HP006 - **TRANSMISSION OF TOXOPLASMA GONDII INFECTION DURING BONE MARROW TRANSPLANTATION IN A MOUSE MODEL**

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Toxoplasma gondii infection may present a fatal outcome in human bone marrow transplant (BMT) recipients, due to the rapid disease course in immunosuppressed individuals. There are many cases reported in literature of toxoplasmosis after BMT, and usually it is the result of reactivation of latent infection rather than due to primary infection. However, there are some cases of toxoplasmosis reported in BMT recipients with negative T. gondii antibody titers, suggesting transmission of infection through bone marrow. The purpose of this study was to determine the possibility of transmission of T. gondii infection through BMT in a mouse model, checking donors under acute and chronic infections. With that intent, C57BL/6 mice were infected i.p. with 100 tachyzoites of RH-RFP strain for acute phase experiments or ME-49-GFP strain for chronic phase. Mice were euthanized after 7, 15 and 30 days of infection and the presence of tachyzoites was evaluated under fluorescent microscopy by analysis of fresh bone marrow. We found tachyzoites in all samples analyzed. Bone marrow samples from infected mice were transplanted via i.p in naïve recipient. The morbidity and mortality were observed during 20 days after transplantation. ELISA was performed with all animals for evaluating T. gondii seroconversion, and all animals transplanted were seropositive. This study suggests that T. gondii positive bone marrow donors are potential source of parasite transmission.
Supported by:CNPQ, CAPES and FAPEMIG **Keywords:**Toxoplasma gondii; bone-marrow transplant; transmission model

HP017 - ***TRYPANOSOMA RANGELI* DETECTION IN THE SKIN OF A VERTEBRATE HOST**

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Trypanosoma rangeli is a non-pathogenic parasite protozoan that infects mammals and triatomines in Latin American countries. Despite much work has been developed, the development of this parasite in the vertebrate host is still unknown. *T. rangeli* infection starts when infected triatomines inject metacyclic trypomastigotes in mammals during a blood meal. These infected mammals can transmit the parasite to other triatomines for several months, which indicates that *T. rangeli* is able to multiply in the vertebrate host. As the bite site is the first place the parasite is in contact with, the present study used qPCR and histological techniques to track down *T. rangeli* in the skin of exposed mice during the first two weeks of infection. Animals were exposed by the bite of infected 5th instar nymphs for qPCR analyses and by deposition of metacyclic trypomastigotes in the skin for histological evaluation. Our results show, for the first time, that *T. rangeli* DNA can be detected in the skin of a mammalian for up to seven days after its inoculation. Histological data showed extracellular parasites in the dermis and hypodermis in the first 24 hours after exposure. Inflammatory infiltrates were observed from 12 hours up to seven days after exposure, with a large presence of mast cells and eosinophils. Fifteen days after exposure, neither parasites nor inflammatory infiltrates were observed at the bite site, which suggests an elimination of the parasites from the skin by the immune system.
Supported by: Fapemig, Fiocruz, CNPq
Keywords: Trypanosoma rangeli; mammal host; skin

HP018 - **IMMUNOCHEMOTERAPY OF LEISHMANIA (LEISHMANIA) AMAZONENSIS-INFECTED BALB/C MICE WITH DPPE 1.2 ASSOCIATED TO THE RECOMBINANT CYSTEINE PROTEINASE RLDCCYS1 PLUS PROPIONIBACTERIUM ACNES.**

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Leishmaniasis affect 12 million people worldwide. The treatment is limited by the toxicity and parasite resistance, validating the development of new leishmanicidal compounds. Previous data from our laboratory showed the effective activity of DPPE 1.2 on L. (L.) amazonensis-infected BALB/c mice followed by immune modulation characterized by increase of T CD4+ and T CD8+ lymphocytes. More recent findings showed that the treatment of L. (L.) amazonensis-infected BALB/c mice with DPPE 1.2 associated either to rLdccys1, or to P. acnes as an adjuvant, resulted in an exacerbation of the leishmanicidal effect. These data led us to test the effect of DPPE 1.2 in association with rLdccys1+ P. acnes on BALB/c mice infected with L. (L.) amazonensis. The treatment with 600 µg/Kg of DPPE 1.2 associated with 7.5 mg/Kg of rLdccys1+15 mg/kg of P. acnes resulted in a parasite reduction of 21,600 fold, while this reduction was of 2,500 fold, 3,600 fold, and 490 fold in animals treated respectively with DPPE 1.2 + P. acnes, DPPE 1.2 + rLdccys, or DPPE 1.2 alone. There was a significant increase of TCD4+ lymphocytes in animals treated with either DPPE 1.2 alone or in association with rLdccys1 or P. acnes or with both, besides a significant increase of TCD8+ lymphocytes in those treated with DPPE1.2 associated with rLdccys1 + P. acnes. Furthermore, the animals treated with DPPE 1.2 associated with rLdccys1 + P. acnes displayed a significant increase of TCD4+ and TCD8+ memory lymphocytes. Although all treatment schedules led to a significant increase of IFN-□ and reduction of TGF-β, these cytokine profiles were more pronounced in animals treated with DPPE 1.2 + rLdccys1 + P. acnes. The present data showed a more effective leishmanicidal effect of DPPE 1.2 when it was associated with rLdccys1 + P. acnes followed by immunomodulatory responses and expression of memory T lymphocytes, encouraging us to explore the potential of this association for the chemotherapy of cutaneous leishmaniasis. **Supported by:**capes **Keywords:**Dppe 1.2; rldccys1; p. acnes

HP019 - **NETOSIS INDUCED BY *LEISHMANIA INFANTUM* AMASTIGOTES**

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Leishmaniasis is a neglected disease which occurs in tropical and subtropical countries as cutaneous, mucocutaneous and visceral forms. The visceral disease (VL) caused by Leishmania infantum (L.i) is characterized by hepatosplenomegaly and hypergammaglobulinemia, being fatal if not treated. Netosis is a neutrophil cell death which occurs by the release of extracellular web-structures composed of chromatin decorated with some cytosolic and granular proteins (NETs), which trap and kill pathogens extracellular traps. NETs are extruded by two mechanisms: the classical which is ROS-dependent and the ROS-independent named early/rapid netosis. Our group showed that Leishmania promastigotes induce NETs release by both mechanisms. However, little is known about the netosis induced by the amastigote forms of the parasite, thus this is the subject of the present work. For this, human neutrophils (10^6) purified from blood of healthy donors were incubated for 10 and 90 min with different numbers of amastigotes and NET-DNA released in the supernatant measured with Picogreen. Our data show that axenic amastigotes induce netosis in a number dependent way. Then we assessed signaling pathways using chemical inhibitors pretreating neutrophils 30 min before the amastigotes stimuli. We demonstrated that previous treatment with the apocynin and DPI (ROS inhibitors) decreased NET-DNA release significantly. Elastase inhibition also significantly decreased NET-DNA induced by amastigotes. We also investigated the correlation between NET-DNA release and amastigote endocytosis. Our data show that the lower endocytic index the higher NET-DNA release. We can conclude that axenic amastigotes induce netosis, and that this mechanism is ROS-mediated and dependent of elastase. Moreover, endocytosis and netosis are independent mechanisms that contribute to host defense against the parasite. **Supported by:**CNPq, CAPES e FAPERJ **Keywords:**Visceral leishmaniasis; leishmania infantum; netosis

HP020 - **ADENOSINE A2B RECEPTOR BLOCKADE CONTROLS LESION DEVELOPMENT IN MICE INFECTED BY *LEISHMANIA AMAZONENSIS***

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L. amazonensis infection is refractory to conventional therapeutics and patients have a lack of antigen-specific T-cell responses that impairs the parasite control. A_{2B} receptors antagonists are able to block the inhibition of dendritic cells by the parasite, and these cells can stimulate the activation of IFN-γ-producing T lymphocytes responsible for control of *Leishmania* infection. To investigate the effect of A_{2B} receptors antagonists on the infection with *L. amazonensis*, C57BL/6J mice were inoculated in the ears with $1.0x10^3$ metacyclic promastigotes, with or without 5µM MRS1754 or PSB1115, and the lesion size was measured weekly. Alternatively, mice were treated ip with 1 mg/kg PSB1115 1 hour before infection. Moreover, the number of parasites in the lesion was estimated by limiting dilution assay and the production of IFN-γ and IL-10 evaluated by ELISA. Our results showed that these treatments reduce lesion size and tissue parasitism. Lymph node cells from treated mice produce higher levels of IFN-γ than control mice, without altering the production of IL-10. In addition, we examined the activation state of dendritic cells on ears and draining lymph nodes. Interestingly, PSB1115 treatment increases the percentage of CD40+ dendritic cells in both tissues. Finally, dendritic cells were stimulated in vitro with heat-killed *L. amazonensis* metacyclic promastigotes in the presence or absence of MRS1754. These cells were inoculated iv in mice previously infected with *L. amazonensis*. MRS1754-treated dendritic cells are able to decrease lesion size and tissue parasitism and increase IFN-γ production. In conclusion, A_{2B} receptors have a significant influence on the *L. amazonensis* infection, probably due to the impairment of dendritic cell activation and inhibition of immune response, suggesting that this receptor can contribute to the *L. amazonensis* pathogenesis. **Supported by:**CNPq, CAPES, FAPEMIG, UFOP.
Keywords: Leishmania amazonensis; adenosine a2b receptor; dendritic cell

HP021 - **SOCS2 HAS A DICHOTOMOUS ROLE IN HEMATOPOIETIC AND NON-HEMATOPOIETIC CELLS BEING ESSENTIAL TO CONTROL OF THE HEART FUNCTION DURING THE EXPERIMENTAL TRYPANOSOMA CRUZI INFECTION**

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Introduction and Method: Chagas disease is an infectious disease caused by the protozoan *Trypanosoma cruzi* whose intensity of immune response it is directly related to the development of chagasic cardiomyopathy. The inflammatory process and damage to the vascular endothelium promote heart failure, arrhythmias, abnormalities of blood flow and alterations in the electrophysiology of cells. The suppressor of cytokine signaling (SOCS) 2 is a very important protein for the regulation of the various intracellular pathways and was shown to be important in the control of cytokine and calcium and potassium influx in cardiomyocytes during *T. cruzi* infection. To further elucidate the role of SOCS2 in cardiomyocytes and immune cells, we investigated the disease profile in C57Bl/6 (WT) and SOCS2 knockout (KO) mice with bone marrow transplanted between them. These animals were infected with *T. cruzi* Y strain and were analyzed the parasitemia, weight loss and electrocardiogram. Results: Deficiency of SOCS2 resulted in decreased parasitemia and increased heart rate when compared with WT. SOCS2 KO mice that received a bone marrow transplant of a WT presented reduction of: i) control of parasitemia; ii) heart rate and QTc interval of the electrocardiogram. Moreover, WT mice that received SOCS2 KO bone marrow, despite to present similar parasitemia and BPM, presented increased of PR and reduction of QTc interval of the electrocardiogram when compared with WT/WT. Conclusions: In summary, our results suggested that SOCS2 in hematopoietic cells is prejudicial in the control of parasite replication. In the other hand, SOCS2 in non-hematopoietic cells is essential for the control of ventricular repolarization time associated with reduction of potassium currents.
Supported by:CAPES, CNPq, FAPEMIG
Keywords: Chagas disease ; electrophysiology ; chimeras

HP022 - **MAST CELLS COUPLE INTRACELLULAR SENSING OF AMASTIGOTES TO INFLAMMATORY NEOVASCULARIZATION IN THE HAMSTER CHEEK POUCH MODEL OF *T. CRUZI* INFECTION**

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Introduction: Mast cell (MC) granules harbor VEGF and chymase, a serine protease that promotes angiogenesis in an implant sponge model established in hamsters. Using a model infection of hamster cheek pouch (HCP) with mammalian cell-culture derived trypomastigotes (TCTs), we have reported that inflammation is propagated by plasma leakage via cross-talk between MCs and the kallikrein-kinin cascade. Increased vascular permeability contributes to angiogenesis because plasma leakage enables the formation of a provisional fibrin matrix necessary for neovascularization. Availability of genetically modified *T.cruz*i (Dm28c-GFP and Dm28-luciferase) led us to inoculate HCP tissue with TCTs or epimastigotes (Epis). Methods and Results: Three methodologies were combined to investigate the outcome of *T. cruzi* infection (i) intravital microscopy (IVM) (ii) In vivo imaging system (IVIS) measurements of luminescence (iii) confocal microscopy. Visual inspection and IVM-analysis at 7 d.p.i. disclosed a marked angiogenesis with increased number of vascular segments. IVIS and IVM measurements revealed that TCT parasitism peaked at 7 d.p.i. Inoculation of Epis into HCPs did not reveal any significant alterations. We sought to determine whether angiogenesis was triggered by TCTs or by intracellular parasites. Hamsters inoculated with TCTs were treated 24 h later with benznidazol (100 mg/kg, 1-5 d.p.i.) that abolished tissue parasitism and neovascularization. Toluidine blue staining showed an increased density of MC in parasitized HCPs (7-14 d.p.i). Proteomic analysis (3 d.p.i) revealed that the levels of chymase were higher in parasitized HCP. Pharmacological studies showed that targeting of chymase with chymostatin inhibited amastigote-driven angiogenesis. Conclusions: Our studies suggest that MC/chymase, acting at the endothelial interface, might couple immunological sensing of intracellular amastigotes to the inflammatory pathways that ultimately promote neovascularization. **Supported by:**CNPq, CAPES, FAPERJ and INBEB **Keywords:**1trypanosoma cruzi; 2angiogenesis ; 3mast cells

HP023 - **"ROLE OF ASCORBIC ACID DURING THE ACUTE PHASE OF CHAGAS DISEASE: SYNERGISTIC EFFECT IN COMBINATION WITH BENZNIDAZOLE AND CARDIOPROTECTION"**

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Nowadays seven million people are infected with *Trypanosoma cruzi* worldwide. Benznidazole (BZ) is the only treatment available in the country and it has low efficacy and serious side effects. Wherefore, the greatest challenge for researchers is the development of new therapies. In addition, Chagas disease involves an intense inflammatory process that is strictly linked to the establishment of oxidative damage, making the process of tissue destruction even more severe. The studies using antioxidants in disease therapy have been gaining the attention of scientists. In the present research ascorbic acid (AA), an important antioxidant, was tested alone and in association with BZ in a subclinical dose (10mg / kg). Our aim in this work was to evaluate if that compound have trypanocidal effect and if it could bring benefits to the current therapy in relation to oxidative stress. Male Swiss mice experimentally infected with Y strain of *T. cruzi* were treated orally during fifteen days. The obtained results suggest that the association between the substances (AA+BZ10) showed synergy in the reduction of the number of circulating parasites. Besides, a decrease in the levels of intracellular reactive oxygen species (ROS) was observed as well as lipoperoxidation in cardiac tissue, evidenced by the dosage of thiobarbituric acid reactive substances (TBARS). It was noted that the antioxidant alone or in association with BZ significantly decreased the parasitism in the heart detected by qPCR. Likewise, histological analyzes revealed a greater reduction in the inflammatory infiltrate in this organ when the substances were administered concomitantly. Thereby, it is concluded that AA could bring benefit to the treatment of this pathology if associated with reduced doses of BZ. Oxidative parameters reveal that, in the long term, preservation of cardiac tissue should occur. **Supported by:**FAPESP/CAPES **Keywords:**Ascorbic acid; chagas disease; oxidative stress

HP024 - **SOLUBLE CD14 AND MHCII AS BIOMARKERS IN CANINE VISCERAL LEISHMANIASIS**

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Canine visceral leishmaniasis (CVL) is a chronic disease caused by Leishmania infantum wich affects dogs and promotes serious injury leading to death. Dogs are the main reservoir of this parasite, having a central role in the transmission to humans. CD14 is a membrane glycoprotein on monocytes and macrophage and can also be found in soluble form (sCD14). sCD14 is able to activate endothelial cells, assist in the recognition of circulating pathogens and inhibit the macrophage activation decreasing inflammatory response. MHC class II molecules (MHCII) are a class of major histocompatibility complex molecules normally found only on antigen-presenting cells as dendritic cells, mononuclear phagocytes and B cells. As seen for CD14, MHCII can be found in serum in its soluble form. sMHC is involved in maintenance of self tolerance, healthy functioning of the central nervous system and changes in the physiological concentrations of sMHC are associated with pathological conditions such as rheumatoid arthritis, asthma, AIDS and chronic hepatitis C. The identification of biomarkers for prognosis or clinical recovery in dogs would provide insight toward disease pathogenesis and may contribute to improved clinical monitoring. The serum levels of sCD14 and sMHCII in control, assymptomatic and symptomatic dogs were determined by ELISA. Futhermore, we compared levels of sCD14 and sMHCII in symptomatic dogs before and after the treatment with immunoterapy. Concentration of sCD14 and sMHCII were higher in symptomatic dogs compared to assymptomatic and control dogs. Futhermore, both soluble molecules levels were higher during disease and decreased after treatment. These results suggest that these molecules could be good biomarkers for CVL. Future studies may explain the role of these soluble molecules in canine visceral leishmaniasis. **Supported by:**FAPEMIG; CNPq; CAPES
Keywords: Leishmaniasis; scd14; smhcii

HP025 - **ANALYSIS OF MUCIN EXPRESSION IN INTESTINAL CELLS INFECTED WITH GIARDIA LAMBLIA**

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The intestinal parasite *Giardia lamblia*, the ethiological agent of giardiasis, affects millions of people every year worldwide. Giardia is an extracellular parasite of the small intestine and, to colonize the duodenum, the parasite has to pass through a layer of mucus that covers the entire intestinal epithelium. The major components of the intestinal mucus are the mucins. All mucins are characterized by mucin domains which have abundant Ser, Thr and Pro amino acid residues that are heavily O-glycosylated. Previous studies demonstrated that some enteropathogenic organisms, such as the bacterium Escherichia coli, modulate the expression of mucins in the gastrointestinal tract. Considering this, in this work we evaluated the effect of G. lamblia infection on the levels of mucin gene expression in Hutu-80 cells (human duodenal adenocacinoma cell). As host cells are maintained in RPMI medium, initially we evaluated the parasite's viability in RPMI by PI-stain and FACS analysis. Our data show that Giardia is viable in RPMI medium up to 4 h, indicating that parasite-host interaction experiments could be conducted under these conditions. Next, we evaluated the expression of different mucins (MUC2, MUC3 and MUC5AC) by RT-PCR, quantitative PCR (qPCR) and immunofluorescence in cells co-incubated with Giardia trophozoites. The results show increased expression levels of MUC2 and MUC5AC in Hutu-80 infected cells when compared to non-infected control cells. Confirming these results, immunofluorescence analysis using a polyclonal antibody against mucin showed an increase in fluorescence intensity after infection of host cells with Giardia for 4 h. In conclusion, we provide evidence that the co-incubation of Hutu-80 cells with trophozoites for 4 h causes an specific upregulation in the transcription of MUC2 and MUC5AC suggesting a modulatory effect of *Giardia lamblia*. Funded by FAPESP (#2016/11096-7).
Supported by:FAPESP
Keywords: Giardia lamblia; mucin; mucin expression

HP026 - **CD11B+ CELLS AND MONOCYTES ARE IMPORTANT BIOMARKERS IN EXPERIMENTAL LEISHMANIA MAJOR INFECTION UNDER IMMUNOSUPPRESSION CONDITIONS.**

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Immunocompromised patients with leishmaniasis, as co-infected with human immunodeficiency virus, present great possibilities of developing the clinical disease and high rate of relapse and mortality. The resolution of Leishmania infections depends on the activation of T cells, which activate macrophages that control the infection. Monocytes play an important role during infection, since they can kill Leishmania parasites and differentiate into macrophages. Monocytes are usually recruited to the site of infection, which leads to their increasing number in blood. The prognostic indicators for this disease are not good and the characterization of immunological or clinical markers can be used as such, and it is important to reduce morbidity and mortality and to guide potential prophylactic and therapeutic measures, especially in these patients at greater risk. Thus, the aim of our work is to analyze the frequency of CD11b+ cells and blood monocytes by flow cytometry and by counting by optical microscopy in immunosuppressed induced animals, after Leishmania major infection. For this, animals were submitted to treatment with synthetic glucocorticoid Dexamethasone or submitted to chronic restraint stress. Dexamethasone treated and long-term stressed animals became more susceptible to parasite infection manifested by the exacerbation of the lesions with intensification of the inflammatory process and areas of necrosis. Monocytes and CD11b + cells showed decreased frequencies soon after immunosuppression, when the lesions were discrete. When the lesions began to grow, immediately the frequency of these cells also increased in the blood, following the progression of the lesion in the following weeks. This assures them as indicative of cellular responsiveness to the performance of exogenous and endogenous glucocorticoids, acting as good clinical markers in experimental infection by L. major.
Supported by:FAPEMIG; CNPq; CAPES **Keywords:**Immunosupression; leishmaniasis; monocytes

HP027 - **DETERMINATION OF MICRORNA PROFILE OF HUMAN MACROPHAGES INFECTED WITH *LEISHMANIA (L.) AMAZONENSIS***

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The establishment of *Leishmania* infection in macrophages can be modulated by non-coding microRNAs (miRNAs) by post-transcriptional regulation of genes through the complementary binding to the 3' UTR of target mRNA. The production of Nitric Oxide (NO) by nitric oxide synthase 2 (NOS2) versus polyamines production during *Leishmania* infection plays a pivotal role in the parasite survival, since NO production is decreased in detriment of a higher production of polyamines, essential for *Leishmania* replication.
Here, we show the level of expression of 84 miRNAs of human macrophages derived from THP0-1 monocytic cell line infected with *L. (L.) amazonensis* wildtype (*La*-WT) or with *L. (L.) amazonensis* arginase knockout (*La-arg*$^-$). After 4 hours of infection with *La*-WT, 39% of macrophages miRNAs and 18% in the *La-arg*$^-$ infection were modulated. After 24 hours this modulation dropped for 7% in *La*-WT infection and remains stable with 11% modulation in *La-arg*$^-$ infection. After 48 hours, 24% of the miRNAs were modulated in *La*-WT and only 3,6% in *La-arg*$^-$ infection. The miRNA expression was variable during time-course of *La*-WT or *La-arg*$^-$ infection, while the absence of arginase activity promoted differential regulation of miRNA. Some modulated miRNAs were upregulated (such as miR-202, miR-381, miR-302, miR-372 or miR-520d) others showed a reduced expression (miR-29a, miR-29b, miR-29c and miR-340) in *La*-WT and/or *La-arg*$^-$. Similar results were observed in THP0-1 macrophages infected with *L. BRAZILiensis* or *L. infantum* in studies conducted in our lab. These miRNAs present complementarity to the 3'UTR of some targets involved in the polyamine/NO pathways.
In conclusion, *L. (L.) amazonensis* infection alters the miRNA profile of human THP0-1 macrophages, parasites absent of arginase activity modulate different miRNAs expression and this can be an indicative of a molecular mechanism for immune response evasion through the modulation of polyamine/NO production by regulating target mRNAs. **Supported by:**FAPESP
Keywords: Gene expression; microrna; arginine metabolism

HP028 - **THE ERGOSOME OR THE STEROLS PATHWAY? AN OMICS APPROACH TO UNDERSTAND AN ANCIENT DRUG CANDIDATE IN *LEISHMANIA MEXICANA*.**

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Drugs targeting ergosterol have been used for decades to treat Leishmaniasis. Nonetheless, their mechanism of action is not fully understood. We hypothesized that the sterols biosynthetic pathway in trypanosomatids may consist of a multi-enzymatic complex. An Omics approach is used to understand and characterize drug resistant Leishmania mexicana promastigotes selected in vitro. Genomic (NGS), Transcriptomic (RNA-seq, qPCR) and Metabolomic (LC-MS and GC-MS) data are correlated with a resistant phenotype of parasites that were infective in murine macrophages, mice and sandflies. We have identified key changes in metabolites and sterols. Complementary in silico modeling data of the interactome network is being used to identify known PPIs between enzymes in this sterol pathway. We also identified structural differences between the PPIs network reported with the Yeast Split-Ubiquitin system and the STRING database. Some enzymes present in S. cerevisiae, which is the pathway of reference for drugs targeting sterols, are not reported in Trypanosomatids and this has led to a bias in drug discovery. Antifungals targeting the membrane of the parasite have their specificity related to an electron affinity which is in turn determined by structural differences between the parasite sterols (ergosterol) and its counterpart (cholesterol) found in human (mammals). Additional biophysical approaches, such as Membrane Biophysics, Spectroscopy, EPR, SAX and others are needed to complement our findings related to the mechanism of action/resistance of antifungals, and provide clues that cannot be determined by molecular approaches alone.
Supported by: Cnpq **Keywords:**Omics; drug discovery; leishmaniasis

HP029 - **TOLL-LIKE RECEPTORS OPERATE IN THE MIRNA AND MRNA TARGETS INVOLVED IN NO/POLYAMINES PRODUCTION OF LEISHMANIA AMAZONENSIS INFECTED MACROPHAGES**

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The Leishmania-modulation of infected macrophages microRNAs (miRNAs) expression acts post-transcriptionally inhibiting Nitric Oxide Sintase (NOS 2) and consequently the production of nitric oxide (NO). This modulation directs L-arginine for production of polyamines by increasing the expression of host arginase I, that results in the survival and replication of the parasite. The *L. (L.) amazonensis* arginase competes with NOS2 for L-arginine during macrophages infection and the absence of its activity proved to be an attenuating factor of infectivity of BALB/c or C57BL/6 (B6) macrophages. The aim of this work is to investigate whether the signaling via MyD88, TLR2 or TLR4 during infection of macrophages can modulate the composition of the host miRNAs and induce the macrophage to phagocyte and eliminate the parasite. The *L. (L.) amazonensis* (La-WT) infection of BALB/c mice Bone Marrow-Derived macrophage (BALB/c-BMDM) modulated 27% of the 84 miRNAs analyzed when compared to uninfected-macrophages, from these, 78% were upregulated (Muxel, 2017). Similarly, La-WT infection of B6-BMDM modulated 32% of miRNA analyzed, while only 50% were upregulated. The absence of MyD88, TLR2 and TLR4 altered the percentage of miRNAs modulated during La-WT infection. However, La-WT infection of B6-BMDM reduced the levels of mRNA expression of TLR signal molecules and cytokines and cytokines receptors, as Irak1, Map3k1, Myd88, Nfrk, Irf3, Fos, Tlr5, Il1b, Il10, Il6ra. The amount of Nos2 mRNA was higher in La-WT infection of WT-BMDM after 4-24h compared to the non-infected control, and was reduced in the absence of MyD88, TLR2 and TLR4. However, the levels of CAT2B and CAT1 were higher only in in La-WT infection of Myd88-BMDM than WT-BMDM infection, and Arg1 levels was increased in TLR2 absence. We concluded that *L. (L.) amazonensis* infection alters the TLR signaling pathway via macrophages miRNA modulation to subvert the host immune responses
Supported by:FAPESP and CNPq
Keywords: Microrna; toll-like receptor; macrophages

HP030 - ***LEISHMANIA AMAZONENSIS* INFECTION INDUCES MIGRATION OF FIBROCYTES**

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The bone marrow harbors the hematopoietic system being responsible for the origin of different cell types found in peripheral blood. Fibrocytes originate in the marrow, express the pan-leukocyte CD45 protein and produce matrix proteins. Parasites of the *Leishmania* genus in mammalian host may lead to an intense inflammatory reaction with infiltration of blood-derived cells into infected tissues. From this information, we suggest that the fibrocytes correspond to one of the migratory groups in the skin, composing the inflammatory set in the response to *L. amazonensis*. In order to analyze the behavior of fibrocytes in the dermis of BALB/c mice during infection, ear skin fragments were removed, included in OCT and immediately frozen in liquid nitrogen for analysis by fluorescence microscopy. Cryostat sections were obtained, fixed and incubated with the CD45 and HSP47 antibodies, they were developed with secondary antibodies complexed to fluorochromes and analyzed under a microscope equipped with epifluorescence Zeiss Axioplan 2. For analysis by transmission electron microscopy, fragments of the ears skin were fixed and post-fixed, dehydrated and included in PolyBed812 resin. After that, ultrathin sections were analyzed in transmission electron microscope/Jeol JEM-1011. Analyzes showed, from 15 days of infection, an inflammatory cellular infiltrate and internalized amastigotes. Fibrocytes identification in the infected tissue was performed using a double labeling for CD45 and HSP47. Fluorescence microscopy analyzes showed the presence of three distinct cell groups: leukocytes/non-fibrocytes (CD45+/HSP47-);Fibroblasts (CD45-/HSP47+) and fibrocytes (CD45+/ HSP47+) after 15 days of infection. With these analyzes, we detected the progressive fibrocytes migration in the inoculum areas of *L. amazonensis*. Initial evaluations of the production of inflammatory mediators by fibrocytes lead us to assume the probable action of this cell on the immune response in Leishmaniasis.
Supported by: Faperj, IOC / Fiocruz **Keywords:**Fibrocytes; leishmania amazonensis; migration

HP031 - **EVALUATION OF THE ROLE OF B CELL IN LEISHMANIA AMAZONENSIS INFECTION USING BALB/XID MICE**

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Leishmaniasis is a neglected disease and *Leishmania amazonensis* is the etiological agent of diffuse cutaneous leishmaniasis in Brazil. This work aims to study the role of B lymphocytes during the course of *L. amazonensis* infection using BALB/Xid mice that present lower frequency of B1 and B2 lymphocyte compared to wild type (WT) mice, mainly B1. Mice were infected on the footpad and euthanized at 60th day post infection. Our results demonstrated that BALB/Xid mice developed smaller lesions in comparison to control. However, the parasite load obtained from infected footpad, spleen and on the lymph nodes were similar in both groups. The number of B cells on draining lymph nodes from infected BALB/Xid mice was lower compared to WT mice; there were no differences in the percentage of TCD4, TCD8 and TCD4 producing IFNg cells between the two of them and the percentage of Treg cells from BALB/Xid mice lymph nodes were higher than WT. We observed an increase in the percentage of B1a and a decrease of B1b cells on the peritoneal cavity of infected WT mice related to naïve WT mice. The BALB/Xid infection did not affect the frequency of those cells compared to naïve BALB/Xid. BALB/Xid mice had lower levels of IgM and IgG1 in the serum when compared to WT probably due to smaller number of B1 and B2 cells respectivly. Cytokines analysis of infected footpad, draining lymph node and spleen showed a lower IL-10 production by BALB/Xid than WT mice, which could be related to low B lymphocytes numbers and IgG1 reduction. We performed in vitro interaction using peritoneal B1 lymphocytes stimulated by LPS in the presence or absence of *L. amazonensis*. B1 lymphocytes were capable to produce higher levels of IL-10 when exposed to *L. amazonensis* plus LPS compared to the positive control. Together, these data indicate that B cells are associated to murine cutaneous leishmaniasis pathogenesis caused by *L. amazonensis* through production of IgG1 and IL-10. **Supported by:**CNPq
Keywords:B cell; leishmaniasis; pathogenic antibody and cytokine

HP032 - **HIGH LEVELS OF PROTECTION IN MICE IMMUNIZED WITH DIFFERENT STRAINS OF *TOXOPLASMA GONDII* IRRADIATED BY GAMMA RADIATION**

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Toxoplasma gondii is an obligate intracellular parasite capable of infecting warm-blooded animals. Toxoplasmosis is a disease that does not present specific symptoms without serious damage to the host, which explains the significant number of affected individuals. We demonstrate that RH strain tachyzoites irradiated at 255Gy, do not cause infection in the host, inducing immunity as a natural infection. In this study, mice were immunized by parenterally route with three biweekly doses of irradiated 255Gy ($Cobalt^{60}$) *T. gondii* tachyzoites RH, VEG and RH+VEG. Two weeks after last dose, we evaluated antibodies responses (IgG, IgM and IgA), IgG subclasses (IgG1, IgG2a and IgG2b) and the protection was measured by numbers of brain cysts, 30 days after challenge. By ELISA all immunized models presented antibodies production in their serum with higher IgG production in all groups immunized with irradiated parasites. IgM and IgA antibodies production was similar and at lower levels in relation IgG antibodies. The evaluation of IgG subclasses was characterized by a TH1 response by increased production of immunoglobulin of type IgG2a and IgG2b. All immunized groups presented significant protection when challenged with ME-49, however, 255Gy RH+VEG group showed higher protection, with three negative animals on brain microscopic analysis and two negative animals by real-time PCR. Our results show that irradiated parasites, in association with the different strains, presents an efficiency immune response with high protection and could help in the design of an efficient vaccine candidate to interrupt the transmission chain of toxoplasmosis. **Supported by:**FAPESP
Keywords: Toxoplasma gondii; ionizing radiation; protection

HP033 - **LEISHMANICIDAL ACTIVITY OF ENDOPHYTIC FUNGI EXTRACTS ISOLATED FROM THE PLANT *ANEMIA TOMENTOSA***

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INTRODUCTION: Leishmaniasis is a neglected disease that affects 12 million people in 89 countries, and causes 70,000 deaths per year mainly in tropical and sub tropical regions. Leishmania sp is the parasit proctozoan responsible for the disease. During its life cycle it has two evolutionary forms: amastigote, an obligatory intracellular parasite that infects mainly macrophages of vertebrate hosts, and promastigotes an intestinal parasite of Phlebotomine sandflies. The disease has two main clinical manifestations, cutaneous and visceral leishmaniasis. Actually, treatment of leishmaniasis is dependent on pentavalent antimonials and amphotericin B, the latter being a natural compound that is synthetized by bacteria, but both present high toxicity, complexity of the route of administration, extensive treatment period, high cost, failure of distribution and resistance of some species. AIM: Evaluate the potential leishmanicida action, in *Leishmania amazonensis*, from the fraction ethyl acetate of crude extract of endophytic fungi isolated from the root (ATR2A), leaf (ATF1,2 e 4A) and spore (ATE1, 2 e 3A) of plant *Anemia tomentosa* collected in Rio de Janeiro State. METHODOLOGY: Promastigotes of *L. amazonensis* were incubated with different concentrations of extracts from endophytic fungi and its cellular viability was measured by MTT assay and analyzed by spectrophotometry after 48 hours. RESULTS: Some fungi extracts demonstrated leishmanicide capacity in a dose-dependent manner, specially ATE1A, ATE2A, ATF1A and ATF2A. The most effective extract, ATE2A, induced 50% parasite death at 20 µg/mL concentration. CONCLUSION: It was concluded that the extracts of endophytic fungi have leishmanicide capacity. More experiments need to be performed to determine the extract with best activity and the subfraction responsible for the activity as well as toxicity in macrophages.
Supported by:CAPES; FAPERJ
Keywords: Leishmanicidal activity; promastigote; natural products

HP034 - **ANTIMALARIAL ACTIVITY OF S-FARNESYLTHIOSALICYLIC ACID ANALOGUES STUDIED IN *PLASMODIUM FALCIPARUM* TRANSFECTED WITH ULTRA BRIGHT NANOLUC**

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Malaria is the most important parasitic disease of humans. As a consequence of increasing parasite resistance to antimalarial compounds, new approaches to drug design are needed. Our group studies the biosynthesis of several secondary isoprenoid compounds in *Plasmodium falciparum*. Previously, our group selected some terpenes and the antineoplasic modified terpene, S-farnesylthiosalicylic (FTS), to be tested on cultures of the intraerythrocytic stages of *P. falciparum*. The IC_{50} value of FTS was found to be 14 µM. FTS and most terpenes tested inhibited the biosynthesis of ubiquinone and dolichol in the schizont stages when ^{3}H farnesyl pyrophosphate was used as precursor. At the same time, treatment of schizont stages with S-farnesylthiosalicylic acid decrease in intensity the band corresponding a p21ras protein. This is the same antineoplasic mechanism which is supposed to be acting in mammal cells. In the present study we synthetized and tested 39 FTS analogues for its antimalarial activity in vitro (24, 48 and 72h of treatment starting at ring stage). By this purpose we used exogenous luciferase based methodology. Exogenous luciferases are a powerful tool that has been applied in studies of several aspects of parasite biology and high througHP0ut growth assays. We used a *Plasmodium falciparum* expressing the NanoLuc (Nluc) assays which previously showed at least 100 times brighter than the commonly used firefly luciferase, a low cost and a better sensibility. IC_{50} values obtained by this methodology and Smilkstein fluorimetry were compared using two antimalarial compounds (chloroquine and artesunate) at different hematocrit and culture conditions. Modifications in some FTS analogues improved its antimalarial activity (IC_{50}: 3-5 µM). Preliminary results in human cells show that the new FTS analogues have promissory therapeutic index. **Supported by:**FAPESP / CNPq / Agencia Nacional de Promocion Científica y Tecnologica-PICT, Argentina.
Keywords: Plasmodium falciparum; terpenes ; antimalarial

HP035 - **ACTIVATION OF TLR3-TRIF SIGNALING PATHWAY CAUSED BY THE PROTOZOAN *NEOSPORA CANINUM***

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Neospora caninum is an intracellular parasite that has the dog as its definitive host and other mammals, especially cattle, as intermediate hosts. This parasite is closely related to *Toxoplasma gondii*, and has been studied in recent decades for causing important disease in veterinary medicine with induction of relevant clinical signs, as abortions in cattle and neuromuscular paralysis in dogs. The aim of this study was to evaluate the specific role of TLR3-TRIF-IRF3 signaling pathway in *N. caninum* infection. For this, were performed genic expression assays on murine macrophages to investigate if *N. caninum* is able to modulate TLR3 and IRF3 expression. For the study of adapter molecule TRIF, were performed *in vitro* experiments with bone marrow derived macrophages (BMDMs) from C57BL/6 wild-type (WT) and TRIF knockout (TRIF-/-) mice stimulated by tachyzoites. In addition, *in vivo* infections were performed in order to investigate the production of cytokines, cellular and tissue parasitism, histological changes during different phases of infection and survival analysis. Differently of *Toxoplasma gondii*, *N. caninum* RNA increases the expression of TLR3 e IRF3. We observed that TRIF-/- BMDMs presented notable defects in inflammatory cytokine production in relation to WT macrophages. Additionally, we found that the concentration of NO, IL-12p40, IFN-y and TNF were decreased in peritoneal fluids and lungs of TRIF-/- mice compared to WT. Higher parasite burden was observed in peritoneal cells, lungs and brain during the acute and chronic phases of infection, which were associated with inflammatory changes in the analyzed tissues. Furthermore, TRIF-/- mice survival rate decreased 2-fold compared to WT. In conclusion, TLR3-TRIF signaling pathway traditionally known to act on viral infections also has important involvement in infection by protozoan *N. caninum*, participating in the support of Th1 immune response. **Supported by:**CAPES, FAPEMIG, CNPq **Keywords:**Tlr3; trif; neospora caninum

HP036 - **COMPARATIVE ANALYSIS OF BIOLOGICAL AND MOLECULAR ASPECTS OF LEISHMANIA INFANTUM ISOLATES**

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Leishmaniases are diseases with a wide variety of clinical manifestations, which are dependent on the *Leishmania* species and the host's immune status. *Leishmania infantum* is one of the species that causes the visceral leishmaniasis (VL) and is present in the Old World (OW). *L. chagasi* is responsible for VL in Latin America, however, was considered identical to *L. infantum* from the molecular point of view. Few studies address the biological differences, as well as the behavior of these strains during infection. Nevertheless, many studies seek a better understanding of host-parasite relationship. Our group has studied this relationship with the focus on the metabolism of L-arginine and mainly the nitric oxide synthase (NOS) and arginase (ARG) enzymes, which can act directly on death, through nitric oxide (NO) production, or in the survival and multiplication of the parasite in macrophages, through the polyamines production. Based on these data, the main objective of this work was to analyze the biological and molecular differences between two *L. infantum strains* (*Li*OW and *Li*NW), as well as to understand the role of arginase/NOS in the parasite-host relationship. Therefore, the *Li*OW strain showed more infective than the *Li*NW strain both *in vivo* (during infection on BALB/c and Swiss Webster mice) and in vitro. In addition, BALB/c mice were more susceptible to infection than Swiss mice. In the spleen and liver cells of the animals infected by both strains, a difference in NOS and ARG activity occurred. In vitro, promastigotes of *Li*OW isolated from BALB/c and Swiss Webster mice showed higher ARG activity than those *Li*NW during the growth curve, however, no difference was observed in intracellular NO production between these strains. With this work, it can be concluded that although *L. chagasi* strains are considered identical to *L. infantum* strains, both have different biological and molecular behavior. **Supported by:**CNPq, FAPERJ, FIOCRUZ **Keywords:**L. infantum strains; nitric oxide synthase; arginase

HP037 - **THE INFLUENCE OF A RNA BINDING PROTEIN ON GENE EXPRESSION CONTROL OF THE AMINO ACID TRANSPORTER AAP3 IN LEISHMANIA (L.) AMAZONENSIS.**

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The RNA binding-proteins (RBPs) are known by its fundamental role in mRNA processing control, mRNA stability as well as in protein translation, by their interaction with the 3'-untranslated regions (3'UTR) of the regulated mRNA. Studies have shown that RBPs can influence the cell metabolism in trypanosomatids in cellular cycle control, differentiation of promastigotes into metacyclics or amastigotes and also in post-transcriptional regulation of gene expression. In an L-arginine starvation midst, the L. amazonensis AAP3 transporter transcript (La-aap3) presents an increase in its half-life resulting on an increase in L-arginine uptake (Castilho-Martins et al 2011). The starvation also led to the increase LinJ 04.0040 transcript, which encodes for a putative RBP (Goldman-Pinkovich et al, 2016) in L. donovani. We performed experiments to determine the L. amazonensis LinJ 04.0040 homolog RBP (La-RBP) expression in L-arginine starved parasites in comparison with the AAP3 expression. We compared the amount of La-AAP3 transporter and La-RBP homolog transcripts expressions during growth curve of L. amazonensis promastigotes. We also submitted mid-log promastigotes of L. amazonensis WT to L-arginine starvation and temperature and pH variations according to promastigotes or amastigotes growth conditions (25°C or 34°C and medium pH 5.5 or 7), to measure the transcripts. We confirmed an increase of the levels of La-aap3 and La-RBP transcripts in stationary phase compared to log phase in WT cells. The levels of La-RBP were lower in stationary phase of the L. amazonensis arginase knockout parasite (La-arg-), than in log phase. The levels of the La-RBP transcript correlated with the increased levels of La-aap3 transcripts in stationary phase WT promastigotes. The levels of La-aap3 mRNA changed on variable pH and temperature conditions and La-RBP also changed in the same conditions. These observations may be an indicative of this RBP functional role in regulating AAP3 expression. **Supported by:**FAPESP
Keywords: Leishmania; starvation; rbp

HP038 - **NITROHETEROCYCLIC DRUGS CURE EXPERIMENTAL TRYPANOSOMA CRUZI INFECTIONS MORE EFFECTIVELY IN THE CHRONIC STAGE THAN IN THE ACUTE STAGE**

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The insect-transmitted protozoan parasite Trypanosoma cruzi is the causative agent of Chagas disease, and infects 5-8 million people in Latin America. Chagas disease is characterised by an acute phase, which is partially resolved by the immune system, but then develops as a chronic life-long infection. There is a consensus that benznidazole and nifurtimox are more effective against the acute stage in both clinical and experimental settings. However, confirmative studies have been restricted by difficulties in demonstrating sterile parasitological cure. To increase the accuracy and reproducibility of drug testing, we developed highly sensitive bioluminescence methodology based on the expression by trypanosomes of a red-shifted luciferase reporter. This in vivo imaging procedure has a limit of detection of 100-1000 parasites, and facilitates the real-time tracking of parasite burden in individual mice during long-term experimental infections. Here, we describe the use of this predictive model to undertake a detailed comparison of the efficacy of the nitroheterocyclic agents against acute and chronic T. cruzi infections. Unexpectedly, we find both drugs are more effective at curing chronic infections, judged by treatment duration and therapeutic dose. This was not associated with factors that differentially influence plasma drug concentrations in the two disease stages. We also observed that fexinidazole and fexinidazole sulfone are more effective than benznidazole and nifurtimox as curative treatments, particularly for acute stage infections. In summary, the application of highly sensitive imaging technology to predictive models of Chagas disease provides new insights into drug efficacy. Of particular importance is the finding that T. cruzi infections are more readily cured in the chronic stage. If these findings are translatable to human patients, they will have important implications for treatment strategies. **Supported by:**Drug for Neglected Disease Iniciative (DNDi) **Keywords:**Trypanosoma cruzi; treatment; nitro drugs

HP039 - ***PLASMODIUM BERGHEI* ANKA INFECTION INDUCES ERYTHROCYTIC APOPTOSIS IN WISTAR RATS**

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Severe malarial anemia is one of the most common complications of malaria and a great problem of public health. Although pathophysiology of anemia in malaria is not fully understood, it is known that premature elimination of non-parasitized RBCs (nRBCs) plays an important role. In this context, we previously showed that lethal experimental infection of Plasmodium yoelii 17XL in mice increases the levels of apoptotic nRBCs, a phenomenon that could be could be related to anemia. However, the high parasite loads in *P. yoelii* infection limited our analysis concerning apoptosis and anemia. In the present study, therefore, we investigate erythrocytic apoptosis during infection of *P. berghei* ANKA in Wistar rats; a model of malarial anemia poorly explored that courses with low parasitemia and acute anemia similar to human malaria. Female rats were intraperitonially inoculated with *P. berghei* ANKA-pRBC (pRBC) expressing GFP (Green Fluorescent Protein) and, then, parasitemia and haemoglobin levels were monitored during 26 days post-infection using flow cytometry and hemoglobinometer, respectively. In parallel, apoptosis of nRBCs was estimated detecting phosphatidylserine exteriorization by flow cytometry using annexin V-APC. As expected, *P. berghei* ANKA infection was marked by low levels of parasitemia that reached a mean peak of 3% on day nine post-infection and solved spontaneously. A significant reduction of the haemoglobin levels (~30%) was also observed on days subsequent to the peak of parasitemia, persisting until day 16 pos-infection. In apoptosis assays, it was possible to observe a significant increase in the levels of nRBC apoptosis, which coincided with the reduction of hemoglobin levels and was positively related to anemia. These results confirm our previous studies that evidenced a proapoptotic effect of malaria infection in nRBCs, as well as suggest that apoptosis can be implicated in the pathogenesis of malarial anemia. **Supported by:**Faperj and CNPq
Keywords: Malaria ; anemia; apoptosis

HP040 - ***PLASMODIUM FALCIPARUM*** **ANTIGENS MODULATE EXPRESSION OF SIGNAL REGULATORY PROTEIN α (SIRPα) ON MONONUCLEAR CELLS**

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Malaria is a vector-borne infectious disease caused by protozoa of the genus *Plasmodium* that infect red blood cells in vertebrate hosts. Parasite toxins released in bloodstream during erythrocytic cycle induce strong systemic pro-inflammatory response that, in turn, can limit parasite development. However, it is known that malaria parasites have evolved multiple mechanisms to evade host immune defenses, including production of antigens that modulate cells of innate immune response. SIRPα is a membrane receptor present on myeloid cells, such as monocytes and dendritic cells, that mediates downregulatory signals leading to inhibition of both phagocytic processes and inflammatory response, events related to effective response against parasites. Thus, the present study attempts to investigate if malaria infection modulates expression of SIRPα in cells of innate imune system. Human peripheral blood mononuclear cells (PBMC) from healthy individuals were incubated in presence of lipopolysaccharide (LPS) or schizont antigens obtained from Plasmodium falciparum in vitro culture and, then, expression of SIRPα was evaluated by flow cytometry using anti-SIRPα monoclonal antibodies. As expected, LPS showed na inhibitory effect on expression of SIRPα in the population of monocytes characterized by cell morphology in flow cytometry analysis. Conversely, P. falciparum antigens induced a significant increase in SIRPα expression on these cells after 24h of incubation, suggesting that malaria parasites could explore inhibitory receptors, such as SIRPα, to suppress antiparasite immune responses. Additional studies with PBMC from malaria patients are being performed to better understand the modulatory effects of malaria infection on SIRPα. **Supported by:**Faperj and CNPq **Keywords:**Malaria ; innate immunity; sirpα

HP041 - **TRYPANOSOMA CRUZI METACYCLIC STAGE-SPECIFIC SURFACE MOLECULE GP90 INHIBITS HOST CELL LYSOSOME SPREADING AND DOWN REGULATES PARASITE INVASION**

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Trypanosoma cruzi metacyclic trypomastigote (MT) forms express the surface molecules gp82 and gp90 that play critical roles in the process of host cell invasion and in oral infection in mice. Both gp82 and gp90 bind to target HeLa cells in a receptor-mediated manner and function as mediator and down regulator of parasite internalization, respectively. This study aimed at investigating the mechanism by which gp90 exerts its down modulatory effect. Highly invasive CL strain MT expressing gp82 and low gp90 levels induced HeLa cell lysosome spreading, an event required for parasite internalization, whereas poorly invasive G strain MT expressing high gp82 and gp90 levels had no effect on lysosome mobilization. A recombinant protein containing the full-length gp82 sequence (r-gp82) induced HeLa cell lysosome spreading from the perinuclear area to the periphery, with a tendency to accumulate at the cell borders, which are the preferential sites for MT invasion.The lysosome mobilization-inducing effect of r-gp82 was counteracted by the native gp90 or by a recombinant protein containing the conserved C-terminal domain of gp90 (r-gp90C). Incubation of HeLa cells with r-gp82 resulted in increased expression of the major lysosome membrane protein LAMP2 and this effect was reversed in the presence of r-gp90C. Both the native gp90 and r-gp90C, at 10 µg/ml, significantly inhibited CL strain MT entry into HeLa cells. Assays using 20-mer synthetic peptides spanning the C terminal domain of gp90 revealed that the sequence GVLYTADKEW is involved in MT-target cell interaction and down regulates parasite invasion. Taken together, these data plus the finding that in a mixed infection CL strain MT internalization was inhibited by G strain MT suggest that the inhibition of target cell lysosome spreading is the mechanism by which the gp90 molecule exerts its down regulatory role. **Supported by:**FAPESP, CNPq
Keywords: Trypanosoma cruzi; lysosome spreading; lysosome membrane protein

HP042 - **HISTONE DEACETYLASE INHIBITORS AS A NOVEL APPROACH FOR THE TREATMENT OF TOXOPLASMOSIS**

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Toxoplasmosis is a cosmopolitan zoonosis, caused by the obligate intracellular protozoa *Toxoplasma gondii*. *T. gondii* infection can cause uveitis, congenital diseases and encephalitis in immunocompromised individuals. The treatment for toxoplasmosis is restricted to few drugs, which are commonly related to several side effects. Besides, the available drugs are only effective against the acute stage of the disease and do not promote the parasitological cure the chronic stage of the disease. Thus, the discovery of new effective treatments for toxoplasmosis is necessary. Recent studies show that the histone deacetylase inhibitors are potential chemotherapeutic agents for the treatment of parasitic infections. In this work, we evaluated the anti-*T. gondii* in vitro effects of four histone deacetylase inhibitors (KV24, KV30, KV46 and KV50). For that, LLC-MK_2 monolayers were infected with tachyzoites of *T. gondii* RH strain and then treated with different concentrations of the compounds for 24. Three compounds showed high activity against *T. gondii* resulting in IC50s at nanomolar range: KV46 (220nM), KV30 (110nM) and KV24 (220µM). Cytotoxicity studies against LLC-MK_2 by the MTS assay also showed that KV46, KV30 and KV24 have high selectivity for *T. gondii*. Analysis of the cellular effect of KV46 against *T. gondii* by transmission electron microscopy showed that the treatment with 3µM KV46 for 24h affected parasite division process and induced subpellicular microtubule disorganization and conversion of tachyzoites to bradyzoites. These results indicate that KV24, KV30 and KV46 are promising for the treatment of toxoplasmosis. **Supported by:**CNPq, Capes and Faperj
Keywords: Toxoplasma gondii; histone deacetylase; chemotherapy

HP043 - **CARACTERIZAçãO DA PROTEíNA QUINASE DO FATOR DE INICIAçãO DA TRADUçãO 2 DE *TRYPANOSOMA CRUZI***

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Trypanosomes have to adapt to diverse environmental conditions. Most of these modifications occur through changes in RNA processing, RNA stability and protein synthesis, which leads to the preferential expression of proteins that act in the stress recovery. This control occurs during protein synthesis initiation through the eukaryotic initiation factor 2 (eIF2), which carries a GTP and the initiator tRNA required for the scanning and addition of the first methionine to the translating mRNA. eIF2-GDP is then released from the newly formed ribosome. To start a new round of translation initiation, the GDP has to be replaced by a GTP, which is inhibited by the phosphorylation of the alpha subunit of eIF2 (eIF2α) by specific protein kinases activated by different cellular stresses. Trypanosomes present three protein kinases (K1, K2 and K3) with characteristics of eIF2-kinases. We previously found that growth and dealing with oxidative agents are dependent of the Trypanosoma cruzi K2 (TcK2). This enzyme has a similar topology when compared with the mammalian kinases know as PERK, for which several inhibitors have been developed. Therefore, the goal of our work was to test these inhibitors against TcK2 as a possible target to treat Chagas' disease. We generated several recombinant proteins harboring the kinase domain of TcK2 in Escherichia coli. We found that some of these recombinants were soluble and displayed phosphorylating activity against mammalian eIF2α, detected by specific monoclonal antibodies. We then optimized a screening assay to test the available and other compounds as potential enzymatic inhibitors, which could be used against proliferative or intracellular forms of T. cruzi. **Supported by:**FAPESP
Keywords: Trypanosoma cruzi; inibidores ; proteína quinase

HP044 - **INHIBITION OF APOPTOSIS AND SURVIVAL OF *TRYPANOSOMA CRUZI*-INFECTED CELLS IS MEDIATED BY LECTIN GALECTIN-3 THROUGH ITS MOBILIZATION BETWEEN CYTOPLASM AND NUCLEUS, INFLUENCING THE BCL-2/BAX APOPTOSIS SIGNALING PATHWAY.**

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Introduction: Studies have shown that Galectin-3 (Gal-3) may participate in pathways of apoptosis. Our recent results demonstrated that Gal-3 may inhibit the apoptosis during the infection by *Trypanosoma cruzi,* the etiologic agent of Chagas disease. Objectives: We carry out experiments to investigate the role of Gal-3 over apoptosis signaling pathways in HeLa cells infected by *T. cruzi.* Methods: HeLa cell, HeLa-shGal3 (depleted of Gal-3 by RNAi), and HeLa-scramble (negative control of RNAi) were infected by *T. cruzi* followed by functional analysis: (1) cell viability assay with or without induction of death, (2) western blot and qPCR to determine the levels of pro- e anti-apoptotic proteins, (3) and immunolocalization of Gal-3 and Bax. Results: Lineage HeLa-shGal3 infected showed a reduced viability, demonstrated that the presence of the parasite promotes proliferative stimuli to cells of Gal-3-dependent manner. Another approach using etoposide as an induction agent of death revealed that *T. cruzi* can mitigate and subvert the pro-death stimuli in lines with endogenous levels of Gal-3 but not HeLa-shGal-3. In the absence of Gal-3, changes occur in kinetics of apoptotic mediators such as Bcl-2 and Bax, with a reduction in the levels of these mediators during infection. Moreover, a concentration of Gal-3 was detected in the cytoplasm during the first hours of infection with translocation to the nucleus 8 hours after infection, we believe that Gal-3 cytoplasmic stocks can associate with Bcl-2 and Bax inhibiting the intrinsic apoptotic pathway in the short-term, whereas nuclear stocks could be signaling for pro-survival pathways later in infection. Conclusions: We believe that parasite appropriates of the signaling pathways of the host cell in its favor, positively regulating the functions of Gal-3 related to survival and apoptosis inhibition, keeping the host cell alive for sufficient time for replication of amastigotes and differentiation of these in trypomastigotes.

Supported by:IFRJ-Prociência

Keywords: Trypanosoma cruzi; apoptosis; host-parasite interaction

HP045 - **ADDITIVE EFFECT OF LOPINAVIR AND MILTEFOSINE ON INTRACELLULAR *LEISHMANIA INFANTUM* AMASTIGOTES**

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The search for new treatments against leishmaniasis is still an urgency due to the high frequency of drug resistance registered in endemics areas, side effects of available chemotherapeutic compounds, and complications caused by co-infection with HIV. An interesting strategy for improving life quality of co-infected patients is drug combination therapy. Studies have shown that drugs association can be very effective, reducing side effects and decreasing the induction of resistance. Lopinavir is an HIV peptidase inhibitor used clinically for AIDS treatment, which also showed activity over other pathogens, such as fungi and protozoa, including Leishmania. Up-to-date, for visceral leishmaniasis treatment, the only drug available for oral treatment is miltefosine, which has been widely used in India, and presents cure rates of approximately 94%. However, after a decade of use, several cases of recurrence, decreased efficacy and side effects of treatment have been described. Here, we investigated the effect of the combined use of lopinavir and miltefosine over the development of intracellular amastigotes of *Leishmania infantum* in mouse peritoneal macrophages for 72 h. The drug interactions were assessed using the isobologram method and fractional inhibitory concentrations index (FICI). The sum of FICI (ΣFICIs) and the overall mean ΣFICI were calculated for drug association. The mean ΣFIC value was 0.78 ±0.39, which indicates an additive effect of the compounds. This data showed that combination of lopinavir and miltefosine is effective against intracellular amastigote of *L. infantum*. These in vitro results are promising and encourage in vivo studies, which may help to improve HIV-Leishmania co-infected patients therapy.

Supported by:CNPq, CAPES, FAPERJ and Fiocruz.

Keywords: Leishmania infantum; lopinavir; miltefosine

HP046 - **CHARACTERIZATION OF *LEISHMANIA* SP. STRAINS CAUSING CANINE VISCERAL LEISHMANIASIS IN DIFFERENT ENDEMIC REGIONS FROM THE MINAS GERAIS STATE**

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Leishmaniasis is a vector-born disease widely spread throughout Brazil's territory caused by parasites of the *Leishmania* genus. In the New World, *L*. (*Leishmania*) *infantum* infection is associated to the visceral clinical manifestation of the disease in dogs and humans, although some recent studies also point a possible role of *L*. *amazonensis* in this form of the disease. Dogs are the main reservoirs of the visceral form, and are mostly asymptomatic or can present a range of symptoms. Thus, the tracking of seropositive dogs followed by the isolation and characterization of *Leishmania* isolates circulating in endemic regions represent important approaches for a better understanding of the biological behavior of these parasites and association that exists between the clinical status of the disease and the *Leishmania* populations circulating in the area. To this aim, dogs from endemic regions of the Minas Gerais state are being clinically evaluated and their blood samples submitted to serological assays such as immunochromatographic (SNAP *Leishmania* – IDEXX, Maine, USA) and ELISA, which employs KDDR as primary antigen to detect *Leishmania* infection. So far, we have analyzed approximately 100 samples from São Joaquim de Bicas city, Minas Gerais state, Brazil, and our results indicate that the prevalence rate assessed using both tests are around 30%. The positive dogs are being clinically followed up and will be submitted to a bone marrow aspiration that will allow the isolation of the parasites in NNN medium. *Leishmania* species implicated in the infection will be identified using a panel of species-specific primers developed by our research group. **Supported by:**FAPEMIG, CAPES, CNPq, UFMG

Keywords: Visceral leishmaniasis; dogs; leishmania sp. strains

HP047 - **ENDOSYMBIONTIC DSRNA VIRUS WORSENS LEISHMANIA INFECTION BY LIMITING NLRP3 INFLAMMASOME ACTIVATION THROUGH A TLR3-TRIF-IFN-β-AUTOPHAGY PATHWAY**

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Introduction: Leishmaniasis is a disease that affects millions of people worldwide. Depending on the immune response and intrinsic parasite factors, individuals infected with L. guyanensis may develop mucocutaneous lesions, a more severe form of the disease. L.g. may harbor an endosymbiontic double stranded RNA virus, the Leishmania RNA virus (LRV). However, its relevance in Leishmaniasis has remained largely unexplored. Thus, the aim of this work is to evaluate whether LRV modulates the innate immune response against Leishmania infection.

Methods and results: Nlrp3-/- deficient mice were previously shown to be more susceptible to Leishmania infection than WT mice. However, we found that NLRP3 is dispensable for infection with L. guyanensis M4147, a strain that harbors high levels of LRV (L.g.+). In order to study the impact of LRV in disease progression, we ramdomly generated a M4147-derived clone that does not contain LRV (L.g.-). Upon infection with these two different clones, we found that LRV favor parasite replication in WT mice and macrophages (BMDMs). Interestingly, Nlrp3-/- mice infected with L.g.- displayed increased lesion size and parasite titers in vivo and in vitro. In BMDMs, LRV induces high levels of cytokines such as type I IFN, and a reduced caspase-1 cleavage and IL-1β release. These effects were not observed in Tlr3-/- and Trif-/- BMDMs. Mechanistically, we determined that autophagy was strongly induced by L.g.+, but not by L.g.-, and addition of poly:IC or IFN-β restored autophagy induction in L.g.- parasites. Accordingly, inflammasome blockage by L.g.+ was null in Atg5-/- macrophages. Finally, we determined that LRV induces NLRP3 and ASC degradation via TLR3 and autophagy.

Conclusion: Mechanistically, LRV induces a TLR3-TRIF-IFNβ-autophagy pathway that impairs inflammasome activation by L. g., showing an evasion mechanism that results in increased parasite replication and survival. **Supported by:**FAPESP

Keywords: Leishmania rna virus; innate immunity; inflammasomes

HP048 - **EXPRESSION OF AMINO ACID PERMEASE 3 IN LEISHMANIA AMAZONENSIS DEPENDS OF L-ARGININE AVAILABILITY AND ARGINASE ACTIVITY**

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Leishmania alternates its life cycle between the invertebrate and the mammalian hosts. These environmental changes submit the parasite to dynamic undergo modifications in morphology, metabolism, cellular signaling and gene expression to allow for rapid adaptation to the new conditions. Leishmania uses the amino acid L-arginine as a substrate for the arginase enzyme, as part of polyamine pathway, which is used by the parasite to replicate and is essential to establish the infection in the mammalian host. L-arginine is not synthesized by the parasite, so the amino acid uptake occurs by an amino acid permease 3 (AAP3), encoded by two gene copies arranged in tandem on the genome (5.1 and 4.7 aap3). In this work, we described the expression of amino acid transporters expression in L. amazonensis wild type (La-WT) and L. amazonensis arginase knockout (La-arg-), by RNA-seq. In addition, we characterize the AAP3 function in parasites maintained in pH and temperature conditions that simulated both the insect and mammalian micro-environment, and also submitted the parasites to amino acid starvation, simulating mid-gut starvation, as a signal for promastigote metacyclogenesis. Our results demonstrated a down-regulation of 5.1 and 4.7 aap3 transcripts, and other 18 amino acids transporter regulated in the comparison of La-WT vs. La-arg- promastigotes and axenic amastigotes. These results suggest that depending on the amino acid pool and arginase activity, Leishmania senses and could use an alternative route for amino acid transport in response to stress signaling. We also characterized the behavior of the parasite in environmental changes of pH and temperature at the mRNA and protein level. In addition, we demonstrated AAP3 localization in the plasma membrane and in the glycosome of L. amazonensis promastigotes, indicating that L-arginine uptake is directed to this organelle as a strategy to control Leishmania infection by inhibiting L-arginine flux into the glycosome. **Supported by:**FAPESP and CNPq
Keywords: Leishmania ; amino acid transport; arginase

HP049 - **HETEROLOGOUS EXPRESSION OF *TRYPANOSOMA CRUZI* MUCINS I AND II BY *TRYPANOSOMA RANGELI*, A NATURAL KNOCKDOWN MODEL FOR MUCIN-LIKE GLYCOPROTEIN STUDIES**

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Mucin-like glycoproteins are abundant molecules on the surface of *T. cruzi*, acting as acceptors of sialic acid transferred from host cell via trans-sialidase. This set of molecules allows the parasite to attach to the host cell membrane prior internalization. *T. rangeli* is a non-pathogenic hemoflagellate protozoan parasite, phylogenetically closer to *T. cruzi*, capable of infecting the same mammalian host species and being transmitted by triatomine vectors. In contrast to *T. cruzi* (CL Brener strain) that presents ~992 genes coding for mucins and has an active trans-sialidase,*T. rangeli* lacks a functional trans-sialidase and presents 15 mucin-coding genes (SC58 strain), but only a single 33 kDa mucin expressed by the trypomastigote form has been detected by mass spectrometry. So far, the variable and complex genomic architecture among *T. cruzi* strains have impaired the studies of these multicopy gene families. Since activity of *T. rangeli* mucins as sialic-acid acceptors is unknown, we thus propose to assess the phenotypic changes on infectivity of *T. rangeli* expressing *T. cruzi* mucins (types I and II). Initially we have characterized in silico the *T. rangeli* mucins, having found 10 distinct ORFs, among which, four have shown the canonical mucin elements (signal peptide,GPI-anchor addition site and O-glycosylation), among which, three seem to be orthologous to *T. cruzi* mucins and could be expressed by *T. rangeli*. Aiming heterologous expression of *Tc*MUC by *T. rangeli,* T. cruzi mucins type I – *Tc*MUCI (TriTrypDB: TcCLB.511149.130) and type II – *Tc*MUCII (TriTrypDB: TcCLB.507357.130) and their respective 3'end UTRs were cloned into pTREX-mRFP plasmids. So far, expression of *Tc*MUCI and II by *T. rangeli* (Choachí strain) was achieved. Antigenicity analysis of *Tc*MUCI and II was performed and three distinct peptides were synthesized to generate a polyclonal antiserum aiming the study of the expression levels and sites.
Supported by:CAPES, CNPq, FINEP and UFSC
Keywords: Mucin; sialic acid; trans-sialidase

HP050 - **THE *AEDES AEGYPTI* FOXO PATHWAY INFLUENCES THE MOSQUITO'S GUT HOMEOSTASIS AND BACTERIAL DIVERSITY**

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Aedes aegypti is the emerging mosquito species in urban areas with the greatest impact on public health, being the only vector able to transmit the arboviruses dengue, zika and chikungunya. For this reason, understanding the molecular mechanisms governing vector-virus interaction is urgently needed. As found in other organisms, the FoxO pathway has important roles in metabolism, reproduction, stress tolerance, life span and immunity. The akt kinase is a negative regulator of the FoxO pathway, which phosphorylates the transcription factor foxO and prevents its nuclear translocation. Considering that classical immunity pathways are widely conserved among insects, the FoxO pathway appears as an important yet unexplored target for the elucidation of antiviral responses mediated by the intestinal microbiota of *A. aegypti*. Our purpose was to evaluate the transcriptional profile in *akt* silenced *A. aegypti*, as well as to analyze, at taxonomic levels, the influence of akt knockdown (KD) on the mosquito microbiota. We have shown that *akt* silencing resulted in the activation of essential genes for the maintenance of the mosquito homeostasis, influencing its intestinal microbiota and antiviral responses. Furthermore, the transcriptome of these *akt* KD mosquitoes also revealed the modulation of 345 genes. Notably, it was observed a strong down-regulation of 11 prophenoloxidases (PPOs), in addition to a potent up-regulation of genes coding to NADH dehydrogenase subunits, compared to the respective control groups. *Akt* silencing led to a decrease in bacteria from the Burkholderiales order, conversely favoring those of the Pseudomonadales, suggesting that the FoxO pathway plays an important role in modulating the diversity of the gut microbiota. This study opens new avenues to the understanding of the molecular mechanisms controlling *A. aegypti* gut homeostasis and its influence on bacterial proliferation and vector competence. **Supported by:** FAPESP
Keywords: Foxo pathway; microbiota; functional genomics

HP051 - **PURIFICATION PROCESS FOR NATIVE HUMAN IGG, IGM AND IGA ON MOLECULAR EXCLUSION CHROMATOGRAPHY FOR PRODUCTION OF SECONDARY ANTIBODIES USEFUL IN ASSAYS OF MUCOSAL ANTIBODY DETECTION FOR TOXOPLASMOSIS**

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Commercial toxoplasmosis serological tests use secondary antibodies (anti- IgG, anti -IgM and anti-IgA) usually produced from myeloma proteins, especially for IgA and IgM conjugates. These reagents are efficient for most assays but the monoclonal origin of immunogen could select some classes of antibodies, especially when alternative antibody sources are used, as in mucosal immunology antibody detection. Purification of native pooled immunogens by using affinity chromatography is complex with different subclasses affinity and also due to acids or chaotrope exposure. Size exclusion chromatography can be an advantageous alternative for purification of class immunoglobulins, allowing a complete set of immunogens useful for the production of secondary antibodies without subclass selection and also without exposure to harsh conditions. Here, we standardize molecular exclusion chromatography purification for human IgG, IgM and IgA immunoglobulins. We used Sephacryl High Resolution chromatography for main purification with subsequent elimination of contaminants by affinity chromatography on selective ligands media, as Protein A for IgG. The purified product was checked by SDS-PAGE and its integrity was tested both in solid-phase immunofluorescent (FISA) or immunoenzymatic tests (ELISA). IgM is easily purified due to its high molecular weight and lower-molecular-weight components (IgA and IgG) are purified by affinity chromatography exclusion, allowing that the antibodies are not exposed to any stressing conditions as acid pH or chaotrope agents. The purification of human native IgG, IgM and IgA complete set of immunoglobulins is an important step for the optimization of serological tests in the control of diseases, such as toxoplasmosis and others infectious diseases, especially when different source of antibodies are used for detection of protective immunity, as salive and other biological fluids. **Supported by:**LIM-49 HCFMUSP
Keywords: Toxoplasmsosis; molecular exclusion chromatography; human immunoglobulins

HP052 - **MORPHOFISIOLOGICAL CHANGES IN THE EXTRACELLULAR MATRIX OF LEISHMANIA INFANTUM-NATURALLY INFECTED DOGS AFFECT SPLENIC LYMPHOID NICHES AND CD4+ T CELL FREQUENCY**

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The spleen is one of the main affected organs in canine visceral leishmaniasis (CVL). Disorganization of the splenic white pulp (SWP) has been associated with immunosuppression and disease progression. This study aims to assess structural and cellular changes in the splenic extracellular matrix of dogs with CVL correlating them with the parasite load and clinical signs. Splenic fragments were collected from 41 naturally infected animals for parasite load quantification through quantitative PCR, histopathological analysis and immunohistochemistry for CD3+, CD4+, CD8+ T cells, CD21+ B cells, Ki-67+, IFN-γ+, IL-10+ expressing cells and MMP-9, ADAM-10 enzymes. Laminin, collagen and fibronectin deposition were evaluated as well. The animals were grouped according to the level of SWP organization into 1- Organized to slightly disorganized (OR-SD, n= 11); 2- Moderate to intense disorganization (MD-ID, n= 30). SWP disorganization was accompanied by reduction in the quantity of lymphoid follicles/mm2 (p> 0.0001). Animals with moderate to intense disorganization showed more clinical signs (p=0.021), higher laminin (p=0.045) and collagen deposition (p=0.036), higher MMP-9 expression (p=0.035) and lower numbers in CD4+ T cells (p=0.027) in the spleen compared to organized to slightly disorganized animals. Positive correlations between CD8 and parasite load (p=0.042, r=0.320), CD8 and CD4 cells (p=0.028 r=0.343), IFN-γ and IL-10 (p=0.001 r=0.497), laminin and fibronectin expression (p= 0.043 and r2 =0,393) were observed. Whereas ADAM-10 was negatively correlated with follicles/mm2 (p=0.022 r=-0.486). The data suggested that the splenic structure and function are drastically altered and compromised during CVL. These alterations on extracellular matrix compounds and immune cells may consequently lead to immunosuppression and severe disease. **Supported by:**CAPES
Keywords: Canine visceral leishmaiasis; spleen; extracellular matrix

HP053 - **CTLA-4, TIM-3 AND APOPTOTIC CELLS CORRELATE WITH SPLENIC DISORGANIZATION AND FAILURE IN CONTROLLING PARASITE LOAD IN DOGS NATURALLY INFECTED WITH *LEISHMANIA INFANTUM***

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In canine visceral leishmaniasis (CVL), the spleen is one of the major affected organs and the disorganization of the splenic microarchitecture has been associated with disease progression, reduction of cytokines and chemokines expression and failure to control parasite load. Such profile is compatible with cellular exhaustion. This study aims to evaluate the in situ expression of exhaustion markers and its relation to histopathology and parasite load. 40 dogs were grouped according to splenic white pulp (SWP) organization and parasite load as: 1- organized/low parasite load (OL, n=10); 2- disorganized/low parasite load (DL, n=22); 3- disorganized/high parasite load (DH, n=8). The parasite quantification through qPCR using ssRNA target, histopathology, immunohistochemistry and TUNEL were performed. CD4, CD8, IFN-γ, IL-10, TIM-3, CTLA-4 and apoptotic cells were detected. The disorganization of SWP occurred together with the reduction of lymphoid follicles/mm2, CD4 cells and disease's worsening. IFN-γ and CTLA-4 cells were detected in OL dogs and reduced according to increase of disorganization and parasite load. TIM-3 was highly expressed and similar among groups. Apoptosis was detected even in organized dogs, increased in DL and reduced in DH dogs. There were correlations between TIM-3 and CTLA-4, IFN-γ and apoptotic cells, CTLA-4 and apoptotic cells. TIM-3+B-cells were detected. The data suggest that: 1. T cells exhaustion can occur in the early stages of infection, since OL animals have shown TIM-3 and CTLA-4 cells; 2. Apoptosis is the mechanism involved in the reduction of lymphoid follicles, CD4, CTLA-4 and IFN-γ expressing cells; 3. There was no difference among groups in TIM-3+ cells, since B-cells express this molecule. The results suggest a potential association between exhaustion markers, apoptosis and splenic disorganization, CD4 lymphocytes reduction, failure of parasite control and the worsening of the disease. **Supported by:**CNPq, FAPERJ, FIOCRUZ.
Keywords: Visceral leishmaniasis; dog; spleen

HP054 - **ROLE OF SAPA REPEATS PRESENT IN TRANS SIALIDASES DURING TRYPANOSOME CRUZI INFECTION**

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Proteins containing amino acid sequences are highly abundant in different intracellular protozoan parasites. It has been suggested that their repeat domains are part of mechanisms designed by these parasites to avoid an effective immune host. Members of group 1 of the Trans sialidase family (TcTS-I), which are GPI-anchored glycoproteins expressed at the surface of T. cruzi trypomastigotes, also exhibit amino acid repeats named SAPA (Shed Acute Parasite Antigen) at their C-terminal domain. TcTS-I are enzymes whose function is to transfer sialic acid residues from host glycoconjugates to mucin-type molecules also present on the surface of the parasite, an activity involved in the invasion of the host cell by the parasite. Besides inducing a strong humoral response early during infection, the presence of SAPA repeats in these proteins increases the half-life of these enzymes, which are released in the bloodstream, thus contributing to parasite virulence. The importance of SAPA repeats during the infection process was also highlighted by comparative genomic studies showing that TcTS-I genes from the avirulent CL-14 T. cruzi clone possesses less SAPA repeats. When we overexpressed in CL-14 a TS gene containing 19 SAPA repeat motifs, we observed an increased capacity of transfected CL-14 amastigotes to differentiate and to release trypomastigotes from infected cells compared to wild type CL-14. To further investigate the role of SAPA repeats during T. cruzi infection, we produced recombinant versions of the trans sialidase containing or not the repetitive domain to evaluate the effect of the presence of this antigen during infection with CL-14. We also plan to investigate the host immune response after mice immunization with different versions of the protein as well as the protective capacity of each recombinant version of this antigen after challenging immunized animals with the virulent T. cruzi CL Brener strain.
Supported by:CNPq
Keywords: Trans sialidase; sapa; cl14

HP055 - **MODULATION OF B-1 CELLS AFTER INFECTION WITH *LEISHMANIA (LEISHMANIA) AMAZONENSIS* PROMASTIGOTES**

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Leishmaniasis is a neglected disease, endemic in several countries caused by protozoa of Leishmania genus. The result of infection depends on the first events that occur in cells of innate immunity. Macrophages are the mainly cells involved in infection but other cell types can be infect by the parasites, such as B-1 cells. These cells are a subtype of B lymphocytes with peculiar characteristics in immunity, including to immunoglobulin production, cytokines, chemokines, migration to inflammatory sites and they are also able to differentiate into mononuclear phagocyte-like cells with phagocytes properties. However, the activation profile of these cells in the presence of Leishmania has not been fully elucidated. In this study, we evaluated the expression of activation markers in B-1 cells after stimulating in vivo. For this purpose, BALB/c mice were intraperitoneally infected with L. (L.) amazonensis promastigotes and after 24 hours total peritoneal cells were collected. B-1 cells were identified by using specific surface markers (CD19 + CD23 -) and then co-stimulatory molecules, MHCII, F4/80 and NO were analyzed in the gating cells. Uninfected mice were used as control. We observed that infected mice not shown changes in co-stimulatory molecules, MHCII, F4/80 in the gate of B-1 cells. On the other hand, the results showed that there was a significant decrease in NO in B-1 cells from infected group, as compared to the uninfected group. We also observed differences in the expression of myeloid and lymphoid restricted transcription factors after stimulation with parasites. Thus, our work suggests that L. (L.) amazonensis promastigotes induced modification in the B-1 cells status which can be related with the parasite pathogenesis.
Supported by:FAPESP
Keywords:B-1 cell; leishmania; activation

HP056 - **MODULATION OF THE GAP JUNCTION AND CYTOSKELETON BY INFECTION WITH *TOXOPLASMA GONDII* AND INFLAMMATION CONDICTION IN IMMUNE SYSTEM**

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Toxoplasma gondii is a protozoan parasite responsible for toxoplasmosis, and it's believed that this parasite has infected one-third of the world population. In immunocompromised individuals toxoplasmosis may be cause problems in the central nervous and visual systems. Some of these complications are associated with the change of intercellular communication mediated by Junctions Communicators. However, there are still systems that aren't fully characterized regarding the junctional communication, including the innate immune system, represented by Macrophages. In view of this, the aim of this study is to evaluate the structural and functional modulation of gap junctions formed by Connexin 43 (Cx43) in macrophage lines and peritoneal macrophages after infection with *Toxoplasma gondii*, and treatments with pro-immune-inflammatory factors. The methodology used is: (1) J774-G8 macrophage cell line culture; (2) Western Blot Assays; (3) Immunofluorescence assays and analysis by confocal microscopy; and (4) Intracellular dye microinjection (functional assessment of gap junctions). The cell cultures are activated with pro-inflammatory immune factors (Tumor Necrosis Factor-α (TNF-α) and interferon-γ (IFN-γ) or infected with the RH strain of Toxoplasma in its tachyzoite form. The results revealed that J774-G8 cells showed significant changes in their profile junctional communication dye injection experiments, when subjected to microenvironments with inflammatory pro-immune factors combined (IFN-γ+ TNF-α) in incubations 48 hours. The Cx43 and Phalloidin proteins interact in the plasma membrane of the J774-G8 lineage, and they undergo a sensitive reduction in the membrane after 72 hours of infection with the parasite *Toxoplasma gondii*. The evaluation of the Cx43 protein expression by immunoelectrophoretic transfer has been shown to be altered (elevated) in J774-G8 macrophage cells infected with the parasite *Toxoplasma gondii* 24 and 48 hours compared to uninfected cells.
Supported by:CAPES, CNPq and FAPERJ **Keywords:**Gap junction; macrophages; toxoplasma gondii

HP057 - **DIFFERENCES IN PRIMARY CULTURE AND CELL LINE INFLUENCES *LEISHMANIA BRAZILIENSIS* INFECTION AND AUTOPHAGY**

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Leishmania (Viannia) braziliensis is the main etiological agent of leishmaniasis in Americas. Autophagy is a constitutive process that occurs in all eukaryotic cells to degradation of intracellular components as macromolecules, organelles or pathogens by lysosomes. Our group is investigating the role of autophagy in parasite infection and we hypothesized that *in vitro* models (as primary and lineage cells) respond differently to infection and autophagy. Thus, we analysed the influence of macrophages´ autophagic pathway during *L. braziliensis* infection. We initially evaluated infection by less and high virulent strain of *L. braziliensis* from different Brazilian endemic areas for 3 and 24h in peritoneal macrophages (*Swiss* mice) and THP0-1 (human acute leukemia monocyte cell line). After 3h of interaction with promastigotes (10 parasites per host cell), non-internalized parasites were removed and host cells were maintained up to 24h. We observed that both cell types were infected by two isolates of *L. braziliensis*, but peritoneal macrophages were more susceptible to infection in early period, reaching up to 80% of infection. Less virulent sample infects 2-fold more than the other strain in peritoneal macrophages during first 3h, distinctly from THP0-1 cells. The infection in less virulent isolate also decreases in 24h in macrophages, inducing host cell lysis, and suggesting short period of reinfection, in contrast to THP0-1 which maintain low infection and parasite/infected cell. We also observed that both strains of *L. braziliensis* decrease LC3-II expression after 24h of THP0-1 infection, suggesting a downregulation of autophagic pathway, also supported by qPCR analysis. Altogether, these results indicate that *L. braziliensis* infection presents different pattern according to host cell type. **Supported by:**CAPES, CNPq, FAPERJ and FIOCRUZ. **Keywords:**Leishmania braziliensis; tHP0-1; autophagy

HP058 - **TRIPLEX SOLID-PHASE FLUORESCENT ASSAY FOR THE SIMULTANEOUS DETECTION OF SPECIFIC ANTI-*T. GONDII* IGG, IGM AND IGA ANTIBODIES FOR TOXOPLASMOSIS ANTENATAL CARE SCREENING**

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Toxoplasmosis screening in pregnant women is mandatory to prevent congenital infections and early therapy for the avoiding disease sequels. Recent studies showed low toxoplasmosis prevalence in children, which will be adults and mothers, resulting in high proportion of seronegative women. For antenatal screening of a treatable infection, we need new quick and inexpensive assays. New Solid Phase Fluorescent Assays (FISA) allows direct antibody quantification in microplates as we reported conjunct anti-*T. gondii* IgG and IgM detection high specificity and low cost due to improved fluorescent conjugates. Here, we evaluated the efficiency of triplex solid-phase fluorescent assay (tFISA) for the simultaneous detection of specific anti-*T. gondii* IgG, IgM and IgA antibodies for toxoplasmosis antenatal care screening. We tested 130 serum samples from adult volunteers at a large public hospital screened previously by IgA ELISA, IgG/IgM Elecsys Toxo and IgM IFAT. Compared to IgG/IgM Elecsys Toxo, IgG tFISA showed excellent concordance (Kappa=0.769), 84.6% sensitivity and 92.3% specificity, while IgM tFISA showed good concordance (Kappa=0.637), lower 61.5% sensitivity and similar 95.6% specificity. Compared to IgM positives samples by IgM Elecsys Toxo and confirmatory IgM IFAT test, IgM tFISA showed excellent concordance (Kappa=0.876), 100% sensitivity and 96.6% specificity with lower false-positives results (n=15) than IgM Elecsys Toxo (n=27), and compared to IgA ELISA, IgA tFISA showed excellent concordance (k=0.922), 91.6% sensitivity and 99.0% specificity. Triplex FISA was reproducible for anti-*T. gondii* IgG (85%), IgM (88%) and IgA (82%) detection. These data suggest that tFISA can be used for toxoplasmosis screening serum conversion in pregnant women useful in antenatal of public health care. **Supported by:**CAPES, FAPESP, LIM-49 HCFMUSP
Keywords: Toxoplasmosis; elisa; solid phase fluorescent assay

HP059 - **EXPRESSION OF INTERLEUKIN 15 DURING TRYPANOSOMA CRUZI AND LEISHMANIA INFECTION**

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Amastins are 170-200 amino acid glycoproteins expressed at the surface of Trypanosoma cruzi and Leishmania spp amastigotes, which may interact with various host cell components. Yeast two-hybrid assays provide evidences indicating that interleukin 15 (IL-15) are among the host cell proteins with which T. cruzi amastins interact during the parasite intracellular development. A role of IL-15 during T. cruzi and Leishmania infection have been also suggested from transcriptome analyses of human fibroblasts and mice macrophages infected with T. cruzi and L. major, respectively, which showed a significant increase in the IL-15 mRNA expression compared to uninfected cells. With the aim to validate the yeast two-hybrid result and to investigate the role of IL-15 during T. cruzi infection, we analysed murine peritoneal macrophages infected with T. cruzi and L. amazonensis as well as L6 myoblasts and HeLa cells infected with T. cruzi, using ELISA and immunofluorescence assays. Although ELISA performed with supernatants collected from T. cruzi infected macrophages 6, 24, 48 and 72 hours post-infection did not reveal the presence of secreted IL-15, analyses of total cell extract showed increased levels of IL-15 production in infected cells compared to uninfected macrophages, with IL-15 production reaching the highest values between 24 and 48 hours post-infection. Immunofluorescence analyses showed an uniformed distribution of IL-15 in the cytoplasm and nuclei of uninfected macrophages whereas, in infected cells, IL-15 is predominantly localized in the cytoplasm. Importantly, not only in infected macrophages but also in L6 and HeLa cells infected with T. cruzi, a clear co-localization of IL-15 with the membrane of amastigotes was observed. A similar co-localization of IL-15 with parasitophorus vacuoles containing amastigotes was observed in macrophages infected with L. amazonensis. **Supported by:**CAPES
Keywords: Il-15; trypanosoma cruzi; leishmania amazonensis

HP060 - **PUTRESCINE TREATMENT DECREASES ACTIVE PENETRATION OF *TOXOPLASMA GONDII*, REVERT NITRIC OXIDE PRODUCTION INHIBITION AND INCREASES LYSOSOMAL FUSION IN ACTIVATED MACROPHAGES**

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Toxoplasma gondii is the causative agent of toxoplasmosis. Part of the tachyzoite population of *T. gondii* exposes phosphatidylserine (PS) on the outer leaflet of the plasma membrane, mimicking apoptotic cells. PS is an anionic phospholipid, when exposed induces the release of transforming growth factor beta1 and reduction of nitric oxide (NO) production in macrophages. The subpopulation that exposes PS (PS^+) actively penetrates into macrophages, resides in a tight-fitting vacuole and reduces NO production. However, the subpopulation that does not expose (PS^-) is phagocytosed, remains in a loose-fitting vacuole and does not reduce NO production. Polyamines are polycationic molecules with affinity for anionic molecules, like PS. This study evaluated the effects of the putrescine polyamine on PS exposed by the PS^+ subpopulation. For this, subpopulations were isolated and PS^+ tachyzoites were treated with different concentrations of putrescine (PUT subpopulations). The viability of the parasite and murine macrophages treated with putrescine was verified by flow cytometry and MTT, respectively. Activated macrophages were infected with the total population and the subpopulations PS^+, PS^- and PUT. Parasite internalization in macrophages, parasitophorous vacuole morphology, NO production in culture supernatants and immunolabeling of vacuoles with anti-LAMP-1 were analyzed. The parasite viability was not affected after treatment. The macrophages viability was altered after treatment with 10 mM of putrescine. Infection with PUT 0.1 mM reduced parasite internalization in macrophages, increased rates of loose-fitting vacuole and positive labeling for LAMP-1 (indicatives of phagocytosis and lysosomal fusion) and reversed the inhibition of NO production compared to the PS^+ subpopulation. The PS^+ subpopulation, after treatment, shows similar behavior as PS^- subpopulation, suggesting putrescine interference in PS exposed and that PS is important in the active penetration process. **Supported by:**FAPERJ, UENF, CAPES, CNPq
Keywords: Toxoplasma gondii; phosphatidylserine; putrescine

HP061 - **CADMIUM TELLURIDE QUANTUM DOTS (CDTE- QDS) INDUCES OXIDATIVE STRESS IN *TRYPANOSOMA CRUZI***

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Quantum dots (QDs) are semiconductor nanoparticles (NPs) have been considered important class of fluorescent probes for studies biological systems. However, to ensure its applicability in live organisms, several tests are needed. Citotoxicity effects such as loss of mitochondrial and plasma membrane integrity, DNA damage and cell death have been reported. In this context, we used of Cadmium Telluride QDs (CdTe - QDs) capped with mercaptoacetic acid synthetized in an aqueous medium in order to identify QDs citotoxicity effects such as reactive oxygen species (ROS) generation in *Trypanosoma cruzi* epimastigotes (Dm28c strain). In this study, we evaluated the possible effects on *T. cruzi* growth curves in a time scale of control and incubated cells with different concentrations of QDs (0,2; 2; 20 and 200 µM) to determine the development cells changes. Intracellular ROS levels in *T. cruzi* cells were measured using the cell-permeable dye H2DCFDA and Electron Paramagnetic Resonance (EPR) technique. The results of the growth curve indicated a decrease in the number of parasites incubated with 20 and 200 µM QDs when compared to parasites incubated with 0,2µM and 2 µM QDs and the control group. These data corroborate with previous results that evidenced alterations in the cell morphology at these concentrations (20 and 200µM). H2DCFDA and EPR showed that parasites treated with 20 and 200 µM QDs demonstrated an increase of ROS level when compared to the untreated cells. To understand the interconnections between the cellular and CdTe - QDs dynamics is important to research all of nanotoxic effects for safety biological and biomedical applications. Thus, we intend to deepen our studies with *T.cruzi* parasite labeled with CdTe QDs and evaluate other toxic effects. **Supported by:** CNPq; CAPES; proppi-uff;
Keywords: Trypanosoma cruzi; cdte quantum dots; nanotoxicology

HP062 - **LIPID METABOLISM MANIPULATION IN LIVER OF SWISS MICE INFECTED WITH *TOXOPLASMA GONDII*.**

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Toxoplasma gondii is a worldwide spread protozoan and is able to infect almost all warm-blood animals. It is one of the main problems in public health in Brazil, especially to pregnant women, since the disease is accompanied by malformations in fetuses. *T. gondii* is able to infect organs such as lungs, heart, liver and brain. According to the literature, parasites lack enzymes and key factors to lipid synthesis and degradation - essential molecules for survival and proliferation of *T. gondii*. This work aims to analyze the liver lipid metabolism during acute infection with *T. gondii*. Two groups of male swiss mice (control and infected, n = 8) were subjected to infection with 10^6 parasites injected intraperitoneally for 48h. After euthanasia, liver was collected, subjected to protein dosage and lipid extraction. Thin layer chromatography technique was used to separate the main lipid classes. The results showed a significant increase of triacylglycerol in infected liver compared to control group (48.01 + 2.51 vs 31.23 + 2.01, p <0.001) and a significant decrease of free fatty acids (16, 3 + 1.08 vs 21.2 + 1.32, p <0.05), free cholesterol (18.07 + 0.91 vs. 23.04 + 0, 83, p <0.05) and total phospholipids (10.41 + 0.72 vs. 15.65 + 0.92, p <0.01) in infected liver compared to control, respectively. The results demonstrated that *T. gondii* early infection is capable of alter the lipid composition in mice liver. These changes may be related to the lipid metabolism manipulation by the parasite in an attempt to obtain these molecules for their development and reproduction. **Supported by:**CNPq, CAPES e FAPERJ
Keywords: Lipid metabolism; toxoplasma gondii; liver

HP063 - **LIPID METABOLISM ANALYSIS IN SWISS MICE BRAINS DURING EARLY INFECTION WITH *TOXOPLASMA GONDII***

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Toxoplasma gondii is an obligate intracellular parasite that causes toxoplasmosis. This disease is a leading cause of illness and mortality among immunosuppressed individuals and during congenital infections. There are several factors contributing to survival and proliferation of this parasite. Lipids are extremely important as a basis for membrane structure, energy reserve, cell signaling and immune system evasion. In addition, *T. gondii* does not have the complete pathways for lipid synthesis and degradation. However there is not enough information about how these parasites acquire the lipids from their hosts during the infection. This work aims to characterize the lipid metabolism on the brains of swiss mice infected with *T. gondii*. We analyzed brain samples from control and infected mice (n=8), 48h post infection. Brain samples were collected, processed and subjected to protein dosage and subsequent lipid extraction. Thin layer chromatography technique was used to separate the main lipid classes. The results showed a significant increase for free cholesterol (37.31 ± 0.85 vs 32.97 ± 1.0, p <0.020) and total phospholipids (15.37 ± 0.70 vs 9.6 ± 0.85; p <0,001) and a significant decrease for esterified cholesterol (33.45 ± 1.0 vs. 42.89 ± 1.6; p <0.003) for the infected group when compared to the control group. Among the phospholipids, there was a significant increase for phosphatidylcholine (39.68 ± 2.0 vs 33.07 ± 1.4, p <0.02) and a significant decrease for phosphatidic acid (3.950 ± 0.39 vs 6.663 ± 0.48, p <0.004) in infected group. The results demonstrated it is that acute *T. gondii* infection is capable of alter lipid metabolism in the brains of swiss mice. Such a change needs to be further studied and therefore the next objectives will be to analyze the gene and protein expression of transcription factors and key enzymes in order to understand how the parasite manipulates the host's lipid metabolism.
Keywords: Brain; metabolism; toxoplasma gondii

HP064 - ***LEISHMANIA AMAZONENSIS* STRAINS MAY IMPAIR THE EXPRESSION OF IL-32γ IN C57BL/6 MICE TRANSGENIC FOR HUMAN IL-32γ**

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Interleukin 32 (IL-32) is a proinflammatory cytokine whose IL-32γ isoform is the most potent. It was detected in lesions of patients with American tegumentary leishmaniasis (ATL). Human IL-32γ transgenic mice (IL-32γTg) were used to understand the role of IL-32 in *L. amazonensis* infection. In addition, the induction of IL-32γ by distinct *Leishmania sp.* in murine macrophages was compared. Wild-type (WT) and IL-32γTg mice were infected with different strains of *L. amazonensis* promastigotes (PH8, M2269 from localized cutaneous leishmaniasis and MAB3 from diffuse cutaneous leishmaniasis) into footpad. Lesion size was weekly evaluated (in mm); parasite load of the infected footpad, spleen and liver was analyzed by limiting dilution; bone marrow-derived macrophages (BMDMs) from non-infected WT and IL-32γTg mice were infected with parasites (5:1) to evaluated macrophage microbicidal activity (*L. amazonensis*) and IL-32γ expression (*L.amazonensis, L. braziliensis, L. infantum chagasi*); IL-32γ mRNA expression was assessed by real-time PCR. The results demonstrated that IL-32γTg mice presented a smaller lesion than WT mice on week 10 after infection with MAB3 and week 11 with PH8 or M2269 infection ($p < 0.05$). Parasite load in the footpad lesion and liver of the IL-32γTg mice was similar to the WT for all isolates. However, in the spleen parasite load was lower in MAB3-infected IL-32γTg mice than in WT. IL-32γ expression was not detected in the infected footpads on week 12 of infection. In addition, there was a lower expression of IL-32γ in BMDMs after 3 h or 24 h of infection with distinct isolates of *L. amazonensis* compared to infection with *L. braziliensis or L. chagasi*. The results suggest that IL-32γ does not contribute for cutaneous healing of the lesions caused by *L. amazonensis*, but can limit the dissemination of MAB3 parasites in the later stages of infection. Moreover, *L. amazonensis* may be impairing the production of IL-32γ during infection. **Supported by:**Caps, CNPQ
Keywords: Interleukin 32γ l. amazonensis

HP065 - **THE NOD2 RECEPTOR IS CRUCIAL FOR BOTH HUMAN INNATE AND ADAPTIVE IMMUNE RESPONSES TOWARDS NEW WORLD *LEISHMANIA* SPECIES**

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American Tegumentary Leishmaniasis is a chronic infection caused by *Leishmania protozoan*. It is not clear which innate receptors are crucial for *Leishmania* recognition. Here, *NOD*-like receptor (NLR) family members and their genetic variants were investigated in immune responses to *L. braziliensis or L. amazonensis.* Using functional genomics, genetic variants in *NOD1 or NOD2* were investigated to influence the production of cytokines by human peripheral blood mononuclear cells (PBMCs) from 200 healthy individuals with a Dutch genetic background exposed to *Leishmania sp*. The *NOD1* and *NOD2* mRNA expression were evaluated by qPCR in PBMCs from healthy individuals; phagolysosome formation was inhibited with Bafilomycin A1; cytokine production from PBMCs bearing the wild-type allele of *NOD2* and from four homozygous individuals for the *NOD2* 3020insC mutation presenting Crohn's disease were also evaluated after *Leishmania sp*. exposure; RICK inhibitor was used to evaluate the *NOD* pathway activation; IL-8 production in HEK-293 cells overexpressing *NOD2* was evaluated after *Leishmania* stimulation; cytokine levels were determined by ELISA and infection index was evaluated in human monocyte derived-macrophages. Polymorphisms in the *NOD2* gene decreased monocyte- and lymphocyte-derived cytokine production after stimulation with *L. amazonensis or L. braziliensis* compared to individuals with a functional *NOD2* receptor. The phagolysosome formation was important for *Leishmania*-induced cytokine production and upregulation of *NOD2* mRNA expression. Furthermore, the RICK inhibition decreased the cytokine production after *Leishmania* sp. stimulation. Additionally, HEK-293 cells overexpressing *NOD2* had increased IL-8 production after *Leishmania* sp. exposure. *NOD2* was crucial to control macrophage infection caused by Leishmania sp. In conclusion, the human *NOD2* receptor is important for Leishmania sp. recognition, the induction of innate and adaptive immune responses, and parasite killing. **Supported by:**Caps, CNPQ
Keywords: Leishmania; functional genomics; nod2

HP066 - **MODULATION OF ARGINASE 1 AND INDUCIBLE NITRIC OXIDE SYNTHASE IN MACROPHAGIC CELLS BY *TOXOPLASMA GONDII***

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Resident macrophages (MØ) are immune cells with low microbicidal capacity. However, when stimulated with interferon-gamma and lipopolysaccharide are polarized in a M1 profile and express inducible nitric oxide (NO) synthase (iNOS), which synthesizes NO, a microbicidal molecule that controls the replication of *Toxoplasma gondii*, a protozoan that causes toxoplasmosis. However, *T. gondii* is able to degrade iNOS, reducing NO production, persisting in M1 MØ. Macrophages stimulated with IL-4 are polarized in M2 profile and express arginase 1 (ARG1). The induction of ARG1 in MØ infected with *T. gondii* is considered an evasion mechanism, since ARG1 competes with iNOS for L-arginine and produces L-ornithine, a crucial molecule for the replication of the parasite. However, the modulation of ARG1 by *T. gondii* in resident or polarized MØ, and their role in parasite control are controversial. Thus, we evaluated the activity of ARG1, NO production and protein expression in peritoneal MØ of Swiss mice and RAW 264.7 cell line infected with *T. gondii* strain RH. ARG1 activity was assayed by measuring urea, NO production by nitrite evaluation and protein expression of iNOS by western blotting. Resident and M1 peritoneal MØ showed a lower ARG1 activity after *T. gondii* infection when compared to uninfected cells. However, resident and M1 RAW 264.7 infected with *T. gondii* showed higher ARG1 activity when compared to uninfected cells. Peritoneal MØ and RAW 264.7 M1 after infection with *T. gondii* showed a reduced NO production compared to uninfected cells. iNOS expression was reduced in both cell lines when in the M1 profile. *T. gondii* infection modulated ARG1 activity depending on the MØ lineage, but NO production as well as iNOS expression were always negatively modulated regardless of the MØ type. Further studies are needed to understand the importance of distinct modulation of ARG1 after *T. gondii* infection in activated MØ of both lineages used. **Supported by:**UENF, FAPERJ, CNPq
Keywords: Toxoplasma gondii; inducible nitric oxide synthase; arginase 1

HP067 - **3D STUDY OF THE EVOLUTION OF THE RESIDUAL BODY OF THE TACHYZOITE FORM OF TOXOPLASMA GONDII**

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Toxoplasma gondii RH strain is the most used to study the acute phase of toxoplasmosis. After invasion of the host cell, the parasite is established within a parasitophorous vacuole (PV), where it is multiplieddivides by endodiogeny. The daughter parasites remain connected by a structure called residual body (RB). Our aim was to analyze by transmission and scanning electron microscopy and by 3-D reconstruction from slice view in dual beam microscopy (FIB), the formation and fate of RB and its relation with parasites inside the rosette in PV. Tachyzoites were harvested from the peritoneal cavity of CF1 mice and interacted with the LLC-MK2 cells (ATCC® CCL-7™) in RPMI + 5% SFB was observed at 7, 24 and 48 hours post infection (HP0i). The 10: 1 parasite / cell ratio for 45 minutes. Samples were chemically fixed with 2.5% Glutaraldehyde in 0.1M Sodium Cacodylate buffer, pH 7.2, post-fixed with1% OsO4 and 1.25% potassium ferrocyanide in the same buffer, dehydrated with acetone or ethanol, and embedded in EPON (for TEM and FIB) or critical point dried (for SEM). Observations were carried out in the Zeiss EM900 TEM, and Auriga ZEISS SEM for High resolution SEM or slice and view series. The sequential views were processed in IMOD, and the models were rendered with 3D MOD of the same IMOD package . In 7HP0i, the first division had happened. In 24 HP0i, the rosette was complete and we counted 32 parasites connected to the RB. Some parasites seemed to be leaving the PV, but the 3D models showed that they were still connected to the RB.In 48 HP0i, the tachyzoites were already individualized and vesicles similar to acidocalcisomes appear to fuse with the RB membrane. We conclude that RB is formed soon in the first division of the parasite and all they remain connected to the RB until the egress. This seems to be the fate of the RB: self-destruction of vesicles accumulated within it as the cycles of division of the parasite progress. **Supported by:**CNPq, FAPERJ
Keywords: Toxoplasma; acidocalcisomes; ultrastructure

HP068 - **INFLUENCE OF THE INOCULATION ROUTE ON MICE INFECTED BY TRYPANOSOMA CRUZI**

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Introduction: The route of inoculation has been shown to influence disease outcome in numerous models of infection. However, the factors that determine route-specific influence on T. cruzi infection remain poorly defined. While it may seem obvious that intradermal inoculation of the skin would best replicate the natural infection, intraperitoneal inoculation remains a favored route of infection in Chagas disease studies. Methods and Results: In this study, the development of experimental Chagas disease was evaluated in mice inoculated with parasites by intradermal (ID) or intraperitoneal (IP) routes. The results show that both inoculation routes were able to produce progressive infection in C57BL/6 mice with a peak of parasitemia at 8 days after infection. However, the levels of parasitemia were significantly higher in mice infected by IP route. Moreover, susceptible BALB/c mice infected by IP route succumbed to infection significantly earlier than those infected by ID route. Studies from our group have demonstrated that, in vitro, T. cruzi needs a signal provided by ROS to proliferate inside macrophages. However, when C57BL/6 WT and Phox KO mice (knockout to NADPH oxidase) were infected by IP route we did not observe differences in parasitemia level. Recently, infections performed by ID route showed that Phox KO mice had lower levels of parasitemia, corroborating our in vitro results that show that a signal induced by ROS is important for the replication of this parasite. The mechanism by which the route of infection affects the outcome of disease remains to be elucidated, since the levels of serum cytokines were not different between animals infected by ID and IP route. Conclusion: These results indicate that the inoculation route can influence the development of the disease, impacting parasite load and infection phenotypes. So, it is important to be taken into account during experimental designing.
Supported by:FAPEMIG, CAPES, CNPq
Keywords: Trypanosoma cruzi; intraperitoneal infection; indradermical infection

HP069 - **IL-32γ: NEW PERSPECTIVES DURING ACUTE AND CHRONIC TOXOPLASMOSIS IN MURINE MODEL.**

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Toxoplasmosis is a zoonosis caused by the obligate intracellular parasite Toxoplasma gondii that belong to the phylum Apicomplexa. The immune response to T. gondii infection involves cellular and humoral immunity. Interleukin 32 (IL-32) is a cytokine with protective effects against microorganisms that help to control bacterial and viral infections. In addition, IL-32 may be involved in the immunopathogenesis of some infectious diseases. In parasitic diseases, the function of this cytokine has only been demonstrated in infections caused by Leishmania. In this context, our project seeks to find the role of IL-32 during acute and chronic toxoplasmosis. For experimental procedures, it was used eight week-old C57BL/6 (wild type) and transgenic mice to IL-32γ (IL-32γ TG). Mice were infected orally with 10 cysts of ME-49 strain. Animals were euthanized on day 8 (acute phase) and day 30 (chronic phase). It was observed that IL-32γ TG mice lose more weight than WT along of infection. No significant difference was observed between groups during the acute phase in the histological analysis. During chronic phase there was no significant difference between inflammatory score in the brain, however the number of cysts detected in IL-32γ TG was significantly higher than WT. During the chronification of the disease, there was 22,2% of survival rate in IL-32 mice while WT group was 66,7. There was no significant difference in production of IgG1 and IgG2a subclasses between IL-32γTG and WT animals in chronic phase. According to these results, it is suggested that IL-32 increase morbidity and parasitic load of animals with T. gondii infection.
Supported by:CAPES, CNPq, FAPEMIG
Keywords:il-32γ toxoplasma gondii

HP070 - **DIRECT SEQUENCING FROM CLINICAL L. CHAGASI ISOLATES**

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Recent studies have shed light onto the level diversity in old world Leishmanania spp infections in Imamura et al., 2016, DOI: 10.7554/eLife.12613. However in comparison very little is known of the natural diversity of Brazilian L. infantum isolates (also known as L. chagasi) and the data that is currently available is derived primarily from parasites that have been maintained in culture. We will be demonstrating the effect of sequencing directly from clinical isolates versus sequencing from parasites sequenced after culture, at both the macrostructure level and single gene copy level.
With this data we have two main objectives, firstly, to demonstrate the benefits of sequencing directly from clinical samples, because these parasites are more likely to reflect the 'real' biology compared to cultured samples, and secondly, to use the data collected from these patients in order to investigate the level of diversity in Brazilian L. chagasi isolates using next generation sequencing data. We have used sequence capture to sequence parasites from DNA extracted from bone marrow of 9 infected patients, and have paired data, sequenced from parasites cultured from the same biopsies. In our unpublished data, we have observed unique ploidy gain and loss events in cultured parasites, showing a highly dynamic ploidy profile in cultured strains, compared to parasites sequenced directly obtained from patients, which have a relatively static ploidy profile. We have also used population genomic approaches, and used an additional 80 genomes, to look at the population structure of L. infantum isolates in Brazil. From this we found Brazillian strains were distinct from European L. infantum. However the level of nucleotide diversity is far lower than we would have expected, with only ~20,000 SNPs within these 80 strains, but fewer than 200 SNPs unique to any individual strain on average.
Keywords: Genomics; sequencing; leishmania

HP071 - **NEW INSIGHTS INTO PRMT7 ROLE IN DIFFERENTIATION AND INFECTIVITY OF *LEISHMANIA MAJOR***

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Gene expression in *Leishmania* spp relies mainly on post-transcriptional regulation. The enzyme Protein aRginine MethylTransferase7 (PRMT7) is an important regulator able to target RNA binding proteins. Previous work has shown that PRMT7 in *Leishmania major* works as a regulator of parasite virulence *in vivo*. The stage-specific expression of this enzyme and its implication in pathogenesis suggests it may have a role in parasite differentiation. The aim of this work is to investigate the impact of PRMT7 depletion in lifecycle progression of the parasite and therefore its impact upon virulence.
Comparative qRT-PCR analysis was performed to measure the effect of *prmt7* gene knockout (Δ*prmt7*) on molecular markers of *L. major* lifecycle stages. As expected, SHERP, known to contribute to metacyclogenesis in the sand fly, was highly up-regulated in wild-type metacyclics. Remarkably, the same was not seen in the Δ*prmt7* mutant line. Parasite infectivity and virulence were assessed *in vivo* using the BALB/c mouse model. Interestingly, infections with either a mixed stationary promastigote population or purified metacyclics resulted in drastically different phenotypes. Only Δ*prmt7* stationary phase parasites resulted in mice developing lesions. In contrast, only wild-type purified metacyclic parasites presented lesions in infected mice. Despite these distinctions, parasite burdens were comparable in all infections. Finally, gene expression was evaluated between developmental stages of *L. major* wild-type and Δ*prmt7* using quantitative RNA-seq and computational analysis is in progress. A similar approach will be used to compare the transcriptomes of stationary phase and purified metacyclics to investigate the basis of our contrasting findings during *in vivo* studies.
This study highlights the key contribution of PRMT7 toward *Leishmania major* differentiation, infectivity and pathology. **Supported by:**FAPESP

Keywords: Leishmania major; protein arginine methyltransferase7 ; parasite virulence

HP072 - ***IN VITRO* AND *IN VIVO* ACTION OF PTEROCARPANQUINONE LQB-118 ON *TRYPANOSOMA CRUZI***

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Chagas disease is caused by the protozoan *Trypanosoma cruzi* and endemic in Latin America. The therapy is very limited, not being effective in the chronic phase of infection. The aim of this work was to study *in vitro* the LQB-118 mechanism of action and evaluate its activity by the *in vivo* infection experimental model. Epimastigotes of *T. cruzi* (Dm28c clone) were treated with LQB-118 (0-5µM) for 96h/27°C and quantified daily by Neubauer chamber. An ultrastructural evaluation of epimastigotes treated 2µM LQB-118 for 24/27°C was performed by transmission electron microscopy. *In vivo*, two assays were conducted using *Swiss* mice infected with 10^3 blood trypomastigotes (Y strain) intraperitoneally. In the first test, mice were treated orally with LQB-118 (20 mg/kg/day) for 11 days from the 7º day post-infection (dpi) and in the second test, the animals were treated with LQB-118 (40 mg/kg/day) for 12 days from 4º dpi. We evaluated the parasitemia, survival rates, biochemical parameters analysis of serum and preliminary histopathology in the heart. *In vitro*, the LQB-118 was able to inhibit significantly the growth epimastigotes in a dose-dependent manner and the IC_{50} in 96h was 0,88 ± 0,08µM. In ultrastructural evaluation, we observed morphological changes in the swelling of the kinetoplast region and vacuoles near the flagellar pocket. *In vivo*, the parasitemia in animals treated in LQB-118 decreased both assays. In the first experiment there was a reduction of parasitemia by 37.8% on the 15th dpi, while in the second test, the parasitemia reduction of 49.47% was observed from the 11th dpi. Serum biochemical analysis showed that there was a creatine decrease in animals treated with LQB-118. Preliminary histological analysis indicated a cardiac damage decrease in the treated animals. These results showed that LQB-118 has activity against *T. cruzi* causing morphological alteration in the parasite and has protective action in vivo during the infection acute phase. **Supported by:**CNPq
Keywords: Trypanosoma cruzi; pterocarpanquinone lqb-118; chemotherapy

HP073 - ***ANNONA MURICATA* L. (GRAVIOLA) EXTRACT ETHANOLIC CONTROL *TOXOPLASMA GONDII* PROLIFERATION *IN VITRO* AND *IN VIVO* INFECTION**

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Toxoplasma gondii is one of the most successful parasites in the world, infecting a wide variety of mammals, including a third of the global human population. Extracts from various morphological parts of *Annona muricata* Linn. (Annonaceae) are widely used medicinally in many parts of the world for the management, control and/or treatment of several human diseases. In this context, we investigated the action of ethanolic extract of *A. muricata* L (EExTAM) treatment during *T. gondii*-infection *in vitro* and *in vivo*. For *in vitro*, NIH/3T3 fibroblast and J774 macrophages cell lines were infected and treated with different concentrations of EExTAM, and hexane, dichloromethane and ethyl acetate fractions, and the parasitism and nitric oxide (NO) production were analyzed. For *in vivo* experiments, C57BL/6 mice were infected with *T. gondii* and treated as described above, and analyzed for parasitism, histology, biochemical and immunological parameters. The EExTAM-treatment was able to control the parasitism in fibroblasts and macrophages, and additionally, increased the NO production by the hematopoietic cells; and H, D and ethyl acetate fractions controlled parasite grow at 20 or 50, 50 and 200 µg/mL concentrations, respectively. *In vivo*, the EExTAM-treatment prolonged the survival, controlled the parasite proliferation in the small intestine and lung on day 8 post-infection (p.i.) and in the brain on day 30 p.i., but not the fractions. The lower parasitism in EExTAM-treated mice was associated with IFN-γ and TNF moderated levels, increased goblet cell numbers in the small intestine and decreased triglycerides and VLDL lipid levels systemically. Therefore, EExTAM could be a good candidate for toxoplasmosis treatment.
Supported by:CAPES, CNPq, FAPEMIG
Keywords: Toxoplasma gondii; annona muricata l.; cytokines

HP074 - ***LEISHMANIA AMAZONENSIS* PROMASTIGOTES INDUCE EXTRACELLULAR TRAPS (ETS) RELEASE IN HUMAN AND MURINE MACROPHAGES**

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ETosis is an innate immune mechanism first described in neutrophils, which occurs with the release of DNA, histones and some cytoplasmic and granular-derived peptides associated in a web-like structure named extracellular traps (ETs). These ETs can provide host protection through microbial degradation and by physically arresting pathogens precluding their dissemination and facilitating their clearance by phagocytosis. ETs play an important role on leishmaniasis, a neglected disease caused by *Leishmania* protozoa. Our group first described NETs formation induced by *Leishmania amazonensis* promastigotes, and NETs' potential to entrap and kill the parasite. It has been shown that other cell types can also release ETs such as eosinophils, mast cells and macrophages in response to pathogens or chemical agents. Here, we evaluated if *L. amazonensis* promastigotes could trigger ETs release in macrophages. For this, we incubated murine (peritoneal, bone marrow or RAW 246-7) and human (hMDM or THP0-1-derived) macrophages with the parasite in different proportions. ETs were detected with DNA PicoGreen assay. Our results demonstrated that the parasite induced ETs formation in all cell types in a concentration-dependent manner, which was also observed by confocal microscopy. Next we investigated signaling pathways by which the protozoa could induce ETs formation in macrophages. Cells were pre-treated with inhibitors of NADPH oxidase, ERK, PAD-4, elastase or myeloperoxidase, and incubated with promastigotes for ETs' detection. None of the inhibitors altered significantly ETs formation. THP0-1 ETs' were not toxic to parasite as measured by the XTT assay. Our results suggest *L. amazonensis* promastigotes can induce ETs formation in murine and human macrophages, but the signaling pathways involved in ETs' formation and ETs' role on parasite control are yet to be revealed.
Supported by:CAPES, FAPERJ and CNPq
Keywords: Extracellular traps; nets; macrophage

HP075 - **IRON ION DEPLETION IMPAIRS ORAL *TOXOPLASMA GONDII* INFECTION**

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The iron is an important constituent of our environment, being necessary either for humans and pathogenic protozoa survival. Iron ion-containing proteins exert a wide range of biological processes such as biodegradation and biosynthesis, as well as immune function, fetal development, and physical and mental well-being. *Toxoplasma gondii* is an obligate intracellular parasite that presents specialized structures (apical complex) involved in the invasion, modulation and creates an environment in which the parasite can acquire nutrient and avoid its death. It is estimated that approximately one third of the world population is infected, being this parasite able to infect warm-blooded animals, becoming a pathogen of zoonotic and veterinary importance. C57BL/6 female mice aged 8 weeks were orally infected with 20 cysts of ME-49 *T. gondii* strain and intraperotineally treated with deferoxamina (300 mg/Kg) or supplemented with iron ferric ammonium citrate (100 mg/Kg) or ferrous sulfate (100 mg/Kg) one day prior infection and for a further 7 days post-infection. The small intestine iron accumulation was evaluated through Perls staining and the histological and immunological parameters were analyzed. It was observed that the infection increases iron ion accumulation in the small intestine. Deferoxamine-treated animals presented lower weight loss in the initial phase of infection than those treated with ferric ammonium citrate or ferrous sulfate. Deferoxamine decreased the parasite burden in the intestine and lung, and the inflammatory alterations in the small intestine. However, the iron ion supplementation of these animals increased the parasite burden in the intestine and lung, and induced about 20% of intestinal length reduction. Together, our results suggest that the parasite needs the iron ion for its metabolism and multiplication and the decrease of iron ion levels contributes to the parasite control. **Supported by:**CAPES, CNPq and FAPEMG
Keywords: Toxoplasma gondii; iron ; small intestine

HP076 - **BRAZILIAN STRAINS OF TOXOPLASMA GONDII ARE CONTROLLED BY AZITHROMYCIN AND MODULATE CYTOKINE PRODUCTION IN HUMAN PLACENTAL EXPLANTS.**

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Toxoplasma gondii is an intracellular parasite that causes severe infection during pregnancy due to the possibility of transplacental transmission of the parasite causing congenital toxoplasmosis. Strains of this parasite are genetically diverse and the genotype has been implicated in disease severity. Uberlândia city, Brazil, has two T. gondii parasite strains named TgChBrUD1 (UD1) and TgChBrUD2 (UD2) that are considered virulent. Thus, the aims of this study were evaluated the Brazilian strains virulence in human villous explants and the efficacy of azithromycin in the control of these strains compared to traditional therapy. Villous were infected with RH, ME49, UD1 or UD2 strains and after 24 h were treated with azithromycin (AZ), spiramycin (ESP) or combination of pyrimethamine plus sulfadiazine (PS). The villous viability was analyzed by LDH assay and morphological analysis. Parasite proliferation and cytokines production were analyzed by qPCR and ELISA, respectively. The treatments were not toxic for villous. UD1 infected villous showed higher parasite burden compared with RH, ME49 or UD2 infected villous. Treatment with either AZ, ESP or PS significantly reduced intracellular proliferation of T. gondii, regardless of the strain. UD1 infected villous produced a larger amount of MIF, IL-6 and TGF-β1 compared with others infected villous. AZ treatment increased MIF production by villous infected with RH or UD2 strain, but in villous infected with ME49 or UD1 strain the MIF production was not altered. On the other hand, the AZ treatment decreased IL-6 production by villous infected with ME49 or UD1 strain. In conclusion, AZ was comparable to conventional treatment against T. gondii Brazilian strains. In addition, UD1 strain was able to replicate more in villous than other strains and modulate important cytokines involved in parasite control, showing that different strains have different strategy to evade of the host immune response and ensure their survival. **Supported by:**Cnpq, Fapemig, Capes
Keywords: Toxoplasma gondii; brazilian strains; azithromycin

HP077 - **THE INVOLVEMENT OF OXIDATIVE STRESS IN TRYPANOCYDAL ACTIVITY OF β-LAPACHONE-DERIVED NAPHTHOIMIDAZOLES**

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Chagas disease, caused by the protozoan *Trypanosoma cruzi*, is a neglected illness that affects millions of people in Latin America and recently in non-endemic countries due to immigration of infected people. Due to the current treatment offers variable efficacy and serious side effects, our group has been working on the trypanocidal effect of quinones and derivatives. β-lapachone, a natural naphthoquinone was active on *T. cruzi* being its action related to free radicals production. Previously, it was demonstrated that three naphthoimidazoles derived from β-lapachone (N1, N2 and N3) were active on all *T. cruzi* stages, and the protozoa mitochondrion plays a crucial role in their mechanism of action, evidencing oxidative metabolism enzymes overexpressed in treated parasites. Here, we analyzed the reactive oxygen species (ROS) production and its consequences in epimastigotes treated with the compounds. Pre-treatment with antioxidants α-tocopherol and uric acid reverted the trypanocidal effect of naphthoimidazoles. Corroborating these data, the treatment with naphthoimidazoles induced an increase in hydrogen peroxide (H_2O_2), especially in N2-treated epimastigotes. Furthermore, the efficiency of the antioxidant system was also evaluated, being H_2O_2 production reduced by the pre-treatment with antioxidants in all conditions. N1, N2 and N3 also compromised the parasite mitochondrial function, decreasing the oxygen uptake, and such phenotype was reverted by pre-incubation with uric acid. Once the chemical structures of naphthoimidazoles not confer a redox potential, our results suggest an alternative pathway for ROS generation in N1, N2 and N3 mode of action. **Supported by:**FAPERJ, CNPq and FIOCRUZ
Keywords: Trypanosoma cruzi; naphthoimidazoles; oxidative stress

HP078 - **EVALUATION OF HUMORAL RESPONSE RELATED TO TH1 FACTORS IN NEOSPORA CANINUM INFECTION**

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Introduction: Neospora caninum is a protozoan, obligate intracellular parasite that belongs to the Apicomplexa phylum and causes neosporosis. This parasite has a significant relevance in veterinary medicine causing neuromuscular disease in dogs and repetitive abortions in cattle herds, contributing to affective and economic losses. Th1-based immune responses against N. caninum is one of the major responsible to control infection, inducing the release of effector molecules that limits parasite replication. In that context, this study aimed to define the role of key Th1-related molecules in the synthesis and functions of antibodies during exposure to N. caninum antigens. Methods: To evaluate the production and function of these antibodies, WT and genetically deficient mice for TLR2, MyD88, IFN-y, IL-12, MHCII and iNOS were immunized with soluble antigens of N. caninum (NLA). Kinetics of serum samples during immunization were measured by indirect and avidity ELISA. Profile of antigen recognition were evaluated by immunoblotting and biological functions assays analyzed by complement-mediated lysis and opsonization. Results: We observed that in the absence of crucial factors in induction of Th1 response (TLR2, IFN-y e IL-12) specific production of IgM and IgG antibodies was not impaired. Mice MyD88-/-, MHCII-/- and iNOS-/- presented decrease in the levels of these antibodies and in antigenic recognition. That reduction in these groups directly reflected in compromised lysis and opsonization properties of the hyperimmune sera. Conclusion: So, we demonstrate that factors related to Th1 immune response as MyD88, MHCII and iNOS, are crucial to induce humoral immune responses against N. caninum. **Supported by:**CAPES
Keywords: Neospora caninum; humoral response; th1

HP079 - **ROLE OF PD-1/PDL-1 COMPLEX IN LEISHMANIASIS: EFFECT OF IMMUNOTHERAPY USING ANTI-PD-1 AND ANTI-PD-L1 IN BALB/C.**

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Leishmaniasis is a neglected disease which treatment presents serial side effects. Leishmania amazonensis is the etiological agent of cutaneous leishmaniasis, diffuse cutaneous and the visceral form as well. The PD-1 (Programmed Death-1) receptor is found in lymphocytes and its ligand (PD-L1) is expressed in antigen-presenting cells such as neutrophils, macrophages and dendritic cells. The formation of PD-1/PD-L1 complex can induce T-cells suppression, which are reinvigorated when the complex formation is blocked by specific antibodies. Our study aimed to evaluate a new therapy against leishmaniasis using anti-PD-1 and anti-PD-L1 monoclonal antibodies [MoAbs] in L. amazonensis infected mice. Results showed that both MoAbs decreased parasite load in the infected mouse paw, draining lymph node and spleen. Following we suggest that the main mechanism that generates this control involved the increase of the IFN-ɣ produced by CD8+ T cells and, partially, by CD4+ T cells. We also evaluated the expression of PD-L1 in murine and human neutrophils infected with promastigotes and amastigotes of L. amazonensis. Our data revealed that the infection rate is similar in murine neutrophils infected with amastigotes and promastigotes, however, in human neutrophils promastigotes are more infective. As for PD-L1 expression, amastigotes stimulate PD-L1 expression in about 90% of murine neutrophils and 8% of humans. Promastigotes stimulate 40% PD-L1 expression in murine neutrophils and around 1% in humans. Remarkably, uninfected neutrophils have basal rates of PD-L1 expression. These results suggest that L. amazonensis is able to subvert the immune response by inducing PD-L1 expression in neutrophils and, consequently, suppressing effector T-cells. **Supported by:**FAPERJ, CNPQ, CAPES
Keywords: Immunotherapy; neutrophil; leishmaniasis

HP080 - **EVALUATION OF OLIGOPEPTIDASE B2 OVEREXPRESSION IN *LEISHMANIA MAJOR***

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Leishmaniasis constitutes a serious global health problem for which there is drugs used to treat are highly toxic, low efficiency and high cost, so the search for new molecular targets is urgent. One of the possible targets would be oligopeptidase B2 , a serine oligopeptidase which has no counterparts in humans. Oligopeptidase B belongs to the prolyl oligopeptidase family of serine proteases. This family only hydrolyse peptides smaller than 30 amino acid residues to the catalytic cleft is restricted by the N-terminal β-propeller domain. After genome sequencing, it has been observed two oligopeptidases B, OPB and OPB2. OPB2 has differences in comparison to OPB, as no conservation in S2 subsite and an C-terminal extension (de Matos Guedes, et al 2008). For a better understanding of the role this OPB2, L.major transgenic parasites overexpressing this enzyme using episomal vector were generated (OVER OPB2). L. major parasites overexpressing OPB2 with mutation in the active site of the enzyme (Ser to Gly) were also developed and called OVER MUT. Further, strains deficient in OPB2 had been developed (OPB2-/-). In this sense, we are studying the OPB2, trying to understand the role of this enzyme on growth, metacyclogenesis and infection in vitro and in vivo, through the use of transgenic parasites. **Supported by:**CNPQ e FAPERJ
Keywords: Leishmaniasis; leishmania major; oligopeptidase b

HP081 - **IN VITRO ACTIVITY OF SOME MONOTERPENES AGAINST LEISHMANIA AMAZONENSIS**

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Background Leishmaniasis are parasitic diseases of high morbidity. Pharmacotherapy has limitations due to high toxicity, long treatment time, high financial cost and emergence of resistant strains, emphasizing the urgency of cheaper, safer and more effective therapies for the treatment of leishmaniasis. Methods In this study we demonstrate the in vitro effect of 3-carene, 1,8-cineole, isoborneol and menthol monoterpenes against *Leishmania amazonensis*. The viability of intracellular amastigotesin DH82 cells treated for 48 h was measured by Enzyme-Linked Immunosorbent Assay. Cytotoxicity in DH82 cells was assessed by the MTT method. Changes in plasma membrane and mitochondrial membrane potential were measured by flow cytometry using propidium iodide and rhodamine 123 probes. Lipid peroxidation was determined by the generation of thiobarbituric acid-reactive substances (TBARS). Cytokines were quantified by commercial kits. Results The compounds menthol, isoborneol, 1,8-cineole and 3-carene exhibited activity against amastigotes, with IC_{50} values of 223.0 µg/mL, 57.1 µg/mL, 14.1 µg/mL, and 2.1 µg/mL, respectively. The trivalent antimoniate used as a negative control had IC_{50} of 97.3 µg/mL.The CC_{50} was related with IC_{50} gained the following selectivity indices ($IS = CC_{50}/IC_{50}$): 1.31 for menthol, 2.33 for isoborneol, 13.95 for 1,8-cineole, 107.3 for 3-carene and 0.7 for trivalent antimoniate. An increase in lipoperoxidation and reduction in mitochondrial membrane potentialwasobserved. However, no change in plasma membrane was identified. Also, a reduction in vitro production of IL-12 and TNF-α cytokines (by peritoneal macrophages of mice (Balc/c) infected with *L. amazonensis*) wasdetected after treatment with 100 µg/mL of 1,8-cineole, isoborneol and 3-carene. Conclusions Our study indicates that antileishmania activity caused by 1,8-cineole, menthol, isoborneol and 3-carene may be related to mitochondrial dysfunction and/or oxidative damage. **Supported by:**CAPES
Keywords: Leishmania; monoterpenes; mitochondrial dysfunction

HP082 - **INFLUENCE OF THE NOTCH SIGNALING PATHWAY ON THE ACTIVATION AND DIFFERENTIATION OF GOBLET CELLS DURING ORAL *TOXOPLASMA GONDII* INFECTION**

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Notch signaling pathway is known as an evolutionarily conserved mechanism that influences embryogenesis, tissue proliferation and homeostasis as well as T cell development, differentiation and function. The intestinal epithelium is characterized by its rapid and constant renewal in which Notch is related with the regulation of cell fate through close-range and cell-cell interactions. The purpose of this study is to elucidate the role of Notch pathway during oral infection with *Toxoplasma gondii* and its interaction with goblet and Paneth cells differentiations. C57BL/6 mice were treated intraperitoneally for eight days with two different concentrations (5 and 10 µM) of a gamma-secretase inhibitor (Dibenzazepine – DBZ), an intracellular multicomponent protease of the Notch pathway. Then, each mouse was orally infected by gavage with 20 ME-49 *T. gondii* cysts. Survival of treated and infected animals was assessed during 30 days. Another group of mice was euthanized at day 8 of infection, and intestines were measured and organs were collected. The intestinal segments were processed and stained with Haematoxilin and eosin or Alcian Blue for quantification of Paneth and goblet cells, respectively. In both concentrations of the inhibitor, the number of goblet and Paneth cells was increased. The tissue parasitism decreased in the small intestine when animals were infected and treated with DBZ. Additionally, the DBZ-treated mice presented normal length of the small intestine and colon similarly to uninfected animals. The levels of IFN-γ and TNF in mouse treated and infected were decrease compared with mice only infected. These results suggest that the inhibition of NOTCH pathway is able to ameliorate the intestinal *T. gondii* infection.
Supported by:CAPES, CNPq e FAPEMIG
Keywords: Toxoplasma gondii; notch; gamma-secretase inhibitor

HP083 - ***LEISHMANIA* INDUCES THE RELEASE OF MAST CELLS EXTRACELLULAR TRAPS**

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During the infection by *Leishmania amazonensis*, the insect vector inoculates promastigotes inside a blood pool, whereby the parasite interacts with different cells and with the extracellular matrix proteins. The mast cells are resident granulocytes that regulate the immune response by releasing different mediators. Recently, it has been demonstrated that mast cells release extracellular traps (MCETs), composed of chromatin, histones and granular proteins, which are capable to ensnare bacteria and also has a bactericidal activity. It has already been shown that *Leishmania* promastigotes are phagocytosed and replicate in these cells, and induce mast cells degranulation as well. Although different studies imply mast cells among the first line of defense against *Leishmania*, little is known about the induction and the role of MCETs in the infection. Therefore our objective is to evaluate if promastigotes of L. amazonensis induces the release of MCETs and if these traps are leishmanicidal. We also want to understand the role of laminin, an extracellular matrix protein, in this process. Here, we used HMC-1, a human mast cell lineage, stimulated with promastigotes and the release of MCETs was analyzed in culture supernatants. Our results shown that promastigotes are capable of induce MCETs release by HMC-1 by two different ETosis mechanism (rapid and classic). In order to simulate the environment that these parasites interact with the host cells, we tested the release of MCETs in the presence of laminin. Our results confirmed that MCETs are released by promastigotes and the presence of laminin, either in their polymerized form or in their free form, amplified the ETosis mechanism. Ours results demonstrated that promastigotes forms of *Leishmania amazonensis* induce the release of MCETs in HMC-1, and this mechanism is potentiated by laminin.
Supported by:CAPES, FAPERJ, CNPq
Keywords: Leishmania; mast cells extracellular traps; laminin

HP084 - **1,8-CINEOL DAMPENS *PLASMODIUM FALCIPARUM* RING FORMATION *IN VITRO*.**

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INTRODUCTION: *Plasmodium falciparum*, the causative agent of malaria in humans, has at least one strain resistant to every known antimalarial. 1,8-cineol (1,8Cin), a monoterpene, has anti-inflammatory and microbicidal effects, however its effect in the erythrocytic cycle of *P. falciparum* has never been evaluated. OBJECTIVE: We aimed to evaluate the effect of 1,8Cin in the erythrocytic cycle of *P. falciparum in vitro*. METHODS: *P. falciparum* was cultured in RPMI 1640 medium supplemented with 0,5% Albumax II, 5% hematocrit (CEP-HUCCF n. 074/10). Parasite schizonts (1% parasitemia) were treated or not with 1,8Cin and ring formation was evaluated by optical microscopy, after 24h incubation. RESULTS: Increasing concentrations of 1,8Cin (10 – 1,000 µg/mL) reduced ring formation in a dose-dependent way, with maximum effect at 1,000 µg/mL and IC50 of 154 µg/mL (n=5). Health erythrocytes incubated in the same conditions with 1,8Cin had no significant hemolytic effect. The inhibitory effect of 1,8Cin seemed to be dependent on parasitemia. We observed that 150 µg/mL 1,8Cin induced 55,7 ± 1,0% and 36,7 ± 7% inhibition of new ring formation at 0,5 and 3% parasitemia, respectively (n=3). To evaluate whether the inhibitory effect of 1,8Cin was reversible, cells treated or not with 150, 500 or 1,000 µg/mL 1,8Cin for 24 h, were washed and recultured without compound. The effect of 1,8Cin was maintained for at least 72h incubation (n=3). Interestingly, cultures treated with 1,000 µg/mL, which apparently lost viability, recovered growth 10 days after compound washing (n=3). At this time point, parasitemia was around 4,4 ± 0,9%, whereas the control reached 11,0 ± 1,4% parasitemia at day 4. CONCLUSION: In conclusion, 1,8-cineol impaired *P. falciparum* erythocytic cycle by inhibiting the generation of new ring forms and no apparent toxic effects to the host. These results could represent new avenues in the development of therapeutic interventions. **Supported by:**CNPq, CAPES, FAPERJ
Keywords: Malaria; plasmodium; antimalarials

HP085 - ***LEISHMANIA AMAZONENSIS* MODULATES MURINE MACROPHAGE MIRNA PROFILE BY THE CROSS-REGULATION BETWEEN HOST NOS2 AND PARASITE-ARGINASE DURING INFECTION**

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The *Leishmania amazonensis* induces modulations on the miRNA profile expression on murine-host cells. Some of these miRNAs have as their targets genes that encode important molecules to the activation of the host immune response. The success of infection or its control is connected to L-arginine. The arginase from parasite uses L-arginine from host to produces polyamines, essential for parasite survival. The L-arginine also is used by host Arginase I, to produce polyamines or by Nitric Oxide Synthase 2 (NOS2), to produce nitric oxide (NO) to kill parasite. The aim of this work is to analyze the role of parasite arginase and host NOS2 in the miRNA profile of L. amazonensis infected macrophages. We observed that bone marrow derived macrophages (BMDM) from C57BL/6 wild type (WT) mice or the nitric oxide synthase 2 mice knockout ($NOS2^{-/-}$) show distinct miRNA profiles after 4 or 24h of infection with *La*-WT or knockout for arginase (*La*-arg^{-}), that correlated with a distinct modulation of host miRNA profile. After 24h, BMDM-WT infected with *La*-WT showed 10.7% of 84 miRNA modulated, 66.6% of them up-regulated. In the infection with *La*-arg^{-}, the amount of modulated miRNAs was 22.6% of modulation, 57.9% up-regulated miRNAs. Despite, the $NOS2^{-/-}$ BMDM infected with *La*-WT infection showed only 7.1% of miRNAs modulated, 50% up-regulated. The infection of BMDM-$NOS2^{-/-}$ with *La*-arg^{-} modulated 3.6%, being all of them up-regulated. The miR-294 and miR-721 were up-regulated in BMDM-WT infected with *La*-WT or *La*-arg^{-} after 4 and 24h. The absence of the NOS2 did not interfered significantly on the expression of these miRNAs. Indeed, the levels of *Nos2* mRNA were higher in BMDM-WT infected with *La*-WT after 4 and 24h, but did not in *La*-arg^{-}. *Cat2B* (Cationic amino acid transporter 2B) level increased after 24h of infection in both conditions. Our data suggest that modulation of miRNAs and mRNA expression is dependent on parasite arginase and host NOS2 in *L. amazonensis* infected macrophages **Supported by:**FAPESP and CNPq **Keywords:**Gene expression; microrna; arginine metabolism

HP086 - **NITRIC OXIDE PRODUCTION BY *LEISHMANIA AMAZONENSIS* DEPENDS ON ARGINASE ACTIVITY**

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The L-arginine conversion to urea and L-ornithine is catalyzed by arginase as essential pathway for *Leishmania (Leishmania) amazonensis (La)* replication and survival in mammalian host. L-arginine is also the substrate of nitric oxide synthase (NOS) to produce nitric oxide (NO). Using RNA-seq, we identified a family of oxidoreductases differentially expressed in promastigotes and axenic amastigotes of *La* wild-type (*La*-WT) and *La* arginase knockout (*La*-arg$^-$). We identified an orthologue of NOS-like oxidoreductase that was up-regulated in *La*-WT promastigotes compared to *La*-arg$^-$, and in the comparison between *La*-WT promastigotes vs. *La*-WT axenic amastigotes. Based on that, we hypothesized that this up-regulation could lead to an increase of NO production during promastigote growth besides being up-regulated in the absence of arginase activity. Our hypothesis was confirmed by NO quantification of parasites labelled by DAF-FM in a flow cytometry assay. We detected an increase in NO production in the mid-stationary and late-stationary growth phases of *La*-WT promastigotes. This increase suggests that NO production could be an important factor in metacyclogenesis triggering; on the other hand, *La*-arg$^-$ showed an earlier increase in NO production compared to *La*-WT, suggesting that NO production is arginase-dependent. Interestingly, axenic amastigotes of both strains produced higher levels of NO than those observed in promastigotes. Our work suggests that NOS-like is expressed in *Leishmania* in the stationary growth phase of both promastigotes and amastigotes, and could be regulated by the internal pool of L-arginine in an arginase-dependent manner. **Supported by:**FAPESP and CNPq
Keywords: Arginine metabolism; rna-seq; flow cytometry

HP087 - **LAMININ AND POLYMERIZED LAMININ INDUCE NEUTROPHIL EXTRACELLULAR TRAPS (NETS) AND MODULATE NETOSIS INDUCED BY LEISHMANIA AMAZONENSIS**

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Leishmania promastigotes are inoculated by the insect vector in a pool of blood and neutrophils are the first phagocytic cells recruited and infected in the early stages of the infection. Upon interaction with the parasites they also extrude structures known as neutrophil extracellular traps (NETs). All these initial steps occur in close contact with proteins from the extracellular matrix (ECM). Here, we aim to study the interaction between neutrophils, Leishmania amazonensis promastigotes, and different isoforms of laminins either, isolated or in association, analyzing the induction of NETs. Initially, we observed that neutrophils stimulated with laminin isoforms (111, 211, 332, 411, 421 and 511) adsorbed or in suspension, released NETs. The same happens when neutrophils interacted with the polymerized form of laminin (Polylaminin) 111, 411 and 511. Our results showed that about 70% of neutrophils express the laminin receptor (VLA-6/CD49f), and pretreatment with anti-α6 integrin antibody (GOH3) decreased NETs release by 32% and 35%, respectively in relation to the stimulation with laminins 411 and 511. NETs release mechanism induced by laminin is dependent on neutrophil elastase and peptidylarginine deiminase 4 (PAD4), enzymes that participates in the chromatin decondensation process. NET release mechanism is dependent on ROS generation when neutrophils were stimulated with laminin 511, but not with the 411 isoform. Finally, we demonstrated that laminins 411 and 511 modulate NETs induced by Leishmania amazonensis promastigotes. **Supported by:** CAPES, FAPERJ AND CNPq
Keywords: Extracellular matrix ; leishmania amazonensis ; neutrophil extracellular traps

HP088 - **NEW RHOPTRY PROTEINS (ROP) OF THE APICOMPLEXAN *NEOSPORA CANINUM*: NCROP15B AND NCROP55**

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Neospora caninum is a coccidian of the Apicomplexa phylum, described as the main cause of parasitic abortion in cattle (neosporosis), due to the high transmission efficiency of the infected cow to its fetus. Apicomplexans interact and invade the host cells through the coordinated protein secretion of the apical complex, composed of organelles called micronemes, rhoptries and dense granules. Rhoptries proteins are associated with the parasitophorous vacuole formation (PV), intracellular environment survival and parasite virulence. In *N. caninum*, only a few rhoptries proteins have been so far identified and characterized, such as ROP40 and NcROP2Fam-1. Thus the aim was to identify and characterize the NcROP15B (NcLiv_011700) and NcROP55 (NcLiv_031550) rhoptries proteins of *N. caninum*. *In silico* analyzes of the NcROP15B and NcROP55 sequences indicated the presence of a domain belonging to the protein kinase family for NcROP15B and NcROP55 and a signal peptide only for NcROP15B. The regions for recombinant expression were amplified by PCR from the *N. caninum* tachyzoite cDNA. The inserts were subcloned (pGEM) and then ligated into pET28 expression plasmid in *E. coli* BL21 (DE3) for IPTG-induced recombinant expression. After purification, the recombinant NcROP15B and NcROP55 were used for immunization of mice. Both anti-ROP15B and anti-ROP55 polyclonal serum reacted with two proteins in the *N. caninum* extract in Western Blot 1D, with approximately 35 KDa, within the predicted for NcROP15B (32 KDa), but below for NcROP55 (47.9 KDa). In confocal immunofluorescence, NcROP-15B and NcROP55 exhibited a perinuclear localization pattern. Further investigation of NcROP-15B and NcROP55 are being performed for a better understanding of these protein kinases associated with the processes of invasion and proliferation of the parasite, targets with great preventive potential.
Supported by:CNPq
Keywords: Rhoptry; neospora caninum; apicomplexa

HP089 - **1,8-CINEOL IMPAIRS THE INTRAERYTROCYTIC DEVELOPMENT OF *PLASMODIUM FALCIPARUM IN VITRO***

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INTRODUCTION: Malaria is a parasitic disease caused by *Plasmodium*. During the erythrocytic phase, *P. falciparum* undergoes a developmental process assuming different forms, within 48 hours. Different antimalarials, such as artemisinin, affect the intracellular development of the parasite. However, parasites are able to develop mechanisms of resistance. 1,8-Cineol (1,8Cin), derived from *Artemisia annua*, has proven anti-inflammatory and microbicidal effects. However, its effect on the development of *Plasmodium sp*. is unknown. OBJECTIVE: Our aim was to evaluate the effect of 1,8-Cineol on the development of *P. falciparum in vitro*. METHODS: Erythrocytes infected with P. *falciparum* (1% parasitemia), were maintained in RPMI-1640 medium supplemented with 0.5% Albumax II and 5% hematocrit (CEP-HUCCF: 074/10). Cultures were synchronized and schizonts were treated daily with 150 µg/mL of 1,8-cineol (IC50) for 96 hours, two consecutive cycles of the parasite. The parasitemia and morphological changes were evaluated daily by light microscopy. RESULTS: In the first 24 h, 1,8Cin reduced parasitemia to 50% when compared to the control (n=3). The number of infected erythrocytes was maintained till the end of treatment (approximately 2,0%), whereas parasitemia in the control increases to 6,5 ± 0,6%. The number of schizonts at the end of the first cycle (48 h) was reduced in the treated group when compared to the control (0.1 ± 0.09% to 0.8 ± 0.15 %). At the end of treatment, cells seems to had a reduction in hemozoin formation and light vacuoles. CONCLUSION: Taken together, our results showed that 1,8-cineol retards the intra-erythrocytic cycle of *P. falciparum*, inducing an increase in time that parasite remains at each stage. These results suggest a potential antimalarial effect, however further studies are needed to describe the observed morphological changes and the delay in parasite intracellular development. **Supported by:**CAPES, CNPq, FAPERJ
Keywords: Malaria; plasmodium; antimalarials

HP090 - **BRADYKININ INCREASES ADHESION OF *PLASMODIUM FALCIPARUM*-INFECTED RED BLOOD CELLS TO ENDOTHELIAL CELLS**

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INTRODUCTION: In our previous work, we have demonstrated that bradykinin (BK) levels are increased in *P. falciparum* culture supernatant. Although its influence in leukocytes adhesion have already been shown, the role of BK in the iRBC adhesion to endothelium have never been reported yet. OBJECTIVE: The objective of this work was to investigate the role of BK in adhesion of iRBC to endothelial cells. METHODS: iRBC-BMEC adhesion was analyzed under light microscopy. Protein expression was determined by immunoblotting. RESULTS: BMEC stimulated with 10^{-7} M BK increased iRBC adhesion in 78%. This effect was completely abolished by 10^{-7} M HOE-140 or 10^{-7} M DALBK, B2 and B1 antagonists, respectively (n=3). Since BK levels are increased in the *P. falciparum* culture supernatant, we tested the effect of this supernatant on iRBC adhesion to BMEC. For this, the culture supernatant was harvested right after the rupture of mature forms and used to treat BMEC cells. After 14 h incubation, the adhesion assay was performed in the presence of iRBC. The parasite supernatant increased the adhesion of iRBC to BMEC in 2.5 folds. In addition, the pretreatment of BMEC with 10^{-7} M HOE-140 or 10^{-7} M DALBK prevented this effect (n=3). Immunoblotting analysis showed that 10^{-7} M BK reduced in 20 % AKT expression (n=4). In relation to vascular dysfunctions, the expression of ZO-1, a tight junction protein was analyzed in BMEC stimulated by 10^{-7} M BK. The treatment reduced in 18 % the expression of ZO-1 in BMEC (n=3). CONCLUSION: Our results suggest that increasing of BK during *P. falciparum* infection stimulates iRBC adhesion to endothelial cells in a B2 and B1 manner, probably increasing adhesion molecules through AKT dependent signaling pathway. **Supported by:**CNPq, CAPES, FAPERJ
Keywords: Malaria; plasmodium; host-parasite interaction

HP091 - **CHARACTERIZATION OF A SURFACE SRS PROTEIN OF *NEOSPORA CANINUM* (SAG-RELATED NCSRS57)**

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Neospora caninum is an obligate intracellular parasite of the Apicomplexa phylum responsible for abortion and loss of fertility in cattle, resulting in significant worldwide losses in the livestock. As any apicomplexan, the adhesion is mediated by surface proteins, mainly the SRS protein superfamily (Surface Antigen Glycoprotein SAG – Related Sequences). This work was carried out with the aim of characterizing a novel SRS surface protein NcSRS57 (SAG3/NcLIV_060660) of *N. caninum*. Our results showed that; among the apicomplexan homologues, NcSRS57 presented higher identity and similarity with Toxoplasma gondii (TgSRS57). NcSRS57 has twelve conserved cysteine residues predicted to form six disulfide bonds distributed in two SRS domains (D1 and D2), constituted by β-sheet sandwiches and α-helices associated with each other. The coding sequence of NcSRS57 (without the signal peptide and without the GPI anchor) was cloned into pET32, however only a 92 bp fragment was translated in contrast to the cloned sequence of 1074 bp, probably due to the presence of a hidden stop codon. This event generated the recombinant protein (rNcSRS57) with a molecular mass lower than expected (19.5 kDa obtained against 31 kDa predicted). On western blot native NcSRS57 was detected with 43 kDa mass. The anti-rNcSRS57 polyclonal antibody resulted in a 16% inhibition on the adhesion/invasion of tachyzoites. On confocal immunofluorescence the native form of NcSRS57 was localized on the whole external surface area of the *N. caninum* tachyzoites. This study increases the knowledge of the parasite *N. caninum*, specially on the search and selection of new targets to be investigated against neosporosis. **Supported by:**CNPq
Keywords: Neospora caninum; srs proteins; ncsrs57

HP092 - ***IN VITRO* ACTIVITY OF SYNTHETIC NAPHTHOQUINONE LQB-166 IN *TRYPANOSOMA CRUZI***

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Endemic in Latin America, Chagas is a disease caused by the protozoan *Trypanosoma cruzi*. The therapy of this disease is limited, toxic, and not effective in the infection chronic phase. The aim of this study is to evaluate the *in vitro* activity of the naphthoquinone LQB-166 (2-hydroxy-3-phenyl-1,4-naphthoquinone) on *Trypanosoma cruzi* and its toxicity on the mammalian lineage cells as VERO and THP0-1 macrophages. *Trypanosoma cruzi* epimastigotes (Dm28c clone) were incubated with different concentrations of LQB-166 (0-100 µM) for 168 h / 27 ° C and counted daily in Neubauer's chamber. To evaluate the toxicity in mammalian cells, VERO cells and THP0-1 macrophages were incubated with different concentrations of LQB-166 (0-3200 µM) for 24h /37°C/ 5% CO2. As a result, LQB-166 was able significantly inhibit($P<0,0001$) the parasite growth in 39.1%, 52, 2% and 70.6% at 25µM, 50µM and 100µM, respectively. The IC50 estimate at 37.4µM with 120h of culture. The LQB-166 showed no toxicity on mammalian cells, the IC50 was estimated at 3013µM for VERO cell and 3044µM for THP0-1 macrophages. These results shown that the synthetic naphthoquinone LQB-166 exhibits a great activity on *Trypanosoma cruzi* epimastigotes forms and does not present toxicity in mammalian cells VERO and THP0-1 macrofages. We are currently analyzing the action of this naphthoquinone on cardiomyocytes (H9C2) and intracellular amastigotes forms of *Trypanosoma cruzi*.
Keywords: Trypanosoma cruzi; lqb-166; mammalian cells

HP093 - **DETECTION OF MMP-2 IN DIFFERENT SPECIES OF LEISHMANIA AND IN INFECTED PERITONEAL MACROPHAGES**

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Metalloproteinases (MMPs) are zinc dependent endopeptidases, which degrade components of the extracellular matrix, thus participating in cell migration, therefore they may help the dissemination of intracellular parasites, such as the protozoan *Leishmania*. Studies have demonstrated the increase of MMP in macrophages infected with *Leishmania*. Thus, the present study investigated the presence of MMP2 in *Leishmania* sp. and during in vitro macrophages (*Mφ*) interaction. First, we investigated the presence of MMP2 in *L. amazonensis* (*La*) and *L. braziliensis* (*Lb*) in different growth phases by flow cytometry, and detected a higher amount of MMP2 in *La* in the stationary phase compared to *Lb* in the same phase, and with the logarithmic phase of *La*. For the infection *Mφ* was stimulated with LPS or without, and infected with *La* or *Lb* for 2h in the presence of the specific inhibitor of MMP2 activity (cis-9-Octadecenoyl-N-hydroxylamide; oleoyl-N-hydroxylamide), the endocytic index (*ind end*) and adhesion index (*ind adh*) were calculated. Thus the decrease of the *ind end* was detected when *Mφ* were infected with *La* in the presence of the MMP2 inhibitor. Whereas, for *Mφ* infected with *Lb* in the presence of the inhibitor the *ind adh* were lower. Through the immunofluorescence technique, was possible to observe MMP2 during the infection, especially the group of *Mφ* only infected, where observed marking near the vacuole parasitophorous of *La* and marking near the plasma membrane when *Mφ* were infected with *Lb* after 2h of infection. Thus, our preliminary results suggest that MMP2 is important for the infection of *La* in Mφ and for adhesion of *Lb* in this Mφ. In addition, the intense labeling of MMP2 near the phagocytosed parasite suggests the importance of this protein during infection. Thus, a higher production of MMP2 in *La* in the stationary growth phase suggests the need for this molecule at the time of infection, indicating a new function for MMP in the parasite-host relationship.
Supported by:CAPES/CNPQ; SVS-MS-IEC; UEPA
Keywords: Leishmania; metalloproteinase; macrophage

HP094 - ***IN VITRO* WOUND HEALING PROCESS IN FIBROBLASTS TREATED WITH CONDITIONED MEDIA FROM MACROPHAGES INFECTED WITH *LEISHMANIA (LEISHMANIA) AMAZONENSIS* OR *LEISHMANIA (V.) BRAZILIENSIS*.**

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American Tegumentary Leishmaniasis (ATL) is caused by species of Leishmania, an intracellular protozoan. The most common cells at the site of the infection are macrophages, even though there are also reports of the presence of both promastigotes and amastigotes in fibroblasts. Furthermore, wound healing plays crucial role in the skin recovery during ATL. The promastigotes of *L. amazonensis (L.a)* and *L. braziliensis (L.b)* were used to infect the macrophages, after that, it was collected the conditioned media (CM) and used to incubate the fibroblast from Balb/c in in vitro scratch assay and images was captured after 24h to observe cell migration. Subsequently, morphological analysis was performed by scanning electron microscope and fluorescence microcopy to detect MMP-9. The cytokine assay was performed using flow cytometry. Our results showed the higher cell migration in the scratch of fibroblast groups incubated CM from macrophages infected with *L.b* compared to fibroblast incubated with conditioned media from not infected macrophages 24h and fibroblast incubated with CM from not infected macrophages and infected with *Leishmania* of 48h. Moreover, the cytokines found in conditioned media from macrophages infected with *Leishmania* were IL-4, IL-6 and TNF-α. After the fibroblast was incubated with CM from macrophages produced only TNF-α production was significant, despite IL-6 was detected in all supernatants. In this study, we observed the morphology and extensions at the leading edge of fibroblast – lamellipod – and filopodia and detection of MMP-9 in all groups, except in fibroblast with CM from not infected macrophages. It was concluded that fibroblast incubated with CM from macrophages not infected and infected with *Leishmania* for 24h showed the higher cell migration than fibroblast incubated with CM from macrophages infected for 48h. The presence of MMP-9 seems to have a beneficial effect for injury control. **Supported by:**CNPq; IEC/SVS/MS; UFPA **Keywords:**Leishmania; fibroblast; macrophages

HP095 - **3'UTR ROLE ON GENE EXPRESSION OF PROTEINASES GROUPS ON PROMASTIGOTE AND AMASTIGOTE FROM *LEISHMANANIA (V) BRAZILIENSIS***

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Leishmaniasis is a parasitic disease caused by protozoa of the genus Leishmania. There is not an effective vaccine yet and its chemotherapy is inadequate. The search for new therapeutic targets is an essential points in Leishmanises combat. Proteases are related to the capacity of these parasites to infect and survive in their hosts and are therefore classified as virulence factors. Leishmania species presents significant distinctions in many protease features, such as occurrence, quantity and conservation, then expression regulation of proteases would be differentially between proteinases according to the circumstances of their microenvironment. The genome from Leishmania is organized in the large groups of genes which are constitutively transcribed by RNA Pol II (ribonucleic acid polymerase II) producing a polycistronic pre-mRNA. The regulation of gene expression in these organisms is almost exclusively in the post-transcriptional level. Studies of complex regulatory networks control gene expression in Leishmania become of great interest. This study aims to understand the factors involved in proteinase gene expression, such as conserved signaling sequences in UTR sequence. It was observed that the amastigotes and promastigotes of *Leishmania braziliensis* expressed differentially *in vitro* genes of the two main protease classes: cysteine and metallo. Furthermore, it was observed that some proteases have sequences could be involved on regulation of gene expression associated life stage of Leishmania. Some sequences were selected and cloning on plasmid gene report to trying to understand the functioning of the control of gene expression of enzymes groups. This study goals understanding the factors involved on regulation of proteinases gene expression and possibly indicate promising targets for the development of new therapeutic strategies against this disease. **Supported by:**Faperj; CNPq; FIOCRUZ **Keywords:**Gene expression; leishmania; proteases

HP096 - **CLONING AND EXPRESSION OF THE ARGINASE ENZYME FROM THE NEW AND OLD WORLD STRAINS OF *LEISHMANIA INFANTUM***

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Visceral leishmaniasis is a public health problem in tropical and subtropical regions of the world, caused by *Leishmania infantum* in the Old World and by *L. infantum (*syn *L. chagasi)* circulating in the New World. L-arginine metabolic pathway was observed in trypanosomatids, as well as in host cells. In this pathway, L-arginine substrate is shared by two enzymes: nitric oxide synthase (NOS), which leads to the production of nitric oxide (NO); And arginase which results in the production of urea and ornithine, essential in the polyamines synthesis. However, there no much data about Leishmania infantum infection in relation to the role of L-arginine metabolism in infectivity. The general objective of this work is to know the genetic and biochemical differences of arginase in New and Old World strains of the same species of *L. infantum*. As first step, the heterologous expression of the *L. infantum* arginase gene is being developed. This enzyme was cloned through primers drawn from gene sequence from the Tritrypdb.org genoma database. The gene was amplified from *L. infantum* and *L. infantum* (syn. *L chagasi)* cultures by PCR, the fragment obtained were purified and sequenced. Once confirming the sequence, no difference was observed between the strains. The fragment was cloned into pET28a generating the plasmid ARGpET28 on DH5alpha cells. Upon confirmation by digestion, it was transfected into BL21 chemo-competent cells. Different expression tests are being performed with IPTG at different temperatures and induction times. Expression analysis was done by gel and westernblot. Cloning was successful being confirmed PCR and corresponding bands were observed with the expression, however, the standardization of the expression the method is being developed.

Supported by:CNPq; FAPERJ; FIOCRUZ

Keywords: Arginase; leishmania infantum; cloning

HP097 - **THE ROLE OF CYCLOPHILIN 19 DURING TRYPANOSOMA CRUZI INFECTION**

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Cyclophilins are ubiquitous and evolutionarily conserved enzymes, known for its peptide-prolyl-cis-trans-isomerase (Ppiase) activity. These enzymes are expressed from prokaryotes to eukaryotes. Normally cyclophilins are intracellular enzymes and their expression is increased under stress situations as cardiomyopathies. In general, cyclophilins act as chaperones stabilizing other proteins and inducing changes in their conformation that results in alterations in signaling and protein-protein interactions. They are also found secreted in some organisms. We have previously shown that cyclophilin 19 (Cyp19) is involved in the interaction of the Trypanosoma cruzi, the protozoan parasite that causes Chagas disease, with the insect vector. Cyp19 was found secreted by the insect stage of T. cruzi, protecting the parasites from antimicrobial peptide concomitantly causing calcineurin signaling in parasites, and driving infectivity. Here we found that Cyp19 is expressed by all lifecycle stages of the parasite and is secreted by culture cell trypomastigotes and intracellular amastigotes. Importantly, preliminary results show that Cyp19 is found in the host cell cytoplasm. Therefore, we further investigated the mechanism of secretion. We also found by mass spectrometry analysis that Cyp19 is acetylated at two lysine residues, including one present in this additional N-terminal domain. As acetylation of CypA, the human homolog of Cyp19 is required for secretion, we suggest that both these acetylations could be involved in the secretion process of T. cruzi. As secreted cyclophilins modulate inflammatory responses, the release of Cyp19 might be relevant for the pathogenesis of Chagas disease.

Supported by:CAPES/CNPQ/FAPESP

Keywords: Cyclophilin 19; trypanosoma cruzi; infection

HP098 - **COMPARATIVE TRANSCRIPTOME PROFILES OF HUMAN CELLS DURING INFECTION WITH VIRULENT AND AVIRULENT STRAINS OF *TRYPANOSOMA CRUZI***

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Gene expression data generated from host-pathogen interaction studies are invaluable to the functional characterization of host defense mechanisms. Previous transcriptome studies have shown differences in the responses to in vitro infection of human foreskin fibroblasts (HFF) with two different strains of Trypanosoma cruzi (Sylvio X-10 and Y strains) that belongs to distinct parasite lineages (TcI and TcII). Here we present a transcriptome analysis of non phagocytic HFF infected with tissue culture trypomastigotes of T. cruzi strains showing opposite virulent phenotypes with the aim of uncovering the factors responsible for such contrasting outcomes of the infection. Total RNA was extracted from HFF cells at 60 and 96 hours post-infection (HP0i) with CL Brener, a cloned virulent strain, and CL-14, a cloned strain that is neither infective nor pathogenic in in vivo models of infection. Differential expression analysis showed that the cells have similar transcriptome profiles when infected with either CL Brener or CL-14 at 60 HP0i. However, gene ontology analysis of differentially expressed genes (DEG) at 96 HP0i revealed that genes categorized under immune response were highly enriched. Although the expression and the total number of DEG genes under this category were more pronounced at 96 HP0i compared to 60 HP0i with both strains, the number of DEG at 96 HP0i with CL Brener is higher when compared to CL-14. Among the highly DEG related to the immune response are several chemokines (CCL5, CXCL10, CXCL11), interleukins (IL8, IL11), interferon 1 beta (IFNB1), a tumor necrosis factor (TNFSF10), colony stimulating factors (CSF3) and innate immune response related genes (RSAD2, OAS1, OAS2, OAS3, OASL). Notably, a group of cytokines and chemokines (CXCL5, CXCL6, IL1A, IL1B, IL24, IL33) were exclusively upregulated in cells infected with CL Brener at 96 HP0i, indicating that these genes may constitute factors that could be correlated with the distinct infection outcomes.
Supported by:CAPES; CNPQ **Keywords:**Trypanosoma cruzi; differential expression; immune response

HP099 - **ANALYSIS OF THYMIC MICROARCHITECTURE AND EXPRESSION OF MICROENVIRONMENT COMPONENTS IN MALNOURISHED MICE INFECTED WITH LEISHMANIA INFANTUM**

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Malnutrition is a risk factor for the development of visceral leishmaniasis (VL). Previous studies from our group on a murine model of protein malnutrition and infection with Leishmania infantum demonstrated that malnourished mice present severe thymic atrophy followed by significant alterations in lymphocyte subpopulations. In addition, chemokine levels were significantly altered in the thymuses of those animals but ex-vivo migration capabilities of T cells were preserved, suggesting that the thymic microenvironment of malnourished and infected animals is particularly compromised. Our hypothesis is that protein malnutrition modifies the composition of thymic extracellular matrix (ECM) and induces microarchitecture remodeling, impairing the correct migration and maturation of T cells, with consecutive lack of control of parasite proliferation in the spleen, accelerating the clinical outcome of severe VL. Hence, this work aims to analyze the effect of protein malnutrition on thymic organization and on the expression and distribution of ECM components in BALB/c mice infected with L. infantum. Localization and distribution of T cell subpopulations will be also evaluated in the thymus of those animals. Histopathological analyses revealed that whereas well-nourished and infected mice (CPi) showed an increase in the cortical zone, malnourished and infected animals (LPi) presented a significant decrease in this region exhibiting a reduction of ~60% cortical region ($p = 0.01$). Quantification of fibronectin and laminin shows significant decreased levels of these molecules in CPi animals. These molecules were also detected in LP and LPi animals. Altogether, the data suggest that malnutrition could affect T cell differentiation, selection and maturation, all of which occur in the thymic cortical zone. Those defects might negatively affect the exit of mature T cells to periphery and ultimately the control of parasite proliferation in peripheral lymphoid organs such as spleen. **Supported by:**CNPq FAPERJ
Keywords: Leishmania infatum; thymus; malnutrition

HP0100 - ***TOXOPLASMA GONDII* DECREASES THE 5-LIPOXYGENASE EXPRESSION IN THE SMALL INTESTINE AS A MANNER TO PROTECT ITSELF**

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Toxoplasma gondii induces Th1 immune response in infected hosts that can lead to serious consequences mainly in immunocompromised individuals and pregnant women. 5-lipoxygenase (5-LO) is required for production of leukotrienes and lipoxins and interferes in others parasitic infections such as *Strongyloides venezuelensis* infection. To investigate the role of 5-LO during experimental toxoplasmosis, C57BL/6 mice were treated with MK886 or with larva-antigen of *S. venezuelensis* (AgSv) to inhibit or induce 5-LO pathway, respectively. Mice were infected with 20 ME49 *T. gondii* cysts and euthanized 7 days post-infection. Parasite itself decreased enzyme expression in small intestine and treatment with MK886 reinforce this reduction during infection, once treated infected-mice presented higher intestinal parasitism. AgSv treatment increased 5-LO expression in small intestine and in parallel decreased parasitism in the organ. Furthermore, MK886 treated-mice presented increased inducible nitric oxide synthase (iNOS) positive cells in small intestine, while AgSv treatment trigged opposite effect. Although the low parasite burden normally detected in the liver, MK886 treatment decreased once more T. gondii proliferation and enhanced iNOS positive cell numbers; however, the AgSv treated-mice presented increased parasite burden and similar iNOS positive cell numbers when compared with control-infected mice. Furthermore, *T. gondii* infection increased glutamate-pyruvate transaminase (GPT) levels, especially in MK886 treated-mice. These data suggest that *T. gondii* decrease 5-LO expression in the small intestine as a manner to subvert the immune response and protect itself in this important site of entry of the parasite; on the other hand, the higher iNOS positive cells detection when 5-LO is dampened could be improving immune response against the parasite in the liver of infected mice. Altogether, 5-LO has different effects in different organs during toxoplasmosis. **Supported by:**CAPES, CNPq, FAPEMIG
Keywords: Toxoplasma gondii; 5-lipoxygenase; mk886

HP0101 - **ANALYSIS OF THE M1/M2 ACTIVATION PROFILE OF C57BL/6 PERITONEAL MACROPHAGES INFECTED WITH *LEISHMANIA (VIANNIA) BRAZILIENSIS*.**

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Leishmaniasis are an infectious diseases caused by different species of protozoa of the genus *Leishmania*. This disease may present a pole of M1 activation, in which macrophages have cytotoxic activity and proinflammatory cytokines. or the M2 pole with higher levels of anti-inflammatory cytokines. *Leishmania (Viannia) braziliensis* develops a mechanism to subvert the microbicidal function and, generally, reprogram the macrophage to a state of M2 activation, that antagonize the properties of M1 macrophages. Therefore, it is important to understand the polarization of the macrophage, since reprogramming of its polarity allows the parasite to escape the innate immune response. Therefore, the aim of the present study was determine the M1/M2 C57BL/6 peritoneal macrophages polarization infected with *Leishmania (Viannia) braziliensis*. Peritoneal macrophages were obtained from lavage of the peritoneal cavity and the interaction between parasite and macrophage was done during 2 hours in an oven. Cytokine and nitrite assays were performed using supernatants from infected macrophages cultures after 24 hours using specific kits, following manufacturers' instructions and read on a cytometer and spectrophotometer. For determination of ROS production, *CellROX*® kit was used and read in fluorimeter. For the immunofluorescence assays the infected macrophages were submitted to the laboratory protocol. A significant statistical difference was observed in the production of the inflammatory cytokines IFN-γ, IL-6 and TNF in the groups of macrophages stimulated with LPS and/or infected with *L. braziliensis*, compared to the control group, as well as ROS and nitrite production. In the immunofluorescence assays the presence of CD86, iNOS and CD210 molecules was observed. The production of inflammatory cytokines, ROS and nitrite, as well the detection of CD86 molecule and iNOS, showed a predominance of M1 profile activation in the macrophages infected with *L. braziliensis*. **Supported by:**CNPq; IEC/SVS/MS; UFPA
Keywords:C57bl/6; polarization m1/m2; leishmania (viannia) braziliensis

HP0102 - **THE EFFECT OF PROTEIN MALNUTRITION ON THE REMODELING OF THE SPLENIC EXTRACELLULAR MATRIX IN MICE INFECTED WITH LEISHMANIA INFANTUM**

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Protein malnutrition is considered a primary risk factor for the development of visceral leishmaniasis (VL). However, the immunological bases of this association are mainly unknown. The aim of this study is to determine the effects of protein malnutrition on the splenic microarchitecture and on the distribution and location of lymphocyte subsets in the spleen of BALB/c mice infected with L. infantum. Animals were fed with control (CP, 14% protein) or low-protein (LP, 4% protein) diet. After seven days of diet, each group was divided in two and one of them was infected with L. infantum (CPi and LPi). Mice were euthanized after 14 days post-infection. We observed that malnutrition induced a significant increase in the splenic parasite load of infected animals and induced a significant decrease in CD4+ cells. In addition, we observed a severe disorganization of splenic microarchitecture in LPi mice including a significant reduction in the number of follicles, and the presence of follicular structures undistinguishable from the red pulp and T cell areas (p = 001). These findings were accompanied by a significant decrease of pro-inflammatory and chemotactic molecules. As such disorganization might be associated with changes in the expression of extracellular matrix (ECM) molecules and these alterations may influence the distribution of lymphocyte populations in the organ, we decided to measure specific ECM components and analyze the distribution of T and B cells. Metallopeptidases, laminin and fibronectin were quantified in the spleen of LPi animals. Immunohistochemical analyses revealed a reduction in the expression of laminin and fibronectin in LPi animals when compared to control mice, suggesting that malnutrition has a significant role in the splenic white pulp disorganization, mediated by ECM alterations.Together, our results indicate that malnutrition intensifies VL severity by compromising T-cell mediated control of parasite proliferation in malnourished animals. **Supported by:**CNPq FAPERJ
Keywords: Leishmania infantum; spleen; malnutrition

HP0103 - **CHARACTERIZATION OF THE EXTRACELLULAR VESICLES OF TOXOPLASMA GONDII**

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T. gondii depends on sequential secretion of molecules to invade and establish the parasitophorous vacuole, during the acute and chronic phases of infection. Extracellular vesicles including exossomes from the endolysosomal system and microvesicles released directly from plasma membrane from pathogens have been recently involved in cell-to-cell communication and in the host immune response modeling. The investigation of vesicles secretion in T. gondii is important to elucidate the maintenance and development of the infection. The aim of this work is to establish a protocol for the isolation of extracellular vesicles of tachyzoites of the RH strain and to characterize their morphology by negative contrast by TEM and by Dynamic Light Scattering analysis. Extracellular tachyzoites were harvested from 44h infected LLC-MK2 cells. In order to exclude cellular debris the material was passed through 8 micrometers millipore filters. The isolated parasites were then incubated for 90 min at 37 ° C in RPMI medium without fetal bovine serum (starvation). The sample was centrifuged at 2000xg for 20 min in order to obtain a debris-free supernatant containing only the microvesicles. This supernatant was ultracentrifuged at 140000xg for 70 min in PBS. DLS analysis showed two populations of circular structures measuring from 50 to 150nm and another from 200 to 800nm. Negative staining confirmed the dimensions and shape of both population of vesicles. In order to find out if the parasites remained viable throughout the experiment, viability tests with propidium iodide were performed and showed that after passage in Millipore filter, 98% parasites were viable and after 1:30 h of starvation, 97,3% kept viability. In conclusion, extracellular vesicles found in the supernatant of T. gondii have origin in parasite secretion process and are not resealing membrane products of parasite or host cell. Enriched vesicle samples are being processed to secretome analysis. **Supported by:**CNPq, CAPES, FAPERJ, FINEP
Keywords: Toxoplasma gondii; vesicles; secretion

HP0104 - **EVALUATION OF LEISHMANICIDAL ACTIVITY OF HYDRAZINE DERIVATIVES**

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INTRODUCTION:Leishmaniasis is a zoonotic disease caused by protozoan parasites of the genus *Leishmania*. It is considered a neglected disease and affects 12 million people in 89 countries causing 70,000 deaths per year mainly in tropical and subtropical regions. During its life cycle, the parasite has two evolutionary forms: amastigotes which survive as an obligatory intracellular parasite infecting mainly macrophages of vertebrate hosts, and promastigotes, which are intestinal parasites of Phlebotomine sandflies. The disease has two main clinical manifestations, cutaneous and visceral leishmaniasis. Actually, treatment for leishmaniasis is dependent on pentavalent antimonials and amphotericin B but both present high toxicity, difficulties for administration, extensive treatment period, high cost, failure of distribution and resistance of some species. Previous results demonstrated that hydrazide derivatives have an inhibitory activity against the enzyme arginase from *Leishmania amazonensis*, leading to parasite death. AIM: Biological assay of 5 hydrazides derivatives from unsubstituted isoteric anhydride in order to evaluate the leishmanicidal activity in promastigote forms of *L. amazonensis*. METHODOLOGY: Promastigotes of *L. amazonensis* were incubated with different concentrations of hydrazides derivatives and their cellular viability was measured by MTT assay and analyzed by spectrophotometry after 24 hours. RESULTS: the hydrazine derivatives 2a, 2b and 2c displayed leishmanicidal activity, being more efficient than the prototypical compound. Respectively the IC50 of theses substances were 1.07 µm, 38.9 µm and 4.95 nM CONCLUSION: hydrazines are small inorganic molecules that display toxic activity in promastigote forms of *L. amazonensis*. More experiments are necessary to determine the toxicity in host cells and to evaluate the mechanism of the parasite´s death. **Supported by:**FAPERJ **Keywords:**Leishmania amazonensis; leishmanicidal; hydrazine

HP0105 - **UNVEILING THE INVASION PROCESS OF NON-PHAGOCYTIC CELLS BY LEISHMANIA AMAZONENSIS.**

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Intracellular parasites of the genus Leishmania, the causative agents of human leishmaniasis, are known to reside inside macrophages after the sandfly vector inoculates parasites into the skin of mammalian hosts. However, macrophages are not the first cells to be infected at the bite site and there have been several reports showing that even non-phagocytic cells can be invaded by the parasites. The aim of this work was to understand the mechanisms involved in this particular type of invasion. Our results show that mouse embryonic fibroblasts (MEF) are invaded by promastigotes of L. amazonensis in vitro. Heat-inactivated and PFA-fixed parasites were not internalized in MEF, showing that infection requires living parasites. Host cell cytoskeleton disassembling by cytochalasin-D not only did not decrease cell infection but actually increased invasion, showing that entry into host cell does not involve any type of induced phagocytosis. Using fluorescence microscopy, flow cytometry and biochemical assays we demonstrated that during invasion, the infective parasites induce host cell plasma membrane damage and Ca2+ influx, which triggers lysosomal recruitment and lysosomal exocytosis at infection site. Cells deficient in lysosomal proteins LAMP1 and 2 and with abnormal lysosomes had a dramatic reduction in invasion. On the other hand, invasion was markedly increased after lysosome mobilization towards the cell periphery by brefeldin-A. Taken together, our results show that like plasma membrane repair, the process of non-phagocytic cells infection by L.amazonensis is driven by calcium influx, facilitated by cytoskeleton disassembling and needs lysosomal exocytosis, similar to what occurs during host cell invasion by T. cruzi. The fast invasion of non-phagocytic cells at the bite site can actually provide a rapid and safe shelter for recent inoculated parasites that eventually end up captured by the macrophages in which infection continues. **Supported by:**FAPEMIG **Keywords:**Leishmania amazonensis; mouse embryonic fibroblasts (mef); invasion

HP0106 - **MOUSE SPECIFIC IGG ANTI-*TOXOPLASMA GONDII* DETECTION BY CAPTURE ASSAY USING PROTEIN A FOR UNKNOWN SAMPLES.**

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Specific IgG detection using solid phase adsorbed antigen are quantitatively expressed by reactivity in known standard dilution of the biological sample, such as sera. Vaccine mouse models do not allow accurate sampling without killing the experimental animal. Surviving mice are essential for understanding specific individual protection after challenge, implying in using alternatives such as tail blood on filter paper or cell supernatants. Those assays presented many problems and several bias and their data are not quantitatively comparable, a need when comparing vaccines efficiency. The use of a fixed amount of an IgG Fc ligand like *Staphylococcus aureus* A protein (SPa), may allow equal amounts of IgG captured without interference from its source. Incubation with labelled antigen quantifies the specific IgG proportion in the whole IgG pool, independent of the biological sample or IgG offer. We standardized the quantitative detection of proportion of specific anti-*T. gondii* IgG in protein A capture assays using *T. gondii* soluble antigen conjugated to biotin for revealing specific IgG. Wells were adsorbed with Protein A at 2.5 µg, 1 µg, and 10 µg / ml. Rabbit's positive and negative were used for capture, after which we applied the biotinylated antigen at dilutions of 1/200, 1/400, 1/800 and 1/1600. Bound antigen were revealed with avidin-peroxidase and OPD reagent. All assays allows the discrimination of positive and negative sera, with the same reactivity, but low Protein A adsorption give low backgrounds. IgG binding to protein A and the antigen/antibody ratio, was constant, independent of the concentration of immunoglobulins in samples. Our results demonstrate that the use of a specific ligand of IgG antibodies may allow comparing the IgG response obtained from experimental models without euthanasia, allowing the challenge of the same immunized mice and the direct correlation of humoral immunity and protection in toxoplasmosis vaccine models. **Supported by:**IMT-FMUSP
Keywords: Toxoplasma gondii; protein a; vaccines

HP0107 - **SYSTEMIC INHIBITION OF THE PROTEASOME INCREASES THE SURVIVAL OF C57BL/6 MICE INFECTED WITH TOXOPLASMA GONDII**

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Toxoplasma gondii is an obligate intracellular parasite that causes toxoplasmosis, capable of multiplying in practically all nucleated cells of its hosts. During infection, T. gondii uses many evasion mechanisms, such as the inhibition of nitric oxide (NO) production, which acts as a potent microbicidal agent. Macrophages activated with interferon-gamma and lipopolysaccharide produce NO via the inducible NO synthase enzyme (iNOS), however, T. gondii infection inhibits NO production by iNOS degradation through the proteasome pathway. Previous studies have shown that pharmacological inhibition of the proteasome prevents degradation of iNOS during infection in vitro. The aim of this study was to verify the relevance of the proteosome pathway in T. gondii infection in vivo. For this, mice were infected with tachyzoites of T. gondii and treated with proteasome inhibitors Bortezomibe and MG132. We evaluated the survival of mice, number of tachyzoites and NO production of peritoneal macrophages from infected animals after different days of treatment. The in vitro treatment of macrophages with Bortezomib was able to reverse the lower NO production caused by the infection. C57BL/6 infected mice treated with Bortezomib showed increased survival, reduction in the amount of tachyzoites and macrophages obtained from the peritoneal showed a tendency of higher NO production. However, the same treatment had no effect in Swiss mice. Despite the different behaviors between the lineages of mice, these results indicate that iNOS degradation by the proteasome may be relevant in T. gondii infection, suggesting the use of proteasome inhibitors as a possible treatment against experimental toxoplasmosis. **Supported by:**CAPES, FAPERJ, CNPQ
Keywords: Toxoplasma gondii; proteasome; nitric oxide

HP0108 - **ROP16 RHOPTRY OF TYPE I *TOXOPLASMA GONDII* INHIBITS THE INFLAMMASOME ACTIVATION BY STAT3 PHOSPHORYLATION IN CONTRAST TO *NEOSPORA* SP.**

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Toxoplasma gondii and *Neospora caninum* are obligate intracellular parasites of the phylum Apicomplexa that comprises a variety of pathogens. The impact of apicomplexan parasites on the economy and human health provides the research and commercial interest in developing therapeutic and prophylactic strategies. Therefore, to explore the feasibility of vaccination and treatments against apicomplexan parasites, it is useful to improve understand and to determine the protective immunity in the infection with those parasites. The activation of the inflammasome in response to infection by intracellular pathogens has recently gained attention. In this study we assessed the inflammasome activation, its structural assembly and functional effector mechanisms in the host response to intracellular parasites of the *Neospora* genus. The results suggested that the Nlrp3/ASC/Caspase-1 pathway is involved in response to *N. caninum* infection and that engagement of this pathway has a crucial role in the restriction of parasite replication. Notably, the activation of the inflammasome by *Neospora* is independent of cell primed and IL-1β production is along signaled through IL-1R and MyD88. In addition, we reported that *Toxoplasma* inhibits this pathway, making necessary a prior signal to active the inflammasome. Our findings indicated that ROP16 rhoptry protein of Type I *T. gondii* could lead the inhibition of this STAT3-dependent pathway. **Supported by:**CAPES, FAPEMIG, CNPq
Keywords: Neospora; rop16; inflammasome

HP0109 - ***PLASMODIUM CHABAUDI* INFECTION MODIFIES THE LIPID METABOLISM IN SWISS MICE: AMPK ENZYME INVOLVED IN REGULATION MECHANISM**

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Malaria is the most important parasitic disease in humans. It is transmitted in 108 countries and in 2015 caused about 216 million cases and 438,000 deaths. Malaria is transmitted through the bite of female mosquitoes of the genus *Anopheles*. *Plasmodium*, as well as other protozoan parasites, manipulates the lipid metabolism of the vertebrate host, since they are deficient in synthesizing all the lipid classes that are necessary for its development and replication. *Plasmodium* loses its ability to synthesize fatty acids when in the intraeritrocytic stage, but it maintains the competence to store lipids within the parasitophorous vacuole. However, the actual mechanism by which this occurs is still unclear. In this work, infection of Swiss mice with *P. chabaudi* presented a totally altered plasma profile, such as hyperproteinemia, hypertriglyceridemia, hypoglycemia and hypocholesterolemia. In addition, the infected liver lipid profile analysis presented an accumulation of the main lipid classes in relative composition, such as triacylglycerol, free fatty acids and free cholesterol. The white adipose tissue also presented an increase in triacylglycerol proportion. The infection by *P. chabaudi* altered the gene and protein expression of enzymes and key factors of the lipid metabolism, directing it to a more lipogenic and less lipolytic profile. The cell energetic metabolism key enzyme AMPK was also regulated by infection, presenting a lower phosphorylation level and lower activity. Together, the results provide new and important information on the lipid metabolism regulation by the malaria parasite, which may contribute to a better understanding of the mechanisms by which this parasite acquires these molecules, which are so important for its development. **Supported by:**Capes, CNPq, Faperj
Keywords: Plasmodium chabaudi; lipid metabolism; ampk

HP0110 - **EXPRESSION PROFILING OF HSP27 ALONG THE *PLASMODIUM BERGHEI* LIFE-CYCLE**

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Malaria transmission depends on the successful development of *Plasmodium* inside the mosquito vector and the mammalian host. Mosquito midgut invasion is critical to succeed in vector colonization. When mosquitoes feed on an infected host, sexual stages of *Plasmodium* are transferred to the mosquito midgut. In the blood meal, parasites develop into motile ookinetes, which migrate towards the midgut epithelium and form oocysts. Sporozoites, the infective forms of malaria parasites, initiate inside oocysts. After oocysts rupture, sporozoites circulate in the hemolymph and invade salivary glands, where later are transmitted to a new host . In previous studies, we found that a small heat shock protein, HSP20, is exclusively expressed in sporozoites and ookinetes. This chaperone can modulate ookinete velocity, as well as speed and directionality of sporozoites, prejudicing malaria natural transmission. We have recently identified a new protein of 27 kDa that share the alpha crystalline domain characteristic of the small heat shock protein family. Parasites expressing HSP27 fused to m-Cherry fluorescent molecule show that this protein is expressed in *Plasmodium* asexual blood stages, particularly in schizonts. In vitro studies mimicking parasite fecundation inside mosquito show that HSP27 is also expressed in some mosquito stages, as zygotes, retorts and ookinetes. The presence of HSP27 protein in *P.berghei* ookinetes was confirmed by western blotting assays using specific antibodies generated against HSP27 recombinant protein produced in bacteria. Preliminary studies to assess the function of this protein using parasites lacking *hsp27* gene, indicate that HSP27 could be dispensable for erythrocytic phase development of malaria parasites. **Supported by:**FAPESP
Keywords: Malaria; plasmodium berghei; small heat shock protein

HP0111 - **IMMUNOGENICITY OF THE RECOMBINANT PROTEIN BASED ON THE NATIVE TRYPANOSOMA CRUZI P21**

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Introduction:Trypanosoma cruzi is the etiologic agent of Chagas' disease that affects million of people in Latin America. Recently our research group described and characterized a 21 kDa T. cruzi protein (P21) that has many activities such upregulation of parasite phagocytosis and host cell actin polimerization. We generated the recombinant form of P21 (rP21) based on the native form secreted by T. cruzi and in this study we aimed to evaluate its immunogenicity. Methods and Results: BALB/C mice were split into four group (n=10). Group 1 was inoculated subcutaneous with 100 µL of phosphate buffer saline. Groups 2, 3 and 4 were inoculated subcutaneous with 100 µL of rP21 in 0.5, 1 and 10 µg/ml respectively, and another two booster immunizations were given at weeks 2 and 3. At the fourth week mice were sacrificed, the blood and the spleen were collected. The blood samples were analysed by ELISA assay and the hemogram. The results showed that the immunization promoted specific immune response, with reactivity index (RI) above 1 in all treatments. Although, there was not any significant effect in the number of leukocytes, lymphocytes, segmented neutrophils, eosinophils, basophils. we observed a significant increase in the number of erythrocytes and hemoglobin. Conclusion: The recombinant form of T. cruzi P21 is immunogenic. This phenotype may play important impact on the immune response in Chagas disease. **Supported by:**FAPEMIG/CNPq/CAPES/UFU
Keywords: Trypanossoma cruzi; protein recombinant p21; immunogenicity

HP0112 - **EVALUATION OF THE TRYPANOCIDAL EFFECT OF VORICONAZOLE ASSOCIATED TO THE ANTI-INFLAMMATORY AND ANTIOXIDANT PROPERTIES OF MELATONIN IN THE CHRONIC CHAGASIC CARDIOMYOPATHY**

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American trypanosomiasis is a parasitic disease caused by the flagellate protozoan *Trypanosoma cruzi*. Chagas disease is endemic in Latin America, where an estimated 10-14 million people are infected, and an emerging disease in Europe and the USA. In the present study, it was investigated the possible beneficial actions of the anti-*Trypanosoma cruzi*, anti-inflammatory and antioxidant combination therapy (Melatonin / Voriconazole) on chronic Chagas cardiomyopathy. Some cytokines and other mediators involved in the inflammatory process characteristic of infection were analyzed. In addition, plasma levels of creatine phosphokinase MB (CK-MB), number of inflammatory foci in the cardiac tissue and analysis of the relative weight of the heart were evaluated as predictors of damage reduction in the myocardium after different treatments. As experimental model, we used *Wistar* rats infected with the Y strain of *T. cruzi*. Some groups were untreated and others treated with Melatonin, Voriconazole, or combination of both. Inflammatory cells and heart tissue parasitism were evaluated by PCR and histological techniques. Some pro-inflammatory and modulatory cytokines were quantified by ELISA technique in the myocardium and serum from experimental animals. The production of nitrite by macrophages and cardiac cells of the experimental groups was also investigated through Griess reaction. The parameters were observed during the chronic stage: (60, 90, and 120) days after infection. Our results indicate that Melatonin + Voriconazole treatment significantly increased the concentration of IL-10 and reduced the concentrations of NO and TNF-α produced by cardiomyocytes. Furthermore, it led to decreased heart weight, serum CK-MB levels and inflammatory foci when compared to the untreated and infected control groups.The study may contribute to a better understanding of the pathogenesis of chronic Chagas heart dysfunction, as well as the anti-*T. cruzi* immunological responses.
Supported by: Fapesp
Keywords: Melatonin; voriconazole; chronic chagas cardiomyopathy

HP0113 - **EFFECTS OF LYSOPHOSPHATIDYLCHOLINE (LPC) ON THE GROWTH, DIFFERENTIATION AND INFECTIVITY OF DIFFERENT SPECIES OF THE GENUS *LEISHMANIA***

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Lipid mediators, including LPC and platelet-activating factor (PAF) have been described as presenting a key role in infection of some parasitic protozoa. Our group demonstrated that *T. cruzi* synthesizes a C18:1-LPC, with the ability of aggregating platelets, similarly to PAF. In the present study, we demonstrate the effects of LPC on *Leishmania. i. chagasi, L. amazonensis* and *L. mexicana* proliferation and differentiation, as well as in the interaction of these parasites with mouse peritoneal macrophages. We observed a 32% increase in proliferation of *L. i. chagasi*, *L. amazonensis* and *L. mexicana* on the 5th day of growth, when the parasites were treated with C18:1-LPC, as compared to the control. Pre-treatment of the parasites with WEB 2086 (a PAF receptor antagonist) reversed the C18:1-LPC effects. Also, the number of differentiated forms exceeded the number of promastigotes on the 8th day after induction of differentiation in the presence of C18:1-LPC, as compared to the control parasites, which present the phenomena on the 13th and 16th day for *L. amazonensis* and *L. i. chagasi* respectively. We also tested the effects of C18:1-LPC on the infection of mouse peritoneal macrophages when parasites were pre-treated for 4 hours with this lipid. Preliminary results indicate an enhancement of the infection when C18:1-LPC-treated parasites were used in the interactions. Here, we have also built a three-dimensional model of a putative *Leishmania* spp PAF receptor (PAFR). A molecular docking study was performed to predict the interactions between the PAFR model and PAF or LPC, and was constructed based upon the amino acid sequence submitted to the Phyre2 server. Subsequently, the validation of the model was done using the software PROCHECK.The docking data suggested that C18:1-LPC is predicted to interact with the PAFR model in a fashion similar to PAF. These results suggest that C18:1-LPC modulates infectivity of *Leishmania* spp via a putative PAF receptor in these parasites.
Supported by:CNPq, CAPES, FAPERJ and INCT-EM
Keywords: Leishmania spp ; lysophosphatidylcholine; paf receptor (pafr)

HP0114 - **LIPID METABOLISM ALTERATIONS IN BALB-C PERITONEAL MACROPHAGES DURING INFECTION WITH *LEISHMANIA (LEISHMANIA) AMAZONENSIS***

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Leishmaniasis is a broad spectrum of neglected tropical diseases caused by protozoa *Leishmania spp.* One million new cases per year are reported. The parasite's life cycle involves its interaction with the vertebrate host, which is intermediated through the bite of infected phlebotomine dipterans. In the vertebrate host the extracellular promastigote phase is phagocytosed by macrophages. The survival of this parasite depends on macrophages immune response molecular mechanisms manipulation and then modifying to the intracellular amastigote phase, initiating proliferation, cell disruption and infection of other macrophages. It's known that these parasites do not have complete pathways for lipid biosynthesis and for this reason uses the lipids of its vertebrate host. This work aims to characterize the lipid metabolism in peritoneal macrophages of Balb-c mice infectaed by *L. (L.) amazonensis*. Time course of radioactive fatty acid incorporation in infected macrophages (0, 1, 4, 8 and 24h) was realized. After time the macrophages were subjected to lipid extraction and the main lipid classes were separated by thin layer chromatography. The spots related to each lipid class were removed and the associated radioactivity determined by liquid scintillation. The results showed a higher fatty acid uptake in infected macrophages compared to the control group. The conversion of complex lipids was higher in infected macrophages in 24h post infection. Lipid classes such as triacylglycerol (2711 ± 980 vs 285 ± 31 cpm; $p<0.05$), diacylglycerol (812 ± 260 vs 224.3 ± 30 cpm; $p<0.01$), fatty acid (3346 ± 1050 vs 524 ± 55 cpm; $p<0.001$) and esterified cholesterol (1651.3 ± 300 vs 73.7 ± 33 cpm; $p<0.01$) were the classes with the most significant changes. Taken together these results demonstrated that *L. (L.) amazonensis* is able to modulate lipid metabolism to its survival and particularly its proliferation in the host cell.
Supported by:FAPERJ, CNPq e CAPES
Keywords: Lipid metabolism; cutaneous leishmaniasis; peritoneal macrophages

HP0115 - **ALTERATIONS OF THE GAP JUNCTIONS IN THE PROCESS OF INFECTION OF *TOXOPLASMA GONDII* ON THE INTESTINAL EPITHELIAL CELL**

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Toxoplasmosis is a disease caused by an obligate intracellular protozoan, *Toxoplasma gondii*. The gastrointestinal tract is therefore a major route of *T. gondii* infection in most cases. For this reason the intestinal epithelium is a cellular model that provides to study the first line of defense against oral infections. The gap junctions, mediated by connexin43 (Cx43), that have the role of mediating the interactions between adjacent cells. In the intestinal epithelial cell gap junction formed by Cx43 may be important in modulating the response of the cell to infectious processes, and by allowing the passage of important agents into the body in the process of infection. Although the invasion of parasites into host cells is a known event, the effects of this infection are not yet well established. In view of this, we investigated the molecular mechanisms that are modified in the intestinal epithelial cell during the infection process and evasion of the protozoan T. gondii. The methodology used is: (1) IEC-6 cell line culture; (2) Infection with the RH strain of Toxoplasma in its tachyzoite form; (3) Western Blot Assays; and (4) Immunofluorescence assays. We intend to reveal with this study the role that these structures played in the infectious process, besides the possible morphofunctional modulation mediated by *T. gondii* within the cellular microenvironment, in order to study the possible alteration of these structures. We observed by immunofluorescence that levels of Cx43 decrease in plasma membrane of the IEC-6 after infection with *T. gondii* parasite in 72 hours of incubation, however Cx43 levels in the cellular cytoplasm are increase. In same experiments we performed Phalloidin labelling and disorganization of cellular cytoskeleton is present. Cx43 protein depends of the cellular cytoskeleton organization, and alteration in this structure interfering in the transport to the cell membrane, and probably alters cell function in intestinal epithelium.
Supported by:CAPES, CNPq and FAPERJ
Keywords: Gap junction; toxoplasma gondii; iec-6

HP0116 - **CHARACTERIZATION OF SV129 MICE AS SUSCEPTIBLE MODEL TO LEISHMANIA AMAZONENSIS**

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Leishmaniasis is a complex of neglected diseases caused by parasites of the genus Leishmania and Leishmania (Leishmania) amazonensis is the specie that causes diffuse cutaneous leishmaniasis in Brazil. In this work, we investigated a new experimental model for the infection by L. amazonensis: SV129 mice. Those animals were first used by SEZARANI H.C. et al. 2006, for a study about the importance of leukotrienes on the control of L. amazonensis infection. Their results showed that SV129 mice are resistant compared to BALB/c in this model of infection, however, only a short time was evaluated. In this work, we investigated the use of SV129 as mice model for leishamaniasis. First, we have infected SV129 mice with 2x105 L. amazonensis in the promastigote form in stationary phase by the subcutaneous route on the posterior right footpad and used infected BALB/c mice as control. We evaluated the lesion growth weekly by pachymetry until 6 months post infection. Our results showed that BALB/c mice more susceptible than SV129 mice, however, SV129 mice were also susceptible, it displayed the same profile as a progressive lesion without control. Infection on SV129 showed delayed lesion growth compared to BALB/c. Differently from BALB/c mice, SV129 mice presented high levels of CD4+ T cells and CD8+ T cells producing Interferon-gamma. Besides, SV129 mice presented high levels of IgG1 and IgG2a, showing a mixed Th1 and Th2 response. In this work, we demonstrated a new susceptible mice model to study Leishmania amazonensis that presented the capacity to produce Interferon-gamma and could be used to vaccine studies.
Supported by: Cnpq e Faperj
Keywords: Leishmaniasis; sv129; ifn-gamma

HP0117 - **RP21 ATTENUATES *T. CRUZI*-INDUCED HEART FIBROSIS**

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The recombinant protein P21 (rP21) is based on the native form secreted by *Trypanosoma cruzi*. rP21 treatment induces angiogenesis inhibition, increases actin cytoskeleton polymerization as well as nonspecific phagocytosis. In this study, we evaluated their role in experimental infection of mice. BALB/C mice were inoculated with 10^6 trypomastigotes from Y strain, treated with rP21 (100 µg/animal) using two different schedules (A and B), then euthanized at 20 days post-infection. In the schedule A, mice were inoculated with parasites and rP21 was administered at the same time, treatment was repeated at 5 and 15 days post-infection; in the schedule B, infected mice were treated at 3, 6 and 9 days post-infection. Applying schedule A, higher parasitemia was observed in rP21 treated group, this is in agreement with previous observations by which rP21 increases parasite internalization. Spleen-derived suspension cells from infected group showed that rP21 treatment resulted in higher expression of CD4 receptor. Collagen measurement, blood vessel and amastigotes nests quantifications indicated that rP21 treatment resulted in low rates of heart fibrosis, larger blood vessels and lesser quantity of amastigotes nests. These results suggest a protective role of rP21 during experimental infection by *T. cruzi*. Applying schedule B, animals treated with PBS showed higher parasitemia and increased parasite burden at heart in all times analyzed, also rP21 treatment induced a greater number of vessels in cardiac tissue. However, the number of nests founded was higher than in the control group indicating that rP21 is responsible for maintenance of the parasite inside the cardiac cell, thus reducing its release into the bloodstream. Therefore, rP21 treatment demonstrated remarkable heart protection in *T. cruzi* infection, since it increases the internalization of the parasite by cells and at the same time decreases the damage in heart tissue. **Supported by:**FAPEMIG, CNPq
Keywords: Trypanosoma cruzi; rp21; experimental infection

HP0118 - **MODULATION OF THE GAP JUNCTION IN A MICROENVIRONMENT OF INFECTION WITH *TRYPANOSOMA CRUZI.***

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Chagas disease is caused by the protozoan Trypanosoma cruzi and is a major health problem in Central and South America. In chagasic infection, the parasite is able to impair the functioning of host cells through changes in cellular communication. However, these junctions are not fully characterized in the Immune System and, in particular, in the macrophages that participate of the innate response. Morphological and functional characterization of gap junctions in macrophages has been the subject of study of various groups, however their regulatory mechanisms still deserve clarification, mainly before pathological changes, such as in infectious and inflammatory processes caused by T. cruzi. Thus, the main objective of this study is a structural and functional modulation of the junctional communication, formed by the connexin43 (Cx43) in macrophages J774-G8, after activation with pro-inflammatory factors and in T. cruzi infection. The experiments were performed is: (1) Western Blot assays; (2) Immunofluorescence assays; and (3) Intracellular dye microinjection in control J774-G8 macrophage cell line, in J774-G8 activated with (Tumor Necrosis Factor-α (TNF-α) plus Interferon-γ (IFN-γ), or in infected cells with the parasite in his trypomastigotes strain Y. Dye injection experiments in J774-G8 treated with factors demonstrated that communication increase between cells. Western blot demonstrated that expression of the Cx43 protein increase in infected cells after 48 hours. After that we performed immunofluorescence experiments in cells infected with T. Cruzi after incubations of up to 48 and 72 hours, and showed that the Cx43 was not present in the plasma membrane and retained in the cytoplasm. In same experiments we performed Phalloidin labelling and disorganization of cellular cytoskeleton is present. Cx43 protein depends of the cellular cytoskeleton organization, and alteration in this structure probably interfering in macrophage cell function in tissue infection.

Supported by:CAPES, CNPq and FAPERJ.

Keywords: Gap junction; trypanosoma cruzi; macrophage

HP0119 - **EVALUATION OF THE LAAG VACCIN ASSOCIATED TO CAF FAMILY ADJUVANTS BY INTRAMUSCULAR ROUTE AGAINST MURINE CUTANEOUS LEISHMANIASIS.**

MACIEL, D.O.[*1]; SANTOS, J.S.[1]; CRUZ, L.F.[1]; GUEDES, H.L.M.[1]; RODRIGUES DE MEDEIROS, J.V.[2]; RAMOS, T.D.[3]

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Leishmaniasis is an infectious disease caused by parasites from genus Leishmania. The most common form is cutaneos, which can progress to more severe manifestations and cause deformations in the skin. The chemotherapy used as a treatment against leishmaniasis is extremely toxic to the patient. Thus, it shows the need of a search for alternative therapies, as vaccines. Adjuvants are substances that are associated to vaccines to enhance or modulate the immunogenicity of the antigen present in the formulation. Our focus is to evaluate the ability of adjuvants (CAF01, CAF04 and CAF09) associated with total antigens of Leishmania amazonensis (LaAg) by intramuscular route to improve immunogenic and protective responses against infection caused by L. amazonensis. The C57BL/6 mice were vaccinated twice by intramuscular route before the infection. The animals were divided into groups treated with the respective vaccines (LaAg, LaAg+CAF01, LaAg+CAF04, LaAg+CAF09) and control group (PBS). After the vaccination, 2x105 promastigotes of the parasite were inoculated in the right footpad of the animals and the lesion growth caused by the infection was measured weekly by pachimetry. The parasitic load of the infected organs was determined by the limiting dilution analysis (LDA). We observe that animals vaccinated with LaAg managed to partially solve the lesion in the progressive phase, while those who were vaccinated with adjuvant-associated vaccines did not show improvement when compared to LaAg. The results indicated that the LaAg, LaAg+CAF04 and LaAg+CAF09 vaccine induced protection in the progressive phase. However, in the chronic phase, the LaAg vaccine and LaAg+CAF04 had a contra-protective effect, which was not observed by the LaAg+CAF09 vaccine. Our results indicate a potential use of the intramuscular route for vaccination using LaAg plus CAF09. Studies to optimize the formulation and to understand the protection mechanism are in progress.

Supported by:CNPq/ Faperj

Keywords: Leishmania; vaccine; adjuvant

HP0120 - **CONTROL OF *TOXOPLASMA GONDII* INFECTION *IN VITRO* AND *IN VIVO* BY *TRICHODERMA STROMATICUM* EXTRACT**

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Trichoderma is a fungus known by inhibit the growth and development of a variety of plant pathogens. Previous studies demonstrated that the fungal spore from *T. stromaticum* downmodulated the response of murine phagocytes by decreasing the production of nitric oxide (NO) and reactive oxygen species (ROS). *Toxoplasma gondii* is an obligate intracellular protozoan parasite that induces a strong Th1 response that activates the production of interferon (IFN)-γ, which in turn activates several innate immune mechanisms, such as NO production. The aim of this study was to verify the effect of *T. stromaticum* extract (ExtTs) in the susceptibility of J774 cells and C57BL/6 mice to *T. gondii* infection. For this purpose, we treated the J774 cells with several concentrations of ExtTs before or after infection with *T. gondii*; or we pretreated the *T. gondii* tachyzoites with ExtTs and added these pretreated parasites in the cells. Furthermore, C57BL/6 mice were infected with *T. gondii* RH strain-pretreated parasites. The parasite intracellular proliferation, nitrite production and cytokine profile were verified in J774 cells, while mortality, weight change and seroconversion were observed in *T. gondii*-infected mice. The pretreatment of tachyzoites with ExtTs reduced significantly the parasite proliferation in J774 cells. In addition, the ExtTs was able to decrease the nitrite production triggered by IFN-γ. Finally, the treatment of the parasites with ExtTs increased survival and body weight of infected mice, and it was not observed the seroconversion of mice. Thus, these results suggested that the parasite is highly susceptible to the ExtTs, indicating that the fungus extract is a good candidate to control *T. gondii* proliferation. **Supported by:**CAPES, CNPq, FAPEMIG
Keywords:J774 macrophage; toxoplasma gondii; trichoderma stromaticum

HP0121 - **SPECIFIC ROLE OF P21 PROTEIN IN DEVELOPMENT OF CHRONIC CHAGASIC CARDIOMYOPHATY**

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Introduction: P21 is a specific protein secreted by all life forms of *Trypanosoma cruzi*. Several studies have shown the importance of this protein in the context of *T. cruzi* infection. In this sense, we proposed to study the specific role of P21 protein in mice in the chronic phase of Chagas disease, comparing with the effect on *Leishmania amazonensis* infection. Methods: Mice infected, separately, with both parasites were treated with the recombinant form of P21 protein every 72 hours for 6 weeks. After this period, the mice were euthanized and the pathophysiology analyzed. In addition, the pharmacokinetics, immunogenicity, tolerance and stability of the rP21 protein were assayed. Results: Treatment of rP21 showed opposite activities in both parasites. In mice infected with *L. amazonensis*, the protein caused progressive increase in the size of the paws, and parasitic load. In contrast, mice infected with *T. cruzi* and treated with rP21 reduced the parasite load on the paws and heart, as well as, increased fibrosis and inhibition of the formation of new blood vessels in injured areas. In addition, rP21 was detected in the serum of the mice after 72 hours of treatment, without prejudice to biological activities. The immunogenicity test showed discrete antibody production. Conclusion: Taken together, the results suggest that P21 has a specific role in developing of chagasic cardiomyopathy pathogenesis. **Supported by:**UFU/ FAPEMIG/ CNPq/ CAPES
Keywords: Trypanosoma cruzi; p21 protein; cardiomyopathy

HP0122 - **B-1 CELLS MODULATE THE MURINE MACROPHAGE RESPONSE TO *LEISHMANIA MAJOR* INFECTION**

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Leishmania major is the causative agent of cutaneous leishmaniasis in the Old World and is transmitted by the bite of the female phlebotomine. In models of susceptibility to *L.major* infection, there is a production of anti-inflammatory mediators, which negatively modulate the response of the vertebrate host, favoring the establishment of infection. Our group recently demonstrated that B-1 CDP (B-1 cell derived phagocyte) cells are easily infected by *L. major* and exhibit high susceptibility to infection and that this mechanism is dependent on prostaglandin E_2/ interleukin-10 production. Based on these data, we investigated the interaction between B-1 cells and *L. major* infected macrophages from BALB/c mice and BALB/c XID mice (a lineage that is genetically depleted of B-1 cells) to elucidate the possible influence of this B-cell population on the progression of infection in vitro. Peritoneal macrophages obtained from BALB/c and BALB/c XID mice were infected with *L. major* and cultured or not in the presence of B-1 cells. Parasitic load were counted and interleukin-10 production was quantified. The levels of the lipid mediator prostaglandin E_2 and the number of lipid bodies was quantified in the cytoplasm of infected macrophages. We report that B-1 cells promote the growth of *L. major* inside peritoneal murine macrophages. We demonstrated that the modulatory effect was independent of physical contact between the cells, suggesting that soluble factor(s) were released into the cultures. We demonstrated in our co-culture system that B-1 cells trigger IL-10 production by *L.major* infected macrophages. Furthermore, the increased secretion of IL-10 was attributed to the presence of the lipid mediator PGE_2 in supernatants of *L. major* infected macrophages. The presence of B-1 cells also favors the production of lipid bodies by infected macrophages. Our results show that elevated levels of PGE_2 and IL-10 produced by B-1 cells increase *L. major* growth. **Supported by:**Capes, CNPq e Faperj **Keywords:**B-1 cells; leishmania major; macrophages

HP0123 - **MAST CELLS INFILTRATION AND CYTOKINES PROFILE IN FETO-MATERNAL INTERFACE OF BALB/C AND C57BL/6 MICE ORALLY INFECTED WITH *TOXOPLASMA GONDII***

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During gestation, foreign fetal antigens challenge the maternal immune system, in which Treg cells will dominate Th17 cells to guarantee fetal survival and the successful of pregnancy. Uterine mast cells (uMCs) are involved in spiral artery remodeling during early pregnancy ensuring the correct blood flow to the fetus and avoiding adverse gestation outcome. *Toxoplasma gondii* infection induces Th1 immune response associated with adverse pregnancy outcome. The aim of this study was to investigate the balance of cytokine profile in serum and placenta/uterus of *T. gondii* infected mice and its relation to the presence of uMCs. For this purpose, C57BL/6 and BALB/c females were orally infected with 5 cysts of *T. gondii* on days 1 and 14 of gestation. Animals were euthanized on days 8 of pregnancy and infection (8dop/8dpi), and 19 of pregnancy and 5 of infection (19dop/5dpi), cytokine levels were measured in serum and placenta/uterus by CBA, uterus was histologically processed and stained by Blue Toluidine for mast cell detection. Our results showed higher TNF and IL-6 levels in C57BL/6 compared with BALB/c mice and an increase of IFN-□ in BALB/c and C57BL/6 at 8dop/8dpi compared with non-pregnant and uninfected animals. BALB/c females showed higher amount of IL-2, IL-4 at 8 dpi. Interestingly, after 19dop/5dpi it was not observed significant differences in TNF, IL-2, IL-4, IL-10 and IL-17 detection in the uterus/placenta of both lineages of infected mice. Additionally, higher amounts of uMCs were detected in the uterus of infected C57BL/6 compared with pregnant uninfected animals. The number os mast cells was not altered by infection in BALB/c mice. Our data suggest that on the first third of gestation the TNF and IL-6 present more pronounced effects in C57BL/6 mice that culminate in poor pregnant outcome in this period of gestation observed in this lineage of mice. Likewise, uMCs appear to be involved in the modulation of inflammation during gestational toxoplasmosis.
Supported by:CAPES, CNPq, FAPEMIG
Keywords: Th1/th2 cytokines; congenital toxoplasmosis; uterine mast cells

HP0124 - **EFFECT OF RECOMBINANT PROTEIN RP21 OF TRYPANOSOMA CRUZI ON TUMOR CELLS INVASION**

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Breast cancer accounts to 28% of new cases of cancer in Brazil.Therefore, novel alternative treatments and therapies are subject of great efforts of researchers in the field. Interestingly, some parasites were identified as having antitumor action, one of them is *Trypanosoma cruzi* which is the etiologic agent of Chagas' disease. The recombinant form of *T. cruzi* protein P21 (rP21) has biological activities, such as induction of actin cytoskeletal polymerization and antiangiogenic capacity, which suggest some potential to modulate the tumor microenvironment. Thus, the present study evaluated whether rP21 had an antitumor effect on breast cancer cells. Thus, breast cancer cells (MDA-MB-231 and Ehrlich) and control cell (MCF-10A) were used to verify the action of the rP21.First, we use different concentrations of protein: 6,25 ug/mL, 12,5ug/mL, 25ug/mL, 50ug/mL and 100ug/mL and differents times: 1, 24, 48 and 72 hours. We analyzed whether the protein interferes with cell viability by MTT. We evaluated whether there was in fact the binding of the protein in the cells using antibody IgY anti-P21 by flow cytometry and after that we did an invasion assay using 100ug/mL of rP21, 10ng/mL of sdf-1α, rP21 togheter with sdf-1α at the same concentrations and using as a positive control the sdf-1α. The actin polymerization was available in the same situations of the invasion assay by flow cytometry. rP21 was not cytotoxic to tumor lines, but we show that it binds to the cell membrane, possibly, to the CXCR-4 receptor. The most interesting is that the treatment with the protein inhibits the invasion of tumor cells, suggesting an antimetastatic potential of the protein. Thus, we are investigating which mechanism of action of the protein that inhibits the invasion of tumor cells. the action on cytoskeletal polymerization seems to respond to the invasion assay, so one possibility is the protein to inhibit the polymerization / depolymerization process influencing the invasion. **Supported by:**CAPES, FAPEMIG, CNPQ
Keywords: Recombinant protein p21; trypanosoma cruzi; tumor cells

HP0125 - **VACCINE USING NCROP4 MAY PROTECT AGAINST *NEOSPORA CANINUM* INFECTION AND REDUCE *TOXOPLASMA GONDII* INDUCED ILEITIS IN MICE**

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Neospora caninum is an obligated intracellular protozoan that has drawn increasing interest due to its association with worldwide repetitive bovine abortions, which cause billionaire losses in livestock economy and dairy industries. NcROP4 is an immunogenic N. caninum rhoptry protein that is important in the invasion and evasion process of host cells. It has already been seen that several proteins of Toxoplasma gondii, a phylogenetically related to N. caninum parasite, are important in modulation of host immune response. Thus, we aimed to evaluated the ability to modulate the immune response induced by NcROP4 for development of vaccine protocols and its capacity as infectious diseases immunotherapy. Here we have identified an immunogenic region of NcROP4 binding to mAb 20D2. Such a region was commercially synthesized and indentified as PepNcROP4. We produced the recombinant NcROP4 protein (rNcROP4) in Eschirichia coli. Both induced IL-10 up-regulation and IL-12 and IFNγ down-regulation and they were used in neosporosis experimental murine immunization protocols. Our results demonstrated that all the immunogens used (NLA, rNcROP4 and PepNcROP4) induced high production of specific antibodies. After challenge, rNcROP4 was shown to induce increase of body weight of immunized animals. Additionally, immunization with this protein reduced parasite burden and considerable survival compared to the other immunogens and control. The regulatory effect of rNcROP4 has been tested in the treatment of T. gondii-induced ileitis. This treatment reduced the inflammatory process in ileum, demonstrating that the therapy with this protein could generate an anti-inflammatory effect during T. gondii infection in mice. Our results showed that the regulatory profile induced by rNcROP4 may be used in treatment of acute inflammation caused by T. gondii infection and the capacity of this protein in protection of N. caninum infection in mice.
Supported by:CNPq, CAPES and FAPEMIG
Keywords: Neospora caninun; rop4; toxoplasma gondii

HP0126 - **AN IRON METALCOMPLEXE COMPOUND IMPROVE THE ANTI-*TOXOPLASMA GONDII* ACTIVITY OF SULFADIAZINE *IN VITRO***

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Toxoplasma gondi, is the toxoplasmosis agent and a protozoa able to infect and replicate in any nucleated cell. Toxoplasmosis is a disease related to severe damages to immunocompromised hosts and the current therapy is limited, and cause side effects. The literature presents many studies of effects of compounds based in metals, known as metalcomplexes, being actives against T.gondii. The dinuclear iron compound [Fe(HP0ClNOL)(SO4)]2-µ-oxo, controled the activity of antioxidant enzymes of Toxoplasma gondii and was secure to the host cells LLCMK2.The present work investigated the activity of new metalcomplexes that have in its structure an iron core coordinated to sulfadiazine against tachyzoites of T. gondii in vitro maintained in LLC-MK2 cells. The analysis of infected and treated cells showed that the compound N4013.1 presented an IC50 value equal to 3.3 µM and reduced significantly the infection rate compared to infected cells that were treated only with sulfadiazine, with metalcomplexes or untreated cells. In addition, the host cell viability was high even after treatment with high concentrations of compound. Electron microscopy analysis showed that treated parasites presented cytoplasmic inclusions similar to amylopectin granules commonly found in bradyzoites. The presence of bradyzoite cysts was confirmed by fluorescence microscopy and Dolichos biflorus lectin staining, specific for cystic wall. Fluorescence microscopy using specific marker LC3B, confirmed the death of parasites by autophagy, however, this index was very low. In conclusion, compound N4013.1 showed a potential effect against the parasite at concentrations in the micromolar range. More studies are needed to find out the type of death suffered by treated parasites.Thus, the new coordinated compound presented a better performance than sulfadiazine alone with selective activity against the parasite and the advantage of safety, being non-toxic to the host cells.
Keywords: Toxoplasma gondii; metalcomplexes; toxoplasmosis

HP0127 - **EOSINOPHILS IN THE CONTEXT OF LEISHMANIA AMAZONENSIS INFECTION**

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Recent studies have demonstrated that eosinophils are recruited to the site of injury in different models of parasite infection, including Leishmania. In previous histological analysis, reduced numbers of eosinophils in the inflammatory infiltrate of BALB / c mice, a strain naturally susceptible to infection by Leishmania amazonensis, where seen as compared with animals infected with L. braziliensis, which are resistant to infection. To better understand the role of those immune cells in injury and inflammatory responses, Leishmania amazonensis-infected BALB / c wild type (WT) and ΔDBLGATA-1 knockout (KO) mice were comparatively evaluated. Animals were infected with parasites in the stationary promastigote phase and the course of infection was evaluated weekly. Cytokine levels (IL-4 and IL-10 and IFN-Y) were assessed in lymphonode cells and tissue damage was evaluated by histological analysis. The results demonstrated that, in the fourth week of infection, KO mice had, on average, bigger footpad lesions (0,48+/-0,09mm X 0,32+/- 0,03 mm), even though the parasite load was the same for both groups. In the sixth week of infection, there was a significant difference in the levels of IL-10 (19 pg/mL x 11pg/mL) cytokines, in KO and WT, respectively. All together our results indicate that although the inflammatory process triggered in the absence of eosinophils have an effect on lesion size, this response is not enough for the effective controlling the parasite replication. **Supported by:**cnpq
Keywords: Leishmania amazonensis; eosinophils; inflamation

HP0128 - **CELL SIGNALING PATHWAYS AND THE MODULATION OF INFLAMMATORY RESPONSES, POST-INFECTION WITH LEISHMANIA AMAZONENSIS**

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Cell signaling pathways are molecular networks that comprise signal effector elements and regulatory proteins, acting together to integrate received stimuli. Inside the macrophages, signaling by these cascades directly affects the production of inflammatory mediators that ultimately trigger mechanisms of resistance or susceptibility to leishmania species. Prominent among these cascades are the mitogen activated protein kinases pathway ERK1/2 and the nuclear-kappa B transcription factor pathway (NF-κB). In this sense, in order to evaluate the modulation of these signaling pathways within the context of leishmaniasis, BALB /c mice were infected with Leishmania amazonensis and the parasite load, the levels of inflammatory cytokines and the expression of ERK ½ and NF-κB were evaluated. Our results in vivo have shown the model of infection by Leishmania amazonensis resulted in suppression of ERK1 / 2 phosphorylation in the fifth week of infection when compared whit control group (0,05 +/- 0,03 AU X 1,00+/- 0,05 AU) and coincided with an increase in lesion size (1,12 mm) and parasite load in the murine host. Similarly, in vitro assays have shown that the infection of bone marrow-derived macrophages also induced a modulation of ERK ½ and p-P65 expression after 24 hours of infection, as well as the increased in the production of cytokines such as IL-10. Together, these data support the idea that L. amazonensis infection triggers the modulation of these cellular signaling pathways by reducing the inflammatory response and favoring parasitism inside the host. Thus, although the role of cell signaling cascades in various inflammatory conditions has already been investigated, studies have not been performed for Leishmania amazonensis infections, which leads us to believe that a better understanding of these signaling mechanisms in the context of leishmaniasis may provide new ideas for the development of more effective therapies. **Supported by:**cnpq
Keywords: Leishmania amazonensis; cell signaling pathways ; erk1/2, nf-κ

HP0129 - **IMMUNOLOGICAL AND CYTOHISTOLOGICAL ANALYSIS OF *TRYPANOSOMA CRUZI* INFECTION DURING PREGNANCY**

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Trypanosoma cruzi is the causative agent of Chagas disease. This trypanosomiasis has become a global public health problem due to migration of Latin Americans to nonendemic countries. In Latin America with the succesful implementation of control domiciliated vector infestation and blood transfusion, the importance of congenital transmission has recently increased. Considering the tight regulation of immune system during gestation, we aimed to investigate the changes in the immune system caused by *T. cruzi* infection in the gestation outcome. *T cruzi* G and Y strains (trypomastigotes form) were used to infect female BALB/c mice before or after mating with non-infected male mice. The presence of vaginal plug was used as indicative of mating. Females were euthanized 8 days after confirmation of vaginal plug. We used three female control groups, only infected, only pregnant and non-infected and non-pregnant females. Two groups were infected before mating and other two were infected 4 days after confirmation of vaginal plug. The uterus and spleen were collected for immunochemistry, immunofluorescence and cytokine analysis. Our results showed that despite the MMP's identification being similarly among groups, *T. cruzi* higher virulent strain can interrupt gestation prior mating; the infection also increased cytokines like IFN-γ, IL-1β and IL-4. In conclusion this work suggests that *T. cruzi* infection can impair gestation outcome, furthermore local response to sistemic infection was able to control the infection allowing pregnancy development in some conditions.
Keywords: Immune system ; pregnancy; trypanosoma cruzi

HP0130 - **INVESTIGATION OF LAAG PLUS SAPONIN AS VACCINE AGAINST LEISHMANIASIS**

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Leishmaniasis is a neglected disease caused by the parasite of the genus *Leishmania.* Despite many efforts, until now, there is no vaccine approved for human use. For this reason, we are working on vaccine development against leishmaniasis. The present study involves the LaAg vaccine, which is composed of the total lysate of *L. amazonensis* promastigotes. Previous studies showed that this vaccine is safety and immunogenic. However, phase III clinical trials did not present vaccine efficacy and emerge the possibility to use this vaccine with adjuvants, which are capable of increasing the specific immune response. The aim of our study is to analyze the efficacy of LaAg associated with adjuvants as Saponin. We vaccinated C57BL6 mice intramuscularly with two doses of 100 µg LaAg bound or not with 100 µg of saponin and the control group received PBS. After seven days of the second dose we infected the right paw with $2x10^5$ promastigotes in the metacyclic phase of the *L. amazonenses* (Josefa strain). We performed weekly measurements by pachymetry and after approximately 56 days of the infection we analyzed the parasite load by limiting dilution assay (LDA) of infected lesion, spleen and lymph node lesion. The results indicated that LaAg plus saponin partially controlled the growth of the lesion compared to the other groups. Concerning to the parasite load, our data did not presented differences in the studied groups except for the SAP group, which worsened the parasitic load. The study on the efficacy of the LaAg plus saponin vaccine is still underway and further experiments are needed for comprehend the mechanism of protection.
Supported by:CNPq
Keywords: Leishmania; saponin; vaccine

HP0131 - **THE EFFECTS OF *TRYPANOSOMA RANGELI* INFECTION ON THE LIPID PROFILE OF *RHODNIUS PROLIXUS* METASTERNAL GLAND**

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Rhodnius prolixus is a bloodsucking hemipteran, one of the triatomines vectors of *Trypanosoma cruzi*, etiologic agent of Chagas disease and also a vector for *Trypanosoma rangeli*. *T. rangeli* infects a large number of mammals, but is not considered pathogenic for these animals. However, this protozoan reduces the survival and development of some triatomines. This parasite spreads in various organs of triatomines and reduces the survival and development of these insects. *R. prolixus* has a pair of exocrine glands in the ventral metathorax called Metasternal Gland (MG) that is involved in sexual communication. Gland cells from MG contain a large number of inclusions which are similar to lipid droplets. Lipids represent a rich source of pheromone precursors in insects. In this way we investigated if *T. rangeli* infection can alter the lipid profile in MGs from R. prolixus. MGs lipids were extracted from females fed on blood and infected females that were fed on blood contaning *T. rangeli* epimastigotes. Our results demonstrated that the sterol profile of MGs from infected females had smaller amounts of cholesterol than the control group. In contrast the amount of squalene in MG of infected insects was greather. Lipid transport in insects is mediated by Lipophorin (Lp). Diacylglycerol is predominant in Lp but cholesterol, phospholipids are also present. Previous work reports that the cholesterol used by *T. rangeli* is not produced by de novo biosynthesis. This parasite uptakes Lp from the hemolymph of *R. prolixus*. Thus the mechanism involved in the cholesterol delivery from hemolymph to MGs might be altered due to parasite infection. The metabolic role of squalene in *R. prolixus* and *T. rangeli* remains unknown. More experiments have to to be conducted to elucidate the role of squalene in parasite vector interaction and the influence of others lipids in MG from *R. prolixus*. **Supported by:**CAPES
Keywords: Rhodnius prolixus; trypanosoma rangeli; metasternal gland

HP0132 - **ANTI-ANGIOGENIC ACTIVITY OF TRYPANOSOMA CRUZI PROTEIN 21 ON CHAGASIC CARDIOMYOPATHY ONSET**

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Introduction: Chagas disease, caused by the parasite *Trypanosoma cruzi*, is an important cause of Chronic Chagasic Cardiomyopathy (CCC). The prospection of innovative therapeutic agents against CCC is a major task. The recombinant form of P21 (rP21), a secreted *T. cruzi* protein involved in host cell invasion and on progression of chronic inflammatory processes have been studied as a potential novel therapeutic target. **Objectives:** Our present work aimed to verify the impact of rP21 on blood vessels formation in vitro and investigate the antiangiogenic activity triggered by rP21.
mso-ansi-language:EN-US">Material and Methods:
mso-ansi-language:EN-US">tEnd cells were treated with different concentrations of rP21 or bacterial extract and viability and cellular adhesion were evaluated by MTT and angiogenesis inhibition by Matrigel tube formation assay. To verify the rP21-proteolytic activity on ECM components, fibrinogen, matrigel and fibronectin was incubated with rP21 or not. The substrate digestion was analyzed by SDS-PAGE. The accumulation and distribution of F-actin was determined by Phalloidin staining using ImageJ software. tEnd cells were incubated with rP21 and the total RNA was extracted using RiboZol and cDNA was synthesized by cDNA Reverse Transcription kit and analyzed by real-time PCR. **Results and Discussion:** We observed that rP21 did not alter cell viability and adhesion, but strongly inhibited vessel formation. Tube formation assay showed that angiogenesis inhibition was dependent of the CXCR4-rP21 binding. Moreover, we found that rP21 significantly increased F-actin levels and this protein was able to modulate expression of genes related to angiogenesis and actin cytoskeleton. However, rP21 showed no significant activity on the matrix components. Thus, the anti-angiogenic activity of rP21 may be due to a cascade of events triggered by CXCR4-rP21 interaction. **Conclusion:** Angiogenesis inhibition by rP21 depends on its binding to CXCR4 receptor and on the modulation of gene expression of both cytoskeleton and anti-angiogenic molecules.
Supported by:FAPEMIG/ CNPq/ CAPES/ UFU
Keywords: Trypanosoma cruzi; chagasic cardiomyophaty; angiogenesis

HP0133 - **ACTIVITY OF PTEROCARPANQUINONE LQB-182 ON LEISHMANIA.(V.) BRAZILIENSIS.**

SILVA, T.[*1]; VILLARIM, R.M.[1]; SIQUEIRA, L.M.[1]; SILVA, A.J.M.[2]; COSTA, P.R.R.[2]; SILVA, S.A.G.[1]
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Pterocarpanquinones are synthetic hybrid molecules created from a naphthoquinone and pterocarpan. The aim of this study was to investigate in vitro and in vivo the activity of pterocarpanquinone LQB-182 against L. (V.) braziliensis. Stationary-phase, L. braziliensis promastigotes were incubated with pterocarpanoquinone LQB-182 (20 µM) for 96h at 26ºC, and and the number of parasites was counted daily in a Neubauer chamber.BALB/c mice peritoneal macrophages were incubated with several concentrations of LQB-182 (0-300 µM) for 72h at 37°C/5%CO2 and cell viability was measured by MTT reduction assay. Monolayers of BALB/c mice peritoneal macrophage were infected with L. braziliensis promastigotes and subjected to treatment with the LQB-182 for 72h/ 37ºC/ 5%CO2, and number of infected cells were counted under optical microscopy. L. braziliensis infected BALB/c mice were treated from the seventh day of infection with LQB-182 administered intralesionally (three times a week) for two weeks.The size of lesion was measured weekly and parasite load estimated by limited diluition. Our analysis in vitro showed LQB-182 activity on promastigotes (IC50=10,98 ± 1,44 µM), intracellular amastigotes (IC50 =17,01±2,46µM) forms and low toxicity to BALB/c peritoneal macrophages (CC50 = 303,6 µM). The nitrite and reactive oxygen species quantification on culture supernatants showed that was no increase of production of this radicals. However, we observed that macrophages treated with LQB-182 phagocytosed more fluorescent beads than untreated controls ($p<0,05$). In vivo, LQB-182 was capable of controlling the lesion size and significantly decrease the parasite load ($p<0,001$) when administrated by intralesional route. The analysis of biochemical parameters showed an increase of hepatic enzymes, above the reference values. This data show that pterocarpanquinone LQB-182 was selectively active in vitro, and also effective in vivo. **Supported by:**CAPES
Keywords: Pterocarpanoquinones; leishmania.(v.) braziliensis; treatment

HP0134 - **MICE WITH STREPTOZOTOCIN-INDUCED DIABETES PRESENT DIFFERENT INFECTION AND INFLAMMATORY PROFILES THAN NON-DIABETIC MICE AFTER CHALLENGE WITH LEISHMANIA BRAZILIENSIS OR LEISHMANIA AMAZONENSIS**

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In the past decades, an increasing amount of evidence has emerge indicating that immunologic and inflammatory mechanisms play a significant role in the development and progression of diabetes. Since different host immunological response has been traditionally associated with the outcome of leishmaniasis, we hypothesized that diabetic mice could have a different infection profile when challenge with Leishmania sp. For this aim we induce diabetes in 8-weeks old C57Bl/6 mice with the administration of streptozotocin (STZ) at the dose of 50 mg/Kg/mouse for 5 days. Three days after the last STZ dose the mice were tested for glucose levels in blood and those with levels higher than 250 mg/dL were considered diabetics. As control, mice at the same age were inoculated with citrate buffer and submitted to the same conditions that mice inoculated with STZ. STZ-induced diabetes and controls mice were then challenge with L. braziliensis or L. amazonensis and had their footpad swelling measured at weeks intervals. For L. braziliensis the parasitism of the footpad as well as the cytokine levels was accessed at 3 and 5 weeks after challenge. In L. amazonensis-infected mice the parasitism of footpad, liver and spleen as well as the cytokine levels were accessed at 5 or 8 weeks after challenge. Results showed that mice with STZ-induced diabetes present significantly lower lesions size than the control group. However, when the parasite titer was evaluated, no significantly differences were observed when comparing STZ-induced diabetic mice and control mice after challenge with either L. amazonensis or L. braziliensis. Diabetic mice also presented a different pattern of induction of TNF-α or IFN-γ when comparing with non-diabetic mice. All together our results indicate that although the inflammatory process associated with diabetes has an effect on lesion size such response is not enough for the effective controlling of the leishmania parasites.
Keywords: Leishmaniases; diabetes; leishmania amazonensis and leishmania braziliensis

HP0135 - **ACTIVITY OF PTEROCARPANQUINONE LQB-182 ON LEISHMANIA.(V.) BRAZILIENSIS.**

SILVA, T.[*1]; VILLARIM, R.M.[1]; SIQUEIRA, L.M.[1]; SILVA, A.J.M.[2]; COSTA, P.R.R.[2]; SILVA, S.A.G.[1]
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Pterocarpanquinones are synthetic hybrid molecules created from a naphthoquinone and pterocarpan. The aim of this study was to investigate in vitro and in vivo the activity of pterocarpanquinone LQB-182 against L. (V.) braziliensis. Stationary-phase, L. braziliensis promastigotes were incubated with pterocarpanoquinone LQB-182 (20 µM) for 96h at 26ºC, and and the number of parasites was counted daily in a Neubauer chamber.BALB/c mice peritoneal macrophages were incubated with several concentrations of LQB-182 (0-300 µM) for 72h at 37°C/5%CO2 and cell viability was measured by MTT reduction assay. Monolayers of BALB/c mice peritoneal macrophage were infected with L. braziliensis promastigotes and subjected to treatment with the LQB-182 for 72h/ 37ºC/ 5%CO2, and number of infected cells were counted under optical microscopy. L. braziliensis infected BALB/c mice were treated from the seventh day of infection with LQB-182 administered intralesionally (three times a week) for two weeks.The size of lesion was measured weekly and parasite load estimated by limited diluition. Our analysis in vitro showed LQB-182 activity on promastigotes (IC50=10,98 ± 1,44 µM), intracellular amastigotes (IC50 =17,01±2,46µM) forms and low toxicity to BALB/c peritoneal macrophages (CC50 = 303,6 µM). The nitrite and reactive oxygen species quantification on culture supernatants showed that was no increase of production of this radicals. However, we observed that macrophages treated with LQB-182 phagocytosed more fluorescent beads than untreated controls ($p<0,05$). In vivo, LQB-182 was capable of controlling the lesion size and significantly decrease the parasite load ($p<0,001$) when administrated by intralesional route. The analysis of biochemical parameters showed an increase of hepatic enzymes, above the reference values. This data show that pterocarpanquinone LQB-182 was selectively active in vitro, and also effective in vivo. **Supported by:**CAPES
Keywords: Pterocarpanoquinones; leishmania.(v.) braziliensis; treatment

HP0136 - **EVALUATION OF HETEROCYCLIC COMPOUNDS ON TRYPANSOMA CRUZI *IN VITRO***

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Chagas disease, caused by Trypanosoma cruzi, is a tropical disease neglected. The treatment has side effects and limited efficacy. Aromatic amidines and their analogues present a broad spectrum of action on protozoa. Our goal was to analyze in vitro biological activity of 11 arilimidamides (AIAs) on T. cruzi. The results obtained for forms of blood incubated at 37°C in RPMI for 2h showed that 8 compounds had the lowest EC50 values (1.70 to 25.36 µM) that the benznidazole (Bz) (EC50>50 µM). When incubated for 24 h, the values EC50 were between 0.94 to 29,5 µM (Bz EC50=13 µM). To assess the possible use of these compounds in the therapy by blood banks, trypomastigotes were incubated with the compounds in the presence of 96% mouse blood at 4°C. The incubation for 2 h resulted in EC50>32 µM. After 24h incubation, 2 compounds showed activity more than gentian violet (30 µM). Tests with epimastigotes demonstrated that 3 AIAs presented EC50 values similar to Bz. However, the evaluation of the amastigote using nontoxic concentrations for mammalian cells (LC50=1.17µM) demonstrated that all 11 AIAs were active greater than 0.39 µM, while Bz showed EC50=3.6µM. Forward current limitations of medications available, it is necessary to continuity of in vitro studies of new trypanocidal agents. **Supported by:**Fiocruz, Capes, CNPq, Faperj, Paef and CPDD
Keywords: Expexperimental chemotherapy; t.cruzi; amidines

HP0137 - **EVALUATION OF PHOSPHODIESTERASES AS POTENTIAL DRUG TARGETS IN *TRYPANOSOMA CRUZI***

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Chagas disease is primarily a disease of South and Central America, caused by the protozoan parasite *Trypanosoma cruzi*. The available drugs benznidazole and nifurtimox are only effective against the acute stage of the disease; there are different strains of the parasites that are innately resistant to current treatment. There is therefore need for new, safer, more affordable, easy to administer and effective drugs. In order to avoid cross-resistance with current therapies, these should have a different mode of action from the existing ones. Cyclic nucleotide phosphodiesterases (PDEs) from trypanosomatids appear to be a good target. With success recorded by targeting human PDEs and also PDEs from T. brucei, we aim to target and evaluate the PDEs of *T. cruzi* as potential drug targets. Since *T. cruzi* is divided into different groups, we decided to use genomic DNA from two strains of *T. cruzi* (Colombiana and Y strain) that differ in their sensitivity to available chemotherapy and geographical locations. Cloning of the PDE genes from the two strains has been completed and we have started complementation assays to evaluate whether TcrPDEB1 and B2 can complement for the activity of TbrPDEB1/2. This work is part of the PDE4NPD (PhosphoDiEsterases for Neglected Parasitic Diseases) consortium. This consortium pursues target repurposing of phosphodiesterase inhibitors to shorten new drug discovery times for neglected parasitic diseases. Therefore, we rely on this consortium for genetic materials and compounds to be tested on our constructs. We intend to build on success stories from human PDE inhibitors (Maurice et al., 2014) and also from validation of *T. brucei* PDEs as drug targets (De Koning et al., 2012) for the evaluation and characterization of TcrPDEs as drug targets. There is sufficient knowledge of the structure of this enzyme family and this can be exploited to design highly efficient parasite-specific PDE inhibitors.
Keywords: Trypanosma cruzi; drug targets; phosphodiesterase