. Afterward, mice were infected with 5×10^5 (low dose infection) or 2×10^6 (high dose infection) of promastigotes on footpad. Lesion development was evaluated by paquimeter and the parasite load by limited-dilution. Using low dose infection, all deficient diet became BALB/c more resistant to infection, presenting a delay in the lesion development and a decrease parasite load. Deficient diet in VitB9 or VitD induced a partial resistance and VitB6 induced a strong resistance. Using high dose infection, no difference was observed on lesion development on BALB/c on deficient diet in VitB9 or VitD, however, deficiente diet on vitamin B6 induced a strong resistance again. The increase of resistance in deficient mice mice is involved with the increase of Th1 cytokines in infected footpad. All these finding together demonstrate the participation of VitD, VitB9 and mainly for VitB6 on disease development in L. amazonensis infection. **Supported by:**CNPq FAPERJ CAPES.

IM043 - **CASPASE-1 DETECTION IN HUMAN CELLS INFECTED BY Y OR COLOMBIAN STRAINS OF *TRYPANOSOMA CRUZI.***

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Trypanosoma cruzi, the etiologic agent of Chagas' disease in vertebrates, infects different types of cells, such as the autonomic nervous system, muscle cells and macrophages where it multiplies leading to a persistent infection. The innate immune response against *Trypanosoma cruzi* comprises several pattern recognition receptors (PRRs). There are various classes of PRRs such as Nod-like receptor (NLRs), a multiprotein complex known as Inflammasome, a large protein complex activated upon cellular stress or microbial infection, which triggers maturation of pro-inflammatory cytokines like interleukin-1βand interleukin-18 through caspase-1 activation. On the one hand, IL-18 and IL-1β activate monocytes, macrophages and neutrophils, and induce cellular immune responses adaptative Th1 and Th17. The pro-IL-1β and pro-IL-18 are secreted as inactive precursors and depend on cleavage by proteases as caspase-1 to their maturation. We have observed in human macrophages infected with *T. cruzi* Y strain a significant production of IL-1β. Thereby, we proposed to compare the production of pro-inflammatory cytokines such as IL-1β and IL-18 and the production of caspase-1, in infection of human peripheral blood mononuclear cell (PBMC) and macrophages by *T. cruzi* strain Y and the Colombian. Human macrophages and PBMC were cultured in RPMI 1640 with 10% human serum and incubated in 37 º C with 5% CO_2. The THP-1 cells were cultured in RPMI 1640 with 10% inactivated fetal bovine serum with addition of PMA at 30 nM for differentiation of macrophages and incubated in 37 º C with 5% CO2. After 24 and 72 hours infection supernatants were centrifuged and the cytokines were measured by ELISA. Our preliminary results with human macrophages infected with *T. cruzi* Y strain showed a significant production of IL-1β when compared with *T. cruzi* Colombian strain. The caspase-1 production was detected in human macrophages infected with *T. cruzi* Y strain and this production seems to be related with an increased amount of added parasites. Similar results were also obtained when THP-1cell lineage was used. There was a slight production of IL-18 in human macrophages infected with *T.cruzi* Y strain. Human PBMC infected with *T. cruzi* in the presence of caspase-1 inhibitor (Z-WEHD-FMK) inhibited the production of IL-1β. These results suggest a correlation between caspase-1 and IL-1β production in *T. cruzi* infected human cells. **Supported by:**IOC/FIOCRUZ

IM044 - **COMPARATIVE STUDY OF INFLAMMATORY ANGIOGENESIS AMONG DIFFERENT STRAINS OF *TRYPANOSOMA CRUZI* DURING ACUTE PHASE OF INFECTION IN C57BL/6 MICE.**

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Trypanosoma cruzi infection infiltrates different inflammatory cells and releases several secretory mediators such as inflammatory cytokines and chemokines at the foci which are involved in immunopathology during infection. Studies have shown that these inflammatory cells and mediators are directly or indirectly associated with stimulating or regulating angiogenesis. Since, angiogenesis is an essential process in physiological and pathological conditions especially during wound healing and inflammation, we focussed this study on the involvement of *T. cruzi* on inflammatory angiogenesis. In this study, C57BL/6 mice (n=10) were infected with three different strains (Colombian, VL10 and Y; 100 parasites) of *T. cruzi*. Blood and heart samples were collected during the acute phase of infection to evaluate inflammation, angiogenesis and their mediators by immunoassays (CCL2, CCL3,CCL5, TNF-α, IL17, IL10), conventional histology and real time-PCR (CCL2, CCL5, TNF-α, IL10, CCR2, CCR5, VEGF, Ang-1 and Ang-2). Histological analysis of heart infected with Colombian strain showed increased amastigote nest area and inflammatory cells followed by VL-10 and Y strain. All infected mice demonstrated increased in inflammatory mediators in plasma when compared with not infected mice. When compared among strains, mice infected with Colombian demonstrated increased cytokine (IL-17) and chemokines (CCL5, CCL3) in plasma and as well as expression in heart, followed by Y strain and then by VL10 strain respectively although equal number of parasites were inoculated for the infection. Moreover, the expression of angiogenic proteins, VEGF, Ang-1 and Ang-2, was greatly reduced in mice infected with Colombian strain and followed by Y and VL10 strains. Although, *T. cruzi* infection is inflammatory, our current findings showed reduced expression of angiogenic mediators suggesting relevance role of parasite in regulating angiogenesis regardless of different degrees of inflamed conditions. **Supported by:**CAPES, CNPq, FAPEMIG, ISID, TWAS, UFOP

IM045 - **EVALUATION OF THE SERODIAGNOSTIC POTENCIAL OF NCROP4, A *NEOSPORA CANINUM* RHOPTRY PROTEIN, IN DIFFERENT MAMMALS SPECIES**

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Neospora caninum, the etiological agent of neosporosis, is an obligate intracellular parasite with worldwide distribution. Since 1990's studies have shown the relationship of this parasitic disease with abortions in cattle herds. The diagnosis of neosporosis presents a limitation on the specificity of the tests used due occurrence of cross-reactivity with other protozoa as *Toxoplasma gondii*. This study aimed to evaluate the diagnostic potential NcROP4, *N. caninum* rhoptry protein, in different mammals species. Thus, we produced a monoclonal antibody called 20D2 against the protein NcROP4. To evaluate the immunodominant mimetope, we developed analysis using phage display technology with two library, one with seven and one with twelve amino acids. Capture enzyme-linked immunosorbent assay (ELISA) was developed using antibody 20D2 to capture NcROP4 detection IgG anti-NcROP4 in the serum of sheep, cattle and dogs. Also used to soluble antigens of *N. caninum* (NLA) and *T. gondii* (STAg) for analysis of cross-reactivity. Subsequently, analysis of the amino acid composition was performed to assess homology between the proteins ROP4 of *T.gondii* (TgROP4) and *N. caninum* (NcROP4), and we also predicted the linear B cell epitopes of both proteins. Our results showed that mAb 20D2 selected 17 phage on library of 7 amino acid and 8 phage on library of 12 amino acid. By ELISA, we demonstrated that NcROP4 is recognized by antibodies of different animals positive to *N. caninum* and *T. gondii*. Through alignment the sequences of NcROP4 and TgROP4, we observed that the two proteins have 36.7% indentity. The predictions of B cell epitopes showed that mAb 20D2 only recognizes sequence NcROP4 and this protein has 23 potential epitope regions, while TgROP4 presented 22 regions. From those potential epitopes, 13 are regions located in similar sequences between the two proteins. We conclude that further studies are needed to determine specific regions among the two proteins. **Supported by:**CNPq, CAPES, FAPEMIG, MAPA, FINEP

IM046 - **ANTIBODIES AGAINST *TOXOPLASMA GONDII* AND *NEOSPORA CANINUM* SHOW DIFFERENT PROFILES OF CROSS-REACTIVITY DEPENDENT ON THE ANTIGENIC FRACTION**

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Toxoplasma gondii and *Neospora caninum* are intracellular that may cause clinical diseases in its wide range of vertebrate hosts. Due to the structural and molecular similarities observed between the two parasites, antibody cross-reactivity may be observed during the execution of serodiagnostic techniques. Thus, the aim of this work was to establish the profile of cross-reactivity between *T. gondii* and *N. caninum*, using the mouse model. Balb/c mice were infected with 1.10^6 tachyzoites of *N. caninum*. For the group of infection with *T. gondii* infection, mice were infected with 20 cysts of strain ME-49. After 7, 15, 30, 60 and 90 days of infection, sera from infected and healthy mice were collected and analyzed by Enzyme-linked immunosorbent assay (ELISA) and Western blotting (WB) using different antigens (Soluble antigens: *N. caninum* - NLA, *T. gondii* – STAg; Excreted-secreted antigens: *N. caninum* - NcESA, *T. gondii* - TgESA; Soluble antigens in the absence of excreted-secreted antigens: *N. caninum* - NLA-ESA, *T. gondii* - STAg-ESA; Surface antigens: *N. caninum* – NcSAg, *T. gondii* - TgSAg), and IFAT (indirect fluorescent antibody test) with fixed parasites, with or without surface permeabilization. By ELISA and IFAT, it was found that IgG antibodies from mice infected with *T. gondii* recognized surface antigens of *N. caninum*, whereas the IFAT showed that IgG antibodies from mice infected with *N. caninum* did not react to antigens of *T. gondii.* By ELISA too, IgG antibodies from mice infected with *N. caninum* recognized surface antigens of *T. gondii* only after 60 days of infection and showed a lower cross-reactivity than other soluble antigens. We also demonstrate that IgG anti-*N. caninum* recognized mainly STAg, TgESA and STAg-ESA, by ELISA e WB. In contrast, the reactivity of anti-*T. gondii* IgG to NLA was significantly reduced if compared to its counterpart. Thus, we conclude that *T. gondii* and *N. caninum* exhibit different patterns of cross-reactivity in the murine model. **Supported by:**CNPq, CAPES, FAPEMIG, MAPA, FINEP

IM047 - **EVALUATION OF *TOXOPLASMA GONDII* INFECTION IN *CALOMYS CALLOSUS* MODEL WITH ATYPICAL STRAIN**

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Uberlândia city has two *T. gondii* parasite isolates named TgChBrUD1 and TgChBrUD2. We aimed to evaluate the rodent *Calomys callosus* susceptibility to the referred strains and its applicability as a model for experimental toxoplasmosis. The advantage of this animal model refers to its similarity of immune response to human hosts. Males or females were infected with tachyzoites and monitored for mortality, weight variation and morbidity, totalizing 20 animals. Immunohistochemical and qPCR, encompassing primers target the high sensitive 529 repeated element, were performed to evaluate the parasitism in spleens, livers and brains. For all tests, we performed GraphPad version 5.0 and $p<0.05$ was considered statistically significant. When either males or females were infected with TgChBrUD1 or TgChBrUD2 isolates, the mortality was early, between 8 days 9 days for both isolate post inoculation (p.i). The mortality p.i reached 100% 12 and 13 days after infection by TgChBrUD1 and TgChBrUD2, respectively. We also showed that females infected by TgChBrUD1 die earlier than infected males. In contrast, males infected by TgChBrUD2 die earlier than infected females. The weight and morbidity of males and females showed few changes after infections. Females infected by TgChBrUD2 had significantly higher parasites number in comparison to TgChBrUD1 infected females in both liver and spleen. However, it was not observed significant differences in the liver, spleen and brain parasitism when males and females were infected by both isolates. Our date showed higher *T. gondii* infection rate in the liver compared to the brain when infected for TgChBrUD1and TgChBrUD2 isolates for both males and females. In conclusion, *C. callosus* are susceptible to both TgChBrUD1and TgChBrUD2 isolates. With this new experimental model infection we expect to highlight insights of other studies to comprehend some aspects of *T. gondii* infection. **Supported by:**FAPEMIG, CNPq e CAPES

IM048 - **DETERMINATION OF THE IMMUNOGLOBULIN CLASS IN DOGS NATURALLY INFECTED WITH *LEISHMANIA (LEISHMANIA) INFANTUM CHAGASI*.**

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Background and Aim: It has been suggested that immunoglobulins class and subclass can be use as marker of the evolution of leishmaniasis in dogs; however, the data reported are conflicting. So, the objective of this study was to evaluate the presence of specific IgA, IgE, IgG and IgM in the sera of dogs naturally infected with *Leishmania (Leishmania) infantum chagasi* from endemic areas in northwest of São Paulo state, Brazil. Material and Methods: Ninety eight dogs from the municipalities of Araçatuba and Presidente Prudente with positive parasitological diagnosis by PCR were assayed in duplicate to ELISA using *L. (L.) chagasi* promastigotes total antigen, anti-dog IgA, IgE, IgG and IgM peroxidase conjugated and TMB to development of the reaction. According to the clinical and laboratorial exams, 49 dogs were classified as symptomatic and 49 as asymptomatic. Ten sera from dogs living in non-endemic area of visceral leishmaniasis were used to the establishment of the reaction cut-off. Results: Positivity was observed in 84.7% of the sera for IgG, 57.1% for IgE, 55.9%for IgA and 19.4% for IgM. The level of IgG and IgA were higher in symptomatic than asymptomatic dogs ($p<0.0001$ and $p=0.0071$, respectively); however, the level of IgM and IgE were lower in symptomatic when compared to asymptomatic dogs ($p=0.0175$ and $p<0.0001$, respectively). Positive correlation was observed between IgG and IgA ($r=0.425$ and $p<0.0001$). Conclusion: The data showed that IgG and IgA are present in the canine visceral leishmaniasis fully manifested disease, while IgM and IgE could be a marker to the presence of infection in asymptomatic dogs. **Supported by:**Supported by CNPq, FAPESP 2012/05847-9 and FAPESP 2012/50285-9.