HP021 - PRODUCTION OF NITRIC OXIDE BY *LEISHMANIA AMAZONENSIS* PROMASTIGOTE

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The L-arginine metabolism is important in host-pathogen interactions. *Leishmania amazonensis* also depends on the interconversion of L-arginine by arginase to L- ornithine, a polyamine precursor essential for the replication and parasite survival in the mammal host. L- arginine is also substrate of nitric oxide synthase to produce nitric oxide (NO). Here, we determined NO production in *Leishmania amazonensis* wild type (WT) and in the arginase knockout (arg-) promastigotes in culture, besides the genetic complemented mutants in which the arginase was target (arg-/+ARG) or not (arg-/+ARGΔSKL) to the glycosome. We analyzed the NO production by DAF-FM labeling in flow cytometer. We verified that late-log and stationary-phase promastigotes of all parasites analyzed produced NO, but the arg- parasites increased in 20% NO production at the 7th day of culture when compared to WT. Besides, the arg- amastigotes-like parasites produce lower levels of NO than that observed in WT. The arg-/+ARG and arg-/+ARGΔSKL amastigote-like parasites did not produced NO. In conclusion, the stationary-phase promastigotes and amastigotes produced NO and the absence of arginase in parasites modulates the NO production by promastigotes, amastigotes and infected-macrophages. Taken together, we can infer the existence of a nitric oxid synthase-like in *Leishmania* that is active in stationary phase of a culture but is also regulated by the amount of internal L-arginine availability. As the arg- mutant is less infective then the WT parasite, we also can infer that NO production is important in the metacyclogenesis of promastigotes, but it is not essential for amastigote reinfection. **Supported by**:FAPESP, CNPq
Keywords: Arginase; l-arginine; flow cytometry

HP022 - THE HETEROLOGOUS EXPRESSION OF CATALASE DECREASES THE LEVELS OF ANTIOXIDANTS ENZYMES WHICH DETOXIFIES HYDROGEN PEROXIDE IN *TRYPANOSOMA CRUZI*

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Trypanosoma cruzi is the causal agent of Chagas disease. After more than a century of the *T. cruzi* discovery, there is still no vaccine against this disease, and the available treatments have severe effects and low efficacy during the chronic phase. Searching for new chemotherapeutic approaches, the antioxidant system of *T. cruzi* has called the researcher's attention due to its importance in the adaptation to oxidative environment in which this parasite is exposed to. The catalase enzyme, which decomposes hydrogen peroxide (H_2O_2) into water and oxygen, is found in virtually all aerobic organisms. However, a homologous sequence to catalase gene was not found in the *T. cruzi* genome. Our hypothesis is that *T. cruzi* may have suppressed the catalase as a strategy to allow that specific enzymes of the antioxidant pathway signalize the oxidative environment. We have showed that *T. cruzi* epimastigotes of CL Brener strain transfected with *E. coli* catalase gene have the same growth rate than the wild type (WT), but it has an increased resistance to H_2O_2. Moreover, to pretreat both parasite types with a low dose of H_2O_2 24 hours before the treatment makes the WT cells as resistant to H_2O_2 as the cells expressing catalase. These results suggest that the pretreatment could prepare the parasites for the oxidative environment, although this is not observed with the same intensity in the presence of catalase. Currently, we have showed the influence of the catalase enzyme on the expression of *T. cruzi* antioxidant enzymes. We have verified that catalase decreases the expression of trypanothione reductase, mitochondrial peroxiredoxin and ascorbate peroxidase. On the other hand, the catalase expression increases the superoxide dismutase levels. These results suggest that catalase causes a change in the *T. cruzi* antioxidant system that leads to an increase in the H_2O_2 level, which it has been shown to be important in low concentrations in the oxidative stress resistance. **Supported by**:CAPES
Keywords: Trypanosoma cruzi; oxidative stress; catalase

HP023 - *LEISHMANIA AMAZONENSIS* DECREASES CD40 EXPRESSION AND IL-12 PRODUCTION BY DENDRITIC CELLS BY A MECHANISM DEPENDENT ON ADENOSINE A2B RECEPTORS AND ACTIVATION OF THE CAMP-PI3K-ERK1/2 PATHWAY

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Leishmania infection can result in a wide spectrum of clinical manifestations and *L. amazonensis* is associated with a lack of antigen-specific T-cell responses. Dendritic cells (DCs) direct the differentiation of T-helper 1 lymphocytes that contribute to the control of *Leishmania* infection. In a previous work, we showed that infection by *L. amazonensis*, but not by *L. braziliensis* or *L. major*, impairs DC responses by activating adenosine A_{2B} receptors ($A_{2B}R$). Here, we evaluated the expression of adenosine receptors and the intracellular events triggered by $A_{2B}R$ in infected cells. With this aim, bone marrow-derived DCs from C57BL/6J mice were infected with metacyclic promastigotes of either *L. amazonensis* , *L. braziliensis* or *L. major*. Fluorescence mi croscopy revealed that *L. amazonensis* infection stimulated the recruitment of $A_{2B}R$ to the surface of infected DCs, with out altering total $A_{2B}R$ protein density, as gauged by Western blotting, nor the expression of A_1, A_{2A} or A_3 adenosine receptors in DCs. Infection by *L. braziliensis* or *L. majo r* did not cause $A_{2B}R$ recruitment to the plasma membrane. We also report that *L. amazonensis* increased cAMP production and ERK1/2 phosphorylation in infected DCs by a mechanism dependent on $A_{2B}R$. Furthermore, *L. amazonensis* infection impaired CD40 expression and IL-12 production by DCs, an IL-10-independent effect prevented by the inhibition of adenylate cyclase, PI3K or ERK1/2 phosphorylation. In conclusion, we propose a new pathway used by *L. amazonensis* ($A_{2B}R$ recruitment → cAMP → PI3K → ERK1/2) to suppress DC activation, which may contribute to the pronounced deficiency of immune response elicited against this parasite infection. **Supported by**:FAPEMIG, CNPq, CAPES-FCT, Ciência sem Fronteiras, FCT **Keywords:**Dendritic cells; leishmania amazonensis; adenosine a2b receptor

HP024 - *LEISHMANIA AMAZONENSIS* INFECTION IN A HUMAN IL-32γ MOUSE MODEL

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IL-32 is a proinflammatory cytokine with different isoforms. IL-32γ isoform has the most potent biological activity and it has been detected in lesions of patients with tegumentary leishmaniasis. Murine cells respond to IL-32, however, mice lack the gene for this cytokine. To understand the role of IL-32 in *Leishmania amazonensis* infection, we used the human IL-32γ transgenic mice. C57BL/6 mice (WT) and C57BL/6 transgenic for IL-32γ human gene (IL-32γTg) mice were infected with *L. amazonensis* promastigotes (PH8 strain) in the ear. The results demonstrated that IL-32γTg mice exhibit a delay in the lesion development during the first 3 weeks of infection, when compared with WT mice. The difference between groups was more evident in the third week of infection, when IL-32γTg mice had a lesion size 5-fold lower than WT mice. From the 5th until 9th week of follow-up, both groups had similar profiles of lesion development. Interestingly, on the 3rd week of infection, the parasite burden in IL-32γTg mouse lesion was 100 times higher than in WT mice. After 3 weeks, IL-32γTg mice maintained the same parasite burden until 9 weeks. In WT mice, however, the number of parasites had grown exponentially during the weeks evaluated. Dissemination of parasites to spleen was observed only in WT mice after 9 weeks of infection. Histological sections of the infected ears were used for detection of IL-32 by immunohistochemistry with anti-IL-32 polyclonal antibody. As expected, IL-32γTg mice showed IL-32 protein expression in different cells whereas this cytokine was not detected in WT mice. Our data suggest that IL-32γ favors infection by *L. amazonensis* in the early stages by allowing parasite growth; however, it can limit the growth and dissemination of parasites at later stages. The mechanisms responsible for the different clinical and parasitological profiles in IL-32γTg and WT mice are under current investigation. **Supported by**:CNPq, FAPEG **Keywords:**Leishmania amazonensis; il-32; mouse model

HP025 - MIMOTOPE-BASED ANTIGENS SELECTED BY PHAGE DISPLAY TECHNOLOGY EMPLOYED FOR THE SERODIAGNOSIS OF CANINE VISCERAL LEISHMANIASIS

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Visceral leishmaniasis (VL) is a disease that is endemic to Brazil, where dogs are the main domestic parasite reservoirs. The serodiagnosis of CVL is hampered by factors related with the sensitivity and/or specificity obtained with the different antigens employed, leading to the occurrence of a large number of false-positive results caused by cross-reactivity with other related parasites. Also, there are two commercially-available Brazilian vaccines (Leish-Tec® and Leishmune®) that can induce the production of Leishmania-specific antibodies in vaccinated animals, causing them to be diagnosed as infected animals in the serological trials. The present study describes a subtractive selection through phage display from polyclonal antibodies of negative and positive sera that resulted in the identification of potential bacteriophage-fused peptides that were highly sensitive and specific to antibodies of CVL. Initially, a negative selection was performed, in which phage clones were adhered to purified IgGs from healthy and T. cruzi-infected dogs to eliminate cross-reactive phages. The remaining supernatant non-adhered phages were submitted to positive selection against IgG of dogs that were infected with Leishmania infantum. Phage clones that adhered to purified IgGs from the CVL-infected serum samples were selected, identified and employed in the serological analysis. Eighteen clones were identified and their reactivity was tested by a phage-ELISA against the serum samples from infected dogs compared to those from vaccinated dogs, experimentally infected dogs with cross-reactive parasites, and healthy controls. Eight clones presented sensitivity, specificity, and positive and negative predictive values of 100%, and they showed no cross-reactivity with T. cruzi- or E. canis-infected dogs, as well as with sera of animals vaccinated with Leish-Tec® or Leishmune®. In conclusion, the identified eight mimotopes could be applied in a phage-ELISA in CVL-monitoring programs. **Supported by**:FAPEMIG, INCT-NanoBiofar, CNPq and PRPq/UFMG
Keywords: Serodiagnosis, canine visceral leishmaniasis; phage display; canine visceral leishmaniasis

HP026 - EFFECT OF MUSHROOM AGARICUS BLAZEI MURRIL IN IMMUNE RESPONSE AND DEVELOPMENT OF EXPERIMENTAL CEREBRAL MALARIA

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Introduction: Cerebral malaria (CM) is debilitating and sometimes fatal. Disease severity has been associated with poor treatment access, therapeutic complexity and drug resistance and, thus, alternative therapies are increasingly necessary. In this study, was investigated the effect of the administration of Agaricus blazei, a mushroom of Brazilian origin in a model of CM caused by Plasmodium berghei, strain ANKA, in mice. Methods and Results: C57BL/6 mice were pre-treated with aqueous extract or fractions of A. blazei, or chloroquine, infected with P. berghei ANKA and then followed by daily administration of A. blazei or chloroquine. Parasitaemia, body weight, survival and clinical signs of the disease were evaluated periodically. The concentration of pro-and anti-inflammatory cytokines, histopathology and in vitro analyses were performed. Mice treated with A. blazei aqueous extract or Fraction C, that shows antioxidant activity, displayed lower parasitaemia, increased survival, reduced weight loss and protection against the development of CM. The administration of A. blazei resulted in reduced levels of TNF, IL-1β and IL-6 production when compared to untreated P. berghei-infected mice. Agaricus blazei (aqueous extract or Fraction C) treated infected mice displayed reduction of brain lesions. Although chloroquine treatment reduced parasitaemia, there was increased production of proinflammatory cytokines and damage in the CNS not observed with A. blazei treatment. Moreover, the in vitro pretreatment of infected erythrocytes followed by in vivo infection resulted in lower parasitaemia, increased survival, and little evidence of clinical signs of disease. Conclusions: This study strongly suggests that the administration of A. blazei (aqueous extract or fraction C) was effective in improving the consequences of CM in mice and may provide novel therapeutic strategies. **Supported by**:CNPQ E FAPEMIG
Keywords: Agaricus blazei murrill; experimental cerebral malaria; anti-malarial therapy

HP027 - PURIFICATION AND USE OF ADJUVANT FRACTIONS OBTAINED FROM AGARICUS BLAZEI MURILL MUSHROOM IN A VACCINE COMPOSITION TO PROTECT AGAINST MURINE VISCERAL LEISHMANIASIS

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The development of effective prophylactic strategies to prevent leishmaniasis has become a high priority. No less important than the choice of an antigen, the association of an adjuvant is necessary to achieve a successful vaccination; however, few adjuvants exist on the market today. In this context, this study evaluated purified fractions from Agaricus blazei as Th1 adjuvants through in vitro assays of their immune stimulation of spleen cells derived from BALB/c mice. The water extract of the mushroom were fractionated, and the obtained fractions were used to stimulate spleen cells derived from naive BALB/c mice. Then, the production of IFN-□ IL-4 and IL-10 was evaluated. Two of the tested six fractions (F2 and F4) were characterized as polysaccharide-rich fractions, and were able to induce high levels of IFN-□ and low levels of IL-4 and IL-10 in the spleen cells. The efficacy of adjuvant action against L. infantum was evaluated in BALB/c mice, with these fractions being administered together with a recombinant protein, LiHyp1, which was previously evaluated as a vaccine candidate against visceral leishmaniasis (VL). The associations between LiHyp1/F2 and LiHyp1/F4 were able to induce an in vivo Th1 response, which was primed by high levels of IFN-□ IL-12, and GM-CSF, by low levels of IL-4 and IL-10; as well as by a predominance of IgG2a antibodies in the vaccinated animals. After infection, the immune profile was maintained, and the vaccines proved to be effective against L. infantum. The immune stimulatory effects in the BALB/c mice proved to be similar when comparing the F2 and F4 fractions with a known Th1 adjuvant (saponin), though animals vaccinated with saponin did present a slight to moderate inflammatory edema on their hind footpads. The F2 and F4 fractions appear to induce a Th1-type immune response and, in this context, they could be evaluated in association with other protective antigens against Leishmania, as well as in other diseases models.
Supported by:FAPEMIG, INCT-NanoBiofar, Minasfungi do Brasil, CNPq and PRPq/UFMG
Keywords: Agaricus blazei murill; adjuvants; vaccine

HP028 - SOCS2 EXPRESSION IN DENDRITIC CELLS IS ESSENTIAL TO CONFER PROTECTIVE IMMUNITY AGAINST TRYPANOSOMA CRUZI INFECTION

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The infection by the parasite Trypanosoma cruzi, induces an inflammatory reaction and the efficacy of the host immune response (IR) is vital during the infection. The SOCS2 (supressor of cytokine signaling) expression is partially mediated by lipoxins (LXA_4) in dendritic cells (DCs). DC is the main antigen-presenting cell (APC) that participates, among others cells subsets, as first line defenders against infections or inflammation. We investigated the role of SOCS2 in DCs function and induction/maintenance of IR during experimental T. cruzi infection. CD11cDTR-Tg mice (DCs depletion), wild type (WT) and SOCS2 (knockout) were infected with T. cruzi. Our data shown an increased parasitemia in depleted DCs mice, highlighting that DCs are vital in the control of T. cruzi infection. Absence of SOCS2 during the innate IR resulted in decreased frequency of DCs producers of IL12 and TNF, but not IL10, without change the Toll-like receptor and MHCII expression. In divergence, a decreased expression of the CD80 costimulatory molecule was observed in SOCS2 deficient DCs. During the adaptive IR the deficiency of SOCS2 in DCs resulted in increased expression of TLR2 and 4, and reduced frequency of DCs expressing MHCII. The adoptive transfer of SOCS2 deficient DCs caused increased parasitemia and changes in the IR shape against T. cruzi infection, mainly: reducing the frequency of NK cells producing IFN-γ and IL17; reducing the frequency of CD8 T producing IFNγ and CD4 producing IL17, despite increasing CD4 cells producing IFNγ; absence of SOCS2 in DCs also resulted in reduction of cells producing IL10 such as CD4 and CD19, besides Treg cells. Here, we demonstrated the pivotal role of SOCS2 in the modulation of DCs function during the innate and adaptive IR throughout T. cruzi infection. Financial support CNPq and FAPEMIG. Keywords: Trypanosoma cruzi, SOCS2, Dendritic Cells **Supported by**:CNPq
Keywords: Trypanosoma cruzi; socs2; dendritic cells

HP029 - DIFFERENTIAL ANALYSIS OF L-ARGININE METABOLISM BETWEEN *LEISHMANIA INFANTUM* STRAINS AND THEIR RELATION WITH INFECTIVITY *IN VITRO*

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Leishmaniasis is a disease causes a variety of clinical manifestations which are dependent upon the species of *Leishmania* and infectivity of the parasite strain. In this sense, the L-arginine metabolism is related with this infectivity, since this amino acid is shared by two enzymes, nitric oxide synthase (NOS) and arginase that may act differently in the death or survival and multiplication of the parasite within macrophages. The nitric oxide (NO) production by NOS and ornithine and urea by arginase are present in the parasite and host cell causing a mix of enzymatic activities. Based in previous observations from our group, that two *L. infantum* strains, one strain from Old World (*Li*OW) and another from New World (*Li*NW) demonstrated different profiles in the *in vivo* infection, the aim of this work was to study the L-arginine metabolism on these parasites. It was studied the NO and urea production during the parasite growth, the infectivity on BALB/c mice peritoneal macrophages and the percentage of metacyclic parasites during growth curve. The evaluation of the parasitic growth curves showed a significant difference between the two strains. The major arginase activity was showed on the third day for both *L. infantum* strains, those values were found at the same time the parasitic growth curves begin to enter in the stationary growth phase. We observe the highest percentage of metacyclic forms in *Li*OW strain, as well as higher infection index *in vitro* compared to the *Li*NW strain in 24h and 72h post-infection. The methodology used to analyze the NO production, was not efficient and at the moment the NO intracellular production is being evaluated. The results up to now indicate that *Li*OW shows higher virulence, according to the high metcyclic forms observed. The metabolism of L-arginine in the parasite can be considered a good therapeutic target, considering that the enzymes may modulate the host response to infection. **Supported by**:CNPq, FAPERJ, FIOCRUZ
Keywords: Leishmania; l-arginine; nitric oxide

HP030 - THE ADAPTER MOLECULE INDUCING INTERFERON-β WITH TIR DOMAIN (TRIF) IS REQUIRED FOR RESISTANCE TO NEOSPORA CANINUM INFECTION IN MICE

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Neospora caninum is an intracellular parasite that has the dog as definitive host and other mammals, especially cattle, as intermediate hosts. Economically, neosporosis is an important disease in veterinary medicine due to serious complications, like abortion in cattle and neuromuscular paralysis in dogs. Further studies are needed to describe the participation of signaling pathways that culminate in the production of inflammatory mediators, enabling the development of preventive and therapeutical methods against this infection. Thus, the aim of this study was to evaluate the role of the adaptor TLR molecule TRIF in neosporosis resistance in vivo and in vitro. For this, C57BL/6 wild-type (WT) and genetically deficient for TRIF (TRIF-/-) mice were infected by N. caninum for survival analysis, cellular and tissue parasitism, histological changes and production of antibodies and cytokines in different phases of infection. Furthermore, in vitro experiments were performed with WT and TRIF-/- bone marrow derived macrophages (BMDMs) infected by N. caninum to measure cytokines. It was seen that TRIF-/- survival rate decreased 2-fold compared to WT mice, while higher parasite burden was observed in peritoneal cells and lungs during the acute phase, and in brain in the chronic phase of infection, in association with inflammatory changes in all analyzed tissues. In relation to the main Th1 cytokines, we have been found that the concentration of IL-12, IFN-γ and TNF-α were decreased in peritoneal lavage and lungs of TRIF-/- mice. Corroborating with in vivo findings, TRIF-/- BMDMs presented notable defects in inflammatory cytokine production. In conclusion, our results show that TRIF is required for resistance against the infection with N. caninum, regulating keys Th1 cytokines production and participating in the control of the tissue parasitism and inflammatory lesions induced against the parasite. **Supported by**:CAPES FAPEMIG CNPQ
Keywords: Neospora caninum; trif; immune response

HP031 - BEYOND BLOOD: FIRST REPORT OF SUGAR MEALS IN KISSING BUGS

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Background. Triatomines, which are the vectors of *Trypanosoma cruzi*, were considered obligatory blood feeders for more than 100 years, since the discovery of Chagas disease. Methods. We offered artificial sugar meals to laboratory-reared *Rhodnius prolixus*, a strict haematophagous insect model *par excellence* and registered feeding by adding colorant to the sugar meals. To assess putative phytophagy, *R. prolixus* was exposed to cherry tomatoes *Solanum lycopersicum* and tomato DNA was detected in the insects by PCR. We also assessed longevity, blood feeding and urine production of fruit-exposed kissing bugs and controls. Wild kissing bugs were collected in Northeast Brazil and tested for presence of plant DNA in gut homogenates by PCR. Results. All instars of *R. prolixus* ingested sugar from artificial sugar meals in laboratory conditions. First instar *R. prolixus* ingested plant tissue from *S. lycopersicum* fruits, and this phytophagy increased the amount of ingested blood ingested and excreted urine. It also decreased mortality after blood feeding. Exposure to *S. lycopersicum* increased longevity and reduced weight loss by desiccation. Field collected *Triatoma brasiliensis* presented DNA from three local plants in their gut contents. Conclusions. We describe in this manuscript the first report of sugar feeding and phytophagy in a species that was considered a strict blood-feeder for over a century. In this respect, local plants may have a nutritional role in the maintenance of triatomine vectors, being not mere shelters for insects and vertebrate hosts as previously believed. The description of sugar and plant meals in triatomines opens new perspectives for the study and control of Chagas Disease.**Supported by**:CAPES/CNPq
Keywords: Triatomine; chagas; phytophagy

HP032 - LOPINAVIR/RITONAVIR, AN HIV ASPARTIC PROTEASE INHIBITOR, MODIFIES SYSTEMIC IMMUNOLOGICAL CELL PROFILE IN LEISHMANIA (L.) AMAZONENSIS-INFECTED BALB/C MICE WHEN ADMINISTRATED ORALLY

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There are few studies about the immunomodulatory properties of the HIV aspartic protease inhibitors on the immune response against *Leishmania*. The aim of the present study was to evaluate the impact of oral treatment with lopinavir/ritonavir (LPV/RTV) (200/50 mg/Kg/day) on the leukocytes during *Leishmania* infection. After 30 days of treatment, blood was collected to determine the total and differential leukocyte count and the serum cytokine levels. Spleen was also obtained to determine the immunophenotypic profile of leukocytes and parasite load. *Leishmania* infection did not influence the total leukocyte count in non-treated mice. However, the parasitic infection increased the number of neutrophils and monocytes and decreased the number of lymphocytes in the blood circulation of these animals. These changes were followed by increased levels of IL-17A cytokine in the sera. In the spleen of non-treated *Leishmania*-infected mice, there was a decrease on the percentage of CD4+ and CD8+ T lymphocytes. Although, there was a higher percentage of activated CD4+ T lymphocytes (CD25+ and CD69+), as well as a higher percentage of B lymphocytes CD80+. LPV/RTV treatment in *Leishmania*-infected mice did not alter blood leukocyte count and serum cytokine levels compared to non-treated Leishmania-infected mice. However, HIV protease inhibitor treatment decreased the percentage of splenic macrophages CD69+, CD8+ T lymphocytes CD28+ and B lymphocytes CD69+ compared to non-treated *Leishmania*-infected mice. These changes were followed by a significant decrease on the parasite load. Our study showed that LPV/RTV treatment decreased activation status of leukocytes and parasite load in the spleen of *Leishmania*-infected BALB/c mice. It is possible that LPV/RTV may help to reduce the leukocyte activation and the parasite load during the HIV/*Leishmania* coinfection, thus collaborating to improve the survival of HIV/*Leishmania* coinfected individuals.**Supported by**:CNPq, CPqRR(FIOCRUZ/MG)
Keywords: Leishmania; hiv ; lopinavir/ritonavir

HP033 - DIFFERENTIAL RELEASE OF SOLUBLE PROTEINS AND EXTRACELLULAR VESICLES BY DIFFERENT *TRYPANOSOMA CRUZI* STRAINS IS ASSOCIATED WITH DIFFERENTIAL ABILITY TO INVADE HOST CELLS

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Trypanosoma cruzi strains vary in their ability to invade host cells. We investigated whether the extracellular vesicles and soluble proteins released by different strains can modulate their infectivity toward target cells, using metacyclic trypomastigote (MT) forms of highly invasive CL strain and the low invader G strain. These strains express similar levels of gp82, a MT-specific molecule that mediates cell invasion, whereas gp90, a MT-specific molecule that down modulates invasion, is expressed at higher levels in G strain. Parasites were incubated for 1 h in full nutrient D10 medium. The conditioned medium (CM), collected after parasite centrifugation, designated as CL-CM and G-CM, was used in invasion assays. Human HeLa cells were incubated for 1 h with CL strain MT in D10, in absence or in the presence of CL-CM or G-CM, and the number of internalized parasites was counted. Invasion of CL strain MT diminished significantly in the presence of G-CM but not of CL-CM. Western blots of G-CM and CL-CM, using monoclonal antibody (mAb) to gp82 or gp90, revealed high levels of both molecules in G-CM and low levels in CL-CM. Incubation of CL strain MT with HeLa cells, plus G-CM and anti-gp90 mAb that does not recognize live parasites, reverted the G-CM inhibitory effect. Vesicles and soluble factors, containing gp82 and gp90, decreased CL strain MT invasion similarly to non fractionated G-CM. As G strain invasion capacity in D10 is very low, assays with this strain were performed in nutrient-free PBS++ solution. Parasites were incubated with HeLa cells in PBS++ or in PBS++ plus D10, G-CM or CL-CM, at 1:50. G-CM, but not CL-CM, significantly reduced G strain MT invasion, in a manner reversible by anti-gp90 mAb nonreactive with live parasites. Our data suggest that the factors released by MT impair parasite-host cell interaction, gp82 competing for the host cell receptor with the molecule expressed on MT surface and gp90 further contributing to down modulate invasion. **Supported by**:CNPq e FAPESP
Keywords: Metacyclic trypomastigote ; vesicles; infectivity

HP034 - EFFECTS OF LALRR17 PROTEIN FROM LEISHMANIA (L.) AMAZONENSIS IN MACROPHAGE INFECTION AND IDENTIFICATION OF ITS POSSIBLE LIGANDS

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Leishmania ssp. are protozoan parasites transmitted to vertebrates by the bite of female phlebotomine insects. They have two main forms in their life cycle: promastigotes and amastigotes. The amastigotes live preferably in macrophages and are able to circumvent the microbicidal activity of these cells, surviving and multiplying inside them. In Leishmania amazonensis this ability is due to several molecules produced by the parasite as, for example, the LaLRR17 protein. LaLRR17 is expressed in promastigotes and amastigotes and was detected in the cytoplasm of infected macrophages. We have shown that L. amazonensis promastigotes overexpressing this protein have increased infectivity in vitro, and that the presence of the recombinant LaLRR17 in different concentrations promotes higher infection of macrophages by wild type parasites. LaLRR17 contains in its central region six leucine-rich repeats (LRRs). The LRR motifs of various organisms are usually involved in protein-protein interactions. It is therefore plausible to assume that LaLRR17 participates in interactions with macrophage molecules, and that these interactions may modulate the response of this host cell. However, we do not know which molecules from the macrophage interact with LaLRR17. Phage display is a technique based on the expression of synthetic peptides or proteins in phage capsids and has been used for various purposes including the identification of protein ligands. We have used a Phage Display peptide library over recombinant LaLRR17 to select for ligands of this virulence factor. The selected phages were sequenced and the sequences obtained will be compared to mouse / human databases to determine the possible ligands of this protein in the host cells and explain its contribution to the parasite virulence. **Supported by**:fapesp
Keywords: Leishmaniasis ; phage display; lrr

HP035 - METACYCLIC STAGE-SPECIFIC SURFACE MOLECULE GP90 PLAYS A MAJOR ROLE IN DOWNMODULATING THE INFECTIVITY OF TRYPANOSOMA CRUZI GENETIC GROUPS TCI AND TCIV IN VITRO AND IN ORAL INFECTION

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T. cruzi genetic groups TcI and TcIV constitute the predominant agent of Chagas disease in regions where outbreaks of oral infection prevail. The mechanisms of host cell invasion by these parasites are poorly understood. We analyzed the insect stage metacyclic trypomastigote (MT) forms of four TcI strains and one TcIV strain isolated from Chagasic patients in different geographic regions, aiming to identify the factors involved in host cell invasion in vitro and in oral infection in mice. Parasites of all strains were poorly infective when given orally into mice. Amastigote nests were either absent or present in small numbers in the target gastric epithelial cells. It is possible that the mucin-containing mucus hindered MT migration toward the gastric epithelium, as judged by the inefficient migration through the gastric mucin-coated transwell filter. Another factor could be the resistance to pepsin degradation of gp90 the MT surface molecule that negatively regulates host cell invasion. The cell invasion capacity of TcI and TcIV strains was examined using human epithelial HeLa cells. When incubated with HeLa cells in nutrient-deprived PBS++, instead of full nutrient DMEM supplemented with 10% FBS (D10), MT of all strains exhibited higher infectivity. To determine whether soluble factors released by the parasites affected invasion, a conditioned medium (CM) was prepared by incubating MT in D10 for 1 h and collecting the supernatant. As compared to invasion in PBS++, or in PBS++ plus D10 (1:50), MT internalization diminished significantly in PBS++ plus CM (1:50). High gp90 levels were detected in CM by western blot analysis. MT incubation with HeLa cells in PBS++ containing CM, plus anti-gp90 monoclonal antibody that does not recognize live parasites, reverted the inhibitory effect of CM. Taken together, these results indicate that the pepsin-resistant gp90 is possibly the major determinant of low infectivity of TcI and TcIV parasites in vitro and in oral infection. **Supported by**:Fapesp e CNPq
Keywords: Gp90; metacyclic trypomastigote; tci

HP036 - ROLE OF TUMOR NECROSIS FACTOR IN IMMUNE RESPONSE CELLULAR DURING *NEOSPORA CANINUM* INFECTION

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The protozoan parasite *Neospora caninum* has been associated to abortions in cattle since the early 1990's, and the infection leads to major economic impact to the segment. Tumor necrosis factor (TNF) is a proinflammatory cytokine produced quickly during the acute phase of infection, playing an important role in the immune response against various pathogens. Considering the importance of this cytokine during acute infectious processes, we aimed to verify the role of TNF during N. caninum infection. Therefore, we analyzed the cellular immune response using C57BL/6 wild type (WT) and genetically deficient mice in receptor 1 of TNF ($TNFR1^{-/-}$). These animals were infected intraperitoneally with *N. caninum* tachyzoites of the Nc-1 strain. We found an increased susceptibility of $TNFR1^{-/-}$ mice. It was evidenced by higher mortality and an enhanced parasite burden in the supernatant of peritoneal lavage and lungs during the acute phase, as well as in the chronic phase in the brain. Tissue inflammation were also analyzed through hematoxylin and eosin staining, showing a higher inflammatory infiltrate in lung and brain tissues in animals $TNFR1^{-/-}$. Additionally, we analyzed the effect induced by the absence of TNF action on the production of Th1 pattern of immune mediators. It was found a significant increase either in IL-12p40 and IFN-γ in peritoneal fluid, sera and lung homogenates of $TNFR1^{-/-}$ mice. This result demonstrates that the production of these cytokines is independent of TNF. Furthermore, we observed that TNF is required for induction of nitric oxide (NO) production, once animals $TNFR1^{-/-}$ showed significantly reduced levels of NO. Overall, we conclude that TNF plays an important role in host resistance against infection by the protozoan *N. caninum*, due to the lack of parasite control in $TNFR1^{-/-}$ animals. **Supported by**:CAPES, CNPq e FAPEMIG
Keywords: Neospora caninum; tnfr1-/-; cytokines

HP037 - ANALYSIS OF ESAG FUNCTIONS IN AFRICAN TRYPANOSOMES

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Bloodstream-form Trypanosoma brucei are covered by a major antigen called variant surface glycoprotein (VSG). VSG is transcribed solely from one of around 15 subtelomeric bloodstream expression sites (BES). Along with VSG, the BES encodes a group of expression site associated genes (ESAGs), the functions for the majority of which remain unknown. We have recently shown that most ESAGs localise to the cell surface, where they may play roles mediating host-parasite interactions. ESAGs form 14 distinct families, encompassing the BES ESAGs and chromosomal-internal genes related to ESAGs (GRESAGs). Here we use endogenous-locus tagging, RNA interference and mutant phenotype analysis to functionally characterise the individual ESAG families, from each of these genomic locations. Our strategy enables the specific silencing of each active BES ESAG gene, as well as a pan-family approach. We show that ablation of just the BES ESAG does not impair parasite proliferation in vitro, while silencing of whole families results in cell growth defects. These findings suggest a redundancy mechanism between the BES ESAGs and their GRESAG counterparts, and that as a family they are essential to the survival of the parasite.**Supported by**:BBSRC
Keywords: Antigenic variation; contingency genes; surface proteins

HP038 - NOVEL FLAGELLAR POCKET PROTEINS OF AFRICAN TRYPANOSOMES

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To survive in the host bloodstream, *Trypanosoma brucei* must perform the critical cellular processes of nutrient uptake and secretion whilst evading the host immune response. These functions are performed at the flagellar pocket, making this the primary interface between the parasite and its host. The overall importance of the pocket for parasite virulence, and the essentiality of two receptors characterised to date, make flagellar pocket proteins attractive therapeutic targets. Our lab has recently defined a surface proteome for bloodstream-form *T. brucei*. This "surfeome" identified several novel flagellar pocket components, and here I present work towards their functional and biochemical characterisation. To establish if these proteins are necessary for surface function and host interaction, RNA interference was used to silence individual GFP-tagged flagellar pocket genes and loss-of-fitness associated with protein ablation was assessed during parasite growth in vitro. In addition, analysis of the glycosylation status shows that the majority of pocket proteins possess both high mannose and paucimannose/complex N-glycans. I am investigating the possible roles played by glycosylation in the sorting of proteins to the flagellar pocket, a specialised surface membrane domain out of sight of the host immune system.**Supported by**:Royal Society and University of Nottingham
Keywords: Flagellar pocket; cell surface; host-parasite interface

HP039 - LEISHMANIA AMAZONENSIS ARGINASE ALTERED MIRNA PROFILE OF INFECTED MACROPHAGES TO MODULATE NO/POLYAMINES

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The microRNAs (miRNAs) are non-coding RNAs (21-24 nt) that can modulate gene expression by the complementary binding of the initial 6 to 9 nucleotides of its 5' region to the 3'UTR of target mRNA. The miRNAs can modulate inflammatory mechanisms of immune response by post-transcriptional regulation of genes involved in those pathways. Recent studies have described the importance of balance in the production of Nitric Oxide (NO) versus polyamines during *Leishmania* infection. Here, we determined that *L. (L.) amazonensis* infection could subvert the miRNAs profile of mouse macrophages as well as the production of NO. We also showed that the activity of arginase of the parasite is involved in macrophage miRNA modulation. We analyzed the miRNA profile of RNA from BALB/c mice Bone Marrow-Derived macrophage (BMDM) infected by *L. (L.) amazonensis* WT or *L. (L.) amazonensis* knockout for arginase (La_arg-). We verified a modulation on 32% of the 84 miRNAs analyzed in BMDM infected with *L. (L.) amazonensis* WT compared to non-infected BMDM, whereas 60% were up-regulated. The infection with the La_arg- mutant increased the percentage of miRNAs modulated to 45% compared to WT infection, but only 35% of the miRNAs were up-regulated. Moreover, we showed the up-regulation of miR-294-3p and miR-721 in *L. (L.) amazonensis* WT infected macrophages, but not in La_arg- infection. In silico analysis indicated nitric oxide sintase-2 coding mRNA (Nos2) as a possible target for those miRNAs. Indeed, the *Nos*2 expression and NO production were higher in macrophages infected by La_arg- and the infectivity was lower, compared to WT. We showed that blocking the binding of these miRNAs to *Nos*2 mRNA, the NOS2 expression and NO production is increased in macrophages during *L. (L.) amazonensis* infection. We concluded that *L. (L.) amazonensis* infection alters the miRNA profile of macrophages to subvert the host immune responses. **Supported by**:FAPESP and CNPq **Keywords:**Microrna; nos2; nos2, no

HP040 - THE VIRULENCE OF LEISHMANIA INFANTUM CHAGASI IS MODULATED BY INTRINSIC LYSOPHOSPHATYDILCHOLINE

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In the New World, the visceral form of leishmaniasis is caused by Leishmania infantum chagasi. Platelet activating factor (PAF, 1-O-alkyl-2-acetyl-sn-glycero-3-phosphocholine) exhibits potent biological activity and is synthesized by a wide variety of cells, including neutrophils, platelets, macrophages and lymphocytes. Previous data from our group showed some physiological roles related to cell differentiation induced by PAF in trypanosomatids. LPC is the major component of oxidized low-density lipoproteins (LDL), being a potent atherogenic initiator and an important factor in inflammatory processes. LPC is involved in Trypanosoma cruzi infectivity towards macrophages and mice. PAF is structurally very similar to LPC. Recently, we showed that T. cruzi synthesizes a bioactive PAF-like lysophosphatydilcholine (C18:1-LPC) that aggregates platelets most likely through binding and activation of PAF receptor (PAFR). Here, we demonstrate that Leishmania i. chagasi synthetizes several LPC species, including C18:1-LPC. We have identified these LPC species by electrospray ionization-tandem mass spectrometry (ESI-MS/MS). We also describe that the crude lipid extract isolated from L. i. chagasi, PAF and C18:1-LPC trigger platelet aggregation. Similarly to PAF, the platelet-aggregating activity of C18:1-LPC was abrogated by the PAFR antagonist WEB 2086. Besides that, we show that when L. i. chagasi promastigotes were kept in the presence of the crude lipid extract isolated from L. i. chagasi, PAF or C18:1-LPC, there was an enhancement of mouse macrophage infection by these parasites, with involvement of the protein kinases PKC and CK2. All these effects were abrogated by WEB 2086. These data suggest that L. i. chagasi contains the components of an autocrine PAF and LPC ligand-receptor system that modulates L. i. chagasi infectivity towards mouse macrophages. **Supported by**:CNPq, FAPERJ, CAPES e INCT-EM.
Keywords: Leishmania infantum chagasi; lysophosphatydilcholine; infectivity

HP041 - ACTIN DYNAMICS REGULATION BY HOST GTPASES DURING CELL INVASION BY EXTRACELLULAR AMASTIGOTES OF *TRYPANOSOMA CRUZI*

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Extracellular amastigotes of *T. cruzi* subvert an actin-dependent phagocytosis like process during invasion of non-phagocytic cells. Participation of actin-related proteins such as gelsolin, integrins, cortactin, protein kinase D1 and others have been previously described. However, detailed mechanisms leading to actin polymerization and EA uptake are still poorly characterized. The aim of this work was to evaluate the role of major host GTPases (Cdc42, Rac1 and RhoA) and their effector proteins (N-WASP and WAVE2) in actin dynamics during EA internalization by HeLa cells. Invasion by AEs was inhibited in HeLa cells depleted for Cdc42, Rac1, N-WASP and WAVE2 but not RhoA. Using GFP-tagged wild type and mutant constructs, we observed recruitment and colocalization of these host proteins with actin at the EA invasion sites, in fixed and/or live cells by confocal microscopy. Similar EA driven actin recruitments were observed for all HeLa depleted groups when evaluating interaction in fixed cell specimens. On the other hand, interactions evaluated by time lapse confocal microscopy revealed a delayed and/or less efficient EA driven actin recruitment by these HeLa lineages. Scanning electron microscopy showed no apparent differences in surface microvilli beneath interacting EAs. In summary, our work demonstrate the regulation of Cd42/N-WASP and Rac1/WAVE2 host signaling pathways in actin dynamics during EA internalization by HeLa cells. **Supported by**:FAPESP, CAPES and CNPq
Keywords: Trypanosoma cruzi; extracellular amastigotes; host cell gtpases

HP042 - *LEISHMANIA* MUTANTS OVEREXPRESSING A PUTATIVE PHOSPHOLIPID TRANSLOCATOR DISPLAY INCREASED PHOSPHATIDYLCHOLINE UPTAKE AND ANNEXIN V BINDING

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The parasites of the genus *Leishmania* present successful strategies to establish infection in the vertebrate host. Among these strategies, some studies point to the exposition of phosphatidylserine (PS) in the outer face of its plasma membrane as a signal to be phagocyted by host macrophage. There is a controversy if this PS exposition is related to apoptosis or apoptotic mimicry but parasites that bind to annexin V are more infective. As there is a consensus that in apoptotic cells annexin V preferably binds to the PS, it was assumed that PS was the signaling molecule in *Leishmania*. However, previous studies of our group were unable to identify the presence of PS in *Leishmania*. Here we aim to identify the phospholipid involved in parasite recognition by the macrophage as well as the translocator responsible for the exposition. Screening of a *Leishmania (L.) amazonensis* genomic library, constructed in vector cLHYG, allowed for the purification of a clone, named 25-36 that was maintained under hygromycin pressure. This clone showed a premature annexin V binding in the mid-log promastigote phase compared to wild type (WT) parasites. FACS based fluorescent lipid uptake assays showed an increased internalization rate of PC in the mutant as compared to WT while the uptake of other lipids was not affected. Partial sequencing of the cosmid DNA indicated the presence of candidate PC translocators. Lipid efflux and toxicity assays will be performed to further characterize the lipid dynamics in the plasma membrane of the mutant. Taken together, our data point to the identification of a lipid translocator or regulator coded in the cosmid DNA that might be used to control the infection. **Supported by**:CAPES, CNPq, FAPESP and Danish National Research Foundation (DNRF85).
Keywords: Leishmania; phospholipids; lipid uptake

HP043 - IN SEARCH OF A RECEPTOR FOR TRYPANOSOMA CRUZI SURFACE MOLECULE GP82 THAT MEDIATES HOST CELL INVASION

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One of the fundamental questions about host cell invasion by the insect stage metacyclic trypomastigotes (MT) of Trypanosoma cruzi refers to the identity of target cell receptors implicated in the process. Previous studies using human epithelial HeLa cells have indicated that MT internalization is mediated by gp82, a MT-specific surface molecule, which is highly conserved among divergent T. cruzi genetic groups and is implicated in the establishment of infection by the oral route, a mode of transmission responsible for frequent outbreaks of acute Chagas disease in recent years. Gp82 binds to target cells in a receptor-dependent manner, triggering calcium signalling and lysosome exocytosis, events that are required for parasite internalization. We aimed to identify the host cell receptor for gp82. The possibility that the receptor for gp82 may mimic a sequence of MT surface molecule gp90, which acts as a negative modulator of cell invasion, emerged fortuitously. To further investigate that question, we performed a series of experiments using MT of CL strain and human epithelial HeLa cells as target cells. Monoclonal antibody (mAb) 5E7 directed to a gp90 epitope, cryptic in live parasites, inhibited MT invasion. Two out of 10 synthetic peptides based on the domain containing the epitope for mAb 5E7, namely peptides p5 and p6, significantly inhibited MT internalization. Binding assay, using the recombinant protein containing the full-length gp82 sequence and the recombinant protein based on gp90 C-terminal domain containing the epitope for mAb 5E7, revealed that the two proteins interact. In western blots of immunoprecipitates of HeLa cell extract with mAb 5E7, a band of ~160 kDa was specifically detected by anti-HeLa cell antibodies but not by antibodies to K562 cells that are resistant to MT invasion. Attempts to identify potential interacting host cell-parasite partners using mAb5E7 affinity column are currently underway. **Supported by**:UNIFESP
Keywords: Trypanosoma; invasion; gp82

HP044 - MONITORING THE NATURAL AND ARTIFICIAL INFECTION OF TRIATOMINES BY QUANTITATIVE REAL TIME PCR

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Chagas disease constitutes the fourth most important tropical disease, supplanted only by malaria, tuberculosis, and schistosomiasis. Microscopical examination is a classic method to describe the natural infection of triatomines by *Trypanosoma cruzi*, but it presents some limitations. In this context, the application of a quantitative Real Time PCR (qPCR) assay to triatomine samples can improve sensitivity, specificity and reproducibility. In this work, we developed a methodology based on qPCR, which is capable to detect and quantify absolute levels of *T. cruzi* in triatomine samples, differentiating total parasites from viable parasites by DNA and mRNA detection. In addition, in order to evaluate *T. cruzi* metacyclogenesis and vectorial competence, we are working on a protocol to distinguish between trypomastigote and epismastigote forms, by using specific mRNA markers, detectable only in trypomastigote forms. Our multiplex qPCR reaction presented a linearity ranging from105 to 0.5 parasite equivalents, for the *T. cruzi* target, and from 3 to 0.00015 triatomine equivalents, for the vector insect target. It was also possible to determine that qPCR Limit of Detection (LOD) was 0,41 parasite equivalents. To validate the assay, 13 samples from field triatomines, positive for *T. cruzi* infection, were evaluated by qPCR, with parasite loads from 10^2 to 10^{11} par. Eq./Insect Eq. Furthermore, to follow *T. cruzi* (Dm28c) development in *Rhodnius prolixus* digestive tract, total DNA and RNA were extracted from insect samples under increasing periods after feeding with blood containing *T. cruzi*. It was possible to observe a time-dependent decreasing on the viable parasite quantity during the time course of the experiment. The qPCR and RT-qPCR assays developed in this study are suitable to determine the infection rate of sylvatic triatomines and they can contribute to understand the vectorial competence of triatomines and the incidence of Chagas disease. **Supported by**:FIOCRUZ - IOC; CNPQ
Keywords: Triatomine; chagas disease; qpcr

HP045 - EZRIN, RADIXIN AND MOESIN (ERM PROTEINS) PLAY DISTINCT ROLES IN HOST CELL INVASION BY *TRYPANOSOMA CRUZI* EXTRACELLULAR AMASTIGOTES

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Cell invasion by *T. cruzi* extracellular amastigotes (EAs) is mediated by complex cellular signaling events regulating actin filaments, the main component in EA uptake. ERM proteins (Ezrin, Radixin and Moesin) are key elements linking actin filaments and plasma membrane, important for maintenance of cell morphology, cell migration, tissue structure and invasion of intracellular pathogens. Therefore, the aim of this study was to evaluate the role of ERM proteins in actin cytoskeleton-plasma membrane interplay during host cell invasion by EAs. Depletion of host Ezrin, Radixin but not Moesin inhibited EAs invasion in HeLa cells. Western blotting assays showed host Ezrin phosphorylation/activation during EA incubation. Using confocal microscopy we observed recruitment and colocalization of GFP-tagged wild type ERMs with actin at EAs invasion sites in live and fixed cells. Additionally, Ezrin opened conformation mutant (active) is also recruited and colocalizes to actin in EA invasion sites but closed conformation mutant does not. Finally, time-lapse confocal microscopy has shown reduced and delayed actin dynamics in Ezrin and Radixin depleted HeLa cells when compared to control and Moesin groups. Altogether, these findings show distinct roles of ERM proteins in actin filaments and plasma membrane interplay during EAs host cell invasion. **Supported by**:FAPESP
Keywords: Trypansoma cruzi; extracellular amastigotes; erm proteins

HP046 - SOCS2 IN THE CENTRAL NERVOUS SYSTEM: MODULATION OF NEUROTROPHIC FACTORS PRODUCTION AND IMMUNE SURVEILLANCE DURING PLASMODIUM BERGHEI ANKA INFECTION

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Introduction: The suppressor of cytokine signaling (SOCS) 2 is a critical protein for regulation of the several intracellular pathways acting in: immune regulation; modulation of neural development and in the nerve growth factor (NGF) response. Neurotrophic factors such as glial cell line-derived neurotrophic factor (GDNF), brain-derived neurotrophic factor (BDNF) and NGF are necessary for the survival and regeneration of the central nervous system and their reduction was associated with cerebral malaria (CM) severity. Rodents infected with P. berghei ANKA (PbA) closely resemble many characteristics of human CM. However, SOCS2 involvement in CM is not known. Method: C57Bl/6 (WT) and SOCS2 deficient (-/-) mice were infected with PbA and parasitemia, clinical signs and survival were monitored daily. The SOCS2 expression was assessed by qPCR. Cytokines production in the brain and spleen was assessed by ELISA and flow cytometry. In the brain, neurotrophic factors was evaluated by ELISA, leukocyte recruitment was evaluated by intravital microscopy; nitric oxide (NO) was assessed by the Griess method and performed histophatological analysis. Results: PbA-infected SOCS2-/- mice showed significantly lower parasitemia and less severity of symptoms associated with CM when compared with WT mice. In the brain of infected SOCS2-/- mice there was an increased production of IL6, IL17, TGFβ and NO. Additionally, there was a decreased level in the brain of PbA-SOCS2-/- mice, but not in spleen, of IL1β, TNFα and IL12 when compared with WT. Interestingly, in the brain of PbA-SOCS2-/- mice, we found a decreased level GDNF and BDNF besides increased microvascular obstruction and decreased leukocyte rolling when compared with WT. Conclusions: These findings demonstrated, for the first time, a role for SOCS2 in modulates neurotrophic factors, beside immune response, which contribute, at least, for the CM immunopathogenesis. **Supported by**:CAPES, CNPq, FAPEMIG
Keywords: Socs2; cerebral malaria; neurotrophin

HP047 - ROLE OF RESOLVIN D1 IN THE DIFFUSE CUTANEOUS LEISHMANIASIS

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Diffuse Cutaneous Leishmaniasis (DCL) is a rare clinical manifestation of tegumentary leishmaniasis caused by *Leishmania amazonensis* wich is characterized by inefficient parasite-specific cellular responses and heavily parasitized macrophages in skin lesions. Previous investigations from our group and others have shown that eicosanoids and their precursors play a crucial role during Leishmania infection. The recently identified lipid mediators named, resolvins have been described to exhibit potent anti-inflammatory and immune-regulatory activities and are critically involved in restoration of tissue homeostasis during the resolution phase of acute inflammation. However, the role of resolvins in leishmaniasis remains unknown. Here, we tested if resolvin D1 (RvD1) exhibit a direct effect in axenic *L. amazonensis* cultures. Moreover, we evaluated the role of RvD1 in *L. amazonensis* infection of human monocyte-derived macrophages in vitro and assessed the circulating levels of this mediator in DCL patients (n=12), individuals with Localized Cutaneous Leishmaniasis (LCL; n=29) and healthy endemic controls (HC; n= 43). We observed that increased concentrations of exogenous RvD1 did not affect *L. amazonensis* growth in vitro. RvD1 supplementation in cultures of *L. amazonensis*- infected human macrophages from healthy blood donors resulted in a dramatic dose dependent increase in intracellular parasite loads. Exploratory assessment of RvD1 plasma concentrations revealed that DCL patients exhibit-significantly higher levels of RvD1 compared to individuals with LCL or HC. These findings demonstrate that RvD1 favors parasite survival in infected human cells and argue that this lipid mediator may influence the anti-inflammatory immune profile observed in the pathogenesis of DCL. **Supported by**: CNPq, FAPESB, FIOCRUZ-BA
Keywords: Diffuse cutaneous leishmaniasis; resolvin d1 (rvd1); leishmania amazonensis

HP048 - INVOLVEMENT OF CASPASE-11 IN THE RESTRICTION OF LEISHMANIA AMAZONENSIS INFECTION.

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Introduction: Leishmaniasis is a disease that affects millions of people worldwide. Although several studies have been performed in order to better understand its pathogenesis, more effective treatments and vaccines haven't been succesfully developed so far. The innate immune response against *Leishmania spp.* has been shown to be coordinated by pattern recognition receptors (*PRRs*), such as the *Toll-like* receptors, C-type lectin receptors and, more recently, NLRP3, a Nod-like receptor that leads to inflammasome activation,was demonstrated to be important in the control of Leishmaniasis. NLRP3 interact with an inflamatory caspase called *caspase-1*, although the functions of another inflammatory caspase, *caspase-11*, is yet obscure. Thus, the aim of this work is to evaluate the contribtuion of *caspase-11* in the recognition and restriction of *L. amazonensis* infection.
Methods and results: Bone marrow-derived monocytes from C57BL/6 (WT) and *caspase-11*$^{-/-}$ mice were used to perform in vitro studies with *Leishmania amazonensis*. After 24h of infection, supernatant was collected and used for ELISA. BMDMs from *caspase-11*$^{-/-}$ mice showed decreased secretion of IL-1β compared to WT mice, and were less capable of killing *L. amazonensis* parasites, as shown by FACS. C57BL/6 and *caspase-11*$^{-/-}$ mice were infected in vivo in the ear with 10^6 total parasites and the lesion was followed by eight weeks, then mice were sacrificed and had their lymph node and ear processed for parasite titer. *Caspase-11*$^{-/-}$ mice had larger lesion and parasite titer in the ears compared to WT mice, although the difference in the latter was not observed in the draining lymph node. Conclusion: Together, our results suggest that, by still unknown mechanisms, *Leishmania amazonensis* triggers *Caspase-11* activation, which could possibly be involved in induction of the non-canonical activation of NLRP3 inflammasome, a feature that we have recently shown to be important in the restriction and control of Leishmaniasis. **Supported by**:FAPESP, CNPq and CAPES
Keywords: Leishmania spp.; caspase-11; inflammasome

HP049 - DEPLETION OF AN EIF2α KINASE OF *TRYPANOSOMA CRUZI* IMPAIRS INTRACELLULAR GROWTH AND INDUCES PARASITE PROTECTION IN MICE

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Regulation of translation initiation through the phosphorylation of the alpha subunit of translation initiation factor 2 (eIF2α) has been shown to play a key role in differentiation (Tonelli et al. 2011, PlosOne 6:e27904). This phosphorylation is partially regulated by the activation of a membrane bound eIF2α kinase (TcK2) when heme levels decrease in the medium (Augusto et al. 2015, PlosPathog 11:e1004618). Heme binds specifically to the catalytic domain of the kinase, inhibiting its activity. Parasites knocked out for the TcK2 lose this differentiation capacity and heme is not stored in endosome, remaining in the cytosol. TcK2 null cells display growth deficiencies, accumulating hydrog en peroxide that drives the generation of reactive oxygen species. Here, we show that the absence of TcK2 impaired intracellular growth of amastigotes in cultured mammalian cells. Metacyclic-trypomastigotes without TcK2 infected normally epithelial, muscle and macrophage cell lines and differentiated into amastigotes that exit much slowly from the phagolysosomes. The produced amastigotes remained viable undergoing protein synthesis for long periods without proliferating in the mammalian cell cytosol. When injected into mice, these metacyclic-trypomastigotes did not produce blood parasitemia but induced full protection against a highly virulent challenge. These results highlight a potential role of TcK2 for vaccination and as a drug target to control Chagas' disease. **Supported by**:FAPESP
Keywords: Kinase; drug target; translation

HP050 - EVALUATION OF XID MICE INFECTION BY *LEISHMANIA AMAZONENSIS*

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Leishmaniasis is one of many neglected diseases in whole world, per year there is around 1.3 million of new cases. This work aims to study the infection of XID mice by *Leishmania amazonensis*. XID mice are deficient on B1 lymphocyte that develops an entirely different role of B2 lymphocyte, being related with innate immune pathway known as innate-like B cells (ILBs). B1 cells produce IL-10 and express high levels of natural and polyreactive antibodies against infections stimulated by pathogen-associated molecular pattern (PAMPs) and its role in unknown in infections by *L. amazonensi*s. We accomplished the experiments using XID mice and wild mice (BALB/c) as control group. The infection was performed inoculating stationary promastigotes of *L. amazonensis* (Josefa) in the footpad of the left paw of mice. The lesion was weekly checked using caliper rule for register the increased thickness of the infected foot. After this period, the animals were euthanized and the infected footpads and spleens were macerated. Using the limiting dilution analysis (LDA), we determined the parasite load. The lymph nodes were macerated and processed for analysis by flow cytometry. Our results shown that lesions from BALB/c were bigger than XID's. However, the parasite load determined on both infected footpad spleens are similar. Besides, we observed an increase on regulatory T cells in XID's lymph nodes, this could explain the smaller lesion found in this group when compared to the control group. We didn't observe differences in effector cells producing IFN-gamma on the lymph nodes, it could explain why we didn't found differences on the parasite load. Studies assessing cytokine production by ELISA of supernantants are in progress. Understanding what mechanism is involved in the increase of regulatory T cells in XIDs will be our next challenge. Finally, our results demonstrated that B1 lymphocytes have a role in the development of the pathogenic process of *L. amazonensis* infection.
Keywords: Leishmaniasis; b1 lymphocyte; leishmania

HP051 - IN VITRO EVALUATION OF 1-ARYL-4-IMIDAZOLYLPYRAZOLE DERIVATIVES AGAINST *TRYPANOSOMA CRUZI*

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Chagas disease, a tropical neglected disease caused by the flagellated protozoan *Trypanosoma cruzi*, is a global public health problem. Despite being endemic in Latin America the current scenario shows the spreading of this disease to non-endemic countries due to blood transfusion and organ transplantation of infected immigrants. The nitroaromatic drugs used for treatment have many side effects and low efficacy in chronic disease. Therefore, the search for new targets and rational drug development are strategies for identifying a new drug. Pyrazole derivatives have been proposed as potential lead compounds for Chagas disease and Leishmaniasis chemotherapy. In this study, we evaluated the effect of a series of synthetic 1-aryl-4-imidazolylpyrazole derivatives against *T. cruzi in vitro*. All substances had low cytotoxicity(CC50>200µM), except compound MSJ05, which had a CC50 of 52.2µM in Vero cells. Pyrazole derivatives showed activity against epimastigotes with IC50 ranging from 9.8 to 190.2µM. Among the nine substances tested, the most potent were selected for screening against intracellular amastigotes, the relevant clinical forms of the parasite. Interestingly, a high activity was observed against amastigotes with IC50 ranging from 3.5 to 23.9µM, resulting in a selectivity index(SI) >10 except for 5-amino-1-(2-bromophenyl)-4-imidazolylpyrazole (MSJ12; SI>8.37). Among the substances tested, 1-(3-chlorophenyl)-4-imidazolylpyrazole (MSJ02) and 1-(3-bromophenyl)-4-imidazolylpyrazole (MSJ14) showed highest SI with values of 17.53 and 22.09, respectively. It is important to highlight that modifications in the aromatic ring differently affects parasite viability and, therefore, can guide rational drug optimization. Additionally, in silico prediction of physicochemical properties demonstrated a good oral bioavailability and druglikeness. Further in vivo studies will be carried out to evaluate the safety and efficacy of these compounds and their potential as drug candidates. **Supported by**:CAPES
Keywords: Chagas disease; 1-aryl-4-imidazolylpyrazole derivatives; chemotherapy

HP052 - PRENATAL AND NEONATAL SEROLOGICAL SCREENINGS TO TOXOPLASMOSIS: RELEVANCE FOR THE DIAGNOSIS OF PRIMARY INFECTION IN PREGNANT WOMEN AND CONGENITAL TOXOPLASMOSIS IN NEWBORNS, AND FOR INDICATION OF SPECIFIC TREATMENT.

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Congenital toxoplasmosis is one of the most serious manifestations of Toxoplasma gondii infection in humans, and may cause abortions or fetal losses, as well as severe ocular and/or cerebral sequelae in newborns. The serological screening of pregnant women and newborns is mainly based on the detection of specific IgG and IgM antibodies and constitutes an important measure to control congenital toxoplasmosis. This study aimed to evaluate the levels of IgG, IgM and IgA antibodies to T. gondii in 173 paired sera from mothers with suspected primary toxoplasmosis in pregnancy and their respective newborns from Clinical Hospital of Federal University of Uberlândia by ELISA, while clinical data were obtained from research in the clinical records of each patient. All samples from mothers and newborns (100%) were positive for IgG, while 83.8% of mothers and 17.1% of newborns were positive for IgM, and 24.3% of mothers and 13.7% of newborns were positive for IgA. From 173 maternal sera tested, 146 (84.4%) were IgM+ and/or IgA+, being 108 (74.0%) from mothers treated with spiramycin (T) and 38 (26.0%) from untreated mothers (NT), from which were born 32 newborns with serology suggestive of congenital toxoplasmosis - IgM+ and/or IgA+ (13 from T and 19 from NT). The analysis of clinical data from 1-year follow-up of these 32 newborns confirmed congenital toxoplasmosis in 22 of them (5 from T and 17 from NT), of which 18 had ocular abnormalities (4 from T and 14 from NT), 13 showed brain damages (4 from T and 9 from NT), 8 presented with hepatosplenomegaly (1 from T and 8 from NT), 2 had hearing loss (both from NT), two came to death (1 from T and 1 from NT) and 3 were asymptomatic (both from NT). Therefore, prenatal and neonatal serological screenings allow the identification of vertically infected newborns, mainly through simultaneous detection of IgM and IgA antibodies; and maternal treatment with spiramycin reduces, but does not avoid vertical transmission of T. gondii. **Supported by**:CAPES, CNPQ, FAPEMIG
Keywords: Congenital toxoplasmosis; serological screening; maternal treatment

HP053 - HYPERVALENT ORGANOTELLURIUM COMPOUNDS AS INHIBITORS OF P. FALCIPARUM CALCIUM-DEPENDENT CYSTEINE PROTEASES

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Hypervalent organotellurium compounds (organotelluranes) have shown several promising applications, including use as potent and selective cysteine protease inhibitors and antiprotozoal agents. Here, we report the antimalarial activities of three organotellurane derivatives (RF05, RF07 and RF19) in two P. falciparum strains (CQS 3D7 and CQR W2), which demonstrated significant decreases in parasitemia in vitro. The inhibition of intracellular P. falciparum catalytic activity by RF05, RF07 and RF19 was determined and the IC50 values were 3.7 ± 1.0 µM, 1.1 ± 0.2 µM and 0.2 ± 0.01 µM, respectively. Using a proteolysis assay performed in the presence of the ER Ca2+-ATPase inhibitor we showed that the main enzymatic targets were cysteine proteases stimulated by calcium (calpains). None of the compounds tested caused haemolysis or a significant decrease in endothelial cell viability in the concentration range used for the inhibition of intracellular proteolysis. Taken together, the results presented suggest promising compounds for the development of antimalarial drugs.
Supported by:Capes
Keywords: Calpain; tellurium compounds; malaria

HP054 - AN IMMUNODOMINANT DOMAIN FROM CCP5A, A LCCL-RELATED PROTEIN FROM *TOXOPLASMA GONDII*, PLAYING A ROLE AS MOLECULAR MARKER FOR OOCYST/SPOROZOITE INFECTION IN HUMANS AND DOMESTIC ANIMALS.

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The current immunoassays are not able to distinguish the infective forms responsible for *Toxoplasma gondii* infection. In order to serologically differentiate between oocyst- and tissue cyst-driven infections, we tested two recombinant proteins (CCp5A and OWP1) from the oocyst/sporozoite of T. gondii against a panel of serum samples from animals (chickens, pigs and mouse) that were either naturally or experimentally infected with different parasite stages. Moreover, we tested sera from humans who have been infected by oocysts during a well-characterized toxoplasmosis outbreak or IgM+/IgG+ pregnant women with an unknown source of toxoplasma infection. Only the sporozoite-specific CCp5A protein was able to differentiate the parasite stage that infected chickens, swine and mice, with exclusive detection in oocyst-infected animals. Furthermore, the CCp5A showed as a potential marker of recent infection by oocyst/sporozoite in pigs and mice. In humans, CCp5A showed higher reactivity with serum samples from an outbreak in comparison with serum from pregnant women. These findings suggest that CCp5A could be a new tool for diagnosis and epidemiological surveys to identify the source of infection in animals and humans. The identification of parasite infective stage can help to design effective strategies to minimize severe complications in immunocompromised people and, particularly, in pregnant women to prevent congenital infection.**Supported by**:CNPq, FAPEMIG, CAPES
Keywords: Toxoplasmosis; oocyst/sporozoite; diagnosis

HP055 - CHARACTERIZATION OF IN VIVO PLASMODIUM FALCIPARUM PROTEOLYSIS IN DIFFERENT LIFE CYCLE STAGES USING Z-F-R-MCA AS SUBSTRATE

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Malaria is still the most serious parasitic disease affecting humankind, causing about one million deaths annually. Parasites from genus *Plasmodium* are the causative agents of malaria, where *P. falciparum* is one of the most important. It has been demonstrated that proteases play crucial roles in *P. falciparum* infection and they have been considered as targets for the development of new therapeutic agents. The aim of this study was to characterize the in vivo proteolysis at different stages of *Plasmodiun falciparum* life cycle using the fluorogenic substrate Z-F-R-AMC. In all stages the substrate was degraded, with higher proteolysis in the trophozoite and schizont stages, as these are the most metabolically active stages during the parasite´s life cycle. The inhibition profile obtained using classic inhibitors such as PMSF, o-Phenantroline, pepstatin A and E64 suggests that the major enzyme activities are that of cysteine proteases, which is in agreement with the in vitro results reported by other authors, who have performed studies of proteolysis with lysates of the parasite and characterized some of the proteases involved in hemoglobin degradation. This is the pioneer in vivo proteolysis study encompassing the whole erytrhocytic cycle of *Plasmodium falciparum*. Studying *in vivo* proteolysis using different substrates sheds light on the main proteolytic specific activity at each parasite stage, which is of most importance in the design of stage-specific drugs targeting proteases. **Supported by**:Capes
Keywords: Proteolysis; malaria; p.falciparum

HP056 - THE ROLE OF EPSTEIN-BARR VIRUS INDUCED 3 (EBI3) IN *NEOSPORA CANINUM* INFECTION

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Neospora caninum is an intracellular obligate parasite that has the dog as definitive host and can cause abortion in infected cattle, this may lead to great economic losses. Epstein-Barr virus induced 3 (EBI3) is a IL-12 family protein that combined with other family members, p28 and p35, results in production of IL-27 and IL-35, respectively.In *Listeria monocytogenes* infection, EBI3 is described as a mediator of Th17 responses. The aim of this work was to characterize the role of EBI3 in mice infected by *N. caninum.* First, we analyzed the survival of two different groups of infected mice, wild type (WT) and deficient in EBI3 (EBI3$^{-/-}$), infected with 1x10^{7}

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days) samples, quantification of nitric oxide (NO), Th1 cytokines (IL-12 and IFN-γ) and tissue parasitism (lungs/7 days and brain/30 days). However, the concentration of typical Th17 cytokines (IL-17A, IL-17F, IL-21 and IL-23) were significantly increased in EBI3$^{-/-}$ mice when compared to WT litter mates. EBI3 also downregulated the production of specific IgG antibodies and its subsets. In conclusion with the data obtained, like in *L. monocytogenes* infection, EBI3 is proving to mediate Th17 response in *N. caninum* infection as well and has no influence on Th1 response. **Supported by**:CNPq, CAPES, FAPEMIG
Keywords: Neospora caninum; ebi3; imune response

HP057 - TEMPERATURE STRESS INDUCES TRYPANOSOMA CRUZI AUTOPHAGY, MITOCHONDRIAL SWELLING AND DESPOLARIZATION

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During its life cycle, T. cruzi undergoes physical and chemical changes, including thermal shifting, one of greatest barriers to the infection. In the natural transition from the invertebrate to the vertebrate host (and vice versa), or even in artificial conditions such as in blood bags and contaminated food, the parasite is exposed to temperature shock. In other models, the exposure to stress led to the mitochondrial remodelling in a process regulated by autophagy. Despite the critical role in T. cruzi infection, the role of autophagy in recycling and reorganization of mitochondria in the parasite is still unclear. In the present work, we evaluated the effects of the temperature stress in the T. cruzi mitochondrion and autophagy. Trypomastigotes (Y strain) were incubated in RPMI up to 144h in a range of temperatures from -20°C to 40°C. Our results show that the temperature variation induced parasite lysis. Extreme temperatures (-20°C and 40°C) showed high trypanocidal potential, leading already after 2h of incubation to a decrease in mitochondrial membrane potential (about 40%), and a slight increase in autophagy, both phenotypes were confirmed by electron microscopy. The temperatures of 4, 28 and 37°C induced time-dependent changes in the mitochondrial potential and in the activation of autophagy. After 2h at 4 and 28°C, autophagosomes formation and Golgi cisternae disruption were observed while at 37°C no alteration was observed. After 24h at 4 and 28°C, autophagosomes were detected, as well as Golgi cisternae disruption, dilation of endoplasmic reticulum and mitochondrial swelling. our data suggest that the thermal shifting promotes the modulation of autophagic and mithocondrial activity, however the cellular and molecular mechanisms involved must be further investigated. **Supported by**:FAPERJ, CNPq and FIOCRUZ. **Keywords:**Trypanosoma cruzi; temperature stress; mitochondria

HP058 - SERODIAGNOSIS OF ACUTE AND CHRONIC TOXOPLASMOSIS IN PREGNANT WOMEN BY ENZYME-LINKED IMMUNOSORBENT ASSAY USING SYNTHETIC PEPTIDES DERIVED FROM TOXOPLASMA GONDII IMMUNODOMINANT ANTIGENS

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The current immunoassays used for diagnosis of human toxoplasmosis usually employ total parasite antigens, that can provide false-positive and false-negative results and a weak differentiation between acute and chronic infection, making the diagnosis of primary and congenital infections a challenging situation. This study aimed to identify B cell epitopes within immunodominant antigens from Toxoplasma gondii using software-based prediction and evaluate the diagnostic performance of synthetic peptides representative from these epitopes in the serodiagnosis of toxoplasmosis in pregnant women. A panel of 22 linear B cell epitopes of antigens from surface (SRS; n=4), rhoptries (ROP; n=2), micronemes (MIC; n=8) and dense granules (GRA; n=8) were identified, and 15 residues from their amino acid sequences were used to synthesize peptides chemically linked to bovine serum albumin backbone. The diagnostic performance of these peptides was evaluated in immunoassays (ELISA) to detect specific IgG antibodies in sera from two groups of pregnant women: G1 (n=42) with suspected acute toxoplasmosis, and G2 (n=42) with chronic phase of infection. All peptides were recognized by IgG antibodies, showing mean absorbances higher than cut off values, high percentages of positivity, and good differentiation from seronegative samples. The peptides derived from SRS (Pep1-Pep4) and ROP (Pep5 and Pep7) antigens showed high mean reactivity and positivity rates. Pep13 (GRA4) and Pep21 (M2AP) were significantly more recognized by sera from G1, while Pep12 (MIC14) was significantly more recognized by sera from G2, characterizing them as potential markers for acute and chronic phases of infection, respectively. Thus, synthetic peptides designed from B cell linear epitope prediction constitute promising antigens in serological assays to diagnose toxoplasmosis and differentiate acute from chronic phases of infection, representing an alternative to the use of total or recombinant antigens. **Supported by**:CNPq, FAPEMIG, CAPES **Keywords:**Toxoplasmosis; synthetic peptides; b cell epitopes

HP059 - LACTOCOCCUS LACTIS HSP65 PRODUCER AS AN ALTERNATIVE THERAPY FOR CUTANEOUS LEISHMANIASIS CAUSED BY LEISHMANIA BRAZILIENSIS

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In cutaneous and mucosal leishmaniasis caused by Leishmania braziliensis, there is induction of a Th1 response that results in a strong inflammatory reaction. The exacerbated inflammation observed in CL lesions might be controlled by anti-inflammatory responses. Lactococcus lactis, are nonpathogenic gram positive bacteria and Lactococcus lactis HSP65 are genetically modified bacteria that produces a heat shock protein (HSP65), which has immunomodulatory activity by inhibiting TNF-α and IFN-γ production and increase IL-10 secretion by T cells. HSP65 also has a strong effect on induction of CD4+ CD25+LAP+ regulatory T cells. In this study, our main objective is to test oral administration of Lactococcus lactis producing HSP65, as potential immunomodulatory treatment in experimental model of cutaneous leishmaniasis. BALB/c mice received by oral route recombinant Lactococcus lactis strains, which produces or not HSP65 for 4 consecutive days. Twelve days after administration, animals were infected with Leishmania braziliensis. Two days before infection, HSP65 group received PAM3CSK4 (TLR2 agonist) intraperitoneally. Evaluation of ear thickness showed that at the peak of inflammation, mice treated with L. lactis HSP65 showed larger lesions compared to control group, not being associated with parasite load, which was higher in control group. On the other hand, we observed a greater amount of parasites in draining lymph nodes of treated animals, as well as higher IL-10 production by this group. Our data suggest that pre-treatment with L. lactis HSP65 may lead to a modulation of inflammatory response, characterized by IL-10 production. It is necessary a more detailed analysis of producing cytokines lymphocytes population (IFN-γ, IL-10) as well as CD4+LAP+ T cells presence. Histopathological evaluation of lesions and measurement of proinflammatory cytokines such as IFN-γ, will be important to clarify modulation mechanism of resulting pre-treatment with L. lactis HSP65. **Supported by**:CAPES
Keywords: Leishmania braziliensis; heat shock protein 65; oral tolerance

HP060 - DIAGNOSIS OF CONGENITAL TOXOPLASMOSIS BY IMMUNOBLOTTING TO DETECT IGG SUBCLASSES SPECIFIC TO *TOXOPLASMA GONDII* ANTIGENS IN PAIRED SERA FROM MOTHERS AND NEWBORNS

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The diagnosis of human infection with Toxoplasma gondii is a critical and challenging task, especially in pregnant women with primary infection and fetuses and newborns with congenital infection, and it is mainly based on the detection of IgG, IgM and IgA antibodies by serological tests. This study aimed to detect total IgG and its subclasses in paired sera from mothers and newborns with serology of recent toxoplasmosis. A total of 28 paired sera was divided into two groups, according to the results obtained by ELISA: GI - mothers with recent infection (IgG+/IgM+) and their newborns with congenital infection (IgG+/IgM+) (n=12 pairs), and GII - mothers with recent infection (IgG+/IgM+) and newborns without congenital infection (IgG+/IgM-) (n=16 pairs). The IgG subclass detection (IgG1, IgG2, IgG3 and IgG4) was performed by immunoblotting using total soluble antigen of T. gondii tachyzoites (STAg) and a fraction of this antigen obtained by precipitation with a solution of ammonium sulfate 40% (STAg 30-40%). When using STAg, IgG1 from GI and GII detected a higher variety of antigenic bands, with similar results in both groups and very similar to the results observed with total IgG; while IgG3 from GI detected a higher variety of bands, with higher frequency than GII. IgG2 and IgG4 recognized a lower variety of bands than IgG1 and IgG3, with sera from GI recognizing a higher frequency of bands than GII. When using STAg 30-40% fraction, all IgG subclasses from GI and GII recognized lower variety and frequency of antigenic bands than STAg, without recognition of differential bands among GI and GII and among mothers and newborns from GI. Overall, the detection of IgG subclasses in paired sera of mothers with suspected primary toxoplasmosis during pregnancy and their newborns, especially IgG3, using STAg and its antigenic fraction STAg 30-40% as antigens, by the immunoblotting technique, constitutes an important tool to diagnose congenital toxoplasmosis. **Supported by**:CAPES, CNPQ, FAPEMIG
Keywords: Congenital toxoplasmosis; igg subclasses; immunoblotting

HP061 - INCREASED OF THE PD-L1 EXPRESSION IN CANINE MACROPHAGES INFECTED WITH *LEISHMANIA* SPP. *IN VITRO*

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Introduction: The leishmaniasis occur in 88 countries and 65 exhibit the visceral form. Dogs are considered the main domestic reservoirs of *L. (L.) chagasi*, because they possess high rate of parasites in the skin, facilitating the contamination of vectors.The progression of the canine infection is caused by failure at cell immunity. However, the mechanisms that lead to the failure of immune response are poorly understood. Recently was described molecule PD-1 (Programmed cell Death 1) and their ligands (PD-L1 e PD-L2), a negative costimulatory molecule, involved with the immune regulation of adaptative response. Objective: The aim of this study was to evaluate PD1 and their ligands expression *in vitro* after *Leishmania* spp. infection macrophage. Materials and Methods: Macrophages differentiated from PBMC of the 10 healthy dogs were cultivated in RPMI 1640 by at 37º and 5% CO_2 at 5%. The macrophage were infected with promastigotes forms of *Leishmania chagasi* (MHOM/BR/00/MER02) (10:1 parasites / macrophage). After 3 days of the incubation at same conditions the cells were collected and stained with human monoclonal antibody anti-CD279 (PD-1), CD274 (PD-L1) and CD273 (PD-L2) conjugated to phycoerythrin (BD Pharmingen™) and their respective isotype controls conjugated to fluorochromes (BD Pharmingen™). The acquisition of data was performed in flow cytometer (BDAccuri™ C5) and the analysis performed using the Cell Quest Pro software (BD Biosciences, CA). Results: PD-L1 increased expression on macrophages infected with *Leishmania* spp. compared to the no infected ($p<0.05$, T test). Conclusion: The increase of the PD-L1 expression in macrophages after infection by *Leishmania* spp. suggest the participation of this molecule in immune suppression and can be related to the increase in parasite burden in these cells. **Supported by**:FAPESP **Keywords:**Leishmania; programmed cell death 1 receptor; macrophages

HP062 - SYSTEMIC AND COMPARTMENTALIZED IMMUNE RESPONSE IN PATIENTS WITH CUTANEOUS LEISHMANIASIS IN THE XAKRIABá INDIGENOUS COMMUNITY, SOUTHEASTERN BRAZIL

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The aim of this study was to characterize immunological biomarkers in peripheral blood as well as in inflammatory infiltrate of cutaneous lesions from patients with CL or with positive Montenegro skin test without injury (TM+sL group) living in the Xakriabá indigenous community, Minas Gerais, Brazil. The results showed that patients of the CL group showed activated profile, with high percentage/Stimulation Index (SI) of CD23, and production of high levels of TNF-alpha by monocytes that non phagocytized and phagocytized promastigotes of Leishmania (Viannia) braziliensis. On the other hand, patients from TM+sL group showed better balance between the production of inflammatory (IL-12, IFN-gamma, IL-17) and modulatory (IL-10, IL-4) cytokines

recent lesion group showed a lower SI of CD23 and CD80, accompanied by lower production of inflammatory (TNF-alpha, IL-17) and modulatory (IL-10, TGF-beta and IL-4) cytokines as compared with late lesion group. The analysis of the localized immune response showed that patients of the recent lesion group presented higher transcript levels of IL12B, IFNG, TNF, IL10, TGFB1, IL4, CCL2, CCL3, CCL5 and CXCL10, as well as higher parasite burden as compared with late lesion group. This study allowed the identification of potential biomarkers associated to the resistance to CL (IL-12, IL-17 and NO) and to the development and maintenance of the lesion (TNF-alpha). **Supported by**:CAPES **Keywords:**Cutaneous leishmaniasis; monocytes; cytokines

HP063 - IMMUNIZATION WITH RECOMBINANT NCROP4 HAVE PROTECTIVE EFFECT BY TO REDUCE THE BRAIN PARASITE LOAD AFTER NEOSPORA CANINUM INFECTION IN MICE

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Neospora caninum is an intracellular protozoan parasite that causes neosporosis that affects several animals and is the primary infectious cause of abortion in cattle. Immunological mechanisms induced by parasite have not been well described. Several proteins from the parasite's compartments as rhoptry have been tested in the vaccine development. Therefore, this study aimed to evaluate the immunogenicity of the rhoptry protein NcROP4 and a derivative peptide to be used as a potential vaccine target. Thus, we employed the phage display technology to determine the binding region of the monoclonal antibody 20D2 to NcROP4. This region was synthetized (PepNcROP4) and correspond to 360-400 amino acids of NcROP4. Recombinant protein (rNcROP4) was produced in Eschirichia coli. Soluble antigen of N. caninum (NLA) was used as positive control. rNcROP4, PepNcROP4 and NLA were inoculated at mice to evaluate the immune response. Peritoneal, bone marrow derived macrophages (BMDM's) and splenocytes were stimulated with these antigens to observe the cytokine profile. These antigens were used to immunization protocols. As control was used a PBS group. After the immunization, the animals were challenged with N. caninum tachyzoites. rNcROP4 and PepNcROP4 induced IL-10 upregulation and IL-12/IFN-γ downregulation in culture cells. Differently, NLA induced IL-12/IFN-γ upregulation. Immunized animals showed increasing antibody anti-N. caninum established after challenge. rNcROP4-immunized animals increased the body weight, while the PepNcROP4 and NLA groups maintained and the PBS group lost weight until 30 days after infection. rNcROP4 and NLA groups showed significantly lower parasitism after 30 days of challenged in brain when compared with other groups. Our results demonstrated that rNcROP4 and PepNcROP4 induced a regulatory profile with IL-10 upregulation in vivo and in vitro, simultaneously with the decrease in brain parasite loads during latent N. caninum infection. **Supported by**:CNPq FAPEMIG CAPES
Keywords: Neospora caninum; vaccine; rhoptry

HP064 - ACID PHOSPHATASE IN *LEISHMANIA AMAZONENSIS* EXTRACELLULAR VESICLES PARTICIPATES IN INFECTION AND ENHANCES ROS PRODUCTION DURING PARASITE- MACROPHAGE INTERACTION

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Secretion of virulence factors is a major mechanism by which pathogens interfere or modulate the cell response. Among Trypanosomatids virulence factors include molecules expressed on the cell surface as well as those secreted or shed into the extracellular medium. Recently, we characterized acid phosphatase activities in *Trypanosoma cruzi* extracelular vesicles (EVs) that are largely responsible for EVs-induced host cell infection. In this study, we characterized phosphatase activities associated with extracellular EVs in procyclic and metacyclic promastigotes forms from *Leishmania amazonensis* and addressed the role that phosphatases activities play in *Leishmania* infection of macrophage and inflammatory mediators production. To obtain EVs, parasites were in incubated in Hank's balanced salt solution and, then, parasite-free supernatant was centrifuged at 4ºC for 2h at 110.000xg to pellet EVs. EVs from procyclic and metacyclic forms presented the same optimum acid phosphatase activity at pH 5.0. The optimum alkaline phospatase activities, was detected at pH 7.5 and 8.5 in procyclic and metacyclic EVs, respectively. To better characterize the nature of secreted phosphatases present in EVs we used phosphatase inhibitors. Sodium fluoride (Ser/Thr and acid phosphatase inhibitor) and *o*-vanadate (irreversible phosphotyrosine and alkaline phosphatase inhibitor) inhibited in 100% phosphatases present in EVs of metacyclic and procyclic. Tartarate (acid phosphatase inhibitor) and zinc chloride (phosphotyrosine phosphatase inhibitor) was less sensitive in EVs from procyclic and metacyclic forms inhibiting 89 and 63%, respectively. The acid phosphatase activity present in the EVs interfered significantly in infection and enhances ROS production during parasite-macrophage interaction, but no effect on NO production by host cells. **Supported by**:CNPq, CAPES, FAPERJ **Keywords:**Exosomes; vesicles; leishmania

HP065 - DEVELOPMENT A NOVEL SEROLOGICAL DIANOSTIC BASED IN THE RECOMBIANTE NCROP4 PROTEIN TO DETERMINE ACUTE OR CHRONIC PHASE DURING NEOSPORA CANINUM INFECTION

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Neospora caninum is an obligate intracellular parasite which is able to infect a wide range of hosts. This parasite has emerged as an important disease of cattle and dogs. The neosporosis diagnosis is classically performed by serological tests such as IFAT, the gold standard, and ELISA. However, there are no commercial tests designed to assess the stage of infection, once the main clinical signs are reported during the acute phase or during infection reactivation. Thus, we aimed to study the potential of NcROP4 protein as a stage marker of N. caninum infection. We evaluated the expression and localization of NcROP4 during infection in HeLa cells to investigate the possible exposure of this protein to antibodies. Subsequently, bioinformatic analysis was developed to predict and determine B-cell epitopes of the NcROP4. Mice and cattle were experimentally infected to investigate the producing of IgG through indirect immunoenzyme assay against recombinant NcROP4 (rNcROP4) during infection. It was observed that the NcROP4 protein is secreted into the cytoplasm of HeLa cells during the invasion process, which allows its extravasation to the extracellular environment after cell lysis. Bioinformatics analysis showed that NcROP4 displays 23 regions that are potential B-cell epitopes and analysis of phage display have showed that mAb 20D2 binds to the region 360-400 amino acids of this protein. Futhermore, in mice was observed high avidity antibodies against rNcROP4 after 30 days of infection (chronic phase) as well as no difference between the IgG subclass (IgG1 and IgG2) in this recognition process. Experimentally infected cattle recognize rNcROP4 after 44 days of infection, featuring the recognizing of chronic phase and produce high avidity antibodies. Thus, we concluded that NcROP4 can be used as chronic phase marker during N. caninum infection, being an additional strategy in the immunological diagnosis of neosporosis. **Supported by**:CNPq FAPEMIG CAPES **Keywords:**Neospora caninum; serodiagnosis; rhoptry

HP066 - EVALUATION OF AN APTAMER-BASED ASSAY TO DETECT A BIOMARKER OF *TRYPANOSOMA CRUZI* INFECTION IN SERUM/PLASMA

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The blood borne pathogen, *Trypanosoma cruzi* is the etiological agent of Chagas disease. Unlike the chronic phase, parasites can be easily detected in blood of infected individuals by microscopy or PCR during the acute phase of the disease. To overcome the difficulty of detecting parasites directly, diagnostic assays detect host anti-*T. cruzi* antibodies as a surrogate marker for infection. However, these assays are not reliable during the initial window period, or to follow cure after drug treatment due to the persistence of parasite specific antibodies. The parasites secrete various antigens in the blood collectively termed as *T. cruzi* Excreted Secreted Antigens (TESA). We utilized in-vitro RNA SELEX methods to develop TESA aptamers (short nucleic acid molecules) and used them as specific ligands in Enzyme Linked Aptamer (ELA) assays to detect TESA biomarkers in serum/plasma of *T. cruzi* infected mice. Our goal was to use ELA assays to follow the level of biomarkers in samples of infected mice treated with Benznidazole (Bz) and imunnossupressed with Cyclophosphamide (Cy) in order to determine if biomarker levels could predict sterile cure. Mice infected with *T. cruzi* Colombiana strain were treated with Bz during the chronic phase. Forty days after treatment mice were immunosuppressed to promote multiplication of remaining parasites, indicating drug failure. Our data showed that before treatment the biomarker levels were higher in infected mice when compared with non-infected. Following treatment, no significant difference was observed in the biomarker levels between Bz treated and non-treated animals suggesting the presence of parasites in treated animals. Following Cy, biomarker levels remained higher in treated animals compared to non-infected controls. In conclusion, ELA assay is a good indicator of treatment failure when the drug is administered during the chronic phase. Further, the ELA assays could be used for the diagnosis of *T cruzi* chronic infections. **Supported by**:FAPEMIG, CNPq, CAPES and FIOCRUZ **Keywords:**Chagas disease; aptamer; diagnosis

HP067 - COMPARATIVE STUDY OF BRADZYZOITE INFECTION OF TOXOPLASMA GONDII OF ME-49 STRAIN IN INTESTINAL AND FIBROBLAST CELL LINES.

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Toxoplasmosis is a worldwide disease caused by the intracellular protozoan Toxoplasma gondii which can infect any nucleated cells of warm-blooded animals. The most common forms of transmission of T. gondii are: the ingestion of undercooked meat containing encysted bradyzoites or food and water contaminated with oocysts. Thus, intestinal cells may be a primary site of invasion in a new host. Bradyzoites are slow replicating forms, and a key event for establishing the pathogenesis of toxoplasmosis is the ability to convert to tachyzoites in the new host. In order to understand the process of conversion, we used bradyzoites of the cystogenic strain of T. gondii ME49 isolated from the brain of infected mice. Bradyzoites were allowed to interact with intestinal cells (IEC-6). The samples were fixed at progressive time intervals and labeled with anti-BAG-1 and anti-SAG-1 antibodies, which respectively recognize bradyzoite and tachyzoite proteins; and with anti-CST1 and DBA-FITC, cyst wall markers. Samples were also observed by scanning electron microscopy (SEM). Past 14 hours of interaction with bradyzoites HFF and IEC-6 cells were positive for BAG-1 and SAG-1, characterizing that conversion to tachyzoites had started. At 24h post infection, almost 100% of the population was double labeled, including dividing parasites. The conversion occurred faster in fibroblasts, 48hpi most of them were marked only for SAG-1. In IEC-6 this was observed at 72hpi. The natural egress of parasites started 96hpi. At 120hpi parasites marked for BAG-1 were still present. Cyst wall proteins were deposited in the membrane of VPs 48 hpi. In SEM, intravacuolar we observed parasites dividing, and, from an early time after the establishment of VP, the intravacuolar network. At 144hpi tissue cysts with parasites immersed in cystic matrix were seen. The data obtained in this study contributes to the understanding of how infection occurs from bradyzoites and the parasite conversion process.

HP068 - THE USE OF MESENCHYMAL STEM CELLS FROM ADIPOSE TISSUE AND BONE MARROW AS AN ALTERNATIVE THERAPY TO MURINE CUTANEOUS LEISHMANIASIS CAUSED BY *LEISHMANIA AMAZONENSIS* IN C57BL/6

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Leishmaniasis is a chronic disease caused by protozoa of the genus Leishmania that may have cutaneous or visceral manifestation depending on the species of the parasite. Cell therapy seeks to restore the structure and function of a tissue by using one cell or a group of cell populations. Against leishmaniasis there is no approved vaccine, chemotherapy is inadequate as there are adverse effects and so far there is no ideal and functional therapy. In this work we aim to evaluate the effect of multipotent mesenchymal stem cells from bone marrow (CT-M) and adipose tissue (CT-A) in the treatment of infection by *Leishmania amazonensis*, responsible for cutaneous and diffuse cutaneous disease. C57BL/6 mices were infected in the right hind paw with $2x10^6$ promastigotes in the stationary phase of *L. amazonensis* (Josefa strain) and treated with two doses of 5 $x10^5$ mesenchymal stem cells CT-M or CT-A, intravenously, at 15 and 21 after the infection. For control, animals were treated with PBS. The infected footpads were assessed weekly using a paquimeter, to check the development of the lesion and draw a clinical profile of the disease. After at least 90 days after infection paws were removed and their parasitic loads were evaluated by limiting dilution. By measuring paw we could see that mice treated with CT-A show a slight tendency to have a lower lesion compared with the animals treated only with PBS, while the animals treated with CT-M does not present many differences in profile compared to the control of injury. In the parasite load in any of the experiments the mice treated with stem cells had no significantly different parasite load of mice treated with PBS. These results suggest that despite a slight tendency of improvement of the injury, through the use of stem cells from adipose tissue, stem cells do not show a significant improvement for use as treatment for a infection by *L. amazonensis*. Next step is combine this therapy with Glucantime therapy. **Supported by**:CNPq, FAPERJ
Keywords: Leishmania; leishmaniasis; stem cell

HP069 - *TRYPANOSOMA CRUZI* ACETYLOME REVEALS THE INVOLVEMENT OF ACETYLATION IN THE REGULATION OF OXIDATIVE STRESS RESPONSE MECHANISMS

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Protein acetylation has emerged as an important posttranslational modification, present in several proteins, involved in a wide range of cellular processes in different organisms. To better understand the function of protein acetylation in *T. cruzi*, we performed a wide survey of protein lysine acetylation in the epimastigote form. A total of 424 lysine-acetylated sites in 266 proteins were identified, number similar to those detected in other protozoan parasites, such as *Plasmodium falciparum*. The major functional groups were protein acetylation was detected in *T. cruzi*, were translation, proteolysis and oxidation/reduction. Oxidative stress response is an important mechanism for multiplication of epimastigotes forms and for the progression of the infection in the mammalian host. A total of 26 proteins related to oxidative stress response were identified in our acetylome, including the mitochondrial Fe-superoxide dismutase (TcFeSOD-A) that catalyzes the dismutation of superoxide radical ($O2^-$) into H_20_2. Acetylation at K68 residue has been described to negatively regulate the human manganese superoxide dismutase (MnSOD). Comparative protein structure analysis showed that the acetylated residue K97 detected for TcFeSOD-A in our acetylome is located in the correspondent region where K68 is in MnSOD-A. The MnSOD acetylation is regulated by SIRT3, a NAD+-dependent lysine deacetylase. In fact, parasites overexpressing the mitochondrial sirtuin, called TcSir2rp3, showed decreased levels of reactive oxygen species and an increased SOD activity when compared with wild type parasites. This effect was not due to increased levels of TcFe-SOD-A expression, suggesting a role of acetylation in the regulation of TcFeSOD-A activity. In addition, parasites overexpressing TcSir2rp3 are more resistance to benznidazol when compared with wild type parasites. Altogether, our data suggest that protein acetylation is important to regulate oxidoreduction mechanisms in *T. cruzi*. **Supported by**:FAPESP
Keywords: Trypanosoma; acetylome; superoxide dismutase

HP070 - MOLECULAR IDENTIFICATION OF PROTEIN KINASE CK2 (AMPLIFICATION, CLONING AND HETEROLOGOUS EXPRESSION) OF L. BRAZILIENSIS AND POLYCLONAL ANTIBODY PRODUCTION.

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Parasites of the genus Leishmania are transmitted by the bite of sand flies and infect cells of the mononuclear phagocyte system in their vertebrate hosts. The study of surface molecules related to the process of parasite-host interaction is of fundamental importance. This study aimed to clone and characterize the L. braziliensis ck2α gene, assess their genomic organization, protein expression in eukaryotic system (L. tarentolae), its influence on the infectivity process and the differential expression of this protein from a virulent strain and other avirulent L. braziliensis. After L. braziliensis genome analysis, species-specific primers were designed and then cloned into the pGEM®-T Easy Vector plasmid transfected in the Top10 bacteria confirmed by PCR with specific primers. The obtained Amplicom shows high level of identity to the gene ck2α L. braziliensis by sequencing and analysis in BLASTn. Subsequently, the plasmid containing the gene ck2α was submitted to specific restriction to release the cassette for ligation with the plasmid Lexsy was also subjected to the restriction enzymes. The ligation cassette containing the gene ck2α with Lexsy plasmid was transfected in the Top10 bacteria and confirmed by PCR. To perform the transfection in L. tarentolae, the plasmid containing ck2α gene was subjected to a new restriction using a specific enzyme for the plasmid linearization and removing the ampicillin resistance gene. The transfection protocol in L. tarentolae was performed as described by Jena Bioscience. The heterologous protein production from a eukaryotic expression system aims to produce a morphologically protein and activity more similar to the natural, thereby creating a vaccination protocol with the objective of reducing the mice infection by L. braziliensis. **Supported by**:FAPERJ
Keywords:L. braziliensis; casein kinase 2 (ck2); eukaryotic expression system lexsy

HP071 - INDUCTION OF HO-1 DECREASES THE PRODUCTION OF REACTIVE OXYGEN SPECIES BY ACTIVATED AND INFECTED MACROPHAGES FROM DOGS

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Introduction: Heme oxygenase-1 (HO-1), enzyme responsible for metabolizing heme from hemoglobin, can impair cellular oxidative metabolism through the unavailability of heme necessary for activation of NADPH oxidase, predisposing host cells to infections. Objective: The aim of this study was to evaluate if inhibition or induction of HO-1 improves the oxidative metabolism from dog macrophages in the presence of *Leishmania chagasi* infection *in vitro*. Methods: PBMC from 6 healthy dogs were isolated and cultured in RPMI 1640 medium for 7 days at 37 ºC and CO_2 5% until differentiation into macrophages. Macrophages ($2x10^6$/mL) were then cultured above round glass cover slip for adherence and infection was performed with promastigotes of *L. chagasi* ($10x10^6$, ratio 5:1). Prior infection, macrophages received no treatment (control) or were treated with HO-1 inducer protoporphyrin IX cobalt chloride 50 µM (CoPP) and inhibitor Sn(IV) mesoporphyrin IX dichloride 50 µM (SnMsP). After four hours, non-phagocyted promastigotes were removed by washing, the treatments were restored and culture was kept for three days at 37 ºC and CO_2 5%. Macrophages were removed and stained with reactive oxygen species (ROS) dye 2',7'-dichlorodihydrofluorescein diacetate 5 µM in the presence or not of phorbol 12-myristate 13-acetate 10 µM (PMA). Using flow cytometer (BD Accuri™ C5), 10,000 events were acquired, macrophage population selected and green mean fluorescence measured. Results: The induction of HO-1 with CoPP decreased ROS production of macrophages infected with *L. chagasi* alone and treated with SnMsP (12,286±5.277 vs. 22,227±8,895 and 19,341±5,048, respectively, Repeated Measures ANOVA with Holm-Sidak's multiple comparisons test, p=0,02) in the presence of PMA stimuli. No difference was observed in non-stimulated cells. Conclusion: Induction of HO-1 impairs macrophage oxidative metabolism from dogs with decreased ROS production, process that could predispose these cells to infection. **Supported by**:FAPESP **Keywords:**Leishmania chagasi; canine; ho-1

HP072 - BLOCKAGE OF CASPASES CONTRIBUTES TO KILL *LEISHMANIA INFANTUM* BY THE AXIS RIPK1-RIPK3-MLKL-DEPENDENT CELL DEATH IN NEUTROPHIL

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Different cell death pathways are associated with pathogen's infection. Lately, necroptosis has been described as a proinflammatory cell death form, which happens when death receptors are activated activating the axis RIPK1-RIPK3-MLKL in the presence of caspase 8 inhibition. Recently, necroptosis has emerged as a key component of resistance against infections. Neutrophils have been implicated in the immunopathogenesis of Leishmaniasis. Here we tested the hypothesis whether blockage of caspase 8 contributes to kill Leishmania parasites by inducing necroptosis. Using mouse neutrophils, pretreated with zVAD (pan caspase inhibitor) or zIETD (caspase 8 inhibitor), we evaluated *L. infantum* parasite viability within those cells. Neutrophils pretreated with zVAD and zIETD presented a significant decrease of parasite burden when compared with untreated cells. The neutrophils pre-treated with zVAD had higher levels of ROS. Interestingly, RIPK1 and RIPK3 inhibition (Nec-1 or GSK872 respectively) abrogated caspase inhibitors-decreased parasite burden. Electron microscopy (EM) remains an important qualitative method to detect cell death morphological features. EM assays revealed that infected-neutrophils pretreated with RIP1 and RIP3 inhibitors do not have alterations in membrane integrity, preserved intracellular content and presented viable Leishmania within parasitophorous vacuoles, in opposition to the groups pre-treated with zVAD only, which exhibited morphological aspects of necroptotic death. Finally, we investigated MLKL role in parasite survival with human neutrophils. MLKL is an important molecule downstream necroptosis pathway. In the presence of NSA inhibitor, human neutrophils showed high parasite burden when compared with controls only pretreated with caspase inhibitors. Our data suggest that inhibition of neutrophil apoptosis by blockage of caspases contributes to eliminate *L. infantum* by stimulate an inflammatory response associated with necroptotic cell death. **Supported by**:FAPESB, CNPq, FIOCRUZ **Keywords:**Leishmania infantum; neutrophils; necroptosis

HP073 - EXPLORING THE TRYPANOSOMA CRUZI GENOME USING PHAGE DISPLAY WHOLE GENOME SHOTGUN

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During its life cycle, the parasite Trypanosoma cruzi has to invade cells of the mammalian hosts to escape the immune system and replicate. To understand the molecular details of these processes is essential for the development of vaccines and other therapeutic alternatives for patients afflicted with Chagas' disease. In 2005, the first T. cruzi genome was published. Similar to other organisms, half of the genes were annotated as hypothetical. This represents a significant gap in our knowledge, but offers an enormous potential, still hidden in the genome of the parasite, for the development of novel therapeutic approaches. In this work, we employed the phage display technology to explore the T. cruzi genome in search of genes important for parasite biology. We have taken advantage of the fact that T. cruzi genes do not contain introns to build a genomic shotgun expression library in filamentous phage. We elected the Sylvio X10 strain as the source of genomic DNA because its genome has been sequenced and it is not a hybrid strain as CL Brener. The produced library contains $4.4x10^8$ clones with an average insert size of 254 bp. Therefore, the sum of all unique inserts results in a total of $1.1x10^{11}$ bp, representing more than 2,500 times the length of the genome of Sylvio X10 strain. Given such high coverage, this library most likely contains inserts for all proteins encoded in the parasite genome. However, due to methodological constraints, not all phage clones contain inserts in the correct frame and orientation. So, it is noteworthy that after three rounds of selection using the library and immobilized cell surface proteins (integrins and extracellular matrix proteins), the number of phage binders displaying in-frame inserts reaches close to a 100%. This indicated that our platform and selection protocols are working. We are currently performing biopannings on cells and in vivo screenings to identify novel proteins important for parasite-host interactions. **Supported by**:FAPESP e CNPq **Keywords:**Phage display; genome shotgun; trypanosoma cruzi

HP074 - *LEISHMANIA* SUBGENUS SEROTYPING EMPLOYING *LSS-1* (*LEISHMANIA SPECIFIC SUBGENUS -1*) PROTEIN OF *LEISHMANIA INFANTUM* AND ITS DERIVED PEPTIDES CONTAINING LINEAR B-CELL EPITOPES

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The overlap in geographic distribution of different species of *Leishmania* parasites that cause distinct clinical manifestation of leishmaniasis, as tegumentary (LT) *L. braziliensis* and visceral (LV) *L. infantum* infections in Brazil, associated with the high level of conservation of proteins between these species, implies that there is high incidence of cross-reactivity when detecting these parasites using serological techniques. In the present work, we evaluated the use of the recombinant protein *LSS-1* (*Leishmania Specific subgenus-1*) of *Leishmania infantum*, as a potential subgenus-specific antigen for serotyping of leishmaniasis infection. *LSS-1* is highly conserved within species of the *L.* (*Leishmania*) subgenus (*L. infantum*, *L. amazonensis*, *L. major*, *L. donovani* and *L. mexicana*) (identity: 81.47-99.41%; similarity: 84.70-99.41%) and more divergent when compared to species of the *L.* (*Viannia*) subgenus (*L. braziliensis* and *L. peruviana*) (identity: 76.32-77.64%; similarity: 80.58-82.35%). Linear B-cell epitope mapping was also performed on *LSS-1* ortholog proteins to identify inter-subgenus polymorphic epitopes. The reactivity of sera from BALB/c mice experimentally infected with *L. infantum*, *L. amazonensis*, *L. major*, *L. donovani*, *L. mexicana*, *L. braziliensis* or *L. peruviana* and from patients naturally infected with *L. infantum* or *L. braziliensis* were evaluated in ELISA assays using *LSS-1* protein and two derived synthetic peptides as antigens. High performance in identifying *Leishmania* subgenus parasites (accuracy: 88.24%) was achieved when using sera from mice experimentally infected and *LSS-1* protein as antigen. For human assays, *LSS-1* also showed good performance (accuracy: 73.33%). Both peptides also presented good performance using both human and mice serum samples, and are potential subgenus-specific epitopes. Our results indicate that *LSS-1* antigen and its derived polymorphic peptides have the potential to be used in serotyping *Leishmania* subgenus infection. **Supported by**:cnpq
Keywords: Leishmania subgenus; leishmania infantum; serodiagnosis

HP075 - HIGH RESOLUTION MELTING ANALYSIS TARGETING HSP70 AS A FAST AND EFFICIENT METHOD FOR *LEISHMANIA* SPECIES DISCRIMINATION

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Leishmaniases are a relevant public health problem in 88 countries worldwide, with about 350 million people under risk of infection. In Brazil, the most common infections are related to seven Leishmania species that affect humans with different clinical forms. Therefore, an accurate diagnostic procedure is extremely relevant in understanding the epidemiological profiles as well as in developing optimized therapeutic protocols. We used heat-shock protein 70 coding gene (hsp70) as target to produce two amplicons that generated distinct dissociation profiles, with temperatures of melting (Tm's) varying among the different Leishmania species. This way, we could discriminate the main Brazilian species: *Leishmania (L.) infantum chagasi, L. (L.) amazonensis, L. (Viannia) lainsoni, L. (V.) braziliensis, L. (V.) guyanensis, L. (V.) naiffi* and *L. (V.) shawi* by High Resolution Melting (HRM) analysis of genomic DNA samples, in the presence or in the absence of mammalian DNA. To evaluate the diagnostic potential of this experimental strategy, we also tested DNA obtained from phlebotomines naturally infected, tissues of mice experimentally infected and clinical human samples. The results from those samples were perfectly in accordance to the ones obtained by applying other techniques, validating the protocol. In conclusion hsp70 PCR-HRM analysis is a robust and faster strategy to detect and discriminate all *Leishmania* species responsible for leishmaniases in Brazil. The method presents high accuracy and sensitivity, detecting less than one parasite per reaction, even in the presence of host DNA.**Supported by**:FAPESP and CNPq
Keywords: Diagnosis; melting profile; hrm

HP076 - *TRYPANOSOMA CRUZI* STRAIN-DEPENDENT MODULATION OF MAMMALIAN PROTEIN KINASE C ISOFORMS DURING HOST CELL INVASION

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Several signaling pathways have been reported to be triggered in the process of mammalian cell invasion by metacyclic trypomastigotes (MT) of *T. cruzi*. Previous studies suggested the participation of protein kinase C (PKC) during MT internalization, based on the changes observed upon inhibition of PKC activity. The PKC family comprises many isoforms that are classified into three subfamilies: classical, novel and atypical. Here we investigated the role of PKC isoforms during cell invasion by MT of genetically divergent T. cruzi strains, CL and G, which differ markedly in their infective properties. Upon five to thirty minutes of interaction, MT of both strains induced activation of PKC in HeLa cells that resulted in increased phosphorylation levels of its substrates. In addition, the profiles of PKC phosphorylation were different in cells infected with G and CL strain MT, suggesting the activation of different PKC isoforms. Inhibition of PKC with a broad-spectrum inhibitor resulted in an increase in cell invasion by CL strain MT but a reduction of cell invasion by G strain MT. On the other hand, inhibition of the classical class of PKC reduced cellular invasion by G strain MT, but did not affect invasion by CL strain MT. As PKCs are upstream to MAP kinases such as ERK, which is differentially activated by MT of G and CL strains, we further verified the impact of PKC on ERK activation. The broad-spectrum inhibitor of PKC abrogated the phosphorylation of ERK in cells infected with MT of CL strain, but not of G strain. Finally, inhibition of ERK pathway reduced in a dose dependent manner cell invasion by CL strain MT. Taken together, these data show that distinct PKC isoforms are triggered during internalization of T. cruzi strains with different invasive capacities.**Supported by**:FAPESP, CNPq - Ciência Sem Fronteira
Keywords: Cell signaling; mapk; metacyclic

HP077 - HOST CELL REORGANIZATION UNDER TOXOPLASMA GONDII CYST FORMATION

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Toxoplasma gondii is the causative agent of toxoplasmosis, a disease spread around the world that can cause abortion, neuronal and ocular damages in immunocompromised patients. The chronic phase is characterized by tissue cysts that contain bradyzoites, a slow division form. In order to better understand the mechanism of cyst formation we looked for modification on host cell structures during this process. We used the Brazilian strain EGS isolated from human, which forms cysts spontaneously in vitro and modified parasites from EGS, expressing stage specific fluorescent proteins. The distribution of actin microfilaments was unaltered, compared to uninfected cells, while microtubules and intermediated filaments were distributed around the cysts, forming a cage-like structure. When infected cells were treated with taxol, a microtubule stabilizer drug, for 72h, the number of cysts increased by 50%. The association of mitochondria to the cyst wall was quantified and compared with mitochondrial association to the parasitophorous vacuole membrane (PVM). This association was 30% less frequent. The intimate association of endoplasmic reticulum with cyst wall was confirmed by electrontomography that showed ER profiles imbricated with cyst wall membrane convolutions. The association of Golgi complex with the cyst wall was not frequent, as occurs with the PVM. When the infected cells were treated with brefeldin A, which impairs the traffic of vesicles from ER to Golgi, the number of cysts increased 10%. Early and late endosomes were observed around cysts, while lysosomes were more frequently distributed in the cyst wall boundary. Endocytic tracers used to track a possible delivery route from host cell cytoplasm to the interior of cysts, as described for the PV, but any tracer used were not observed inside the cysts. This work helps to elucidate cyst formation mechanisms showing how T. gondii exploits host metabolism to maintain bradyzoites. **Supported by**:Faperj, Capes, CNPq
Keywords: Bradizoitas; cisto; conversão

HP078 - BLOCKING PLASMODIUM DEVELOPMENT IN MOSQUITOS USING GENETICALLY MODIFIED SYMBIOTIC BACTERIA

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About 1 million people die of malaria every year. Although current malaria control strategies targeting the mosquito vector and the parasite have helped alleviate the malaria burden in many endemic areas, the emergence and rapid spreading of insecticide-resistant mosquitoes and drug-resistant parasites undermine such efforts. A major bottleneck of Plasmodium development occurs in the lumen of the mosquito midgut, making this stage a prime target for parasite intervention. We are exploring an alternative strategy (paratransgenesis) to render mosquitoes resistant to the parasite by engineering symbiotic midgut bacteria to secrete anti-malarial effector molecules. Bacteria that stably colonize the mosquito and are efficiently transmitted to the next generation are crucial properties for the introduction of recombinant bacteria into field mosquito populations. Recently, we identified a novel bacterial strain of the genus Serratia that has promising properties for introduction into field mosquito populations: a) Serratia efficiently colonizes both the mosquito midgut and ovaries; b) Serratia is vertically transmitted from infected females to larval progeny; c) Serratia is sexually transmitted from male to female mosquitoes. We also investigated whether the growth of human parasite P. falciparum in the vector is affected by effector molecules secreted by Serratia bacteria. Five-day old A. gambiae females were fed Serratia expressing effector genes for one day, fasted for the following day, and then infected by P. falciparum GFP-3D7 strain through membrane-feeding on day-8 post emergence. Eight days after P. falciparum infection, mosquito midguts were dissected and GFP-3D7 oocysts were counted. Serratia expressing various effector genes (Scorpine, Fv-shiva, and a poly-effector gene) significantly inhibited parasite development. **Supported by**:CNPq
Keywords: Plasmodium; serratia; paratransgenesis

HP079 - BALANCE BETWEEN NETOSIS AND PHAGOCYTOSIS BY MOUSE NEUTROPHILS IS ASSOCIATED WITH L. MAJOR RESISTANCE AND SUSCEPTIBILITY PHENOTYPES.

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Leishmania is inoculated in a pool of blood in the vertebrate host and neutrophils (NØ) are the major leukocytes in blood and rapidly recruited to the skin wound, but the role of NØ on the pathogenesis of leishmaniasis is still controversial. Neutrophil extracellular traps (NETs) are composed by a chromatin scaffold decorated with cytoplasmic and granular proteins, released by a process termed netosis. Leishmania promastigotes induce NET release and are ensnared and killed by NETs, however, the influence of netosis on the infection outcome is unclear. Thus, we evaluate here the role of netosis in mice resistance and susceptibility to Leishmania major (LM) infection. Bone-marrow isolated NØs from susceptible (NØ-S) and resistant (NØ-R) mice were stimulated with LM and NETs measured as extracellular dsDNA and elastase activity. After 2h stimulation, NØ-S released significantly more NETs and elastase than NØ-R. However, after 4h stimulation, both NØs released equivalent amounts of NET-DNA. Addition of DNAse to the LM-NØ interaction significantly increased LM survival on NØ-S cultures but had little effect on NØ-R. To evaluate differences on NET composition we performed fluorescence microscopy and indeed we observed reduced elastase staining of NØ-R derived NETs. The same pattern of NET release was obtained after LM interaction with casein-elicited NØs, NØ-R poorly generated NETs compared to NØ-S. CD11b labeling by flow cytometry after 90min of stimulation showed that LM induces the same level of NØ activation on both strains. Moreover, LM binds more efficiently to NØ-R measured by CFSE labeled-LM than to NØ-S. Furthermore, ethidium bromide quenching of extracellular attached parasites showed greater phagocytosis of LM by NØ-R as well. Thus, our data points that a balance between neutrophil phagocytosis and NET generation is associated with resistance and susceptible phenotypes of LM in mice infection. **Supported by**:FAPERJ e CNPq **Keywords:**Leishmania; neutrophils; netosis

HP080 - THE INHIBITOR OF SERINE PEPTIDASES 2 (ISP2) OF TRYPANOSOMA BRUCEI RHODESIENSE IS REQUIRED FOR THE INFLAMMATORY RESPONSE OF MACROPHAGES AND PARASITE SURVIVAL IN MICE.

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Protozoan Inhibitors of Serine Peptidases (ISP1, ISP2 and ISP3) are related to bacterial ecotin, a 13 kDa protein isolated from the periplasm Escherichia coli. Ecotins inhibit the activity of serine peptidases from the S1A family, such as neutrophil elastase (NE) and cathepsin G. In protozoa, ISP genes have been identified in Leishmania, T. brucei and T. cruzi, however only ISP2 was found in the three parasites, and the encoded protein proven to be a functional inhibitor. We reported previously that ISP2 is a crucial virulence factor which protects L. major from the microbicidal response of macrophages via the inhibition of host cell serine peptidases, but the function of ISP2 in T. brucei remains unknown. We generated T. b. rhodesiense IL1852 bloodstream form (BSF) null mutant lines for ISP2 (Δisp2), and confirmed lack of ISP2 expression by Western Blot. Mutant and wild type parasites grew similarly in vitro, but C57BL/6 mice infected with Δisp2 displayed lower blood parasitemia and enhanced survival, when compared to those infected with wild type. To obtain further insight into the possible modulation of the macrophage response, we measured cytokines released upon the interaction between the parasites and macrophages in vitro, for 18hs. We detected high levels of KC, MIP2 and TNF□ and moderated levels of IL6, upon exposure to WT or re-expressor parasites, while there was a drastic reduction in cytokine production by macrophages exposed to Δisp2. Surprisingly, the down modulation of chemokine release by Δisp2 was unaffected by addition of synthetic inhibitors of NE or cathepsin G. These observations indicate that in T. rhodesiense, ISP2 plays an important role for parasite virulence and participates in the modulation of innate responses via a mechanism that is apparently dissociated from the inhibition of host serine peptidases. **Supported by**:CAPES, FAPERJ, CNPq **Keywords:**T. brucei; isp; cytokines

HP081 - ULTRASTRUCTURAL STUDY OF *TRYPANOSOMA CRUZI* TREATED WITH OF CLOTRIMAZOLE AND KETOCONAZOLE-TRIPHENYLPHOSPHINE-η6-*P*-CYMENE RU(II) COMPLEXES

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Chagas disease caused by the protozoan *Trypanosoma cruzi* is endemic in South America with overall prevalence estimated in 10-16 millions of cases. The reference drugs benznidazole and nifurtimox are used to treat the acute and chronic form. Nevertheless, their efficacy against established chronic infections, which are the most common presentation of the disease, is much lower. In the research of new drugs, metal complexes appear to be a promising new approach. A successful strategy has been developed based on the synthesis of complexes combining ligands bearing anti-parasite activity and metals of pharmacological significance. Two complexes with general formula [Ru(η^6-*p*-cymene)(PPh3)(X)Cl]PF6 and Ruthenium in association with clotrimazole (1) [LC-PCTZ] or ketoconazole (2) [LC-PKTZ] were synthesized

trypomastigote, the lethal dose (LD_{50}) was 50 nM after 5 hours of incubation. Analyses by light and electron microscopy revealed that the compounds causes rounding and torsion of the cell body. Thin sections of treated epimastigotes observed by transmission electron microscopy showed intense mitochondria swelling, detachment of plasma membrane of parasite cell body and flagellum and formation of vacuoles similar to autofagosomos. Taken together, our observations show that metal-complex analogs are promising selective inhibitors of *T. cruzi* proliferation. Further studies will evaluate their potential activity. **Supported by**:CNPq, CAPES e FAPERJ **Keywords:**Trypanosoma cruzi; metal complex; chemotherapy

HP082 - *LEISHMANIA* PHOTOINACTIVATION APPLIED TO THE IMMUNOPROPHYLAXIS OF CUTANEOUS LEISHMANIASIS.

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Leishmaniasis is a global parasitic disease caused by different species of the genus *Leishmania*. Due to the lack of effective treatment and vaccines, emerging strategies such as photodynamic therapy (PDT) are considered an alternative regarding vaccine development. PDT uses photosensitizers that are taken up by cells, which upon exposure to light produce cytolytic reactive oxygen species (ROS) and singlet oxygen. These in turn, can photoinactivate *Leishmania* forms while preserving their coat surface proteins. This study aims to generate photoinactivated *Leishmania* parasites in order to develop an attenuated vaccine against experimental cutaneous leishmaniasis. *L. braziliensis* and *L. amazonensis* promastigotes were exposed to alluminium (AlPh) or silicon-phthalocyanines (Pc1 or Pc2) and a X ray viewer light. Both species incorporated the photosensitizers (1 uM) but only Pc1 and Pc2 effectively photoinactivated parasites without dark toxicity, as judged by MTT assay, live/dead staining and growth in culture medium. Photoinactivation of *L. braziliensis* with Pc1 or Pc2 (1uM) was incomplete as parasites proliferated in culture medium. However, exposure of *L. amazonensis* to Pc1 or Pc2, followed by light, rendered parasites unviable as seen by MTT, positive staining for live/dead and lack of growth in Schneider medium. Photoinactivated *L. amazonensis* were phagocytosed by BMDM but rare parasites were observed inside macrophages after 18h. Collectively, these results indicate that *L. amazonensis* but not *L. braziliensis* can be effectively photoinactivatived with Pc1 and Pc2 1 uM. Experiments are under way to evaluate the protective capacity of photoinactivated *L. amazonensis* in an experimental model of cutaneous leishmaniasis. **Supported by**:CNPq **Keywords:**L. amazonensis; photoinactivation; attenuated vaccine

HP083 - HEME-DRIVEN OXIDATIVE MECHANISMS CONTRIBUTE TO PERSISTENCE OF *LEISHMANIA INFANTUM* IN HUMAN NEUTROPHILS

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Visceral Leishmaniasis (VL) is often associated with hematologic manifestations that possibly interfere with neutrophil behavior. Free heme, which is released by oxidized hemoglobin in hemolytic conditions, is a pro-inflammatory molecule capable of inducing migration and activation of neutrophils. Here, we examine a direct link between heme and human neutrophil activation in the context of *Leishmania infantum* infection. Using an *in vitro* model with human neutrophils, we observed that heme enhances intracellular *Leishmania* survival while decreasing viability of host cells, which were associated with increases in production of reactive oxygen species and release of neutrophilic enzymes such as myeloperoxidase, matrix metalloproteinase-9 and neutrophil elastase. When cell cultures were incubated with protoporphyrin IX and Fe^{+2}, key components of the heme molecule, the parasite growth was increased whereas the activation status of neutrophils was no longer affected. Importantly, stimulation of infected neutrophils with heme triggered dramatic increases in transcription of heme oxygenase-1 (HO-1) as well as in superoxide dismutase-1 (SOD-1) enzymatic activity, which may affect the oxidative responses in these cells. In an exploratory study in VL patients from an endemic area in Brazil, we observed a positive correlation between plasma concentrations of heme and HO-1 and a negative correlation between heme values and peripheral blood neutrophils counts. These correlations were not observed in uninfected endemic controls, who exhibited lower circulating levels of both heme and HO-1. These findings indicate that heme promotes intracellular *L. infantum* survival via subversion of neutrophil function and oxidative status. This study opens new perspectives for the understanding of immunopathogenic mechanisms involving neutrophils and its implications in VL-associated inflammatory balance. **Keywords:**Leishmania; neutrophil; heme

HP084 - CIRCULATING ANTIGEN DETECTION IN EXPERIMENTAL VISCERAL LEISHMANIASIS

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Visceral leishmaniasis (VL) is an important disease in resurgence in Brazil. Their diagnosis is suspected by clinical picture and few unspecific laboratory tests, with subsequent invasive spleen or bone marrow aspirates for visualization of the parasite by specialist, however that can bring bleeding risk. Circulating immune complexes (CIC) or antigen detection could be confirmatory of active disease, without risk or trained observer. Several reported attempts for detection of antigen or CIC in VL showed conflicting results. We devised to use affinity purified antibodies in a Leishmania antigen capture ELISA using dissociation of CIC, in order to verify if those tools could solve that detection in experimental infection of hamster with L. (L.) infantum chagasi. Affinity purified anti-Leishmania IgG (aIgG) was obtained using antigen coated magnetic particles. Coating antigen was purified from proteins extracts of culture promastigote. This antigen preparation was selected by reactivity in ELISA dilution assays. Capture antigen assay was standardized using cross word dilutions of aIgG, antigen and in house prepared biotinylated aIgG, detecting between 100- 10µg/ml Leishmania antigen. Serum samples from L. (L.) infantum chagasi infected hamsters were obtained at 15, 30, 45, 60 and 90 days after infection. Higher positivity and higher levels around 200-300ng/ml was found only in early stages of the infection (30 days) despite latter periods show also some positive results. Circulating antigen do not correlate with specific IgG levels or spleen parasite burden. This fact could be explained by CIC removal after splenic enlargement and higher clearing of antigen or complexes. These data could explain why reports of circulating antigen detection give conflicting results that could be due to different stages of disease at sampling. Despite promising and non-invasive, antigen detection are probably ineffective for diagnosis of VL, without technology improvement. **Keywords:**Visceral leishmaniasis ; antigen detection ; circulating antigen

HP085 - ROLE OF *LEISHMANIA INFANTUM* LIPOPHOSPHOGLYCAN DURING MACROPHAGE ACTIVATION

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Lipophosphoglycan (LPG) is the major surface glycoconjugate of *Leishmania* promastigotes and is associated with virulence. During infection, LPG can modulate the immunological and microbicide mechanisms of the cell host. In this study, we investigated the role of different LPG fractions from *L. infantum* Ba262 and BH46. Both glicydic and lipid fractions from LPG induced lipid body (LB) formation, PGE_2 production and nitric oxide release in murine macrophages. To address the role of LPG during interaction between host cell and parasite, we characterized the cell biology of Wide Type (WT) *L. infantum* compared to those genetically modified parasites that do not express LPG. Herein, we used knockout parasites of lpg1 gene (KO) and gene restored (ADD BACK). In this sense, we characterized growth of promastigotes in axenic cultures as well as ultrastructural aspects of these parasites by transmission and scannig electron microscopy (TEM and SEM). KO and ADD BACK parasites presented different growth curve patterns compared to WT parasites. On the other hand, morphological differences were not observed in the parasite surface by SEM or intracellularly by TEM. Altogether, our data showed that LPG or its fractions trigger macrophage activation. In the future, we intend to use genetically modified parasites to assess the role of LPG present on the parasite surface in host cell-KO parasite interaction assays to better understand the mechanisms of host cell activation and the outcome of infection. **Supported by**:Fundação de Amparo à Pesquisa do Estado da Bahia (Fapesb)

Keywords:L. infantum knockout; lipophosphoglycan (lpg); lipid bodies (lb)

HP086 - INTER-STRAIN CONSERVATION AND ANTIGENICITY OF MASP MULTIGENE FAMILY OF *TRYPANOSOMA CRUZI*

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Mucin-Associated Surface Protein (MASP) is a multigene family encoding surface proteins largely expanded in the genome of *Trypanosoma cruzi*. MASP proteins are highly polymorphic and mainly expressed at the surface of trypomastigote stage, suggesting its participation in host-parasite interactions. We speculate that MASP may be involved in host cell invasion and/or in immune evasion mechanisms. To better characterize the immune response against this polymorphic family, we have clustered MASP protein sequences within seven subgroups based on their sequence similarity and selected representative members of each subgroup to generate recombinant proteins. Sequence conservation analysis of MASP family among *T. cruzi* strains from different DTUs revealed that this family also displays high level of inter-strain polymorphism. The antigenic profile of each MASP subgroup was evaluated by ELISA using four sera panels. First, sera from mice infected with *T. cruzi* during the acute and chronic phases were used to evaluate the recognition profile along the infection. Second, sera from mice infected with *T. cruzi* strains from different DTUs, collected 5, 10, 15, 20, 25 and 30 days post-infection. Third, sera from mice infected with *T. cruzi* from the 2, 6, 10, 19 and 22 passages were used to investigate whether MASP antigenic profile changes during successive passages in mice. Fourth, sera from patients with Chagas' disease reactivation after heart transplantation were used to analyze if the MASP antigenic profile changes in distinct human patients. Altogether, our results shows that the MASP members are differently recognized during the acute phase of *T. cruzi* infection and that the recognition profile is highly variable within and between strains, suggesting that there is a diversification of the humoral immune response against this family. **Supported by**:CNPq, FAPEMIG, CAPES, INCTV

Keywords: Masp; antigenicity; conservation

HP087 - SEROPREVALENCE OF TOXOPLASMA GONDII ANTIBODIES IN WILD ANIMAL AND BIRDS FROM ZOO ANIMALS,TRIPOLI, LIBYA

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Toxoplasmosis is a worldwide zoonosis caused by an obligate intracellular protozoan parasite, Toxoplasma gondii, that affects all warm-blooded animals, including wild animals. In this study, The seroprevalence of Toxoplasma gondii was investigated in wild animals and avian from

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seropositivity in captive animals and order (P- value> 0.05). **Supported by**:Libyan National Center forInfectious Diseases Prevention and Control
Keywords: Toxoplasma gondii; seroprevalence, wild animal; tripoli, libya

HP088 - SOCS2 "ORCHASTRATES" THE MACROPHAGES EFFECTOR FUNCTIONS DURING *TRYPANOSOMA CRUZI* INFECTION

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Introduction: Infection by *Trypanosoma cruzi* activates innate immune mechanisms mediated by dendritic cells and macrophages (MØ), which drive a Th1-type adaptive response to control infection. Previously, we showed that *in vitro*, Suppressor of cytokine signaling 2 (SOCS2) modulates MØ response to *T. cruzi* antigens through the control of Mitogen Activated Protein Kinases (MAPK).
Methods and Results: To explore SOCS2's *in vivo* function, we analyzed splenic MØ activities in *T. cruzi* infected mice. We infected C57bl/6 (WT) and SOCS2-/- mice with 10^3 blood trypomastigotes (Y strain) and analyzed splenic MØ at 3, 5, 9, 15 days post infection (dpi) by Flow cytometry. During the initial stages of *T. cruzi* infection (0-9 dpi), we found a lower frequency of MØ in the spleen of SOCS2$^{-/-}$ mice when compared to WT mice. However, at 15 dpi SOCS2$^{-/-}$ MØ increased significantly over WT levels. MØ expressing Toll-like receptor TLR2 were significantly higher at 15 dpi in SOCS2$^{-/-}$ mice while TLR4^{+} MØ did not increase over infection as observed in WT mice. Analysis of MHCII^{+} MØ showed that although the majority of splenic MØ expresses MHCII constitutively, *T. cruzi* infection increases the frequency of these cells. Our results showed that SOCS2 deficiency reduced MHCII^{+} and CD80^{+} expression in MØ. We analyzed MØ production of IL12 and IL10. We found that in WT mice, *T. cruzi* induces an increase in the frequency of IL12^{+} MØ at 9 and 15 dpi while in SOCS2$^{-/-}$ mice IL12^{+} MØ remained stable. Importantly, in WT mice IL10^{+} MØ were reduced in a time-dependent manner by *T. cruzi* infection. In contrast, SOCS2$^{-/-}$ mice frequency of IL10^{+} MØ was significantly higher at basal levels and increased at 5 dpi followed by a drop that reach even lower levels than WT mice at 15 dpi.
Conclusion: SOCS2 might be involved in TLR2 and TLR4 expression and/or traffic, expression of antigen presentation molecules and participates in the control of IL12 and IL10 production in splenic MØ during *T. cruzi* infection.
Supported by:CNPq/FAPEMIG
Keywords: Trypanosoma cruzi; macrophages; socs2

HP089 - RAGE ACTIVATION, HIGH FAT DIET AND METFORMIN IN ACUTE TRYPANOSOMA CRUZI INFECTION.

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Chagas disease, caused by the parasite Trypanosoma cruzi, is a major cause of mortality in the endemic regions of Latin America. In humans the disease has three phases, acute, indeterminate, and chronic. We are studying the pathogenesis of acute Chagas' disease in the context of a high fat diet (HFD) to model the effect of the obesity epidemic on this endemic infection. We had previously demonstrated that during acute murine infection T. cruzi alters liver morphology and function. HFD improved survival and liver function compared with regular diet (RD) during acute infection.
Irrespective of diet, metformin, a glucose lowering drug, increased survival during infection. We hypothesized that HFD and metformin could target energy signaling pathways in the liver, therefore, inmice infected with T. cruzi, we investigated the effect of metformin and HFD on the AMP-activated protein kinase (AMPK) signaling pathway, inflammatory signaling and on levels of receptors for Advanced Glycation End Products (RAGE). We used immunoblot analysis to measure phosphorylated AMPK (active AMPK) and its target proteins mammalian target of rapamycin (mTOR), fatty acid synthase (FAS), Glut 4 and cholesterol efflux (ABCA1). Infection significantly increased pAMPK and HFD caused a synergistic increase. Metformin had no significant effect on pAMPK or its targets during acute infection irrespective of the diet. Neither metformin nor HFD had effects on inflammatory signaling, however, both metformin and HFD decreased the levels of liver RAGE. We infer that metformin treatment may regulate RAGE production during acute infection without targeting inflammatory signaling. This suggests that drugs that reduce RAGE production could represent a new approach to increase survival during acute Chagas disease. **Supported by**:National Institutes of Health (USA)

HP090 - EVIDENCE OF EARLY HIGH AVIDITY IGG PRODUCTION IN EXPERIMENTAL VISCERAL LEISHMANIASIS

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High production of policlonal antibodies is one of hallmarks of visceral leishmaniasis. Those antibodies levels are usually non-related to the parasite burden or its control by host immune system, with disease progression in their presence. Affinity of antibodies to antigens is dependent of immune cell synapses, especially from follicular dendritic cell selection of high avidity clones at lymphoid follicle. We are interested in the quality of those antibodies that could be easily detected in ELISA avidity assays, using several chaotropes as pH, Urea or SCN for elution. We study the specific IgG production and avidity in the experimental model of hamster infection with L. (L.) infantum chagasi, with follow up with sera collection and spleen parasite burden. ELISA shows IgG production after 15 days of infection with progressive increase until 60 days of infection remaining higher thereafter, but without correlation with spleen parasite burden. Most of those antibodies are resistant to Urea elution resulting in avidity levels as more than 60% resistant to 8 M Urea elution, unregard of time of infection. Using pH or SCN, those elution curves showed only highly affinity antibodies. We immunized rabbits with complete adjuvant and promastigote antigen, and tested in similar pH or SCN elution, which showed low avidity antibodies similar to T.gondii antigens, completely diverse from infection. Our data implies that the production of antibodies in L. (L.) infantum chagasi experimental infection appear to use pathways of antibody affinity maturation and plasma cell commitment diverse from usual adaptive T dependent process. Those altered immune response could be related to antigen exposure in infected animal with diverse processing than usual adaptive immune response and must be clarified in order to understand the mechanism of immune response in visceral leishmaniasis. **Supported by**:CNPq; PIBIC; LIMHCFMUSP
Keywords: Visceral leishmaniasis; avidity; antibodies

HP091 - IRON CONTRIBUTES TO *LEISHMANIA* SPP. INFECTION EFFICIENCY

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CBA mouse macrophages (MΦ) control the infection by *L. major*, while are permissive to *L. amazonensis*. Proteomic studies were carried out to compare protein expression in *L. major*- or *L. amazonensis*-infected MΦ. Using proteomics, we observed that proteins involved in iron metabolism, like the transferrin receptor (CD71) and heme oxigenase-1 (HO-1) were positively modulated in *L. amazonensis*-infected MΦ. This is the first attempt that evaluate in a comparative model of resistance and susceptibility the contribution of iron to parasite infection in vitro. Using ELISA, we confirmed higher expression of HO-1 in *L. amazonensis*- (18.34 ng/mL) compared to *L. major*-infected CBA MΦ (7.07 ng/mL). Using FACS analysis, CD71 showed to be highly expressed in *L. amazonensis*- (MFI 2.103) than in *L. major*-infected MΦ (MFI 472). In addition, using fluorescence microscopy, we observed that *L. amazonensis*-infected MΦ bind and takeup HoloTf in a higher extend than *L. major*-infected cells. Unexpectedly, no significant differences were observed in intracellular iron concentrations between these two types of infected cells. To further evaluate the role iron plays in the outcome of *Leishmania* infection, we modulated iron availability to *Leishmania*-infected cells using iron chelates or iron supplements. Iron depletion reduces in 90% the percentage of *L. amazonensis*-infected MΦ and in 70% the percentage of *L. major*-infected MΦ. On the other hand, iron supplementation caused an increase in the percentage of *L. amazonensis*-infected cells from 69.64 to 82.79%, as well as in the intracellular viability of both *Leishmania* species. In sum, these data indicate that although in comparison to *L. major*, *L. amazonensis* modulated MΦ proteins involved in iron metabolism in a higher extend, this metal seems to favor the survival of both parasite species in CBA MΦ. These findings point out that the fine contribution of iron in intracellular survival of each *Leishmania* species deserves further investigation. **Supported by**: FIOCRUZ, FAPESB, INCT-DT, CNPq **Keywords:**Macrophage; leishmania; iron

HP092 - COMPARISON OF VIRULENCE AND IMUNE RESPONSE OF NC-1 AND NC-LIV ISOLATES OF *NEOSPORA CANINUM* PROTOZOAN

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Neospora caninum is a protozoan of the Apicomplexa phylum that affects several domestic and wild animals. This parasite cause neuromuscular commitment canines and abortion in bovines due to vertical transmission, respectively. A considerable economic loss in cattle production is due to this abortion. Additionally, it was found that different isolates of the parasite can induce distinct immune responses. Thus, this study aimed to compare the immune response profile and virulence triggered by Nc-1 and Nc-Liv strains of *N. caninum*. Both isolates were cultivated in HeLa cells and inoculated intraperitoneally in mice C57BL/6. For the survival assays, mice were infected with 1x107 tachyzoites of Nc-1 or Nc-Liv strains. It was observed a survival of 50% in the group infected with Nc-Liv. Mortality was not recorded in mice infected with Nc-1. Additionally, we found an increased weight loss in animals infected with Nc-Liv in comparison with the group infected with Nc-1. During acute phase of infection (7 days) a significant difference in peritoneal parasitism was identified, as well as in brain parasitism in the chronic phase of disease (30 days), evidenced by an enhanced number of DNA genomic copies found in each tissue. However, no significant differences were found in samples of lungs and liver during acute infection. The pattern of cytokine production demonstrated an induction of cytokines of Th1 response (IL-12, IFN-γ e NO) in a similar concentration in samples from the peritoneal fluids, lungs, livers (7 days) and brains (30 days). Nevertheless, the serum concentration of cytokines showed that mice infected with Nc-Liv also induced cytokines from Th2 (IL-4) e Th17 (IL-23 e IL-17A) profile. Moreover, an enhanced neutrophil recruitment was observed by Nc-Liv strain in comparison Nc-1.Thus, we conclude that Nc-Liv isolate possess an increased virulence in relation to Nc-1, which may be due to the induction of a mixed Th1/Th2/Th17 pattern of cytokines during the infection. **Supported by**:CAPES
Keywords: Neospora caninum; isolates; nc-1 and nc-liv

HP093 - ANALYSIS OF THE PARTICIPATION AND LOCALIZATION OF CD100 DURING INFECTION OF MACROPHAGES BY LEISHMANIA (LEISHMANIA) AMAZONENSIS

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Leishmaniasis is caused by trypanosomatids of the genus Leishmania that infect preferentially macrophages. Several factors influence the form and the severity of the disease, especially the species of Leishmania and the host immune response. Studies based on models of animals susceptible and resistant to Leishmania show how the host immune system affects the survival of the parasite. Considering the importance of macrophage activation status in Leishmania infection, the potential role of CD100 in the modulation of macrophage activation and our previous data that recombinant soluble CD100 (sCD100) increases infectivity by the parasite, we aim to characterize the effects of CD100 in the infection by Leishmania (L.) amazonensis. We found that CD100 transcript abundance does not change in macrophages after infection with amastigotes and promastigotes of L. (L.) amazonensis, and we will now analyze the distribution of CD100 protein in the macrophage throughout infection by immunofluorescence. Interestingly, both promastigotes and amastigotes were more infective in the presence of sCD100. CD72 receptor was confirmed on murine macrophages by Western blot and immunofluorescence, and we showed that this receptor is responsible for sCD100 increase in infection. To try to elucidate the pathway modulated by sCD100 we evaluated ERK phosphorylation and tyrosine phosphatase activity in infected macrophages, but did not observe any changes in the presence of sCD100. We then analyzed the effect of sCD100 on phagocytosis index and showed that the increase of infection is due, totally or partially, to the increase of parasite phagocytosis. However, actin polymerization was apparently not modulated by CD100. In vitro experiments indicated similar infection rates between wild type and knockout macrophages. Interestingly, infected knockout animals developed significantly smaller lesions than wild type, suggesting that sCD100 from other cells may influence lesion formation.
Supported by:FAPESP
Keywords: Leishmaniasis; cd100; macrophage

HP094 - INORGANIC COMPOUND WITH COPPER CORE AFFECTS THE ENDODIOGENY OF TOXOPLASMA GONDII

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Toxoplasma gondii, the agent of Toxoplasmosis, is an obligate intracellular protozoan able to infect a wide range of vertebrate cells including nonprofessional and professional phagocytes. Therefore, the drugs to control this parasite must reach intracellular compartments of the host cell. The most usual therapy for Toxoplasmosis is the combination of sulfadiazine and pyrimethamine, although this treatment is associated with adverse reactions. Because of this, the development of new drugs is necessary. The goal of this work was to test the in vitro anti-Toxoplasma activities of new compounds with a metal core of copper. Here we describe the cytotoxic effects of these compounds on T. gondii infecting LLC-MK2 host cells at a treatment time of 48 hours. Quantification of the parasite was performed by optical microscopy (analysis of cells' infection index). The copper compounds B2310 and B10109 after a 10 µM-treatment were able to arrest the infection index to a very low rate, and a 25 µM-treatment almost eliminated the infection of the host cells. This remarkable effect caused by the B10109 was irreversible as verified by re-infection in vitro. Mice infected with treated parasites presented a delay on death. Ultraestructural analysis showed changes after treatment: (a) B2310 affected the parasite's division after 48 hours of treatment with 10 µM of the compound; (b) infected cells treated with B10109 present parasitophorous vacuoles with degraded parasites. The mode of action of these compounds will be studied in more details but it may be possible that the induction of lysosomes fusion to the parasitophorous vacuole is occurring after B10109 treatment. **Keywords:**Toxoplasma gondii; inorganic compounds; copper core

HP095 - EXTRACELLULAR VESICLES SECRETED BY *TOXOPLASMA GONDII*

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Extracellular vesicles including exossomes from the endolysosomal system and microvesicles released directly from plasma membrane have been recently involved in cell to cell communication and in the manipulation of the host cell by pathogens in the establishment of infection. Exosomes, for instance, carry genetic material as miRNA and mRNA while microvesicles, which are larger, could carry completely functional proteins. *Toxoplasma gondii* depends on sequential secretion of molecules to invade and establish the parasitophorous vacuole, during the acute and chronic phases of infection. Our aim is to evaluate the secretion of extracellular vesicles on both tachyzoite and bradyzoite stages of *T. gondii* and to further isolate these microvesicles. In this purpose, we employed electron transmission microscopy to explore the secretion of these parasites. Parasites were obtained by cell culture and mouse brain cysts In bradyzoites, lateral microvesicles measuring approximately 70nm and larger vesicles, in the range of 500nm released from the basal portion of the parasite, were observed by TEM. In order to explore the morphology and biochemical nature of these vesicles we carried out the isolation of extracellular vesicles. Parasites were cultivated for 48 hours in vitro, isolated from the host cell by serial needle passages and purified in a 3µm filter. They were maintained in RPMI medium for 4h without FBS in order to stimulate secretion. Then the supernatant was harvested and submitted to a series of ultracentrifugation steps. The vesicles were obtained and analyzed by Dynamic Light Scattering, which showed two populations of circular structures measuring 75nm and 350nm and by negative staining in TEM, which confirmed the dimensions of both population of vesicles. The improvement of the isolation protocol to obtain a higher quantity of vesicles is in progress and will permit the elucidation of this interesting mechanism of communication between the parasite and host cell. **Supported by**:CNPq, CAPES e FAPERJ
Keywords: Microvesicles; dynamic light scattering; negative staining

HP096 - EFFECT OF N'N'-DIARYLUREAS IN LEISHMANIA AMAZONENSIS PARASITES

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Phosphorylation of translation initiator factor eIF2□ lead to an attenuation of cellular protein synthesis through inhibition of the eIF2.GTP.tRNAiMet ternary complex translation initiator. Much has been discussed about the role of eIF2 in regulation of the translation in tumor cells. In human myeloma cells, this factor acts like as growth regulator through the translation attenuation and consequent induction of apoptosis. eIF2 phosphorilation is mediated by four different kinases,HRI(heme regulated inhibitor),PERK(protein kinase R-like kinase),GCN2(general control nonderepressible 2) and PKR(protein kinase R). It has been reported that phosphorylation of eIF2 is important for the differentiation of Leishmania parasites, mediated by the activation of PERK.

The N'N'-Diarylureas are potent activators of the eIF2 kinases. Due to this ability, such compounds have been extensively tested in the control of tumor cell proliferation by attenuating translation and inducing apoptosis in cancer cells.

Our group have been studing the effects of N'N'-Diarylureas in the viability of Leishmania parasites. Since these drugs were defined as important activators of eIF2α, this could result in attenuation of the translational process of the parasite, as well as change their differentiation mechanisms. We focused on two variations of N'N'-Diarylureas, I-17 and RYF-14, and determined the inhibition concentrations (IC50) to promastigotes of L. amazonensis and RAW 264.7 cell lines through MTT assays(Cell Titer proliferative Assay) and subsequent calculation of the survival rate of cells using GraphPad Prism 5.0. Preliminary experiments using Infection index tests indicate a reduction of infection in RAW cells treated with I-17 and RYF-14, suggesting an effect of these compounds in the control of infection. New experiments will be performed to investigate the pathways that can be induced by these compounds and lead to a reduction of infection, such as nitric oxide. **Supported by**:CNPQ
Keywords:N'n'-diarylureas; eif2 phosphorylation; l. amazonensis

HP097 - *TRYPANOSOMA CRUZI* INDUCES NEUTROPHIL EXTRACELLULAR TRAPS (NET) FORMATION IN HUMAN NEUTROPHILS

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Early interactions between parasites and cells of the innate immune system are foreseen as determinants for parasite survival and disease outcome. *Trypanosoma cruzi*, the causative protozoan of Chagas disease has a complex life cycle with intra and extracellular forms. Neutrophils are the major population of circulating leukocytes, which upon various stimuli migrate to different tissues. Activation of these cells could lead to the release of neutrophil extracellular traps (NETs), scaffolds of decondensed chromatin associated with some granular and cytosolic proteins with microbicidal properties. The aim of the present study was to investigate the capacity of different developmental forms of *T. cruzi* to induce NET release on human neutrophils. Thus, isolated human neutrophils were incubated with *T. cruzi*, and NET release measured in the culture supernatants by picogreen assay. Our results demonstrated that blood trypomastigotes at 5 parasites: 1 neutrophil ratio induce NETs. Moreover, using scanning electron microscopy and propidium iodide labeling we demonstrated that NETs are able to trap and kill blood trypomastigotes. Culture trypomastigotes were also able to induce NET extrusion and a bell shape curve of NET release was observed testing different parasite:neutrophil ratios. Epimastigotes do not induce NET release from human neutrophils. Therefore, our results presents a novel microorganism, *Trypanosoma cruzi* in differents developmental forms, capable of induce NET release in human neutrophils.
Supported by: CNPq, FAPERJ and CAPES
Keywords: Trypanosoma cruzi; neutrophils; net

HP098 - TREATMENT WITH PUTRESCINE REDUCES THE ACTIVE PENETRATION CAPACITY OF *TOXOPLASMA GONDII* IN MURINE MACROPHAGES

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Toxoplasmosis is caused by *Toxoplasma gondii*, an obligate intracellular protozoan. 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 found in the inner leaflet of the plasma membrane of normal cells. Apoptotic cells expose PS that releases transforming growth factor-beta1 in macrophages reducing nitric oxide (NO) production. NO is a microbicidal agent. The subpopulation that exposes PS (PS^+) invades the host cell by active penetration and becomes located in tight-fitting vacuoles. The subpopulation that does not expose PS (PS^-) enters host cells by phagocytosis and remains in loose-fitting vacuoles. Only the PS^+ subpopulation inhibits NO production. Polyamines are polycationic molecules with important physiological roles; its cationic profile gives affinity to anionic molecules like PS. The aim of this study was to evaluate if the polyamine putrescine is able to block PS exposed by the PS^+ subpopulation and alters the entry process in macrophages. Subpopulations of *T. gondii* were separated with magnetic column. The PS^+ subpopulation was treated with putrescine (PUT) or not and in addition PS^- and the total population were used to infect activated macrophages. The entry process was analyzed by light microscopy. The viability of macrophages and tachyzoites after treatment with putrescine was verified by MTT and flow cytometry, respectively, and did not change. Reduction in the internalization rate and increase in loose-fitting vacuoles were detected when the PUT subpopulation was compared with PS^+ infection. This work show how putrescine affects the PS exposed by *T. gondii* and further indicates the importance of PS in the active penetration process.**Supported by**:FAPERJ, UENF, CAPES
Keywords: Toxoplasma gondii; phosphatidylserine; putrescine

HP099 - EFFECT OF THE TREATMENT WITH THE PALADACYCLE COMPLEX DPPE 1.1 ASSOCIATED TO DPPE 1.2 ON *LEISHMANIA (LEISHMANIA) AMAZONENSIS* INFECTION IN VITRO.

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Leishmaniasis affect 12 million people worldwide and 350 million are at risk of infection. Troubles in therapy of the various forms of leishmaniasis and parasite resistance to drugs currently used have led to new treatment schedules by association of compounds aiming to prevent or delay parasite resistance, besides improving the efficacy and reducing the course and toxicity of treatment. Previous data from our laboratory showed the leishmanicidal effect of two palladacycle complexes, DPPE 1.1 and DPPE 1.2, on in vitro and in vivo infection by *Leishmania (Leishmania) amazonensis*, as well as the immune modulation in treated animals. In the present study the action of DPPE 1.1 associated to DPPE 1.2 on *L. (L.) amazonensis* infection in vitro was evaluated. Bone marrow macrophages were infected with *L. (L.) amazonensis* for 24 h and treated with several combinations of DPPE 1.1 plus DPPE 1.2. Controls were carried out with several concentrations of either DPPE 1.1 or DPPE 1.2 alone. The infection index values of treated cultures was calculated 72 h after treatment and used for an isobologram construction. The data showed that the IC50 of DPPE 1.1 associated to DPPE 1.2 was 2 times lower than the IC50 of the each palladacycle compound alone and the isobolographic analysis showed a synergistic action of the two compounds. These results support further studies of this association therapy in vivo and experiments with *L. (L.) amazonensis*-infected BALB/c are currently in progress. **Supported by**:CAPES
Keywords: Leishmania; synergism; palladacycle complex

HP100 - IMMUNE RESPONSE TO LEISHMANIA GUYANENSIS IS BIASED TO TH1 AND PROTECTS BALB/C MICE AGAINST INFECTION WITH LEISHMANIA MAJOR

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Cross-immunity in experimental leishmaniasis models of infection are already reported in monkeys, man and mouse, and concomitant immunity is well accepted. These models are useful for understanding the mechanisms used by the host to restrain parasite growth and confer resistance, with implications in future attempts to vaccines, epidemiology and diagnosis. BALB/c mice are widely studied models to understand the mechanisms of resistance and susceptibility to leishmaniasis. When infected with L. major, BALB/c mice develop progressive lesions and are unable to control the infection. This outcome is attributed to a Th2-type response, with high levels of IL-4, which contrasts with C57BL/6 mice, resistant to the infection due to a Th1-type response, with high levels of IFN-γ, which induces the production of nitric oxide by macrophages. On the other hand, when infected with L. guyanensis, BALB/c mice spontaneously heal lesions and completely eliminate the parasites. The mechanism by which BALB/c mice eliminate L. guyanensis and resist infection is poorly understood. In this study, we measured the production of IFN-γ and IL-4 by the draining lymph nodes of BALB/c mice infected with L. guyanensis. Our results show that, unlike L. major-infected BALB/c mice, L. guyanensis-infected animals produce IFN-γ, but not IL-4, showing a bias to Th1-type rather to Th2-type immune response. In order to verify whether this immune response was efficient to control L. major infection, BALB/c mice, previously or concomitantly infected with L. guyanensis, were also infected with L. major. We verified that infection with L. guyanensis restrains lesion induced by L. major and also partially controls parasite burden. These results indicate that the Th1-type biased immune response induced by L. guyanensis is able to down-regulate the Th2-type response usually induced by L. major in BALB/c mice.
Supported by:CNPq, CAPES, FAPEMIG
Keywords: Leishmania; immune response; resistance/susceptibility

HP101 - IDENTIFICATION OF NEW INHIBITORS OF THE ENZYME ARGINASE FOR THE *LEISHMANIASIS AMAZONENSIS* INFECTION

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Cutaneous Leishmaniasis, caused by Leishmania parasites, affects millions of individuals worldwide. Despite its side-effect and cytotoxicity, antimonial compounds remain the first choice treatment to leishmaniais. In order to discover novel leishmanicial compounds in silico approaches (pharmacophore models) were employed to identify putative inhibitors of arginase, a key enzyme in polyamines biosynthesis that is essential for parasite proliferation. Herein, we report the in vitro assays for three of the compounds selected by this approach, against both promastigote and amastigote stages of *Leishmania amazonensis*. First, cell viability (toxicity) was assessed by MTT assay, using different compound concentrations (0.1, 1, 10,100µM/ml and 1M/ml of S783579, M5171 or A4021). After, 24h cytotoxic effects on human cells was observed. Next, stationary-phase *L. amazonensis* promastigotes were incubated with each compound (0.1, 1, 10,100µM/ml and 1M/ml) for 120h. No reduction in parasite viability was observed. On the other hand, all compounds showed antileishmanial activity (100µM/ml) for 72h, as measured by promastigote viability, when the assays were carried out with human monocyte-derived macrophages, infected with *L. amazonensis* amastigotes. This results show the effectiveness of our in silico approach regarding the selection of compounds that reduce *L. amazonensi*s replication. Moreover, the low cell toxicity and chemical complexity of S783579, M5171 e A4021 make them good candidates for lead optimization and future drug design efforts towards novel treatments for cutaneous leishmaniasis caused by *L. amazonensis*. **Supported by**:FAPESB
Keywords: Leishmania amazonensis; inhibitors; arginase

HP102 - *TRYPANOSOMA CRUZI* TRYPAREDOXIN II INTERACTS WITH DISTINCT PEROXIREDOXINS UNDER PHYSIOLOGICAL OR OXIDATIVE STRESS CONDITIONS

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The antioxidant system centered on trypanothione is critical for the survival of *Trypanosoma cruzi*. Only two tryparedoxins were identified in the parasite´s genome, tryparedoxin I (TcTPNI) and II (TcTPNII). TcTPNII can transfer reducing equivalents to cytosolic and mitochondrial tryparedoxin peroxidases, TcCPx and TcMPx, respectively, supporting its functionality as a classic tryparedoxin. The aim of this study was to evaluate the interactome of TcTPNII in *T. cruzi* epimastigotes under physiological and oxidative stress conditions. In this sense, parasites expressing Histag-TcTPNII (transfected with pTex-HisTcTPNII) or parasites transfected with pTEx (control) were submitted or not to H_2O_2-treatment and afterwards to the pulldown technique. Upon two-dimensional electrophoresis, only the proteins present exclusively on the His-TcTPNII gels were sent to analysis on a Q-Tof Premier API mass spectrometer (Waters). The biological function of the proteins identified were response to stimulus (3), cell organization and biogenesis (5), metabolic process (22) and others with unknown functions (7). In relation to cellular component, the proteins found were related to the nucleus (1), cytoskeleton (1), cytoplasm (11), membrane (1), cytosol (1), ribosome (6) whilst for some proteins (18) their intracellular localization could not be established. One of the highlights of these analyses was finding TcCPx or TcMPx as a TcTPNII partner under physiological or oxidative stress conditions, respectively. The involvement and significance of the proteins identified interacting to TcTPNII will be discussed. **Supported by**:FAPESP, CNPq and Capes
Keywords: Tryparedoxin; antioxidant pathways; reactive oxygen species

HP103 - HOW *TRYPANOSOMA BRUCEI* HANDLES WITH DNA STRAND BREAK PROMOTED BY PHLEOMYCIN

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A major challenge of the cell cycle is to faithfully transmit the genetic information from mother cell to daughter cells. For that purpose, cell has several checkpoint pathways to ensure the integrity of the DNA during replication and chromosomes segregation. In this study, we evaluated the response in a not synchronized *T.brucei* prociclic culture against a stress generated by 5 µg/mL of phleomycin during two hours. This treatment led to block of the DNA replication in more than 80% of the cells in S phase with a complete recovery five hours after phleomycin removal. Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) and Flow Cytometry essays, revealed DNA break (DNA Strand Break-DSB) profiles until two hours after phleomycin removal. Furthermore, indirect immunofluorescence (IIF) using the replication protein A (RPA) showed a positive punctuated pattern (DNA damage response) two hours after phleomycin removal, whereas that punctuated pattern disappears after three hours. Therefore, we suggest that damage repair occurs two hours after washing. However, DNA replication was only recovered 5 hours after phleomycin removal. These results lead us to propose that a signaling pathway inhibiting DNA replication in the DBS presence exists in *T.brucei* prociclic form. We are now investigating whether this signaling pathway is controling DNA replication origin activation and replication fork elongation. We also intend to evaluate if the ATM protein (Ataxia Telangiectasia Mutated) and ATR (ATM-and Rad3-related) are involved in this signaling pathway. Bolsa CNPq numero do processo: 140206/2015-9.
Keywords: Cell cycle arrest; dna strand break ; check point

HP104 - AMASTIN KNOCKDOWN IN LEISHMANIA BRAZILIENSIS AFFECTS PARASITE-MACROPHAGE INTERACTION AND RESULTS IN IMPAIRED VIABILITY OF INTRACELLULAR AMASTIGOTES

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Leishmaniasis, a human parasitic disease with manifestations ranging from cutaneous ulcerations to fatal visceral infection, is caused by several Leishmania species. These protozoan parasites replicate as extracellular, flagellated promastigotes in the gut of a sandfly vector and as amastigotes inside the parasitophorous vacuole of vertebrate host macrophages. Amastins are surface glycoproteins encoded by large gene families present in the genomes of several trypanosomatids and highly expressed in the intracellular amastigote stages of Trypanosoma cruzi and Leishmania spp. Here, we showed that the genome of L. braziliensis contains 52 amastin genes belonging to all four previously described amastin subfamilies, alfa, beta, gama and delta and that the expression of members of all subfamilies is upregulated in L. braziliensis amastigotes. Although primary sequence alignments showed no homology to any known protein sequence, homology searches based on secondary structure predictions indicate that amastins are related to a group of proteins that are components of eukaryotic tight junction complexes named claudins. By knocking-down the expression of delta-amastins in L. braziliensis, their essential role during infection became evident. delta-amastin knockdown parasites showed impaired growth after in vitro infection of mouse macrophages and completely failed to produce infection when inoculated in BALB/c mice, an attenuated phenotype that was reverted by the re-expression of an RNAi-resistant amastin gene. Further highlighting their essential role in host-parasite interactions, electron microscopy analyses of macrophages infected with amastin knockdown parasites showed drastic alterations in the tight contact that is established between the surface of wild type amastigotes and the membrane of the parasitophorous vacuole.**Supported by**:FAPEMIG - CNPq
Keywords: Leishmania braziliensis; amastin; macrophage

HP105 - ORAL ADMINISTRATION OF GW788388, A TGF-β SIGNALING PATHWAY INHIBITOR, REVERSES CARDIAC DAMAGE DURING CHRONIC PHASE OF CHAGAS DISEASE

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Studies published by our group demonstrated the involvement of TGF-β in Chagas cardiomyopathy development in T. cruzi-infected animals during the acute phase of Chagas disease. Activation of TGF-β signaling pathway was observed in the cardiac tissue of infected animals during the acute phase, favoring the increase of extracellular matrix proteins expression. TGF-β is the most import protein involved in fibrosis process. The aim of this study is investigate the effect of GW788388 treatment in TGF-β signaling pathway during the chronic experimental model of Chagas disease. To this end, animals C57Bl/6 were infected with T. cruzi colombian strain (10^2) and treated orally with 3mg/kg GW788388 after 120 days post–infection (dpi) in two treatment schemes: once a week or three times a week during 30 days. Electrocardiograms were performed after 120 and 150 dpi, before and after the treatment. The hearts of infected animals treated or not with GW788388 were collected and total proteins were extracted for the investigation of fibronectin and type I collagen expression by Western blot methods. In addition, collagen deposition was measured in the cardiac tissue of animals by histological methods. Also, circulating levels of TGF-β were evaluated by ELISA. Our data suggested that the chronic model presents 100% of cardiac damage after 120 dpi. GW788388 treatment improved the electrocardiographic state of infected animals: reduced the bradycardia, the PR interval and P wave duration. Furthermore, GW788388 treatment, three times a week, was able to reverse collagen expression in the heart of infected animals. We also observed that TGF-b circulating levels were increased due to T. cruzi infection and GW788388 treatment reverse these levels significantly. To date, the results are promisor and suggested a new possibility of fibrosis treatment in the chronic phase of Chagas disease.
Supported by:INSERM-FIOCRUZ / CNPq / FAPERJ / DECIT
Keywords: Chagas disease; fibrosis; tgf-β

HP106 - GENERATION OF *LEISHMANIA INFANTUM* LIPOPHOSPHOGLYCAN KNOCKOUTS

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Lipophosphoglycan (LPG) is the major surface glycoconjugate of *Leishmania* promastigotes and is associated with virulence. The LPG1 gene encodes a Gal-tranferase responsible for addition of lateral chain of glycoconjugates on LPG. LPG is known for its role in induction of immune response and protection of parasites. Recently in this study, we used targeting constructs and optimized the transfection conditions to delete the *L. infantum* LPG1 gene. Initially BA262 LD50 was determined for each antibiotic used for selection. After two rounds of transfection by electroporation, parasites from BA262 strain were selected by antibiotics (Hygromycin B 50 µg/ml and G418 70µg/ml). Five clones of total KOs and heterozygotes parasites were isolated and tested for expression of the LPG1 gene. After deletion, the LPG1 gene was rescued by transfection in pLeish Zeo encoding the LGP1 gene (ADDBACK) and the selection was performed using Zeocin (100µg/ml). The gene ablation and restoration in BA262 strain LPG1KO and ADDBACK was evaluated by Western blot, agglutination test and immunofluorescence. The parasites obtained will be used to infect mice and human macrophages and neutrophils to evaluate the role of L. infantum BA262 LPG in the induction of inflammatory mediators and signaling pathways. This will be important to understand host-parasite interactions and for development of strategies to control Leishmania infection. **Supported by**:CNPQ
Keywords: l. infantum knockout; lipophosphoglycan (lpg); lpg1

HP107 - OXIDATIVE AND ENERGETIC EVALUATION OF *STRIGOMONAS CULICIS* WILD TYPE, WILD TYPE RESISTANT AND APOSYMBIOTIC STRAINS

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Strigomonas culicis is a monoxenic protozoan found in the midgut of several mosquitoes, presenting a life cycle restricted to the epimastigote form. Among its peculiarities, there is the presence of an endosymbiotic bacterium, but its role is not completely clear, being related to the supply of heme and iron, key molecules in energy and oxidative metabolisms. These protozoa colonize the midgut of hematophagous insects, a reactive oxygen species enriched environment, however *S. culicis* antioxidant mechanisms involved were not elucidated, being crucial for the comprehension of protozoa-insect interactions. In this work, we analyzed *S. culicis* oxidative and energy metabolisms, comparing three different strains: wild type, aposymbiotic and H_2O_2-resistant wild type. Aposymbiotic strain were more susceptible to pro-oxidants, being more dependent of fermentative metabolism, while wild type showed higher resistance to these molecules, especially those that produce an increase in H_2O_2 levels, suggesting a dependence of the mitochondrion as main energy source. As expected, resistant wild type strain showed a greater resistance to the oxidative challenge, being more dependent to oxidative phosphorylation. Supporting this hypothesis, the resistant strain demonstrated higher oxygen consumption and an increased activities of citrate synthase and mitochondrial complex IV. The resistance induction also led to increase of iron and heme intracellular levels, as well as a greater colonization of Aedes aegypti gut ex vivo. Despite physiological changes, the H_2O_2-resistance did not affect the wild type strain morphology, showing typical ultrastructural aspects, including the mitochondria. **Supported by**:CNPq
Keywords: Strigomonas culicis; bioenergetics; oxidative stress

HP108 - *TITYUS SERRULATUS*' VENOM *VERSUS* CHAGAS' DISEASE: A NEW PROMISING DRUG FOR AN OLD HEALTH PROBLEM

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Introduction: Chagas disease (CD), caused by *Trypanosoma cruzi* (Tc), is a worldwide disease which for decades has few drugs available with high toxicity/many side effects. Tityus serrulatus venom (TsV) and its fractions are studied around their immunomodulatory effects [induction of inflammatory mediators' production, as nitric oxide (NO) and proinflammatory cytokines by macrophages(MO)], crucial to control Tc growth/dissemination. Mitogen-activated protein kinases (MAPK) are involved in many cellular programs, also being activated during Tc infection and by TsV stimulus. Our goal was to unveil the immunomodulatory mechanisms and determine the potential effects of TsV and its fraction 7 (Ts7) during Tc infection. **Methods and Results:** C57Bl/6 mice peritoneal MO were stimulated with Tc antigen(AgTc-10mg/ml) and/or TsV(400mg/ml). Cell lysates MAPK were analyzed by western blot. MO were stimulated with Ts7(100|50|25mg/ml) and supernatant was collected after 24/48h for NO. TsV and Ts7 augmented MO microbicidal potential through higher NO production, which resulted in less trypomastigotes release and impairment of amastigotes replication, without interfere with Tc uptake. TsV and Ts7 induced proinflammatory (IL6,IL12,TNFa,IFNg) and decreased the anti-inflammatory IL10 levels produced by infected MO. We found TsV synergizes with AgTc regulating p38 MAPK, increasing its activation in a time-dependent way. Tc pre-stimulation with TsV suggested the venom could act directly on Tc disturbing its capacity to do the trypomastigote-to-amastigote morphogenesis. Ts7 was the main fraction inducing great NO amounts, suggesting a group of key molecules present in TsV able to modulate the immune response to Tc infection. Peptides derived from the same chromatography peak as Ts7 were also tested for NO induction unveiling a new target(TsIV-Asn) to TsV immunomodulatory potential. **Conclusion:** Results showed TsV as a potent immunoregulator and candidate as a new drug design for CD treatment. **Supported by**:CNPq; CAPES; FAPEMIG
Keywords: Trypanosoma cruzi; nitric oxide; tityus serrulatus

HP109 - HOST AUTOPHAGY INDUCTION DECREASES PHAGOCYTIC CAPABILITY IN MACROPHAGES BUT NOT IN HEART MUSCLE CELLS DURING *TRYPANOSOMA CRUZI* INFECTION

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Chagas disease is caused by *Trypanosoma cruzi* and cardiomyopathy is one of the most important clinical manifestations. Many pathways are related to parasite internalization, including phagocytosis and autophagy. In the literature, studies have been described autophagy in this interface, but its role is still unclear in *T. cruzi*–host cell infection. Regarding autophagy, a pathway that leads to degradation of cellular components and signaling, it is suggested that exacerbation downregulates scavenger receptors in phagocytic cells. In this work, we evaluated the participation of autophagy induction in *T. cruzi* internalization by macrophages and heart muscle cells (HMC). First, bloodstream trypomastigotes (Y strain) were incubated for 24h in starvation medium, labelled with TMRE and TO-PRO 3 iodide to evaluate parasite´s viability. Then, macrophages and HMC were infected with these incubated parasites. Starvation did not change parasite viability and infection in both cells, indicating that this stimulus do not affect the parasite, unlike rapamycin, another autophagy inducer also tested. Therefore, starvation was used to exacerbate autophagy in host cells 2h before infection (MOI 50:1) for 90 min, continuing induction during interaction. Autophagy decreases infection 50% in macrophages, but not in HMC. To associate phagocytic capability to autophagy modulation, we incubated starved cells with dead parasites and Zymosan particles (MOI 10:1) for different times. Starved HMC did not interact with inert particles, endorsing low phagocytic profile. While starved macrophages reduced internalized dead parasites and Zymosan, 2-fold comparing to control. However, when we analyzed 24h-interaction pointed more particles present in starved cells, also suggesting an influence on degradation. Our data reinforces an autophagic role in *T. cruzi* infection, indicates that professional and non-professional phagocytic cells respond to autophagy through different pathways. **Supported by**:CAPES,FAPERJ, CNPq and FIOCRUZ
Keywords: Trypanosoma cruzi; autophagy; phagocytosis

HP110 - EVALUATION OF MONOCYTE SUBSETS AND CYTOKINES IN CUTANEOUS AND MUCOSAL LEISHMANIASIS

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Monocytes can play a role in the immunopathogenesis of cutaneous (CL) and mucosal (ML) leishmaniasis. Monocytes are identified as CD14hiCD16-, classical; CD14hiCD16+, intermediates; and CD14loCD16+, non-classical monocytes.The aim of this study was to assess the proportions of monocytes subsets and tumor necrosis factor (TNF) and interleukin 10 (IL - 10) production in whole blood cultures from CL and ML patients, before and after treatment. Peripheral blood from patients (31 CL and ML 23) and controls (n = 54) was used to identify monocytes and for whole blood cultures to measure cytokines. Cultures were stimulated with toll-like receptor agonists (Pam3Cys and LPS, for TLR2 and TLR4, respectively) or *L. braziliensis* antigens (AG). An increase in the percentage of CD16+ monocytes, especially CD14loCD16+monocyte subset, was detected in CL but not in ML patients, before treatment. After treatment, the percentages of these monocytes in CL patients back to similar levels of those from controls. There was also a reduction in the percentages of CD16+ monocytes after treatment of ML patients. TNF and IL-10 levels were similar in cultures from patients and controls. Among the stimuli used, only the AG did not induce significant amounts of IL-10 in patient cultures. After treatment, TNF concentrations decreased in CL cultures, except when the stimulus was Pam3Cys, which increased TNF levels. The IL-10 concentrations were not significantly altered after treatment of CL patients. In ML cultures no significant differences were detected between the concentrations of TNF and IL-10 produced before and after treatment. Data indicate that percentages of CD16+ monocytes are increased in CL. Also, they suggest that monocytes from CL or ML patients showed a decreased capacity to produce IL-10 in response to AG, what can hamper the control of the inflammatory response; and the ability of monocytes to be activated through TLR2 can be suppressed in active CL. **Supported by**:CNPq, FAPEG, CAPES
Keywords: Monocytes; leishmaniasis; cytokines

HP111 - LAMININ ISOFORMS INDUCE NEUTROPHIL EXTRACELLULAR TRAPS (NETS) AND MODULATE NETOSIS INDUCED BY *LEISHMANIA AMAZONENSIS*

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Neutrophils upon various stimuli such as Leishmania amazonensis (La), release neutrophil extracellular traps (NETs), scaffolds of decondensed chromatin associated with granular and cytosolic proteins, in a process named NETosis. Leishmania promastigotes (La) are inoculated by the insect vector in a pool of blood, in close contact with neutrophils and proteins of the extracellular matrix (ECM) such as laminin. Here, we studied the interaction of human neutrophils isolated from human blood with laminin isoforms, alone or in combination with La. Firstly, the expression of α6 integrin, a major laminin receptor (VLA-6/CD49f), was shown to be expressed in around 70% of the human neutrophils used. Next we evaluate if neutrophils stimulated with laminin isoforms (111, 211, 332, 411, 421 and 511) would be capable of inducing the release of NETs. Our results show that all isoforms of laminin were able to induce NET release, in a process involving the recognition of laminin isoforms by VLA-6. Interestingly, the laminin isoforms 411 and 511 were able to modulate NETosis induced by La promastigotes. We also evaluate possible differences between NETs induction by neutrophils by plastic adhered-laminin or laminin in suspension. Our results revealed that neutrophils stimulated by laminin isoforms, both adsorbed or in suspension, were able to release NETs. NET release by both 411 and 511 laminin isoforms is dependent on neutrophil elastase, an enzyme that participates in the chromatin decondensation process. Interestingly, we evidenced that the α1, α4 and α5 laminin chains colocalize with NETs induced by PMA or La. Together, our data show that laminin induces the release of NETs and that isoforms 411 and 511 modulate NETose induced by La. We thank the Hemotherapy Services of Hospital Universitario Clementino Fraga Filho, UFRJ. **Supported by** FAPERJ, CAPES and CNPq.**Keywords:**Neutrophils; extracellular matrix; leishmania amazonensis

HP112 - EFFECT OF BROWN SPIDER PHOSPHOLIPASE-D RECOMBINANT TOXIN IN LEISHMANIA (L.) AMAZONENSIS AND LEISHMANIA (V.) BRAZILIENSIS

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Phospholipases-D are enzymes that cleave the phosphodiester linkage between the phosphate and headgroup of certain phospholipids, forming an alcohol (often choline) and a cyclic phosphate. This specifity restricts the action of toxins to phospholipid presenting a hydroxyl moiety, including ceramide-based lipids like sphingomyelin and phosphatidylcholine.
Several studies indicate that trypanosomatid infections depend of their surface glycoconjugates and lipid derivatives. Moreover, data on the action of new recombinant toxins will provide new grounds for the search of new targets and therapeutic drugs.
In this work we analyze the ultrastructure of amastigotes and promastigotes of L. amazonensis and L. braziliensis treated for three hours with a Loxosceles intermedia recombinant phospholipase-D (LiRecDT1), and also a mutated form of the enzyme that contains an alanine substituting a histidine residue at position 12 in the conserved catalytic domain of LiRecDT1 (LiRecDT1H12A). It is known that this mutation reduces drastically sphingomyelinase activity in an endothelial cell line derived from the rabbit aorta, but there are no studies of its action in parasites. In LiRecDT1 treated L. amazonensis amastigotes we demonstrated, by electron micrographs, an increase of vesicle numbers in the cellular body of parasites, and in some cases disruption of the plasma membrane, in the region of vesicles accumulation. In LiRecDT1treated L. braziliensis amastigotes we noted loss of cytoplasmic material. For both Leishmania species, amastigotes appear to be more sensitive than promastigotes.
In dot blot assays we demonstrated the interaction of LiRecDT1 with inositol phosphorylceramide, the major sphingolipid expressed in Leishmania promastigotes, that could explain the morphological changes showed above. These results may bring new insights and also corroborate previous studies considering inositol phosphorylceramide as a potential target in leishmaniosis therapy. **Supported by**:CAPES; CNPq
Keywords: Phospholipase-d; leishmania; inositol phosphorylceramide

HP113 - MICROBIOTA INFLUENCES THE INFLAMMATORY RESPONSE DURING ORAL INFECTION WITH TRYPANOSOMA CRUZI, PROMOTING UPREGULATION OR TOLERANCE INDUCED

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Chagas' disease is caused by the protozoan Trypanosoma cruzi. Oral infection can cause a distinct outcome of the disease, when compared with other routes of infection. Development of innate immune response is closely related to the wide variety of indigenous bacteria. These bacteria are closely related to epithelial cells and components of the immune system. Thus, in conventional (microbiota-bearing) animals, the indigenous microbiota maintains a "physiological" inflammatory state which is drastically reduced in animals maintained under germ-free (GF) condition. So, our hypothesis is that the microbiota influences the outcome of oral infections by T. cruzi. In order to test this hypothesis we infected GF and conventional CV Swiss mice with T. cruzi intragastrically. Our results showed that (CV) mice presented higher parasitemia levels than GF mice on the 9th, 14th and 18th days post-infection. We evaluated the hepatic damage and observed higher levels of ALT and AST in infected CV mice than in GF mice. Our next step was to evaluate the profile of the immune response in both groups. We observed higher levels of IL-12p70 and IL-17 in gut homogenates (colon) of infected GF mice compared to not infected GF, to infected CV mice and not infected CV mice. The same profile was observed for IL-10. The cytokine production in the mesenteric limph node reflected that of the gut. We also quantify monocytes and lymphocytes in the gut and mesenteric limph node. We found higher numbers of dendritic cells (DC) and Foxp3+ cells in CV mice compared to GF mice and no differences in numbers of macrophages, CD4+ and CD8+ lymphocytes were found in gut. Regarding the mesenteric limph node, we observed high numbers of DC, CD4+ and CD8+ lymphocytes in conventional mice. We conclude that the microbiota promotes parasite growth and pathology during oral infection with T. cruzi, probably by promoting a pro-inflammatory state that would recruit host cells and cause tissue damage. **Supported by**:CNPq, CAPES, FAPEMIG, REDOXOMA, REDE DE PESQUISA EM DOENÇAS INFECCIOSAS HUMANAS E ANIMAIS DE MG **Keywords:**Microbiota; trypanosoma cruzi; germ free mice

HP114 - CLASSICALLY ACTIVATED MACROPHAGES AFTER *TOXOPLASMA GONDII* INFECTION: GENE EXPRESSION AND ENZYME ACTIVITY OF INDUCIBLE NITRIC OXIDE SYNTHASE AND ARGINASE 1.

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Classically activated macrophages after interferon-gamma (IFN-γ) and lipopolysaccharide (LPS) treatment express inducible nitric oxide (NO) synthase (iNOS) and produce NO, a microbicide molecule able to control *Toxoplasma gondii* replication. However, *T. gondii* is able to subvert the cell-autonomous immunity persisting in activated macrophages. One of these strategies is the inhibition of NO production caused by iNOS degradation. Alternatively activated macrophages with IL-4 and IL-10 express arginase 1 (ARG1). Induction of ARG1 by macrophages after *T. gondii* infection has been described as an evasion mechanism, because ARG1 is important for the synthesis of polyamines necessary for cell division. However, induction of ARG1 in macrophages infected with different strains of *T. gondii* is still controversial. Thus, the kinetics of iNOS and ARG1 gene expression and enzymatic activity in macrophages infected with two strains of *T. gondii* was performed. Peritoneal macrophage cultured with DMEM supplemented with FBS activated for 24h with LPS and IFN-γ were infected with *T. gondii* of either RH or ME-49 strains. ARG1 activity was measured based on the reaction of α-isonitrosopropiophenon, and NO production by the Griess reagent. RT-PCR was used to determine mRNA expression of iNOS and ARG1. Activated macrophages infected with the ME-49 strain of *T. gondii* exhibited constant ARG1 activity similar to uninfected macrophages; macrophages infected with the RH strain presented reduced activity of ARG1 with infection time. Curiously, RT-PCR results of macrophages infected with the RH strain of *T. gondii* suggests less ARG1 and iNOS mRNA expression when compared with uninfected macrophages. Both strains were able to reduce NO production, but ME-49 infected macrophages regained NO production after 6h of infection. These results are important to better understand the evasion mechanisms used by *T. gondii* to persist in activated macrophages. **Supported by**:UENF, CAPES, CNPq, FAPERJ.
Keywords: Toxoplasma gondii; inducible nitric oxide synthase; arginase 1

HP115 - EVALUATION OF IMMUNE RESPONSES IN BALB/C MICE INFECTED WITH LEISHMANIA (LEISHMANIA) AMAZONENSIS AND TREATED WITH DPPE 1.2 ASSOCIATED TO THE RECOMBINANT CYSTEINE PROTEINASE RLDCCYS1

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Previous data from our laboratory showed in vitro and in vivo activity of the palladacycle complex DPPE 1.2 against L. (L.) amazonensis. The decrease of parasite burden was followed by immune modulation of treated animals characterized by increase of CD4+ and CD8+ lymphocytes in BALB/c lesions. Our data also showed a significant protection mediated by Th1 lymphocytes against homologous infection in BALB/c mice previously immunized with a recombinant cysteine proteinase from L. (L.) chagasi, rLdccys1. Cross reactivity between rLdccys1 and a cysteine proteinase of 30 kDa from L. (L.) amazonensis was also previously demonstrated. Taken together these findings led us to associate DPPE 1.2 to rLdccys1 for the treatment of BALB/c mice infected with L. (L.) amazonensis. Our results showed a significant decrease of lesion size in animals treated either with 13.4 mg/Kg/animal of DPPE 1.2 alone or associated to 200 µg of rLdccys1. In both groups there was a reduction of 99% of parasite load, whereas this reduction was of 75% in mice treated with rLdccys1 alone compared to controls which received PBS. A significant reduction of active TGF-β was also observed in foot lesions of all treated mice. Although the difference was not significant, lower concentration of TGF- β was found in foot lesions of BALB/c mice treated with DPPE 1.2 plus rLdccys1 compared to that from animals treated with DPPE 1.2 alone. Analysis of cytokine profile in foot lesions of treated mice will be performed by a Cytometric Bead Array and expression of CD4+ and CD8+ lymphocytes isolated from inguinal and popliteal lymph nodes will be evaluated by FACS. In vitro cytotoxicity assays will also be carried out by use of L. L.) amazonensis-infected macrophages as target cells. **Supported by**:FAPESP
Keywords: Leishmania amazonensis; dppe 1.2; rldccys1

HP116 - DIVIDING STAGES OF *TRYPANOSOMA CRUZI* PRESENT MONO- AND DIMETHYLATION IN LYSINE 76 OF HISTONE H3 WHILE TRIMETHYLATION IS HELD IN NON-DIVIDING PARASITE STAGES

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In several eukaryotic cells, Dot1 methyltransferases catalyzes the states of methylation of the lysine 79 in the histone H3. These methylations in lysine 79 are homologous to the methylation of the lysine 76 (H3K76) in *Trypanosoma brucei*, which regulate parasite replication. Trypanosomes have two Dot1 homologues, Dot1A and Dot1B. Dot1A is essential for growth and catalyzes mono- (H3K76me1) and dimethylation (H3K76me2) found during late G2 and M cell cycle phases. However, Dot1B catalyzes H3K76 trimethylation (H3K76me3) and its depletion is tolerated, although parasites showed aneuploidy, cell cycle alterations and impaired differentiation. Because *Trypanosoma cruzi* alternate between proliferative stages and non-proliferative and infective forms, we decided to investigate the presence of H3K76me1, H3K76me2 and H3K76me3 in these different parasite stages by using antibodies for different H3K76 methylations. We found by immunofluorescence and western blot analysis that H3K76me1 is detectable only in early mitosis, and that H3K76me2 appeared only during mitosis and cytokinesis phases of epimastigotes and of amastigotes growing in mammalian cells. On the other hand, H3K76me3 was observed throughout all cell cycle and in non-dividing trypomastigotes. These results were confirmed by mass spectrometry showing that all modifications were more abundant in epimastigote compared to trypomastigotes. In addition, we observed a high ratio of H3K76me1 and H3K76me2 (6.5 and 3.0) in epimastigotes and trypomastigotes compared to H3K76me3 (0.78). We also detected variable expression of the Dot1 enzymes by real time-PCR in the different parasite stages. We found higher levels of Dot1A compared to Dot1B in the proliferative stages. We concluded that H3K76 methylation is involved in the control of replication. Further studies may help to understand the role of this histone modification during the differentiation to trypomastigote forms of *T. cruzi*. **Supported by** FAPESP. **Keywords:**Trypanosoma; cell cycle; histone methylation

HP117 - EVALUATION OF RLC36 PROTEIN POTENTIAL ROLE IN LEISHMANIA INFANTUM (SYN. LEISHMANIA CHAGASI) LIFE CYCLE.

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Leishmaniasis are diseases caused by Leishmania genus protozoan and endanger 350 million people worldwide. Visceral leishmaniasis, the most severe clinical form, is caused in Brazil by L. infantum (syn. L. chagasi) and has the dog as the main reservoir near the human beings. Recently, we have evaluated antigenic features of a L. infantum gene called Lc36, which codes for a protein of unknown function (739aa) (DEL CISTIA, M.L., 2013; INPI-BR 10 2014 022868-3, 2014). This study aims to perform functional characterization of this gene in order to understand its role in the parasite life cycle. Bioinformatics analysis using GeneDB and TriTrypDB databases, and PSORT and BLAST tools showed that the protein rLc36 presents a possible nuclear localization signal and a DNA polymerase conserved domain known homologue to Saccharomyces cerevisiae TRF4 (COG5260) which are involved in Poly (A) polymerase activity and/or DNA repair. Quantitative PCR assays are being developed to assess the gene expression levels in the parasite life cycle and revealed, in preliminary results, that the gene is possible expressed only in amastigote, the form responsible for the disease clinical manifestations. Lc36 gene was amplified and then cloned into the expression vector pXG-'GFP+ at BamHI and EcoRV sites for leishmania transfection and topological protein characterization by GFP-tag (green fluorescent protein), as well as expression vector pET28a+ at NcoI and HindIII sites, for biochemical analysis with recombinant protein purified by 6-histidine-tag from Escherichia coli. The recombinant clones are currently being confirmed by sequencing prior to further functional experiments. **Supported by**:CAPES
Keywords: Leishmania infantum; antigenic response; protein function

HP118 - EFFECT OF TEMPERATURE AND PH ON VIABILITY AND SPHINGOLIPID PROFILE OF LEISHMANIA (VIANNIA) BRAZILIENSIS: AN INTEGRATED STUDY

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Leishmania (Viannia) braziliensis is the etiologic agent of cutaneous, mucosal and mucocutaneous leishmaniasis. Recent studies have demonstrated the importance of sphingolipid metabolism in *L. braziliensis* biology (Castro et al., 2013). The aim of this study was to analyze the effect of temperature and pH in *L. braziliensis* viability and sphingolipid profile. Parasites viability was determined by flow cytometry. It was verified that change of temperature of promastigote cultures maintained in medium pH 7.2 from 23°C to 35°C or from 23˚C to 36.5°C leads to a significantly decrease in cell viability after 24 h (viability decreased 35% and 90%, at 35˚C and 36.5˚C, respectively), it was also observed that the parasite presented a more rounded shape. On the other hand, in cultures kept at 23°C, a change of pH from 7.2 to 5.5 did not lead to any significant difference in cell viability or morphology. Also when both pH and temperature were changed from 7.2 to 5.5 and from 23°C to 35°C, it was not observed any significant differences in viability. Conversely, when parasites were cultivated in pH 5.5 at 36.5°C, a remarkable decrease of 48% on parasite viability was observed after 24 hours. Along these studies we also analyzed two different molecules: free ceramide and inositolphosphorylceramide (IPC). These molecules were extracted from promastigotes with organic solvents and the extracts were submitted to alkaline hydrolysis to remove glycerophospholipids. The intact ceramides and other sphingolipids were purified by combination of DEAE A-25 Sephadex chromatography, silicic acid chromatography and silica gel chromatography. Purified ceramide and IPC were identified by high performance thin layer chromatography and mass spectrometry. Ceramide structural details of parasite sphingolipids of control promastigotes grown at pH 7.2 at 23˚C, and stressed parasites (cultured at pH 5.5 at 23˚C and 35˚C) are currently under study. **Supported by**:FAPESP, CAPES, CNPq
Keywords: Leishmania; sphingolipid; stress

HP119 - THE ABILITY OF MONOCYTES FROM INDIVIDUALS WITH SUBCLINICAL *LEISHMANIA BRAZILIENSIS* INFECTION TO KILL PARASITES IS INDEPENDENT OF NITRIC OXIDE AND REACTIVE OXYGEN SPECIES.

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Human Cutaneous leishmaniasis (CL) caused by *Leishmania braziliensis*, presents an exaggerated Th1 response that is associated with ulcer development. Approximately 10% of individuals in an area of transmission of *L. braziliensis*, despite exposure to the parasite, did not show evidence of clinical disease and are considered to have a subclinical(SC) form of the disease. Macrophages are the primary cells infected by Leishmania parasites and two distinct groups of oxidants are important in the control of Leishmania by these cells: reactive oxygen species (ROS) and nitric oxide (NO). However a role for these mechanisms in human CL it is not established. The aim of this study is to evaluate the role of ROS and NO in the control of *L. braziliensis* infection by monocytes from CL patients and SC individuals infected with *L. braziliensis.* Monocytes from CL patients (n = 25) and SC individuals (n = 09), were infected with *L. braziliensis* at 5:1 ratio. The determination of the production of oxidative radicals by flow cytometry was performed by oxidation of DHR -123. The intracellular production of NO and ROS was determined by specific intracellular probes (DAF-FM diacetate and 2DCFDA-HCM). After infection with *L. braziliensis*, the expression of the oxidative burst by monocytes from CL patients was higher when compared to monocytes from SC individuals. The production of ROS was higher than the production of NO by monocytes from CL patients. However, the NO production was higher in monocytes from CL patients when compared with cells of SC individuals. Also, there is a positive correlation between the production of NO and lesion size in patients with CL. After treatment CL patients had a significant decrease of NO and the ROS production by monocytes infected with *L. braziliensis*. The production of NO seems to be more related to lesion development rather than protection. The leishmania killing by monocytes of CL patients is not dependent of ROS or NO. **Supported by**:National Institute of Health (NIH) grant AI30639-20 **Keywords:**Leishmania braziliensis; american tegumentary leishmaniasis; oxidative burst

HP120 - THE ROLE OF TLR9 IN THE INFECTION BY *LEISHMANIA AMAZONENSIS*

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Leishmania amazonensis (La) is the etiological agent of diffuse cutaneous Leishmaniasis. The genomic DNA of L. amazonensis is able to activate TLR9. In order to evaluate the importance of TLR9 in the course of infection by L. amazonensis, animals C57BL6 (WT) and deficient in TLR9 (TLR9 -/-) were studied. We compared the in vitro infection of La in macrophages from WT and TLR9-/-, we observed no difference. For in vivo studies, the animals were infected on the footpad with 2x105 promastigotes of La. To evaluate the initial response, the mice were euthanized 7 days post infection (DPI) and the parasite load were determined by limiting dilution (LDA). It was also observed no difference between groups. To determine the clinical profile the lesion growth was followed by means of pachymetry once a week and performed the LDA at the peak of infection and late stage. The TLR9 -/- showed increased in the lesion development compared to the WT, with the biggest difference in the peak of infection, however, with no change in late stage. Determination of the parasite load by LDA at the peak of infection (60 DPI) demonstrated that the animals TLR9 -/- showed higher parasite load compared to WT. No difference was observed in the late phase (120 DPI). After evaluate these results, a hypothesis of deficiency at the production of effector T cells that controls the infection was suggested. In order to test this hypothesis, T cells were assessed by FACS at peak of infection. We noted that the draining lymph node of the animal TLR9 -/- had the same number of TCD4+IFN-gamma+ as the WT, however, presented a deficiency in the production of TCD8+IFN-gamma+, which are essential for infection control. Probably, there was a failure in cross-presentation Of TLR9 -/- animals. At this time, we are studying this mechanism. Our results demonstrate the importance of TLR9 receptor and we are dedicated to understand in which cellular mechanisms it is involved. **Supported by**:FAPERJ; CNPQ **Keywords:**Leishmania amazonensis; tlr9; tcd8+

HP121 - NEUTROPHIL EXTRACELLULAR TRAPS (NETS) IMPAIR MONOCYTES DIFFERENTIATION INTO DENDRITIC CELLS

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Leishmania infection starts when an infected vector bites a vertebrate host, delivering parasites into the dermis, rapidly recruiting neutrophils and monocytes. Neutrophils are among the first line of defense against invading microorganisms, which can be eliminated by phagocytosis, degranulation or by a newly described mechanism named NETosis. It has been shown that Leishmania parasites induce the release and are killed by neutrophil extracellular traps (NETs). Following neutrophil influx, monocytes arrive at the site of parasite inoculation, and it has been proposed that monocytes-derived dendritic cells (mo-DCs) are essential for the development of a Th1 protective immune response against intracellular parasites. Here we investigated the impact of NETs on monocyte differentiation into dendritic cells and in their response to the parasites. NET-enriched supernatants, generated by activating human neutrophils with Leishmania, were added to monocytes plus IL-4 and GM-CSF. Dendritic cells differentiation in those cultures was monitored by distinctive surface molecules expression, cytokines production and parasite killing by monocytes treated with NETs. NET-treated monocytes were unable to fully differentiate into dendritic cells, and DNase treatment of NETs rescued the differentiation. NETs closely interact with monocytes and downregulate the expression of the IL-4 receptor, a mechanism that may explain the impaired differentiation of NET-treated monocytes. Monocytes treated with NETs differentiated into macrophages as determined by the increased expression of CD68, CD32 and CD163 and decrease in the expression of CD80. In addition, NET-treated monocytes were less efficient in killing the Leishmania. Therefore, our results show that NETs interfere with the monocytes response to the parasites, diminishing the formation of monocytes-derived dendritic cells and stimulating macrophage differentiation. **Supported by**:CAPES, FAPERJ e CNPq **Keywords:**Leishmania amazonensis; neutrophil extracellular traps; monocytes and dendritic cells

HP122 - *TITYUS SERRULATUS* (YELLOW SCORPION) VENOM AVOID IMMUNOSSUPRESSION CAUSED BY *TOXOPLASMA GONDII* INFECTION IN MACROPHAGES

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INTRODUCTION: *Toxoplasma gondii* (Tg), causer of Toxoplasmosis, is an intracellular parasite which “fools” the immune system. Chronically infected individuals can suffer relapse, leading to injuries in the organism. Inflammatory mediators released during infection are important to control the replication of the parasite, avoiding cases of disease reactivation. The venom of the yellow scorpion *Tityus serrulatus* (TsV) induces production of inflammatory mediators by immune cells, however, nothing is known about the immunomodulatory effects of TsV and its fractions in macrophages (MΦ) infected with Tg. METHODS AND RESULTS: Peritoneal MΦ from C57BL/6 mice were plated, infected at 1:1 (Tg:cell) ratio and incubated with TsV (100µg/ml) or its fractions: Ts6 (100µg/ml, Ts7 (50µg/ml). Parasite’s uptake and replication assays were done by counting cells after 4h, 24h and 48h of infection/stimulus, respectively. Cytotoxicity test (MTT) was measured at 24h and 48h after stimulation with TsV, Ts6 and Ts7. Supernatants were collected at 24h and 48h for measurement of nitric oxide (NO) (Griess) and cytokines (ELISA). We showed that Ts6, but not TsV nor Ts7, interfered with the parasite’s uptake. Tg infection induced reduction of NO levels produced by TsV, but remaining amount was still sufficient to control parasite's replication in MΦ as compared with infected non-stimulated cells at 24h after incubation. Similarly, Ts6 and Ts7 were also able to control parasite’s replication until 48h of incubation. Moreover, TsV, Ts6 and Ts7 showed to be potent inducers of cytokines (IFN-γ, IL-12, TNF-α and IL-6), contributing to MΦ microbicidal response. Of note, the parasite did not subvert the levels of cytokines induced by the compounds. TsV and its fractions were not prejudicial to the viability of MΦ (MTT assay). CONCLUSION: Our data suggest that, TsV, Ts6 and Ts7 have potential effect in the control of Tg infection in MΦ, mainly avoiding the immunosuppression induced by the parasite. **Supported by**:CNPq/FAPEMIG/CAPES
Keywords: Toxoplasma gondi; nitric oxide; tityus serrulatus

HP123 - EVALUATION OF REINFECTION OF BALB/C MICE WITH VIRULENT *TOXOPLASMA GONDII* STRAIN

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Toxoplasmosis is usually asymptomatic in immunocompetent individuals, but can cause severe symptoms in AIDS patients and congenitally infected fetuses. Several authors have reported that primary infection with *T. gondii* causes an effective immunity, preventing the host against reinfection. However, cases of congenital toxoplasmosis were reported in immunocompetent women chronically infected, indicating the possibility of reinfection in humans. Recent studies have demonstrated the occurrence of reinfection of BALB/c mice by recombinant Brazilian strains and experimental studies have shown that animals chronically infected with ME 49 (type II) strain are not reinfected when challenged with virulent RH strain (type I), but there is no information about induced protection by primary infection with avirulent or intermediate virulence strains. In this study, we evaluated the infection of Balb/c mice with genetically distinct *T.gondii* strains, using ELISA with strain -specific synthetic peptides and real time PCR. Our data show that humoral immune response induced by the primary infection with VEG or ME49 strain was unable to prevent re-infection by virulent RH strain since the humoral response was specific for epitopes of secondary strain as demonstrated by ELISA with strain-specific synthetic.These results show that serotyping with synthetic peptides is a promising tool for the study of the protective response induced by infection with different *T.gondii* genotypes, contributing to therapeutic or vaccine approaches for toxoplasmosis.
Keywords: Toxoplasma gondii; toxoplasmosis; serotyping

HP124 - PURIFICATION OF GLYCOPROTEINS FROM NEOSPORA CANINUM IN AFFINITY COLUMN AND THE EVALUATION OF THE PROTECTOR EFFECT IN MURINE MODEL

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Introduction: *Neospora caninum* is an obligate, intracellular, coccidian, protozoan parasite of the phylum Apicomplexa, and Sporozoa, Neosporidia, and Neospora genera that naturally infects dogs and cattle. The study of the surface compounds of the parasite is very important because involved the interaction between the parasite and the host cells. The aims this study was to purify glycoproteins to *N. caninum* with affinity by Concanavalin A (Con-A) and analyze the protective effect in in vivo model. Methods: The soluble tachyzoite antigen (NLA) were separated by chromatography on Con-A affinity column and the protein profile were visualized by SDS-PAGE. C57Bl/6 mice were immunized with NLA and glycoprotein fraction and challenged with lethal dose of 3 x 107 tachyzoites/animal. Results: The purification has an efficiency of 9,72% to the bound fraction and the SDS-PAGE profile showed proteins with relative molecular masses of 27, 30-34, 41-42, 45-47, 85, 95 and 117 kDa. The indirect immunofluorescence results indicated that these glycoproteins were predominantly in the cellular surface of the tachyzoites. The protective effect of the glycoproteins of *N. caninum* in C57Bl/6 mice demonstrated that in 7 days, all animals from the control group and NLA group died and 40% from glycoprotein group survive. Conclusions: Our results suggest that glycoproteins from *N. caninum* purified by Con-A column demonstrate a potential protective effect against neosporosis. **Supported by**:CAPES
Keywords: Glycoproteins; neospora caninum; protective effect

HP125 - EVALUATION OF DOT ELISA FOR *TOXOPLASMA GONDII* MONITORING IN MEAT DESTINATED FOR HUMAN CONSUMPTION

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Toxoplasmosis is one of the most prevalent systemic parasitic infections in the world, affecting approximately one billion people and a significant part of the herds destined for human consumption. Ingestion of raw or undercooked meat containing viable cysts of *Toxoplasma gondii* is one of the main means of transmission of infection, with the occurrence of several outbreaks of human toxoplasmosis. Currently, there is no national monitoring program of meat quality to this protozoan, as industrial inspection is just macroscopic. In previous studies, we demonstrated by enzyme immunoassay (ELISA) that the meat juice, fluid obtained by thawing of meat, allows anti-IgG *T. gondii* detection in commercial cuts of rabbit meat. In this study, we improved this approach by developing and standardization of DOT ELISA, test that allows easy implementation and automation, enabling its use as a method of monitoring commercial cuts in slaughterhouses and industrial establishments. For standardization of the test were used 38 samples of rabbit meat juice, from experimental models of infection and commercial cuts of rabbit meat obtained from retail markets. Our results showed that the technique has good reproducibility, sensitivity and specificity, since the results were similar to the results of the ELISA (gold standard). The data obtained from this study will be of great value to the development of rapid commercial assays as immunochromatographic tests that may be used in surveillance and control programs of meat quality for human consumption.
Keywords: Toxoplasma gondii; meat juice; foodborne diseases

HP126 - CONFIRMATION OF INFECTION IN DOG FOR *L. (L.) INFANTUM CHAGASI* IN SOUTH OF MINAS GERAIS

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Visceral leishmaniasis (VL) is a chronic, systemic disease, which main domestic reservoir of the etiologic agent is the dog. The control programs recommend the conducting of serological surveys for the identification of positive dogs. This way, it was made a serological and molecular investigation of the disease from dogs (CVL) in Alfenas-MG, located in a non-endemic area, but with large flow of people from different regions of the country, some of them endemic for this disease. The detection of Leishmania (L.) infantum chagasi infection was performed by serological methods (DPP® Bio-Manguinhos; Elisa and RIFI, both in house), besides molecular methods, with conventional PCR (RV1/RV2) and qPCR (Linj31, Ldon and DNApol) in 87 dogs, from municipal kennel and veterinary clinics. A total of 91 samples (87 of whole blood samples, two spleen and two liver fragments) were analyzed by molecular tests. From the 87 analyzed serum samples, eight (9.0%) were reagents for LVC in DPP®, but only two (2.3%) were confirmed by ELISA and RIFI; and two (2.3%) were indeterminate. The spleen and liver samples were collected from the two seropositive animals, after euthanasia, and they were all positive by parasitological procedures and both molecular methods, conventional PCR and qPCR, while the two whole blood samples (2.2%) were positive only in qPCR. The sequencing of the DNA extracted from spleen of seropositive dogs showed total identity with L. chagasi sequence (AJ 000304.1 access). Positive serology results can be related to cross reactions with other parasites, since all the animals in the Kennel were asymptomatic. However, the first cases of CVL, from the city of Paraguaçu, were confirmed in dogs attended in veterinary clinics of Alfenas. Thus, the conduction of canine surveys in silent municipalities is a way to monitor the progression of the disease by avoiding the occurrence of outbreaks.
Supported by:CAPES, FAPEMIG, CNPq, FINEP, FMV-USP, UNIFAL-MG
Keywords: Canine visceral leishmaniasis; l.l.infantum; autochthonous cases

HP127 - E-NTPDASE OF *LEISHMANIA* MODULATES THE PRODUCTION OF NO AND INFLAMMATORY CYTOKINES BY MACROPHAGES INDEPENDENTLY OF ECTONUCLEOTIDASE ACTIVITY

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We have demonstrated that *Leishmania* uses E-NTPDase to facilitate the accumulation of adenosine, an anti-inflammatory molecule that participates in modulation of the immune response in infected cells. We decided to investigate whether the enzyme expression on the parasites also has an effect on the subversion of the immune response, independent of enzyme activity. For this, J774 cells were incubated with recombinant E-NTPDase of *Leishmania* (rNTPDase), with or without ectonucleotidase activity, for 3 h. Cells were washed and incubated for 48 h in the presence of IFNγ/LPS. The supernatant was used for measurement of NO by Greiss method and cytokines by ELISA. We found that the active enzyme inhibits the production of NO, TNF-α and IL-12 more effectively than the inactive enzyme. However, even in the absence of activity, rNTPDase is capable of reducing the production of NO and inflammatory cytokines in a dose dependent manner. The denaturation of inactive rNTPDase reverses the inhibitory capacity of the inflammatory response in stimulated macrophages. To prove that the rNTPDase has an immunomodulatory function, activity independent, 5 µg of inactive enzyme was pre-incubated with DIDS, an inhibitor of ectonucleotidase activity. Even in the presence of DIDS, the rNTPDase maintained the ability to inhibit around 40% production of NO, and about 60% the production of TNF-α and IL-12. More interestingly, when the inactive rNTPDase was preincubated with anti-NTPDase, to block the binding to the cell, the enzyme lost the ability to inhibit the production of NO and inflammatory cytokines, confirming the direct role of the *Leishmania* E-NTPDase in modulating the immune response in stimulated macrophages. These data indicate that E-NTPDase can promote *Leishmania* infection by inhibiting the production of inflammatory cytokines and NO, regardless ectonucleotidase activity. Currently, we are investigating the mechanisms involved in this process. **Supported by**:CNPq, CAPES, FAPEMIG
Keywords:E-ntpdase; macrophage; cytokines

HP128 - ABSENCE OF IGG3 AGAINST LEISHMANIA AT THE DIAGNOSIS IS RELATED TO THE EARLY HEALING.

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American Cutaneous Leishmaniasis (ACL) caused by *Leishmania spp* can spontaneously heal. However, in some patients, the lesion remains for months or even years after treatment. The aim of this study was to associate the antibody response to *Leishmania* antigens to the delay in the healing of the lesions. Serum from 15 patients diagnosed with ACL was obtained at diagnosis time, 6; 12 and 18 months after the starting treatment. Serum from 19 healthy subjects was used as control. The reactivity of IgG or IgG3 to crude extract of *Leishmania* was evaluated by ELISA. The IgG or IgG3 response to *Leishmania* antigens of patient's sera was higher than controls. Of seven patients that healed within one month after starting treatment, five had the IgG3 response similar or lower than controls. On the other hand, all patients that healed after 1 month of treatment showed IgG3 reactivity higher than controls. The IgG response to *Leishmania* in the group of patients that healed in one month was significantly higher than the controls. These data suggest that the absence of IgG3 response at the time of diagnosis is a good prognosis for healing of ACL. **Supported by**:DECIT/SCTIE/MS, CNPq, FAPEG, CAPES
Keywords: Leishmania; igg3; elisa

HP129 - DETECTION OF PARASITES OF THE SARCOCYSTIDAE FAMILY IN RETAIL BEEF BY HISTOLOGICAL AND MOLECULAR METHODS

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Foodborne diseases are an important cause of morbidity and mortality in human populations, reflecting directly on affected individuals' welfare, as well as overcrowding public health services. Considering foodborne parasites, protozoan of the Sarcocystidae family, such as Toxoplasma gondii and Sarcocystis spp, must be highlighted. In spite of great efforts by the developed countries, there is not an efficient sanitary control or a laboratorial certification program to detect cysts of these parasites in the meat of different species of animals destined to human consumption. The control of Toxoplasmosis by the sanitary inspection services in the slaughter lines is unviable, hence the T. gondii cysts are microscopic, therefore, invisible to the naked eye, differently of other meat borne zoonosis, like the Taeniasis. In addition to that, the usage of standard operational procedures and manipulation hygiene in the processes of slaughterhouses do not avoid the human infection by the ingestion of raw or undercooked meat, once this meat is already contaminated with T. gondii cysts by the animal previous infection. Even though Sarcocystis spp cysts are bigger than T. gondii cysts, they are not always detectable in muscular tissue during slaughter inspection, putting in risk the meat ready to be consumed. We propose to evaluate the efficiency of molecular methods, such as PCR and nested PCR, impaired with the histological procedures of HE staining and Laser Capture Microdissection in differential diagnosis of these parasitic diseases. As preliminary results, cysts obtained by microdissection of bovine hearts destined to human consumption were PCR positive for the Sarcocystidae family, proving the viability of this method. The results achieved by this project could contribute with new diagnostic approaches to the monitoring of meat quality, which is of great value to help with political decision making and allocation of resources destined to Food Safety.

Keywords: Toxoplasma; sarcocystis; sarcocystidae

HP130 - MIRNAS AND GENE EXPRESSION NETWORKS IN HEART OF TRYPANOSOMA CRUZI-INFECTED MICE

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Chagas disease is caused by the parasite Trypanosoma cruzi, and it begins with a short acute phase characterized by high parasitaemia followed by a life-long chronic phase with scarce parasitism. Cardiac involvement is the most prominent manifestation, as 30% of infected subjects will develop heart conduction defects leading to arrhythmias, myocarditis, fibrosis and

to investigate predicted and experimentally validated miRNA targets. With this approach we constructed CCC specific miRNA-Targets co-regulatory networks and found several hubs which dysregulated expression was validated by qRT-PCR. Conclusion: The identified networks may be potential regulatory mechanisms and also provide details of how miRNAs regulate gene expression during the establishment of each CCC clinical parameter: myocardium inflammation, arrhythmias, heart hypertrophy and fibrosis. **Supported by**:FAPESP

Keywords: Chagas disease; microrna; transcriptomics

HP131 - ROS ACT AS SIGNALING MOLECULES INDUCING ENHANCED REPLICATION OF *T. CRUZI IN VIVO*

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Introduction: Although some studies have suggested that ROS produced during the respiratory burst have an important role in *T. cruzi* control, other authors have demonstrated that ROS is important to cellular signaling and proliferation of this parasite. Previous results of our group showed that *T. cruzi* proliferation is significantly reduced when ROS production is inhibited in host cell. Objective: The aim of this study was to clarify the role of ROS during *in vitro* and *in vivo T. cruzi* infection. Methods and results: To evaluate the role of ROS in macrophage infection, these cells obtained from C57BL/6 WT and Phox KO (deficient in the gp91phox subunit of NADPH oxidase) mice were infected with parasites treated with different concentrations of H_2O_2. We found no differences in infection index of C57BL/6 WT macrophages using up to 200µM H_2O_2. Lower concentrations of H_2O_2 (50µM and 100µM) promoted replication of parasites in Phox KO macrophages, while 200µM H_2O_2 brought the parasitism back down. In a higher concentration (300 µM) H_2O_2 was toxic to parasites in both macrophages. Our next step was to evaluate if the treatment of the parasite with H_2O_2 could affect T. cruzi capacity to infect mice. Then we treated blood trypomastigotes of Y strain of *T. cruzi* with 100µM of H_2O_2 for 30 minutes, infected C57BL/6 WT mice by intraperitoneal injection of 10^3 blood trypomastigotes and followed the course of infection. Our results indicate that C57BL/6 WT mice infected with treated parasites presented significantly higher parasitemia compared with animals infected with control non treated parasites and this difference was more prominent at 8 days post-infection. Conclusions: Our results suggest that low concentrations of ROS contribute to growth and signaling events in *T. cruzi*, although the exact mechanism by which low oxidant production enhances infection remains to be elucidated.
Supported by:INCT Redoxoma, FAPEMIG, CAPES, CNPq, and REDE DE PESQUISA EM DOENÇAS INFECCIOSAS HUMANAS E ANIMAIS M
Keywords: Trypanosoma cruzi; reactive oxygen species; chagas disease

HP132 - EFFECT OF *TRYPANOSOMA RANGELI* ON PROTEASES INVOLVED IN *RHODNIUS PROLIXUS* EMBRIOGENESIS.

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Trypanosoma rangeli is a trypanosomatid that is considered pathogenic to its insect vector. Due to its hemolymphatic cycle, this parasite disseminates for several insect organs and can promote molecular changes which may interfere with the reproductive success. In this paper, we investigated the proteolytic changes in reproductive organs of *Rhodnius prolixus* infected by *T. rangeli.* In order to confirm the parasite infection, a multiplex polymerase chain reaction (PCR) was used as a tool, using specific primers for *T. rangeli*. The results demonstrated the presence of parasite cells in all the organs tested: midgut, fat body and ovary. To investigate the aspartic peptidase activity from fat body and ovarian follicle of *R. prolixus* was used a peptidic fluorogenic substrate (7- methoxicoumarin-4-acetyl-Gly-Lys-Pro-Ile-Leu-Phe-Phe-Arg-Leu-Lys(DNP)-D-Arg MIDA) and pepstatin A, a classical aspartic peptidase inhibitor. Additionally, we also investigated the cysteine peptidase activity using the peptidic substrate Z-Phe-Arg-AMC and E-64, a typical cysteine peptidase inhibitor. Our results showed a reduction in aspartic peptidase activity and a significant increase in the cysteine peptidase activity in the fat body and ovary from infected insects compared to control the non-infected ones. Further studies are needed to determine to what extent *T. rangeli* can change those level of aspartic peptidase activity, enzyme involved in the ovarian follicle degeneration and cysteine peptidase activity that is involved in autophagic cell death process in the *R. prolixus* embriogenesis. **Supported by**:Faperj, Fiocruz e Proppi-UFF
Keywords: Aspartic peptidase; cysteine peptidase; embriogenesis

HP133 - *TRYPANOSOMA CRUZI* NEEDS A SIGNAL PROVIDED BY REACTIVE OXYGEN SPECIES TO INFECT MACROPHAGES

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Background: During *Trypanosoma cruzi* infection, macrophages produce reactive oxygen species (ROS) in a process called respiratory burst. Several works have aimed to elucidate the role of ROS during *T. cruzi* infection and the results obtained are sometimes contradictory. Methods and Results: In order to investigate the contribution of ROS in *T. cruzi* infection, we utilized mice deficient in the gp91phox subunit of NADPH oxidase (Phox KO) and performed infections using macrophages. We show that macrophages from these mice produced less ROS than cells from C57BL/6 WT mice upon infection. Phox KO macrophages showed reduced parasitism after 48 hours of infection with Y and CL Brenner strains of *T. cruzi*, as compared to C57BL/6 WT macrophages. In addition, the number of trypomastigotes released in the supernatant of C57BL/6 WT macrophages was greater than in Phox KO macrophages after infection with both strains. This difference is not related to the uptake of parasites, since we demonstrated that both macrophages presented the same parasite uptake. Besides, this difference could not be attributed to NO production, which was similar in both macrophages stimulated with IFN-γ/LPS. To investigate if ROS were responsible for the lack of growth in Phox KO macrophages we inhibited ROS production by C57BL/6 WT macrophages using different antioxidants (SOD-PEG, CAT-PEG, NAC and apocynin) and the parasite replication was reduced. To evaluate if *T. cruzi* needs a signal provided by ROS produced by macrophages to thrive in this host cell we treated Phox KO macrophages or antioxidant treated-macrophages with H_2O_2 and observed that the parasitism could be recovered. To clarify further this issue, we treated *T. cruzi* with H_2O_2 before infection and showed that parasites treated with H_2O_2 can infect Phox KO macrophages similarly to C57BL/6 WT macrophages. Conclusions: This study indicates that ROS contributes to *T. cruzi* growth inside macrophages and increases overall parasitism. **Supported by**:INCT Redoxoma, FAPEMIG, CAPES, CNPq and REDE DE PESQUISA EM DOENÇAS INFECCIOSAS HUMANAS E ANIMAIS MG **Keywords:**Trypanosoma cruzi; reactive oxygen species; phox ko

HP134 - ANTI-*LEISHMANIA* SPP. EFFECT OF HYDROALCOHOLIC EXTRACT OF *ZINGIBER OFFICINALE*

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Leishmaniasis is a major neglected disease that, although affects mostly subtropical countries, is also endemic at several countries in Southern Europe. Clinical manifestations of the disease vary from ulcerated lesions on the skin and mucosa, to damage on internal organs. The drugs currently in use to treat leishmaniasis include pentavalent antimonials and amphotericin B. However, these have limited efficacy and high toxicity. On this matter, several studies have shown that plant extracts may represent a promising source of substances with diverse biological activities, including anti-Leishmania. The ginger (Zingibre officinale) hydroalcoholic extract has been described as biologically active, having been associated with anticancer and antimicrobial properties. The present study intends to evaluate the effects of ginger hydroalcoholic extract over the proliferation of L. amazonensis and L. infantum. Ginger rhizomes were dehydrated and macerated in hydroalcoolic solution (20 ethanol : 1 watter) for 5 days. After that, the solvent was removed by evaporation and the obtained dried extract was stored at 4°C protected from light. Axenic promastigote forms (106 cells/ml) of L. amazonensis and L. infantum were treated with different concentrations of the extracts for 120 hours, in order to establish the minimal inhibitory concentrations. The cytotoxicity test was performed on macrophage lineage RAW 264.7. Cell viability was assessed by the resazurin reduction assay. Apart from that, to evaluate its effects on intracellular amastigotes, macrophages previously infected with parasites were treated for 48 hours with the hydroalcoholic extract. The crude extract of Z. officinale was able to completely inhibit the growth of L. amazonensis and L. infantum at concentrations of 125 and 64.5 mg/ml, respectively. The hydroalcoholic extract showed no cytotoxicity over RAW 264.7 macrophages at the highest concentration evaluated in this study (CC50>500/ml). Finally, the treatment of macrophages infected with L. amazonensis and L. infantum was able to significantly reduce the number of amastigotes in these cells. Our results show the potential of the hydroalcoholic extract of ginger as a source of substances with anti-Leishmania spp activity. Future studies are still necessary to identify these substances. **Supported by**:FAPERJ **Keywords:**Atividade leishmanicida; zingibre officinale; l. amazonensis

HP135 - *IN VITRO* AND *IN VIVO* TRYPANOCIDAL ACTIVITY OF THE PTEROCARPANQUINONE LQB-118

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Chagas disease is a neglected disease endemic in Latin America with great socioeconomic impact. The therapy is very limited and is not effective in the chronic phase of infection. Previous studies from our group show that pterocarpanquinone LQB-118 has *in vitro* effect on trypomastigotes and intracellular amastigotes of *Trypanosoma cruzi* (Dm28c clone). The aim of this study was delineate the mechanism of action of LQB-118 on *T.cruzi in vitro* and evaluate its activity *in vivo* using experimental model of infection. Epimastigotes the parasite (Dm28c clone) were incubated in the presence or not of LQB-118 (0-5µM/ml) for 96 h/28°C and counted daily in Neubauer chamber. To evaluate whether LQB-118 toxicity for the parasite was related to changes in the production of reactive oxygen species (ROS), epimastigotes were incubated in the presence or not of LQB-118 (0-5µM/ml) for 3h and 24h/28°C and subsequently labeled with H2DCFDA probe. For the *in vivo* assay, Swiss mice were infected intraperitoneally with 103 blood trypomastigotes (Y strain). Three groups were used: (1) infected and untreated; (2) infected and treated orally with LQB-118 (20 mg/kg body weight/day, for 11 days) and (3) not infected and untreated. Parasitemia, body weight and survival were evaluated. The LQB-118 inhibit significantly ($p<0.0001$) the growth of epimastigote in a dose-dependent manner and the IC50 was estimated at 2,41± 0,35µM (48h) and 0,83±0,08µM (96h). The parasites showed morphological changes, mainly in the highest concentration tested LQB-118 (5µM). We observe that LQB-118 increase parasite ROS production from 3h incubation. Infected mice treated with LQB-118 showed reduced 37.9% parasitaemia compared to the untreated control (group 1), but there was no significant difference in the survival rate or the weight of the animals. Histological analysis of the hearts of infected animals is in progress. These results show that LQB-118 has the potential against *T. cruzi.* **Supported by**:FAPERJ; CNPQ **Keywords:**Trypanosoma cruzi; in vivo; lqb-118

HP136 - *LEISHMANIA* ACTIVATE THE NLRP3 INFLAMMASOME THROUGH DECTIN-1-SYK KINASE

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Nlrp3 is an important innate immune sensor of microbes and cellular damage. It regulates the assembly of the inflammasome, a molecular platform that contains active caspase-1. Nlrp3 can be activated by different stimulus such as reactive oxygen species (ROS) generation, efflux of k^+, lysosomal damage or after sensing intracellular pathogens. Recently, our group showed that the Nlrp3 inflammasome is a major innate immune platform for the restriction of *Leishmania* infection. However, little is known about the upstream mechanisms that regulate the Nlrp3 inflammasome activation. It is reported that Dectin-1 receptor participate in the Nlrp3 inflammasome activation in response to fungal infections by mechanisms dependent on the production of ROS and Syk kinase activation. Thus, we evaluate the role of Dectin-1/Syk signaling pathway in the Nlrp3 inflammasome activation during *L. amazonensis* infection. Using Asc, Nlrp3 and caspase-1-deficient macrophage, *L. amazonensis* infection induced Nlrp3-dependent caspase-1 activation by ROS-dependent mechanisms. Besides, ROS production in the early stages of infection was critical to inflammasome activation. The treatment with ROS inhibitors induced a diminished leishmanicidal activity in macrophages. Moreover, in the absence of Dectin-1 or after Syk inhibition, macrophages failed to produce ROS, activate caspase-1, process IL-1β and control of parasite replication. *In vivo* infection demonstrated that Dectin-1 activation was crucial for efficient restriction of parasite replication and resolution of cutaneous lesions after infection with *L. amazonensis*. Altogether, our data demonstrate that *Leishmania* trigger the Nlrp3 inflammasome by mechanisms dependent of Dectin-1/Syk pathway, contributing to control of infection. Thus, we elucidate the major signaling platform for the host resistance against *Leishmania* infection and describe the molecular mechanisms that explain the Nlrp3 inflammasome activation during *Leishmania* infection. **Supported by**:FAPESP/CRID, FAEPA, INCTV/CNPq
Keywords: Leishmania; inflammasome; dectin-1

HP137 - IMMMUNOCYTOCHEMISTRY AND QUANTITATIVE EVALUATION OF CECAL LESIONS PRODUCED BY *ENTAMOEBA DISPAR* IN ASSOCIATION WITH *SALMONELLA* TYPHIMURIUM.

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In the face of evidence that *E. dispar* is capable of producing significant experimental lesions in the presence of bacteria and the observation of high frequency co-intestinal infections that affect millions of people each year, we decided to conduct a preliminary analysis of possible interference of amoebic infection by *S*. Typhimurium. Female wistar rats with age about 25 days were divided into groups and subjected to intragastric intubation *S*. Typhimurium and co-infected with MCR strain of *E. dispar* (Ed-Co), while others were inoculated with the strain of *E. dispa*r MCR (Ed) or were only subjected to intragastric intubation with *S*. Typhimurium (ST). After seven days of infection occurred the necropsy of the animals and the cecum was collected for histopathologic and morphometric analysis of the lesions. Wistar rats infected with *E. dispar* only showed intestinal lesions characterized by zones of destruction and inflammation of the mucosa and submucosa. Already in Wistar rats infected with *E. dispar* and co-infected with *S*. typhimurium were observed increased intestinal necrosis and increased intensity of the inflammatory response. Morphometrically we confirm the development of larger area of lesion of cecal mucosa in animals infected with *E. dispar* associated with *S*. typhimurium (139.822±30.089μm^2) when compared to animals infected only with *E. dispar* (50.657±5.131 μm^2) (P<0,0001). Our results show that animals infected with *S*. typhimurium was not capable of causing the destruction of colonic mucosa just being checked for the presence of epithelial shedding zones. Since inflammation and intestinal necrosis area caused only by *E. dispar*, it was lower than that observed in the animals co-infected *E. dispar*/*S*. Thphimurium. Molecular biology studies yet to be conducted will determine whether the bacteria favored the expression of virulence amebic factors and/or, simply, the epithelial lesions of bacterial origin favored the adhesion and invasion of trophozoites. **Supported by**:CNPq e FAPEMIG
Keywords: Entamoeba dispar; salmonella typhimurium a; amoebic colitis

HP138 - THYMIC MICROENVIROMENT IS ALTERED DURING PROTEIN MALNUTRITION AND *LEISHMANIA INFANTUM* INFECTION

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The thymus is one of the most affected organs during malnutrition displaying thymic atrophy and thymocyte depletion. Interestingly, several infectious diseases share these characteristics with the malnutrition effects. We have reported that protein malnutrition and *L.infantum*-infection leads to thymic atrophy and reduced cellularity, suggesting an altered intrathymic environment. The microenvironment inside the thymus plays a crucial role in several biological aspects of the thymocytes. However, the proteins information of this environment is mainly restricted to cytokines, chemokines, extracellular matrix proteins, metalloproteases, some hormones and galectins, but the soluble proteins secreted in the microenvironment are widely unknown. A proteomic analysis of the interstitial fluid (IF) of the thymus in protein restricted mice infected with *L. infantum* was undertaken in this study to define the intrathymic mechanisms that drive thymic response during PM and infection. We compared four groups of mixed IF samples: animals fed 14% protein (CP: iTRAQ 114), animals fed 4% protein (LP: iTRAQ 115), animals fed 14% protein and infected (CPi: iTRAQ 116), and animals fed 4% protein and infected (LPi: iTRAQ 117). Samples were analyzed in a LTQ-Orbitrap-XL. A total of 632 proteins were identified and the fold changes in protein quantification were calculated from the intensities of iTRAQ reporter ions between the CP and CPi, LP, LPi using the PatternLab plataform. A total of 280 proteins presenting a change in abundance were screened in the IF of the thymus of LPi mice, of which 34 proteins were upregulated and 67 proteins were downregulated. Our result revealed the proteins secreted in the thymic microenviroment in physiological and pathological conditions, such as PM and *L. infantum* infection. The LPi protein profile was functionally related to cellular migration and essential thymic aspects, as thymopoiese, which can impact in the thymic dysfuntion previously observed. **Supported by**:TWAS, CNPq, Colciencias
Keywords: Leishmania infantum; malnutrition; thymus

HP139 - THE ROLE OF IL-18 IN THE DEVELOPMENT OF CUTANEOUS LESIONS: MODEL OF INFECTION WITH L. AMAZONENSIS

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In infection by Leishmania amazonensis the classic model of polarization Th1 and Th2, found in the model of infection with Leishmania major, does not apply. What is described is a mixture of responses by changing the profiles of susceptibility and resistance in mice models. The IL-18 is a pro-inflammatory cytokine that has a dubious role, polarizes T lymphocytes to Th1 phenotype because it has the ability to induce the production of IFN-γ by NK cells and T lymphocytes, however, depending on the environment in which it finds, can induce a Th2 phenotype. Our work aims to identify the role of the IL-18 in the course of infection and development of skin lesions in mice infected by L. amazonensis. Our data showed that the wild-type animals (C57BL/6) have larger lesions than those of IL-18 KO since the beginning of the infection. In addition, the times analyzed, the two strains have the same parasite burden. On the cytokine profile, we found considerable levels of IL-4, IFN-γ, TNF and IL-10, demonstrating that there is no polarization of T helper cells to a specific response type. Analyzing the cellular profile, we note that the wild-type lesions tends to have a larger population of important inflammatory cells, such as NK cells, monocytes, macrophages and neutrophils. We have also seen that bone marrow-derived macrophages do not express significant levels of IL-18 receptor in front of various stimulations. However, during the course of infection we identified a massive percentage of T cells CD4+ and CD8+ expressing the receptor for IL-18 after the fourth week of infection. This coincides with the appearance of the lesion exacerbated in wild-type mice and can be associated with the peak production of IL-18 seen by ELISA. Our data suggests that the IL-18 is partially involved in susceptibility to infection by L. amazonensis. This is likely due to the interaction of IL-18 on T lymphocytes, and, perhaps, is involved in cell migration and tissue repair. **Supported by**:CNPq, CAPES e FAPEMIG
Keywords: Leishmania amazonensis; il-18; inflamação

HP140 - INTERACTION BETWEEN *TRICHOMONAS VAGINALIS* AND IEC-6 ACTIVATED WITH INTERFERON γ: PHOSPHATIDYLSERINE EXPOSURE AND MODULATION OF NO PRODUCTION ON THE HOST

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Trichomoniasis is the non-viral sexually transmitted infection most common worldwide nowadays, overcoming gonorrhea, syphilis and acquired immunodeficiency syndrome (AIDS). Caused by the etiological agent *Trichomonas vaginalis*, mandatory extracellular parasite of the human urogenital tract. This infection begins when *T. vaginalis* initiate adherence to host cell in the vaginal epithelial mucosa. Although several studies, little is known about the infection mechanism of this parasite, which can lead to severe cases of Trichomoniasis, leading to miscarriage or infertility. Thus, studies of *T. vaginalis* infection mechanism are needed. Phosphatidylserine (PS) is a phospholipid membrane located on the internal face of the plasma membrane on cells under normal conditions. PS exposure to the external face of the membrane signals programmed cell death by apoptosis. Furthermore, it has been described in subpopulations of *Toxoplasma gondii* and other protozoa, the occurrence of the apoptotic mimicry process, which is the evasion mechanism of the immune response of the host, where PS exposure by *T. gondii* induces TGF-β1 secretion from infected macrophages activated by interferon-γ leading to iNOS degradation, inhibition of nitric oxide (NO) production and thus this infection persists in the host cells. In this study it was found by flow cytometry that 37% of *T. vaginalis* population exposed PS on their surface. Interaction of *T. vaginalis* to IEC activated with interferon γ were performed and NO production was evaluated in IEC supernatant activated after *T. vaginalis* infection, with *T. vaginalis* incubated with Annexin-V or IEC alone (control). These data suggest that *T. vaginalis* was able to infect IEC activated, causing degradation of the NO microbicide system, through apoptotic mimicry, thus allowing the permanence of infection, indicating that *T. vaginalis* has the same escape mechanism presented in other protozoa. **Supported by**:CAPES e FAPERJ
Keywords: Trichomonas vaginalis; phosphatidylserine; nitric oxide

HP141 - INVOLVEMENT OF SSP-4 FROM *TRYPANOSOMA CRUZI* EXTRACELLULAR AMASTIGOTES IN HOST CELL INVASION

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Trypanosoma cruzi extracellular amastigotes (EA) display the stage-specific surface component Ssp-4, an 84 kDa glycoprotein attached to the membrane by a GPI anchor. Ssp-4 is gradually released into the extracellular environment by EA. However, structural and functional aspects from this glycoprotein are still unknown. We have elucidated the amino acid sequence from Ssp-4 and in silico analysis confirms that this protein is secreted and highly expressed in the amastigote form. We have shown that the carbohydrate epitopes attached to Ssp-4, recognized by monoclonal antibodies (mAb) 2C2 and 1D9, are differently expressed in EA from different *T. cruzi* strains. EA that are more infective present a higher reactivity with mAb 1D9. Conversely, EA that are less infective expressed a higher reactivity with mAb 2C2. We observed that EAs from CL strain (less infective) expressed higher levels of Ssp-4 mRNA transcripts in comparison with G strain EA (more infective strain). We also confirmed that EA are able to secrete vesicular structures (100-200 nm) coated with Ssp-4 when adhered to poly-L-lysine, nonprofessional-phagocytic cells and at actin-rich invasion sites. We have shown that vesicles shed from a less infective strain (Y strain) are able to modulate invasion from a the infective G strain. However, Y strain EA infectivity was only enhanced when incubated with vesicle-free supernatant.
Supported by:FAPESP, CNpQ, CAPES
Keywords: Extracellular amastigotes; ssp-4; vesicles

HP142 - DYNAMICS OF PSEUDOCYSTS FORMATION IN *TRICHOMONAS VAGINALIS* AND IRON DEPLETION EFFECTS ON CELL PROLIFERATION AND PLASTICITY OF PARASITE

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Trichomonas vaginalis is a protozoan that causes human trichomoniasis, the most common nonviral sexually transmitted disease. Iron is an essential element for this parasite, responsible for survival, growth, proteases expression, and plasticity. The purpose of this work was to investigate the iron depletion effects on cell proliferation, cell plasticity and dynamics of pseudocysts formation of *Trichomonas vaginalis*. The parasites cultivated in complete iron-sufficient and iron-depleted medium were analysed by differential interference contrast light microscopy, scanning and transmission electron microscopy. Iron depletion was achieved by the addition of 180mM of 2,2-dipyridyl chelator to the medium. Statistical analyzes were performed using firstly the transformation of the arc sine of the square root and then the simple t test. Iron depletion stopped the proliferation of *T. vaginalis*. It also induced morphological changes of trophozoites to spherical non-motile cells, whose flagella were internalized. The formation of pseudocysts was dependent on the exposure time of parasites in the iron-depleted medium, while in 48 hours 97% consisted of pseudocysts and 3% of trophozoites. On the other hand, the parasites maintained in complete iron-sufficient medium consisted almost exclusively by 96% of trophozoites. The conversion of trophozoites into pseudocysts was reversible when iron was restored in the culture medium. Trophozoites maintained in complete iron-sufficient medium were highly plastic, being able to divide while the iron depletion induced the arrest of cell division in different phases of the cell cycle. Our data not only demonstrate that iron has a pivotal role in the biology of this microorganism, but also may indicate the direction to elucidate the mechanisms by which this metal regulates the expression of proteases and how it interferes in the virulence of this protozoan. **Supported by**:UFSJ
Keywords: Pseudocysts; iron; depletion

HP143 - IN VITRO HEALING PROCESS EVALUATION INDUCED BY DIFFERENT LEISHMANIA SPECIES FROM DIFFERENT CLINICAL FORMS

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Cutaneous leishmaniasis in Brazil are caused by different species, being the most common species Leishmania amazonensis and L. braziliensis. These species are responsible for generating different clinical forms with different immunological response and pathologies. Leishmania is an obligate intracellular parasite, mainly parasitizing macrophages; however there are reports of the presence of promastigotes and amastigotes in fibroblasts, which serve as safe shelter for the parasite in leishmaniasis latent forms. Thus, the aim of the study was determine in vitro response induced by different species of Leishmania in the wound healing model using murine fibroblasts. The proliferative capacity was analyzed after 24 h and 48 h after infection. After 24h of infection, a significant decrease in the lesion area was observed in fibroblasts infected with L. braziliensis (18.75%) compared to control (0.95%).After 48 hours of infection, there were no significant differences in the lesion area of infected and non-infected cells. Among different cytokines, only IL-6 was detected. After 24h, was observed a high production of IL-6 in the cells infected with L. braziliensis (2031.94 pg / mL) and L.amazonensis (2465.79 pg / mL) compared to control (600.29 pg / ml).Therefore, collagen production was more intense nearby injured regions of in vitro lesion and after Leishmania infection. The scanning electron microscopy analysis showed several cytoplasmic projections like pseudopod, and lamellipodia and filopodia, during the first minutes of interaction with different species of Leishmania. These projections appear to be involved in phagocytosis process of the parasite by fibroblasts. The results suggest there were differences in the immune responses generated by two Leishmania species during in vitro wound healing model considering cytokine production, fibroblast proliferation and wound healing induction with collagen production.
Supported by:IEC/SVS/MS; PIBIC/CNPq/IEC
Keywords: Leishmania; wound healing; murine fibroblasts

HP144 - ROLE OF TLR ADAPTOR PROTEINS MYD88 AND TRIF ON THE HOST CD200 INDUCTION BY *L. (L.) AMAZONENSIS*

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Leishmania, the causative agent of leishmaniasis, can survive inside macrophage parasitophorous vacuoles, subverting the immune response. Macrophages have on their surface membrane Toll-like receptors (TLRs) - the first defensive system against invasive microorganisms. TLR-ligand interaction triggers activation of a MyD88-dependent signaling pathway or a MyD88-independent pathway (that uses the adaptor protein TRIF), stimulating the immune response. Recent work showed that *L. (L.) amazonensis*, a causative agent of the cutaneous leishmaniasis, is capable of avoiding macrophage responses through the induction of CD200 in the host cell. CD200 is a cell surface glycoprotein from the immunoglobulin superfamily, which is expressed in several cell types. When in contact with its receptor CD200R, an inhibitory signal that prevents local macrophage activation is generated. Early study in *Neisseria* model showed the link between TLRs and CD200, and the participation of MyD88 in this event. In this context, the aim of this work is to investigate if the expression of the CD200 molecule in macrophages induced by *L. (L.) amazonensis* is TLR-dependent. For this, bone marrow macrophages (BMMs) from WT (wild type), MyD88$^{-/-}$ and TRIF$^{-/-}$ mice were infected with *L. (L.) amazonensis* amastigotes. We observed that the parasite induces an increase in the CD200 transcript levels 3 h after infection only in WT macrophages. Six hours after infection, the transcript levels increased in the other infected macrophages from knock out mice, comparable with WT. Western blot analysis showed an increase in CD200 protein levels only in BMMs from WT 1 h after infection. Therefore, our results demonstrate that signaling mediated by TLR adaptor proteins MyD88 and TRIF is required to induce CD200 in infected macrophages by *L. (L.) amazonensis* in initial stages. Further experiments will be performed to demonstrate the role of TLRs on the macrophage CD200 induction by *L. (L.) amazonensis.*
Supported by:FAPESP **Keywords:**Leishmania; toll like receptor; cd200

HP145 - INTRADERMAL IMMUNIZATION OF LEISHMANIA DONOVANI CENTRIN KNOCK-OUT PARASITES IN COMBINATION WITH SALIVARY PROTEIN LJM19 FROM SAND FLY VECTOR INDUCES DURABLE PROTECTIVE IMMUNE RESPONSE IN HAMSTERS.

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Visceral leishmaniasis is a neglected tropical disease, fatal if untreated. There is no vaccine available against leishmaniasis. Parasite persistence is thought to be important for an effective protective response that may be achieved by immunization with gene-deleted live attenuated parasites that do not cause disease. We have previously reported a genetically modified live attenuated parasite, with a cell division specific centrin1 gene deleted in mice, hamsters and dogs, presenting good immunogenicity and protection. In this study we tested the association of LdCen-/- parasites and salivary protein from sand fly vectors (LJM19). We immunized golden hamsters intradermally to mimic a feasible mode of immunization, with parasites and protein (LdCen-/-+Ljm19- G1), comparing to LdCen-/- (intracardiac-G2) and control groups (LJM19-G3 or BSA-G4). After 15 days of immunization, no parasites were detected in spleen and liver by serial dilution suggesting that attenuated parasites are safe and do not persist. The G1 presented higher levels of IgG, IgG1 and IgG2, and higher IgG2/IgG1 ratio, and higher expression of IFN-γ, iNOS and IL-12/IL-23p40 as measured by RT-PCR, after immunization. After 1 month of infection, group 1 presented lower number of parasites in ear and lymph node (LN), higher levels of IgG2 and IgG2/IgG1 ratio, higher expression of type 1 cytokines and lower expression of type 2 cytokines in LN, spleen and liver. In addition, the evaluation 9 months after infection have shown lower parasite load in LN, spleen and liver, and presented a non-inflamed spleen. Further this study suggests that under conditions of natural challenge, vaccination using genetically modified live attenuated parasite (LdCen-/-) in combination with sand fly salivary protein (LJM19) has the capability to confer complete and long lasting protection against visceral leishmaniasis. **Supported by**:FDA/HHS/USA
Keywords: Visceral leishmaniasis; attenuated parasites; vaccine

HP146 - INVOLVEMENT OF INDUCIBLE NITRIC OXIDE SYNTHASE IN MODULATING THE HOST IMMUNE RESPONSE AGAINST NEOSPORA CANINUM INFECTION

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Neospora caninum is an obligate intracellular parasite that is capable of infecting a wide range of hosts. Since its first description, N. caninum infection has emerged as an important cause of neuromuscular disease in dogs and abortion in cattle worldwide, leading to significant economic losses in beef and dairy cattle industries. Inducible Nitric Oxide Synthase (iNOS) is the most important enzyme responsible for the generation of Nitric Oxide (NO), which is classically described as an important effector mechanism in the killing of intracellular pathogens. Here, we aimed to evaluate the role of iNOS during N. caninum infection. For that purpose, we have followed the production kinetics of the major elicited cytokines, mortality rate, inflammation and parasitism after parenteral infections with live tachyzoites, using mouse models with a targeted genetic disruption of enzyme gene (iNOS-/-), along with its wild type C57BL/6 counterparts. Our results obtained from experimentally infected mice indicate that WT mice had a lower mortality rate, parasitism and inflammation if compared to iNOS-/- mice, along with lower early Th1 cytokine profile levels and Th2/Th17 cytokines in intermediate stages of infection. These results indicate that iNOS is an important resistance mechanism in N. caninum infection, by inducing the control of acute and chronic parasitism, cytokine production and consequent exacerbation of the inflammatory responses. **Supported by**:CAPES, CNPq and FAPEMIG.
Keywords: Neospora caninum; inos; cytokine

HP147 - DETECTION OF GELATINASES IN LEISHMANIA AMAZONENSIS AND LEISHMANIA BRAZILIENSIS PROMASTIGOTES AND DURING PERITONEAL MACROPHAGES INFECTION

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Leishmania is an obligate intracellular parasite that invades phagocytes, especially macrophages. Molecules produced by parasite such as gp63 or by the host, as metalloproteinases (MMP), degrading components of the extracellular matrix (ECM), promoting phagocytes arrival to the site of parasite infection and helping the parasite dissemination. It is reported in literature the presence of MMP-9 in trypanosomatid similar to mammalian. Though, there is no report of the MMP-like in Leishmania promastigotes and few studies showing increased of gelatinases activity or secretion during Leishmania infection. Thus, the present study investigated the presence of gelatinases (MMP-2 and MMP-9) in Leishmania promastigotes provided from two different clinical cases (cutaneous leishmaniasis-CL and mucocutaneous leishmaniasis-MCL) and during the infection of peritoneal macrophages. Promastigotes from logarithmic and stationary phases presented, for the first time, the presence of MMP-2 and MMP-9 both in Leishmania amazonensis and L. braziliensis observed by immunofluorescence. However, analysis by flow cytometry showed an increase in the presence of MMP-2 only in L.amazonensis in growth phase. Macrophages infected with L. amazonensis and L. braziliensis showed the presence of MMP-9 diffused to cytoplasm and close to the parasites when compared to control (macrophages without infection) that showed intense labeling close to nuclei. Thus, the gelatinases may also act as prognostic markers for leishmaniasis disease and the presence of MMP may help the parasite to disseminate in the host. However, other studies are caring out to determine its role during infection and development of disseminated and mucocutaneous skin lesions. Furthermore, MMP could constitute a new target molecule for the development of new drugs.**Supported by**:IEC/SVS/MS; PIBIC/CNPq/IEC **Keywords:**Metalloproteases; leishmania amazonensis; leishmania braziliensis

HP148 - EVALUATION OF THE IMMUNE RESPONSE PROFILES TH17 AND TH22 DURING NEOSPORA CANINUM INFECTION

BARROS, P.S.C.[*1]; FERREIRA, F.B.[1]; MOTA, C.M.[1]; RAMOS, E.L.P.[1]; SANTANA, S.S.[1]; SILVA, M.V.[1]; MIRANDA, V.S.[1]; COSTA, L.C.G.P.[1]; SPIRANDELLI DA COSTA, M.S.[1]; MAIA, L.P.[1]; SILVA, V.R.[1]; SANTIAGO, F.M.[1]; MINEO, J.R.[1]; MINEO, T.W.P.[1]
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Neospora caninum is an obligate intracellular parasite that is capable of infecting a wide range of hosts. Since its first description, N. caninum infection has emerged as an important cause of neuromuscular disease in dogs and abortion in cattle worldwide, leading to significant economic losses in beef and dairy cattle industries. Th17 cells are responsible for the production of the cytokines IL-17, IL-21, IL-22 and IL-23 that are essential components of the immune response against different pathogens. IL-22 is the most important cytokine produced by Th22 cells. This cytokine is one of the best-studied members of the IL-10 family of cytokines and plays an important role in the regeneration of the epithelium and defense of barrier tissues such as the intestine, oral mucosa, skin, and lung. This study aimed to evaluate the role of the cytokines IL-17, IL-22 and IL-23 during N. caninum infection. For that purpose, we have infected WT C57BL/6, IL-17-/-, IL-22-/- and IL-23-/- mice with 2x107 live tachyzoites of N. caninum by the oral route. We evaluated the morbidity and survival rates, tissue inflammation (H&E) and parasitism (pPCR), along with cytokine production (ELISA). Our results demonstrate that all WT and IL-17-/- mice survived the challenge while 20% of IL-22-/- mice and 40% of IL-23-/- mice did not survive. In addition, IL-22-/- and IL-23-/- mice showed greater weight loss compared with WT and IL-17-/- mice. H&E staining demonstrated increased inflammation in hepatic and pulmonary tissue from IL-22-/- and IL-23-/- mice, if compared with WT and IL-17-/- mice. However, qPCR analysis showed higher parasitism in tissues from WT mice compared with IL-17-/-, IL-22-/- and IL-23-/- mice. Similarly, WT mice showed higher levels of IFN-□, IL-10 and IL-12 cytokines compared with IL-17-/-, IL-22-/- and IL-23-/- mice. In conclusion, our preliminary results demonstrate that IL-22 and IL-23 cytokines are important in the resistance against the infection caused by N. caninum. **Keywords:**Neospora caninum; th17; th22

HP149 - IDENTIFICATION OF A SECRETED ECTO-NUCLEOSIDE TRIPHOSPHATE DIPHOSPHOHYDROLASE FROM *TRYPANOSOMA BRUCEI*

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Trypanosoma brucei brucei is causative agent of animal African trypanosomiasis, also called nagana, a disease related to the human sleeping sickness. These diseases are transmitted to mammalian hosts by tsetse fly. During their digenetic life cycle these parasites possesses two main stages: the bloodstream form, in the mammalian host, and the procyclic form that lives in the insect vector. Parasitic protozoa, such as *Trypanosoma* species, are thought to express a number of surface and secreted nucleoside triphosphate diphosphohydrolase (NTPDases) of the CD39 family which hydrolyze a broad range of nucleotides tri- and/or diphosphates. Several studies have shown a correlation between NTPDase activity and infectivity of parasites. The *T. brucei* genome presents two putative NTPDase enzyme, homologues for NTPD 1 and NTPD 2. In a previous work, our group characterized a magnesium-dependent E-NTPDase activity on the cell surface of living Trypanosoma brucei, demonstrating the presence of at least 1 NTPDase enzyme. Here we identified a NTPDase that is secreted from the parasite into the culture supernatant. We isolated this enzyme and demonstrated its ability to hydrolyze ATP in the presence of magnesium. In addition, we did not observe ecto-phosphatase activity. Western blot analysis of culture supernatant showed that the anti-CD39 antibody recognized one polypeptide about 50 kDa. We currently are evaluating the biochemical characteristics and the role of this secreted NTPDase as virulence factor in this pathogen. **Supported by**:CAPES/FAPERJ
Keywords: Trypanosoma brucei; nucleoside triphosphate diphosphohydrolase ; ntpdase

HP150 - THE ACTIVITY OF HOST NEUTROPHIL ELASTASE IS REQUIRED FOR THE INTRACELLULAR GROWTH OF *L. DONOVANI* AND VISCERAL PARASITEMIA VIA TOLL-RELATED INDUCTION OF INTERFERON β.

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In visceral leishmaniasis, the molecular mechanisms that determine tissue tropism for L. donovani and L. chagasi and visceral parasitism remain elusive. Leishmania sp has three genes similar to bacterial ecotin: ISP1, ISP2 and ISP3. Ecotin is a serine peptidase inhibitor of S1A family enzymes such as neutrophil elastase (NE). We have reported that, in L. major, ISP2 plays a role in controlling the activity of NE in macrophages to guarantee parasite survival and intracellular growth. ISP2 prevents triggering of a pathway associated with NE, Toll-like receptor 2 (TLR2) and TLR4. We describe here that ISP2 is not expressed by L. donovani, leading to unregulated NE activity during macrophage infection. The addition of a synthetic NE inhibitor to infection assays of mouse peritoneal macrophages in vitro diminished intracellular growth of L. donovani. The parasites survived poorly in macrophages derived from elastase knock-out mice (ela-/-) or in macrophages from TLR2 or TLR4 knock-out mice. We detected co-localization between NE, TLR4 and the parasite in the parasitophorous vacuole. Infected ela-/- mice displayed significant lower parasite burden in the liver and the spleen, which was accompanied by elevated levels of nitric oxide and reduced levels of TGFβ in the liver, and of IL10 in the spleen, as compared to infected C57B6 mice. We generated transgenic L. donovani lines expressing L. major ISP2, as confirmed by Western blot. *L. donovani*:ISP2 lines had impaired intracellular growth in murine macrophages in vitro. C57B6 mice infected with *L. donovani*:ISP2 showed decreased parasite burden in the spleen, as compared to those infected with WT parasites. Addition of exogenous NE or of IFNβ recovered the intracellular growth of L. donovani in $ela^{-/-}$ macrophages, or of transgenic ISP2-parasites in C57B6 macrophages. We propose that *L. donovani* utilizes the host NE-TLR molecular machinery to generate IFNβ that contributes to parasite growth and successful infection. **Supported by**:CNPQ
Keywords: Macrophages; l. donovani; infection

HP151 - ENDOTHELIAL BARRIER BREAKDOWN IN EXPERIMENTAL CHAGAS DISEASE: ACTIVATION OF THE KALLIKREIN-KININ SYSTEM COUPLES INFLAMMATORY EDEMA TO HEART PARASITISM AND ASSOCIATED PATHOLOGY.

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We have previously reported that TCTs (Dm28c) upregulate T cell effector (type 1) development through the coupling between innate immunity (TLR2/CXCR2) and the Kallikrein-Kinin System (KKS). Here we tested the hypothesis that trypomastigotes co-opt the KKS to generate peptides (bradykinin, endothelins) that enhance invasion of cardiovascular cells through interdependent activation of B2R, B1R, ETaR and ETbR. Methods: Assisted by high-resolution echocardiography, we injected mice intracardiacally with TCTs or PBS and measured heart edema, T. cruzi DNA and heart histopathology. Intravital microscopy was used to measure leakage of dextran-FITC in hamster cheek pouches (HCP). Results: Comparison of plasma leakage between steady-state HCP versus (i) histamine-primed ("leaky") or (ii) LPS-treated tissues topically exposed to TCTs revealed that BK-induced inflammatory cascades (MC/KKS pathway) potentiated the proinflammatory phenotype of TCTs. Pharmacological studies linked endothelial barrier breakdown to mast cell (MC)-driven activation of the KKS. Using the model of intracardiac infection, we found that T. cruzi DNA (3 dpi) was sharply reduced in the heart of MC-deficient mice or WT mice pretreated either with the MC stabilizer cromoglycate, Factor XII inhibitor (infestin-4) or GPCR antagonists [B2R, B1R and ETaR/ETbR]. Remarkably, mice pretreated with these GPCR blockers were redundantly protected from myocarditis/fibrosis (30 dpi). Notably, B1R-/- infected mice (i.p.) showed reduced intracardiac parasitism (14 d p.i.) and a mild chronic myocarditis/fibrosis (90 dpi). Targeting of B1R (from 15-60 d p.i.) with the novel B1R antagonist R954 reduced the number of intracardiac pseudocysts in Colombiana-infected mice. Conclusion: Collectively, we suggest that endothelial barrier stabilizers, such as B1R blockers, might attenuate myocarditis/fibrosis by inhibiting MC/KKS-dependent edema and the ensuing release of infection-promoting peptides in the chagasic heart. **Supported by**:CNPQ, FAPERJ, CAPES, INBEB **Keywords:**Bradykinin; chagas disease; inflammatory edema

HP152 - ENTERIC NITRERGIC NEURONS ARE SELLECTIVELY PRESERVED IN CHRONIC INFECTION WITH TRYPANOSOMA CRUZI

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Chagas disease (CD) progresses chronically with alterations in the enteric nervous system which have been linked to the development of enlarged esophagus and/or colon. The role of nitric oxide derived neurons mediating changes in intestinal inflammatory disorders has been investigated. However there are not studies conducted in mouse models of chronic CD. This work aims to study the impact of inflammation and denervation induced by T. cruzi in a potential selective neuronal loss in the enteric nervous system. We used functional and structural markers in order to assess the nNOS expression in myenteric PGP 9.5 positive neurons in the acute and chronic phases of the experimental disease. For this, colon consecutive sections of non-infected and infected animals, acute and chronic, were marked by PGP and nNOS by immunohistochemistry (IHC) and evaluated for morphology by light microscopy. Photographic documentation the images were analyzed and statistical performed according to the distribution of values in each group followed by the T or Mann-Whitney tests. The values of $p \leq 0.05$ were considered significant. Our results demonstrate a reduction in the total number of neurons only in the chronic phase of infected animals relative to their uninfected controls. The expression of nNOS in the acute phase keeps unchanged while in the chronic phase the nitrergic neurons appear in greater numbers representing the majority of the preserved neurons compared to uninfected animals. These data indirectly suggest a relative preservation of this neuronal type in relation to other existing classes. As a perspective, our group will confirm this finding by double IHC and will investigate the expression and the role of excitatory neurons in this model. Our data show that nitrergic neurons are relatively preserved in experimental intestinal CD, which may be one explanation for the functional changes that characterize the megacolon. **Supported by**:UFMG, CNPq, CAPES and FAPEMIG. **Keywords:**Chagas disease; neurons; nitric oxide

HP153 - MODULATION OF HOST IMMUNE RESPONSE BY AMINO ACID REPEATS PRESENT IN TRYPANOSOMA CRUZI ANTIGENS

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Several Trypanosoma cruzi antigens contain amino acid repeats identified as targets of the host immune response. Ribosomal proteins containing an AKP rich repetitive domain and members of the trans-sialidase (TS) family containing the Shed Acute Parasite Antigen (SAPA) repeats are amongst the most imunogenic T. cruzi antigens. We investigated the role of amino acid repeats present in the T. cruzi ribosomal protein L7a, by immunizing mice with recombinant versions of the complete protein (TcRpL7a) as well as truncated versions containing only the repeat (TcRpL7aRep) or non-repetitive domains (TcRpL7aΔRep). Mice immunized with TcRpL7a produced high levels of IgG antibodies against the complete protein and the repeat domain, whereas immunization with TcRpL7aΔRep or TcRpL7aRep produced very low levels or did not produce IgG antibodies against this antigen. Furthermore, mice immunized with TcRpL7a produced high levels of IFN-γ, whereas low levels or no IFN-γ were detected in splenocyte cultures from mice immunized with truncated versions of the protein. After challenging with trypomastigotes, mice immunized with the TcRpL7a were partially protected, whereas immunization with TcRpL7aΔRep did not alter parasitemia compared to controls. Strikingly, immunization with TcRpL7aRep displayed an exacerbated parasitemia and 100% mortality, indicating that these repeats modulate the immune response favoring the parasite. To examine whether repeat domains interfere with the macrophage response towards parasite infection, infection rates and NO production were determined in macrophages cultivated in the presence or absence of the repeats. Preliminary results showed no detectable differences in infection rates nor NO production in in vitro cultured peritoneal mice macrophages. To verify whether SAPA repeats also exert this immune modulatory effect, recombinant versions of the complete TS, TS without SAPA and only SAPA repeats will be tested in mice immunization experiments.**Keywords:**L7a; trans sialidase; antigens-repeats

HP154 - MOLECULAR CLONING AND OVEREXPRESSION OF OLIGOPEPTIDASE B-2 FROM 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. L. major is a protozoa parasite that causes cutaneous leishmaniasis. 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 (OPB2-OVX). L. major parasites overexpressing OPB2 with mutation in the active site of the enzyme (serine to alanine) were also developed and called OPB2-MUT. OPB2-OVX showed slow growth, compared to WT and OPB2-MUT. OPB2-OVX increased the percentage of purified metacyclic as assessed by binding to PNA-lectin, as compared to WT or OPB2-MUT. In addition, OPB2-OVX parasites displayed high infectivity to murine macrophages in vitro. We evaluated the production of reactive oxygen species (ROS) production 15 minutes after infection and we measured the production of nitrite in the supernatants treated with LPS 48h after infection. We observed that OPB2-OVX reduced the production of ROS and NO compared to WT and OPB2-MUT. These results indicate that this enzyme may be related to the L. major metacyclogenesis. However, the function of OPB2 needs to be investigated.**Supported by**:CNPQ **Keywords:**Oligopeptidase b 2; serine proteases; laishmaniasis

HP155 - THE ROLE OF PHARMACOLOGICAL INHIBITOR MTOR RAPAMYCIN IN RESPONSE OF MEMORY CD8+T CELLS INDUCED BY GENETIC IMMUNIZATION AGAINST *TRYPANOSOMA CRUZI*

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Genetic vaccination known as heterologous prime-boost regimen consists in the use of plasmid DNA (priming) followed by replication defective human recombinant adenovirus type 5 (boosting), both of which carrying the gene encoding the protein ASP-2, is a powerful strategy to elicit effector memory $CD8^+$ T cells (TEM) against intracellular protozoan parasite *Trypanosoma cruzi*. In order to intensify the memory response induced by immunization, we analyzed the role of pharmacological inhibitor mTOR (protein target of rapamycin in mammals) rapamycin, which is already known be able to increase the $CD8^+$ memory T cell frequencies in viral infection models. To test that, C57BL/6 mice were immunized and treated with rapamycin each day for 35 days. On day 35 and 95, we harvested the spleen and the phenotyping and functional assays of specific $CD8^+$ T cell were analyzed. Preliminary results show that treatment with rapamycin increased the frequency of specific $CD8^+$T cells, and also the percentage of polyfunctional $CD8^+$T cells that degranulate and secrete IFN-γ and TNF-α were higher when compared to the control group (only immunized). This augment was also observed on day 95. In addition, highly susceptible A/Sn mice immunized with 10 fold less plasmid/adenovirus and treated with rapamycin displayed 100% of survival when compared with control mice only immunized and infected (40%). Taken together, these data suggest that rapamycin treatment increase the specific $CD8^+$ T cell immune response and also the protective immune response generated by the heterologous prime-boost protocol. Additional experiments are necessary to investigate the mechanisms involved in this strategy. **Supported by**:FAPESP, CNPQ E CAPES **Keywords:**Rapamycin; cd8+ t cell; trypanosoma cruzi

HP156 - DIVERSITY OF VACCINE CANDIDATE AMA1 OF PLASMODIUM VIVAX ISOLATES FROM BRAZIL

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In Brazil, P. vivax accounts for 83.6% of malaria cases and is the most common species of Plasmodium. Recent emergence of drug-resistant strains and complications of P. vivax infection leads to intensified research on alternative control methods such as development of vaccines. AMA1 is a protein that is involved in the invasion of Apicomplexa parasites to the host cells. This protein contains four regions, as follows, pro-sequence, a rich cysteine ectodomain, a transmembrane domain and a cytoplasmic region. In the ectodomain, the disulfide bridges formed by cysteine residues, allow separate into three domains, called I, II and III. Among the four regions, domain I present the highest rate of mutation and genetic diversity, while the domain II has a higher degree of amino acid conservation and is also the most immunogenic region. As such genetic diversity can jeopardize the effectiveness of a vaccine containing this antigen, here we characterized the diversity of AMA1 protein from P. vivax Brazilian isolates. Blood samples were collected from patients infected with P. vivax in Manaus, Amazonas State, Brazil, between 2011-2014. Genomic DNA was isolated from whole blood. Four overlaping fragments from Pvama1 gene were amplified, which corresponds to the whole gene. PCR fragments were sequenced. Electropherograms were subjected to quality analysis and the four fragments were assembling. Multiple aligned was performed using ClustalX, which also generated phylogenetic trees. The dendrogram was constructed using the neighbor-joining algorithm and Kimura model. It was possible amplify the first fragment only for 65% of samples. The fragment 2, which corresponds to AMA1 domain I was the most polymorphic, followed by fragment 3 and fragment 4. Phylogenetic tree from global sequences analysis showed different clusters whereas sequences from Brazilian isolates were forming mainly in two clusters. Limited variability was found encouraging the use of AMA1 as a vaccine candidate. **Supported by**:CNPq **Keywords:**Malaria; ama1; diversity

HP157 - NLRP3-INDUCED AUTOPHAGY IN RESPONSE TO *TRYPANOSOMA CRUZI* IS AN IMPORTANT INNATE IMMUNE MECHANISM

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Introduction: Cellular immune response is an essential mechanism to control the intracellular protozoan *T. cruzi* causative agent of Chagas disease. Recently, it has been demonstrated that NLRP3 inflammasome is an important innate immune component against *T. cruzi* and other intracellular pathogens. Interestingly, autophagy has also been implicated in the response to intracellular pathogens. Moreover, it has been described a crosstalk between autophagy and inflammasomes in many infections. Since the role of autophagy in *T.cruzi* infection is unknown, the aim of this work was to evaluate the importance of this pathway in the control of infected macrophages as well the contribution of NLRP3 in the regulation of autophagy. Methods and Results: Peritoneal macrophages (PMs) from C57BL/6, NLRP3-/- or caspase-1-/- mice were infected with *T. cruzi* tripomastigotes. Later, PMs were treated with pharmacological autophagy inhibitors (LY294002 or wortmannin). After 1, 6, 24 and 48h of infection, the number of intracellular amastigotes was evaluated by fluorescence microscopy and the LC3II expression was measured by western blot (WB) or immunofluorescence (IF). Both WB and IF showed an LC3II protein expression increased in PMs from C57BL/6, indicating that *T. cruzi* was able to induce autophagy. Moreover, the treatment with the inhibitors increased the number of intracellular parasites, suggesting that autophagy contributes to trypanocidal macrophages capacity. However, the NLRP3-/- cells failed to induce autophagy in response to *T. cruzi,* in agreement, the treatment didn't change the number of intracellular parasites. Interestingly, caspase-1-/- PMs maintained the capacity to induce autophagy, similar to wild type cells. Conclusion: The NLRP3-induced autophagy in response to *T. cruzi* infection represents a novel control mechanism. This process might be independent of caspase-1, suggesting a new role for NLRP3 molecule in regulating innate immune responses. **Supported by**:CAPES,CNPq e FAPESP **Keywords:**T. cruzi; autophagy; inflammasome

HP158 - IMMUNOHISTOCHEMICAL ASPECTS OF HEPATIC INTRALOBULAR GRANULOMAS IN DOGS NATURALLY INFECTED WITH LEISHMANIA INFANTUM

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Canine Visceral Leishmaniasis (CVL), in Brazil, is a zoonosis and a systematic chronic disease caused by protozoa of the genus Leishmania. In dogs, the chronic hepatitis is well characterized by a presence of intralobular granulomas constituted by epithelioid cells, macrophages, lymphocytes and plasma cells. Macrophages could appear parasitized or not with amastigotes forms of Leishmania. The aim of this study was to characterize by immunohistochemistry the hepatic granuloma cells of twenty-five dogs, both sexes, naturally infected with Leishmania infantum. Dogs were clinically defined in groups of 8 asymptomatic animals without any clinical manifestation and 17 symptomatic with clinical signs of the disease as lymphadenopathy, skin lesions and weight loss. Fourteen dogs with negative serological and parasitological exams to Leishmania were used as controls. Dogs were euthanized with a lethal dose of thiopental (1 ml / kg) and after necropsy, fragments of liver were collected and fixed in 10% buffered formalin. Microscopic and morphometric analyzes showed significantly higher granulomas in symptomatic compared to asymptomatic animals, but no differences between the groups regarding the number of granulomas and the presence of Leishmania amastigotes. We also observed a greater number of calprotectin positive cells (cytoplasmic antigen termed "L1" that indicates that immature macrophages) in the granulomas of symptomatic animals. On the other hand, there is a higher expression of iNOS in granulomas of asymptomatic dogs than symptomatic ones. These findings suggest that the granulomatous inflammatory response in livers of symptomatic dogs occurs extensively where they are constituted by immature macrophages. Further analysis should be performed to evaluate the phenotype and quantification of other cell types to better characterize the hepatic intralobular granulomas in CVL.
Keywords: Granuloma; leishmania; inos

HP159 - IMBALANCE TOWARDS A TH2 PROFILE IS CAUSED BY TRYPANOSOMA CRUZI TRANS-SIALIDASE IN ACUTE STAGE OF CHAGAS DISEASE

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The protozoan parasite *Trypanosoma cruzi* needs to acquire sialic acid to invade mammalian cells. For this, the parasite expresses the *trans*-Sialidase (TS), an enzyme able to transfer sialyl residues from host glycoproteins to its own mucins. Interestingly, two TS isoforms are known in T. cruzi, the enzymatically active (TS) isoform and the catalytically inactive (iTS) isoform, which has a mutation in the catalytic tyrosine but retains the ability to bind the substrate sugars. Previously we demonstrated that TS induces thymocyte depletion, thrombocytopenia, and absence of germinal centers in secondary lymphoid organs. This phenomena that can be prevented by the passive transfer of TS neutralizing antibody (13G9 mAb) to the infected mice. The resolution of the *T. cruzi* infection is dependent on the Th1 response, however both Th1 and Th2 clones are elicited during the infection. Due to the ability of TS to manipulate the immune system, we decided to explore their effect on T helper responses. We found that both TS and TSi decreased the IFN-gamma secretion while increasing the IL4 secretion in mouse-derived DO11.10 splenocytes stimulated with $OVA_{323-339}$. Furthermore, in DO11.10 splenocytes transferred to OVA-immunized Balb/c mice both TS and TSi decreased the IL2 and IFN-gamma secretion with a concomitant increase of IL4. Similarly when we tested this model during *T. cruzi* infection we observed a significant amount of IFN-gamma but also a high level of IL4 and low of IL2. Interestingly, we could observe that the increase of IL4 and decrease of IL2 was prevented by passive transfer of 13G9 mAb. Finally, using neutralizing antibodies and transgenic mouse models we found that the antigen-presenting cell through IL10 regulates these phenomena. These results strongly support the idea that TS and TSi modulate Th1 differentiation and promote a non-protective Th2 response in the acute stage of Chagas' disease. **Supported by**:NIH, CONICET, ANPCyT **Keywords:**Trypanosoma cruzi; trans-sialidase; t helper

HP160 - METALLIC COMPOUNDS AGAINST CHAGAS DISEASE: NEW COMPOUNDS AND ESTABLISHMENT OF A NEW BIOLOGICAL MODEL

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Chagas'disease caused by the protozoan Trypanosoma cruzi, is a neglected disease that affects millions of people mainly in Latin America. The drugs benznidazole and nifurtimox are used to treat the acute and early chronic forms of the disease, but their efficacies against established chronic infection, the most common presentation of the disease, are lower and variable. Because this, it becomes clear the urgency of identification of new alternatives for Chagas' treatment. T. dionisii is a nonpathogenic trypanosomatid, isolated from that shows a high ability to infect mammalian cells (exploiting the same routes used by T. cruzi) and shares epitopes and antigenic components that are recognized by monoclonal antibodies against T. cruzi. Besides, since 1977 it has been suggested that T. dionisii could be a non-pathogenic model for development of new compounds against T. cruzi. Based on this, we decided to use T. dionisii's intracellular amastigotes to test the effect of new metallic compounds and compare the results obtained with T. cruz intracellular amastigotes. We also compared the effect of the metal compounds with that obtained withbenznidazole. Metallic compounds based on iron, zinc, alluminium, copper, platinum and berillyum introduce advantages over common drugs because they have different possibilities of ligation with the ligand and three-dimensional configuration, increasing the recognition of and interaction with the molecular target. Metallic compounds showed activity against S. aureus and L. mexicana. We used six metallic compounds (FEHPSO4, B10109, A11312, FeHC, ZnBe and B2310) against T. cruzi and T. dionisii using high content analysis methodology to assay the effects. Our results show that both Trypanosoma species are sensitive to the compounds in the same extent thus suggesting that T. dionisii can be used as an in vitro model of T. cruzi infection. FeHC and ZnBe show IC50 lower than that observed with benznidazole treatment. **Supported by**:CNPq, FAPERJ, INMETRO **Keywords:**Chagas disease; metallic compounds ; new biological model

HP161 - NOVEL CHEMOTHERAPEUTIC AGENTS TARGETING LEISHMANIA BRAZILIENSISTRYPANOTHIONE SYNTHETASE FOR THE TREATMENT OF CUTANEOUS LEISHMANIASIS

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Leishmaniasis is caused by Leishmania parasites and in Brazil, Cutaneous Leishmaniasis (CL) is caused mainly by Leishmania braziliensis. Currently, the treatment of CL is based on pentavalent antimonials, Amphotericin B or Pentamidin all of which have limitations such as toxicity, high cost, long therapeutic scheme and resistance. Thus, the development of new anti-leishmania drugs is urgently needed. Enzymes involved in the redox metabolism of trypanosomatids such as trypanotione synthetase (TryS) are interesting targets for drug development as they are essential for the parasite metabolism, are uniquely present in the parasites or have little similarity with host enzymes. The aim of this study is to evaluate the therapeutic effect of TryS inibitors on L. braziliensis infection. Experiments performed with L. braziliensis-infected macrophages showed that targeted Trys-inhibition using N5-substituted paullones (benzo[2,3]azepino[4,5-b]indol-6-ones) induced amastigote killing and was also effective against promastigotes with EC50 ranging from 0.14 to 16.2 µM. Compounds also displayed high selectivity indices towards intracellular parasites. Lastly, experiments performed with human macrophages also showed that N5-substituted paullones (benzo[2,3]azepino[4,5-b]indol-6-ones) induce parasite killing in vitro, inicating the potential of such compounds as new chemotherapy candidates. Experiments are under way to further characterize macrophage response to parasite killing. **Supported by**:CPqGM – FIOCRUZ-BA AND INSTITUTE PASTEUR INTERNATIONAL NETWORK

Keywords: Leishmania braziliensis; redox metabolism; trypanotione synthetase

HP162 - SARCOPLASMIC RETICULUM INVOLVING IN THE NUTRIENTS ACQUISITION DURING *TRYPANOSOMA CRUZI*-CARDIOMYOCITES INTERACTION.

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The survival strategies of Trypanosoma cruzi (T cruzi) in host cell require adaptations to hostile environment during its intracellular cycle. After evasion of parasitophorous vacuole, the acquisition of host cell metabolites is a key strategy to this survival. In many models of microbes host cell interactions the nutrients acquisition occurs through modulation of intracellular trafficking of host cell using endoplasmic reticulum elements. Sarcoplasmic reticulum(SR), an organelle involved in many cellular process the interface between T. cruzi and host cell SR may in some way benefit the intracellular parasite development.

To examine SR involving in T.cruzi-cardiomyocytes interaction we performed PDI(protein dissulfide isomerase) indirect imunofluorescence, the cells were fixed 4% PFA, permeabilized, incubated with anti-PDI, washed and incubated with anti-rabbit Alexa 546.Ultrastructural analysis of glucose-6-phosphatase (G6pase) was performed as described by Wachstein and Meisel (1959) and potassium iodide(KI)-osmium tetroxide(OsO4) impregnation, the cells were fixed, washed and post-fixed with 1% KI-1%OsO4.

Immunofluorescence analysis revealed a staining for PDI, visualized in the nuclear envelope(NE) and SR after 24h of infection and at late times of infection(72h) around intracellular parasites. G6pase analysis revealed an electron-dense product in NE, SR and in the cytoplasmic around intracellular parasites at late times. The reaction was detected at the Golgi apparatus, transport vesicles and flagellar pocket of the intracellular parasites. The KI-OsO4 impregnation revealed a reaction in NE and SR profiles of host cell located around intracellular parasites at late times of infection. In intracellular parasites, the reaction was detected at NE, ER tubules, transport vesicles and Golgi cisternae.

These data suggest alteration of the G6pase distribution during T. cruzi intracellular development and the incorporation of SR elements by intracellular parasite. **Supported by**:FAPERJ & CNPq

Keywords: Trypanosoma cruzi; protein dissulfide isomerase; glucose-6-phosphatase

HP163 - IMMUNOCYTOCHEMICAL AS AN ALTERNATIVE METHOD FOR DIAGNOSTIC OF *TRICHOMONAS VAGINALIS*

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Trichomonas vaginalis is the parasite responsible for the most common sexually transmitted infection in the world. Several studies suggest that trichomoniasis may be an important cofactor in the transmission and acquisition of HIV. In this context, laboratory research is essential to a proper diagnosis of the disease, because it leads to appropriate treatment and facilitates the control. This work is based on the fact that the immunodiagnosis can efficiently contribute to complement existing parasitologic diagnostics and increase the confidence index for the disease. Female BALB / c mice were inoculated intravaginally with trophozoites of *T. vaginalis* and on the 4th day post-infection all animals were sacrificed for the collection of vaginal content by scraping with the aid of a sterile scalpel. Microscope slides with this material were made for standardization of immunohistochemistry reaction. The slides were fixed with alcohol (70%), incubated with polyclonal serum anti-T. vaginalis, then with biotinylated IgG and peroxidase conjugated streptavidin. After standardization, the collecting of vaginal material of HIV positive pregnant women was carried out with the help of sterile swabs for diagnosis by immunocytochemistry. Our results showed that immunocytochemistry perfectly detected the presence of the parasite in samples of vaginal content of both animals and infected women, presenting specific labelling and absence of background. This study proved that this technique is an alternative and effective procedure for the identification of the parasite, signaling that can be employed to assist existing diagnostic methods for the identification of trichomoniasis. **Supported by**:Capes, Fapemig e Cnpq **Keywords:**Trichomonas vaginalis; trichomoniasis; immunocytochemistry

HP164 - MURINE EOSINOPHILS CONTROL LEISHMANIA AMAZONENSIS INFECTION IN VITRO

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Eosinophils are infiltrate associated with allergic inflammation and host defense against helminths. They act as effector cells capable of producing and secreting a wide variety of mediators. Although it has already been described that eosinophils accumulate in response to infection with *L. amazonensis* promastigotes, which can be killed by eosinophils in vitro, these need to be better characterized and the mechanisms involved identified and eosinophils can also affect macrophage ability to control *L. amazonensis* infection. BALB/c mice bone marrow derived-eosinophils with a purity > 90%. 1 x 10^5 cells eosinophils co-cultured with *L. amazonensis* promastigotes Josefa in the stationary phase in ratio of promastigotes:eosinophils (5:1 or 1:1). The quantification of the parasite load carried on day 1 and 6 of co-culture showed that, in both conditions (5:1 or 1:1), eosinophils were able to kill promastigotes, reducing the parasite load compared with the control group. Stimulated eosinophils with INF-γ, IL-4 or IL-5 (100 ng/mL) in ratio of 1:1 and no differences on eosinophils-driven leshmanicidal activity were observed in comparison with eosinophils without stimulation. 4 x 10^5 cells peritoneal cavity-derived macrophages were infected with 8 x 10^5 promastigotes and 4 x 105 cells eosinophils were co-cultured. Eosinophils reduced both the percentage of infected macrophages and the parasite load. Based on the well-known ability of IL-5 to potentiate eosinophil effector functions, we incubate eosinophils with IL-5 (10 ng/ml), but IL-5 stimulation did not alter the magnitude of eosinophil-driven effect on infected macrophages. Of interest, IL-5 itself unexpectedly reduced the percentage of infected macrophage as well as the parasite load within macrophages, displaying a non-described cytotoxic effect. We observed anti-leishmanial activity of eosinophils against promastigote and amastigote however comprehension of mechanism involved is an ongoing study. **Supported by**:CAPES
Keywords: Eosinophil; leishmania amazonensis; macrophage

HP165 - NLRP3 INFLAMMASOME AND IL-1β ARE CORRELATED WITH CHRONIC CHAGASIC CARDIOMYOPATHY

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The aim of this study was evaluate the association between innate immune receptors (Toll like receptor/TLR1-9, nucleotide-binding domain-like receptor protein 3/NLRP3) and cytokines produced by antigen presenting cell (APC) in patients with different clinical forms of chronic Chagas' disease. Fifty-four patients were submitted to clinical evaluation, including electrocardiogram (ECG) mapping and chest X-ray, 2D-echocardiogram (ECHO), 24h Holter examination, esophagus and colon contrast X-ray. Patients were classified in indeterminate (n=18), cardiac (n=17), digestive (n=9) and cardiodigestive (n=10) clinical forms. The mRNA expression of innate immune receptors (TLR1-9 and NLRP3), cytokines (IL-1β, IL-6, IL-12, IL-18, IL-23, TNF-α, and IFN-γ), and adapter molecules (Myd88 and TRIF) were analyzed by real time PCR in peripheral blood mononuclear cells of chagasic patients and correlated with cardiac function (left ventricular ejection fraction-LVEF and cardiothoracic index-CI). Patients with different clinical forms of Chagas disease showed similar expression of TLR1-9. However, patients with cardiac form showed higher mRNA expression of NLRP3, IL-1β, IL-12, TNF-α and IFN-γ than those with indeterminate form. In addition, a negative correlation between NLRP3 and IL-1β mRNA expression with LVEF, and positive correlation with CI were observed. Altogether, the data suggest that increased NLRP3 expression in patients with cardiac form may induce enhance of cytokines expression and contribute to increase of cardiac dysfunction.
Supported by:CNPq, CAPES, MCTI/CNPq/MS-SCTIE-Decit
Keywords: Chagas' disease; nalp3-il-1b; cardiomyopathy

HP166 - GENERATION AND MOLECULAR CHARACTERIZATION OF TUNICAMYCIN-RESISTENT *LEISHMANIA BRAZILIENSIS*

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Despite the importance of leishmaniasis as one of the most prevalent human parasitosis in the world, few advances were made in its treatment. Tunicamycin (TM), an antibiotic isolated from Streptomyces lysosuperficus that blocks the formation of N-acetylglucosamine, has been used to study the mechanisms of virulence and drug resistance in *Leishmania*. TM-resistant strains have increased expression of one or more genes related to glycosylation pathway and reports in the literature on changes in the pattern of virulence are controversial. Our proposal was to extend previous studies and to generate a strain of *L. braziliensis* H3227 resistant to high doses of Tunicamycin (40 µg/mL) and investigate the host-parasite relationship. The in vitro and in vivo infection experiments showed virulence increase of TM-resistant line. We have also showed differences on the cytokines profile produced by murine macrophages stimulated with antigens derived from TM-resistant parasites. Analyzed by transmission electron microscopy TM-resistant parasites showed ultrastructural changes on Golgi apparatus and endoplasmic reticulum have been analyzed by scanning electron microscopy. We have also evaluated changes in the molecular karyotype and metabolome which justify the differences observed on host-parasite relationship and could be reveal new antigens to be further explored as new vaccine candidates or targets for drug development. **Supported by**:CNPq
Keywords: Leishmania; metabolômica; virulência

HP167 - ASSESSMENT OF THE ROLE AUTOPHAGY PATHWAY PLAYS IN *LEISHMANIA* INFECTION

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CBA macrophages (MΦ) control *Leishmania major* infection yet are permissive to Leishmania amazonensis. Few studies have been conducted to assess the role autophagy plays in *Leishmania* infection. Herein, we took the advantage of the CBA model to compare the involvement of autophagy in *L. amazonensis* and *L. major* infection in vitro. Initially, we demonstrated by PCR array that *L. amazonensis* and *L. major* infection differentially altered the expression of autophagy genes. Thus, we evaluated by western-blot whether *Leishmania* infection interferes with the expression of the autophagy marker, LC3-II. We found that *L. amazonensis*-infected MΦ expressed 1.5 times and *L. major*-infected MΦ 2 times more LC3-II than control uninfected MΦ. Next, we characterized *L. amazonensis*- and *L. major*-induced parasitophorous vacuoles (PVs) regarding autophagic features. We found that in comparison with *L. amazonensis*, *L. major* colocalized in a higher extend with the markers of acidic degradative compartments, LysoTracker (1.5 times) and DQ-BSA (3 times). On the other hand, LC3 colocalized 2 times more with *L. amazonensis* inside PVs than with L. major. These findings demonstrate that *L. amazonensis* PVs in comparison to those from *L. major* interact in a higher extend with the autophagic pathway. Then, we investigated the role autophagy plays in parasite entry in RAW MΦ cell-line overexpressing LC3 and in a heterozygous-null MΦ mutant for beclin-1 (beclin +/- MΦ), a protein that plays a central role in autophagy. We found that entry of *Leishmania* spp. was reduced about 3 times in LC3-overexpressing RAW cell-line in comparison to control RAW cells, yet in beclin +/- MΦ, *L. amazonensis* or *L. major* phagocytosis was favored in 1.2 or 1.6 times, respectively. These results suggest that autophagy modulates *Leishmania* phagocytosis. Further studies are underway to clarify the role autophagy plays in PVs biogenesis, as well as in parasite infection by modulating *Leishmania* phagocytosis.
Supported by:FIOCRUZ, UFBA, CNPq, CAPES, FAPESB, INCT-DT
Keywords: Leishmania; parasitophorous vacuoles; autophagy

HP168 - NEW ROLES FOR DYNAMIN IN *TOXOPLASMA GONDII* INFECTION?

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The obligate intracellular protozoan parasite *Toxoplasma gondii* actively invades virtually all warm-blooded nucleated cells in a process that results in a nonfusogenic vacuole, inside which parasites continuously replicate, until signaling to egress is triggered. In this work we approached the role of the GTPase dynamin, largely known for execute the detaching of plasma membrane nascent vesicles, in order to investigate its role in the process of *T. gondii* interaction with the host cell. Although invasion of *T. gondii* is considered an active process, the parasitophorous vacuole (PV) has to detach from the host cell plasma membrane before translocation to the perinuclear sites of the infected cell. An immunofluorescence assay was performed with the aim to investigate if dynamin participates on this pinch off, and also on the translocation of the PV and parasite egress. Cells were fixed at different moments post infection, being half of the samples permeabilized with 0.1% Triton X-100 in PBS, but all of them were pre-incubated with 50 mM ammonium chloride and 3% BSA in PBS, pH 8.0, and incubated, first with the primary antibody anti-dynamin, and then with secondary antibody goat anti-mouse IgG (H+L) conjugated to AlexaFluor 488. Actin was stained with phalloidin-red. After washing with PBS and mounting with prolong antifade, slides were examined in a Zeiss 510 LSM. The same moments of *T. gondii* cellular cycle were investigated by cryoimmuno microscopy, labeling dynamin with 15 nm, gold-labeled with anti-mouse IgG, and observed in a FEI Tecnai electron microscope. The detection of dynamin during invasion is an indication of a role in the PV's detaching from host cell plasma membrane, in a similar way to that described for endocytosis. However, *T. gondii* egress seemed independent of dynamin participation, whereas its presence during *T. gondii* development is indicative of undescribed roles for this molecule in the tachyzoite's cell cycle. **Supported by**:CNPq and Inmetro
Keywords: Toxoplasma gondii; electron microscopy; dynamin

HP169 - THE ROLE OF MUTT AND CATALASE ENZYMES IN *T. CRUZI* INFECTIVITY

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Introduction: To deal with oxidative stress to which *T. cruzi* is submitted in its life cycle, this parasite has an efficiently regulated antioxidant machinery, despite the absence of catalase, an enzyme that promotes H_2O_2 detoxification. Enzymes of the oxidized guanine DNA repair system (GO), responsible for repairing DNA damage caused by 8-oxoG, are also found in this parasite. Objective: Our aim was to investigate the importance of MTH (responsible for removing 8-oxo-dGTP from the nucleotide pool) and catalase in *T. cruzi* infection, using parasites overexpressing the TcMTH enzyme (homologous to MutT of bacteria) and heterologously expressing *Escherichia coli* EcCat. Methods and Results: Macrophages from C57BL/6 WT or Phox KO (deficient in the gp91phox subunit of NADPH oxidase) mice were infected with *T. cruzi* and the parasite burden was analyzed by optical microscopy. Our results show that recombinant parasites (TcMTH and EcCat) presented improved growth in these cells when compared with TcWT. Furthermore, modified parasites had increased parasitemia in WT mice. *In vitro*, EcCat multiplied better than TcMTH. However, *in vivo*, TcMTH parasitemia was increased as compared to EcCat. The infection in Phox KO macrophages is reduced compared to the infection in WT macrophages. However all the parasites increased infectivity in Phox KO macrophages after treatment with H_2O_2. After H_2O_2 treatment, TcWT and TcMTH increase 3,67 and 4,21 times, respectively, the parasitism in Phox KO macrophages, while EcCat increases only 2,65 times. Conclusions: Our results indicates that the overexpression of MTH increase parasite resistance and its capacity to respond to H_2O_2 signaling, while overexpression of EcCat makes the parasite more resistant, but reduces its capacity to respond to ROS signal. The higher parasitemia observed in mice infected with TcMTH parasites indicates that capacity to be a signaling parasite is more important than the ability to be only resistant. **Supported by**:INCT Redoxoma, FAPEMIG, CAPES, CNPq, REDE DE PESQUISA EM DOENÇAS INFECCIOSAS HUMANAS E ANIMAIS - MG
Keywords: Trypanosoma cruzi; catalase; tcmth

HP170 - METABOLOMIC FINGERPRINT AS AN ALTERNATIVE TOOL FOR CHEMICAL TAXONOMY OF LEISHMANIA

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Different species of Leishmania can cause different types of syndromes and injury to mammalian hosts. Genomic analysis of *L. major*, *L. infantum* and *L. braziliensis* have shown only a modest number of species-specific genes, suggesting that the differences between the species might be due to post-transcriptional and/or post-translational mechanisms. Here we have applied a metabolomics fingerprint approach to characterize four species of Leishmania (*L. major*, L. infantum, *L. braziliensis* e *L. amazonensis*), associated to distinct sets of clinical manifestations. A multiplatform assay was used, based on gas chromatography, liquid chromatography and capilar electrophoresis, coupled to mass spectrometry. Features were tested in multivariate and univariate statistical analysis, for selection of those that presented at least two statistical significance indexes. Selected features were analyzed by chemoinformatic software for putative identification of metabolites that were confirmed by MS-MS fragmentation profile or by comparison to chemical standards. Chemometric analysis revealed significant metabolic differences that can be useful to characterize different species of *Leishmania*. Moreover, they may reflect in the associated species distinct host interaction and disease manifestation. In vivo and *in vitro* assays are underway to investigate this hypothesis that could reveal key pathways involved in the pathogenesis of *Leishmania* infection. Metabolomics has great potential to be used as taxonomic tool and could be applied to improve the diagnosis, treatment and leishmaniasis epidemiological investigations. **Supported by**:CAPES, FAPEMIG, CNPq, MS-DECIT
Keywords: Metabolomics; leishmania; virulence

HP171 - ARYL HYDROCARBON RECEPTOR (AHR) CONTROLLING INFLAMMATION AND REACTIVE OXYGEN SPECIES DURING EXPERIMENTAL *TRYPANOSOMA CRUZI* INFECTION

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Introduction: *Trypanosoma cruzi* is a protozoan parasite that causes Chagas disease. Host derived- Reactive oxygen species(ROS) has a controversial role in *T. cruzi* biology and recent research indicates that the transcription factor aryl hydrocarbon receptor(AhR) is involved in the control of ROS metabolism. Moreover, AhR is an important mediator in innate and acquired immune responses. In this work we studied the function of AhR in ROS production during *T. cruzi* experimental infection.
Methods and Results: WT and AhR KO peritoneal macrophages(MΦ) were infected with *T. cruzi*. We measured intracellular parasite growth by counting amastigotes and released trypomastigotes. First, we found no difference in the uptake of parasite by AhR KO and WT cells. However, the amount of amastigotes 48h post infection(PI) and trypomastigotes were significantly lower in AhR KO MΦ when compared with WT. A real time chemiluminescence assay was done for ROS detection in MΦ. We observed that AhR KO MΦ produced similar levels of ROS than WT cells at basal levels. However, a dramatic decrease in MΦ ROS production either stimulated with Zymosan or infected by *T. cruzi* was observed in AhR KO Mø when compared to WT. *In vivo* AhR function during *T. cruzi* infection was analyzed by studying cell populations producing IFN-γ, IL-12, IL-17A and IL-10 by flow cytometry and total cytokines mRNA levels by qPCR. We found that absence of AhR resulted in substantial increased amount of Th1, Th2, Th17 and related cytokines in spleen at 10 days PI.
Conclusion: Our results demonstrated that AhR mediates Mø susceptibility to *T. cruzi* and is important for ROS production. This suggests that Mø derived-ROS could be necessary for the parasite replication. *In vivo*, AhR is involved in the regulation of cytokine-mediated immune response of several cell populations against *T. cruzi* demonstrating the importance of the molecular mechanism downstream of AhR in controlling the intensity of immune responses to infections. **Supported by**:CNPq, FAPEMIG e CAPES **Keywords:**Trypanosoma cruzi; aryl hydrocarbon receptor; reactive oxygen species

HP172 - THE ROLE OF ANNEXIN A1 IN THE CONTEXT OF *LEISHMANIA BRAZILIENSIS* INFECTION

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Annexin A1 (calcium-dependent phospholipid-binding protein with 37-kDa) has significant roles on modulation of inflammatory responses. AnxA1 influences the control on the process of leukocyte recruitment to inflammatory sites, modulating the generation of proinflammatory mediators, including those derived from activation of PLA2, COX-2, and iNOS, as well as of the anti-inflammatory cytokine IL-10. Recent evidence suggests that AnxA1 may control leukocyte apoptosis in vitro and favor their removal by macrophage phagocytosis. In this study, we have demonstrated for the first time that AnxA1 levels are significantly increased in sera of patients with mucosal leishmaniasis (ML), as compared to cutaneous leishmaniasis (CL) patients and control subjects. We then hypothesized that AnxA1 may be important for control of inflammatory responses in *L. (V.) braziliensis* infection. Although extremely susceptible to other *Leishmania* species, BALB/c mouse is resistant to *L. (V.) braziliensis* infection, controlling both the inflammatory responses and parasite replication, being an adequate model to investigate resolution of inflammatory responses in this context. We, therefore, compared the course of *L. (V.) braziliensis* infection in BALB/c and AnxA1 deficient mice and investigated the kinetics of inflammatory and pro-resolutive responses. In wild type (WT) mice ANX A1 expression is modulated during infection and is associated with control of tissue parasitism and inflammatory responses. Our results also demonstrate higher ERK ½ and NFk-B activation in ANX-KO animals, as compared to WT mice, after 8 weeks of infection. Curiously, flow cytometry analysis showed increased activation of T lymphocytes in the first weeks of infection in ANX-KO animals. Although the role of AnxA1 in various inflammatory conditions has been already investigated, as far as well know, this is the first performed in Leishmania infection and may provide new insights for the development of leishmaniasis effective therapies. **Supported by**:CAPES, CNPq and FAPEMIG **Keywords:**Leishmania braziliensis; inflammation; annexin a1

HP173 - HOST CELL INVASION BY INSECT AND MAMMALIAN STAGE DEVELOPMENTAL FORMS OF TRYPANOSOMA CRUZI ARE DIFFERENTIALLY REGULATED BY LYSOSOME BIOGENESIS/SCATTERING

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A fundamental question that remains to be elucidated concerning the host cell invasion by Trypanosoma cruzi is whether the insect-borne and mammalian stage parasites use similar mechanisms for internalization. To address that question, we used in vitro-generated metacyclic trypomastigotes (MT) and tissue culture trypomastigotes (TCT), as counterparts of insect-derived and bloodstream parasites respectively, and analyzed their infectivity under diverse conditions. Incubation of parasites with HeLa cells for 1 h in nutrient-free PBS++, a condition that triggered lysosome biogenesis and scattering toward the cell periphery, resulted in higher MT invasion and lower TCT internalization than in full nutrient medium. Sucrose-induced lysosomal biogenesis rendered HeLa cells more susceptible to MT and more resistant to TCT, when compared to untreated cells. HeLa cells treated with rapamycin, an allosteric inhibitor of mammalian target of rapamycin complex 1 (mTORC1), exhibited perinuclear lysosome accumulation and were more refractory to MT but more susceptible to TCT invasion. Confocal microscopy images revealed that metacyclic forms were internalized within a vacuole decorated with mTOR co-localized with lysosomal protein LAMP2 whereas very few TCT exhibited these markers. MT, but not TCT, induced mTOR dephosphorylation and the nuclear translocation of TFEB, a lysosome biogenesis regulator associated with mTORC1, leading to lysosomal biogenesis/scattering, events barely detectable upon TCT incubation with HeLa cells. Interaction of HeLa cells with a recombinant protein based on gp82, the MT surface molecule that promote cell invasion, resulted in mTOR/LAMP2 association at the cell periphery, translocation of TFEB to the nucleus and lysosomal biogenesis/scattering. Taken together, our data indicated that MT invasion is mainly lysosome-dependent and involves mTORC1 whereas TCT internalization is predominantly lysosome-independent and is stimulated by mTORC1 inhibition **Supported by**:FAPESP and CNPq
Keywords: Metacyclic trypomastigotes; lysosomal biogenesis ; transcription factor eb

HP174 - KINETIC ASSESSMENT OF PRO-INFLAMMATORY MARKERS IN LESIONS OF MICE BALB/C INFECTED WITH *LEISHMANIA BRAZILIENSIS* OR *LEISHMANIA AMAZONENSES*

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Considering the cell signaling pathways involved in the inflammatory responses in *Leishmania* infection, and the lack of studies on the inflammation pro-resolving mediators, this study aimed to characterize the kinetics of inflammatory response after infection of BALB/c mice by *L. braziliensis* or *L. amazonensis*. For this purpose, mice were inoculated with promastigotes of *L. braziliensis* or *L. amazonensis*, and the expression ERK1/2, caspase 3, NF-KB, annexin A1 was evaluated. Lipoxin A4 was also evaluated in mice infected by *L. braziliensis*. Cytokine levels (IFN-γ and IL-4) were assessed in lympho node cells. As expected BALB/c mice were resistant to infection by *L. braziliensis*, controlling tissue parasitism and inflammation, twelve weeks post-infection. IFN-γ levels picked at 2 weeks post-infection. Histologically, inflammatory cells' infiltration occurred early and was self-limited. In contrast, BALB/c mice which was susceptible to *L. amazonensis*, developed progressive lesions with increasing parasitism after infection. The IFN-γ production was observed only nine weeks after infection and, histologically, there was an exacerbated inflammation with heavily infected macrophages and necrotic areas. There was activation of ERK1/2 and NF-κB after infection by L. braziliensis, with subsequent return to their baseline levels, 12 weeks post-infection.There was also production of lipoxin A4 during the course of infection. In contrast, during infection by *L. amazonensis*, no changes were observed in the activation of ERK1/2 and NF-κB or annexin A1 in the first 4 weeks, as compared to control mice. However, activation of ERK1/2 in the fifth week post-infection, a pattern reversed in the ninth week. Together, these data confirm the great difference between the immune response triggered in infection by *L. braziliensis* or *L. amazonensis*, which is partly due to differential handling capacity of inflammatory signaling pathways for each species of parasite. **Supported by**:CAPES, FAPEMIG, CNPq **Keywords:**Leishmania braziliensis; l. amazonensis; inflammation

HP175 - PROFILE OF THE RESIDENT MICROBIOTA IN THE SKIN AND ITS ROLE IN CUTANEOUS LEISHMANIASIS

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Leishmaniasis is an infectious disease caused by various types of parasite of the genus *Leishmania* spp and is a vast source of research, due to the fact that it is a disease in which there is still no cure, and can bring severe clinical manifestations to host, such as cutaneous lesions, the clinical form which we will use as the focus of our study. Several studies have shown that the microbiota can stimulate the immune system. A report from our group has demonstrated that the microbiota play an important role during the infection with *Leishmania major.* The focus of our study is to characterize the resident microbiota in the skin of mice infected with *Leishmania amazonensis* in order to discover whether they can be beneficial or harmful to the host, identifying specific microbes that may have an effect. C57BL/6 mice were infected with 10^4 *L. amazonensis* in both ears. We followed the course of infection for 6 weeks. Ears were collected at every week and bacterial DNA was extracted using DNA extraction kits. Swabs of each ear were performed and plated in agar plates. Gram stains of each colony found were analyzed on the microscope. Our preliminary results indicated that there is a change in the profile of the skin microbiota during infection, with an increase of total bacteria in infected mice. How these changes may influence the immune response and the pathology during infection by *L. amazonensis* is not yet known. Experiments are being conducted to further investigate these effects. **Supported by**:CAPES
Keywords: Microbiota; leishmania; skin

HP176 - THE ROLE OF INNATE IMMUNE RECEPTOR DECTIN-1 DURING EXPERIMENTAL MALARIA

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Introduction: Malaria is an important disease that affects millions of people worldwide. The study of immune receptors during this infection is relevant for the development of therapeutic and prophylactic measures against infections induced by *Plasmodium spp*. Here we show that the absence of Dectin-1 confers protection against an experimental model of cerebral malaria. Methods and Results: Female wild type (WT) and genetically deficient in Dectin-1 ($D1^{-/-}$) C57BL/6 mice were infected with *P. berghei* for the measurement of survival, parasitism/parasitaemia, brain inflammation, blood biochemistry and evaluation of the cytokine profile. Experiments with P. berghei demonstrated that WT and $D1^{-/-}$ mice display similar morbidity scores. However, $D1^{-/-}$ mice showed increased survival and decreased parasitaemia. After three days of infection, no differences were observed in the concentration of IL-12p40, although $D1^{-/-}$ mice produced less IFN-γ. $D1^{-/-}$ mice also showed less serum concentration of ALT and AST hepatic enzymes, increased glycemic profile, while no differences were observed in urea concentration. In accordance, $D1^{-/-}$ mice presented decreased parasitism in the liver. Additionally, histological analysis of brain sections demonstrated that $D1^{-/-}$ mice presented minor inflammatory alterations, which were not compatible with cerebral malaria. On the other hand, the central nervous system of WT mice were severely affected by the infection, with the presence of notable perivascular cuffs, necrosis and vessel obstruction. Conclusion: Our results demonstrated that Dectin-1 receptor may be a target for the development of prophylactic and therapeutic protocols against malaria. **Supported by**:CAPES, Fapemig, CNPq
Keywords: Clec7a; p. berghei; immune response

HP177 - DRIED SPOT FC-TRIPLEX CHAGAS/LEISH-IGG1 METHODOLOGY FOR THE DIFFERENTIAL DIAGNOSIS OF CHAGAS DISEASE

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We have recently reported a nonconventional serological approach, referred as FC-TRIPLEX Chagas/Leish IgG1(TRIPLEX) for detection of anti-Trypanosoma cruzi IgG1 reactivity in a single flow cytometry platform, which showed an outstanding accuracy and performance indexes for the differential diagnosis of CD. In this work, we intended to standardize the Dried Spot FC-TRIPLEX Chagas/Leish IgG1 (DS-TRIPLEX), aiming to upgrade the TRIPLEX to be applied in air-dried samples after long-term storage at room temperature (RT). Our results demonstrated that the DS-TRIPLEX* can yield equivalent results as compared to TRIPLEX, but requiring proper sample serial dilutions. One month after storage, equivalent results could be observed using the 2-folds lower serial dilution for DS-TRIPLEX (1:1,000-8,000) as compared to TRIPLEX (1:2,000-16,000). One-year after RT-storage, although equivalent results could still be observed in both methods, the serial dilution for DS-TRIPLEX decreased drastically to 500-folds lower (1:4-1:32) in order to yield equivalent results to TRIPLEX. It is possible that the heat/humidity oscillation and the vulnerability to protein degradation by contaminant microorganisms may have contributed to the drastic drop observed in the antibody elution from air-dried samples during one-year of storage at RT. In conclusion, our data suggest that DS-TRIPLEX represent an alternative method to be applied in CD confirmatory diagnosis using air-dried samples. This upgrade added a reliable gain to the TRIPLEX, especially regarding its application in field studies or in demands from blood banks located in distant geographic areas or those without flow cytometry facilities that required sample shipment to reference laboratory to confirm non-negative result obtained during CD serological screening. Further studies, testing distinct storage conditions are currently under investigation, in order to optimize the antibody elution from long-term stored air-dried samples. **Supported by**:FAPEMIG, CNPq, CAPES and FIOCRUZ **Keywords:**Chagas disease; cytometry; diagnosis

HP178 - NOVEL ROLES OF PEROXIREDOXINS FROM*TRYPANOSOMA CRUZI*

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Trypanosoma cruzi, the causative agent of Chagas disease, is a flagellated protozoan with a complex life being able to invade almost any kind of nucleated cell. During invasion process, both surface and secreted proteins from *T. cruzi* play essential roles in host-parasite interaction and infection. Peroxiredoxins are antioxidant enzymesthrough their peroxidase activity, which is has been recently demonstrated are secreted by damaged cells but also by some parasites undernormal conditions. Secreted peroxiredoxins have additional functions, like immunomodulation, signaling, survival, among others.

In this work we analyzed the secretion of cytosolic tryparedoxin peroxidase from *T. cruzi* (Tc-cTXNPx) by western blot and immunoelectron microscopy in epimastigotes and cell derived trypomastigotes. We found that Tc-cTXNPX is secreted in both parasite stages in extracellular vesicles but also it isdetectedin the vesicle free fraction. Parasitic peroxiredoxin is able to enter host cells through vesiclesbut also the protein alone is capable of interact and enter cells by endocytosis. Interestingly,the interaction of recombinant Tc-cTXNPx with human epithelial cells promotes cell proliferation through ERK activation. We also observed that Tc-cTXNPx enhances the expression of LDL receptoras well as parasite´s infectivity. Our results suggest that Tc-cTXNPxplay a relevant role in parasite-host interaction and parasite invasion.

Finally we found that Tc-cTXNPxis also endocytosedby murine macrophages promoting the secretion of proinflammatory cytokines(IL6 and IL12)indicating that in this cell model the protein also plays animmunomodulatoryrole. **Supported by**:Universidad de la República
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