

HP017 - ACANTHAMOEBA CASTELLANI: PROTEIN PROFILE OF TROPHOZOITES RE-ISOLATED FROM CORNEAL LESION EXPERIMENTALLY INDUCED IN RATS IS DISTINCT OF THE LONG-TERM CULTURE.

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The free-living amoebae of the genus *Acanthamoeba* are opportunistic pathogens that eventually cause serious diseases in humans. The most usual infection caused by these protozoan is *Acanthamoeba keratitis* (AK), a corneal involvement characterized by a severe inflammation which may result in blindness. The knowledge about the proteins involved in this pathophysiological process can be helpful to develop diagnostic and therapeutic strategies for AK. Thus, our proposal is to conduct a proteomic study to identify proteins expressed differently by *Acanthamoeba* trophozoites in long-term culture or re-isolated from corneal lesion induced in an animal model of AK. An *Acanthamoeba castellanii* strain from a patient with AK and maintained in an axenic culture (proteose-peptone yeast extract, glucose - PYG medium), since 2007 was used in the experiments. Wistar rats (45 days old, n= 8) were used to induce AK by intrastromal inoculation of 105 trophozoites. After six days, the corneas were removed from euthanized animals and fragments were inoculated in PYG medium. Two animals presented lesions and one re-isolated culture was obtained. Protein extract from the long-term and re-isolated culture, processed by sonication, was subjected to one-dimensional SDS-PAGE. Activity of somatic proteases was also determined by gel zymography technique, using gelatin as substrate. In SDS-PAGE, seven additional bands were detected in the re-isolated sample and at least two bands were absent, compared to the long-term culture. Serine proteases from about 40 to 145 kDa were observed in zymography of long-term culture sample. Re-isolated sample showed similar zymography profile, except by the absence of a 145 kDa band. These preliminary results indicated that *Acanthamoeba* trophozoites can modulate the protein expression during the infectious process. The next step is a proteomic 2D and mass spectrometry analysis which may unveil the identity of the proteins involved in this process.

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Keywords:Proteomic; *acanthamoeba*; amoebic keratitis

HP018 - HIGH PREVALENCE OF TOXOPLASMA GONDII IN SAUSAGES FROM ERECHIM SOUTHERN BRAZIL

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Infections caused by *T. gondii* affect one third of the world population. The most important way of transmission is the ingestion of cysts in the raw or undercooked meat. In southern BRA the high prevalence of blindness caused by *T. gondii* is also attributed by the ingestion of pork meat and contaminated water. Our purpose is to determine the frequency of *T. gondii* in salami and sausage from Erechim/RS. A total of 118 samples (sausage, n=59 and salami, n=59) were collected from 8 different local meat processors from January to October 2015. We extracted DNA from the samples and Real Time PCR (qPCR) technique was performed to detect *T. gondii* DNA using B1 marker. The results demonstrated that among the total number of samples, 23.7% (28/118) were positive for *T. gondii* infection. Besides, among the 59 sausages and 59 salamis samples, 40.7% (24/59) and 6.8% (4/59) respectively were qPCR positive for B1 marker. Seven of the 8 meat processors tested positive for the presence of toxoplasma DNA. Our study indicates a high prevalence of *T. gondii* DNA mainly in sausage samples from Southern Brazil. **Supported by:**CNPq

Keywords:Toxoplasma gondii; embutidos; pcr em tempo real

HP019 - ANTIGENS CONTAINING REPEAT DOMAINS AS VIRULENCE FACTORS THAT CAUSE IMMUNOSUPPRESSION DURING TRYPANOSOMA CRUZI INFECTION

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Repeat domains are patterns of two or more amino acids present in many proteins that are highly abundant in *T. cruzi*, where they are thought to be involved in immune evasion mechanisms. Here, we show evidence for a role of the repeat domains present in a *T. cruzi* ribosomal protein named TcL7a after immunization of mice with the recombinant full length protein and its truncated versions; Tc Δ Rep (protein without the repeat domain) and TcRep (protein containing only the repeat domain). Immunization with the full length protein induces secretion of antibodies against the repeat domain and protects against a challenge infection with 5000 trypomastigotes of the CL Brener strain. The truncated version Tc Δ Rep induces lower antibody production and lower protection compared with the full length L7a. In contrast, immunization with TcRep not only results in no antibody production against L7a but also exacerbates the parasitemia and increases mortality rates. However, qPCR assays showed that heart parasitism was not affected by the immunization with TcRep. To elucidate the mechanism behind the observed effects of TcRep immunizations we performed in vitro and in vivo experiments. We found that NO production by macrophages as well as parasite uptake after stimulation macrophages with IFN were not affected by incubation with TcRep and that incubation of mice splenocytes with TcRep did not induce non specific antibody production. In contrast, ELISA with sera from immunized and non-immunized mice that were challenged with trypomastigotes, taken at the peak of parasitemia, showed that the immunized animals developed a lower antibody response as determined by the levels of specific IgM and IgG antibodies against total *T. cruzi* protein extract compared to controls, non-immunized infected mice. Taken together, our results indicate that this *T. cruzi* antigen containing a repeat domain contribute to establish parasite infection by negatively modulating the host humoral immune response. **Keywords:** Repetitive domains; immunoevasion; humoral immunity

HP020 - ROLE OF SKIN MICROBIOTA IN IMMUNE RESPONSES DURING EXPERIMENTAL CUTANEOUS LEISHMANIASIS

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Leishmaniasis is an infectious disease caused by various types of parasite of the genus *Leishmania* spp. 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 evaluate the role of the skin resident microbiota in mice infected with *Leishmania amazonensis*. Swiss NIH germfree mice were monoassociated with *Staphylococcus aureus* or *Corynebacterium accolens* and three days later were infected with 10^4 *L. amazonensis* in both ears. Ears were collected at 24 hours and two weeks after the infection and immune cells were characterized by flow cytometry. Parasite load was determined two weeks after infection. We found no difference in numbers of CD11b⁺ cells, macrophages and CD8⁺ T cells between the groups two weeks after infection. Mice monoassociated with *C. accolens* presented lower numbers of CD4⁺ T cells when compared with germfree mice. Monoassociation with *S. aureus* or *C. accolens* led to reduced numbers of Treg cells (Foxp3⁺). There was no difference in numbers of neutrophils 24 hours after infection. After two weeks of infection neutrophils were found infiltrating again the ear of germfree mice. Interestingly, this new influx of neutrophils was not observed in monoassociated mice. Parasite load two weeks after infection was similar between the groups. Our results indicate that bacteria from the skin microbiota can influence immune responses during *L. amazonensis* infection. Mice monoassociated with different skin bacteria displayed reduced inflammatory infiltrate at the early stages of the infection. How these changes may influence the pathology during the infection is not yet known. Experiments are being conducted to further investigate these effects.

Supported by: CAPES **Keywords:** Leishmania; microbiota; skin

HP021 - ROLE OF ALANINE RACEMASE AND FREE D- ALANINE IN TRYPANOSOMA CRUZI

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L-amino acids has been shown to be relevant in many *Trypanosoma cruzi* biological processes. However, little is known about the functions of D-amino acids on the biology of the parasite. The proline racemase (PR) was firstly describe in *T. cruzi*, as a potent mitogen. PR is also involved in the infectivity of the host cells by the parasite. The occurrence of D-Alanine and an Alanine Racemase (AR) activity was also described in *Leishmania amazonensis*. We identified by the first time in a pathogenic protozoa a gene encoding a putative AR (TcAR), a pyridoxal 5'-phosphate dependent enzyme that catalyzes the racemization between L-and D-alanine. We cloned and expressed TcAR and characterized its recombinant product (rTcAR). rTcAR showed a MW of 43kDa, and Vmax and Km values of 21.5 ± 6.04 mM and 60 ± 6.7 μ mol/min/mg respectively. Immunofluorescence with a specific anti-rTcAR serum showed a cytoplasmic localization for TcAR. Specific activity was higher in insect stages compared to mammalian stages. Interestingly, both D- and L-alanine were able to maintain the parasites viability under metabolic stress conditions. In addition, we showed that D-Alanine is a substrate of an ATP-dependent alanine ligase and is excreted in the extracellular medium. We also selected an AR inhibitor, C3, which was able to reduce rTcAR activity, epimastigotes growth, as well as to disrupt plasma membrane, to induce DNA damage, deregulation of ROS, cytosolic Ca²⁺ and mitochondrial membrane potential in epimastigote. Furthermore, C3 impaired the intracellular life cycle of *T. cruzi* with a high selectivity index (<300). The results demonstrated that TcAr is functional and is a relevant target for chagas therapy, as there is no homologous gene in mammalian host. The role of TcAR and free D-alanine, as well as the enzymatic characterization and its participation in the cell bioenergetics have to be investigate more in detail to elucidate their relevance in *T. cruzi* biology.

Supported by:CNPQ FAPESP **Keywords:**Trypanosoma cruzi; d-alanine; racemase

HP022 - LAMP INVOLVEMENT WITH PLASMA MEMBRANE REPAIR AND T. CRUZI HOST CELL INFECTION.

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Trypanosoma cruzi enters host cells by subverting the mechanism of cell membrane repair. In this process, the parasite induces small injuries in the host cell membrane leading to calcium entry, which in turn induces lysosomal exocytosis. These lysosomal exocytic events are followed by compensatory endocytosis that drives parasites into host cells. We have previously shown that absence of both LAMP-1 and 2, major components of lysosomal membranes and also highly sialylated proteins, decreases invasion of *T. cruzi* into host cells. We have also shown that parasite adhesion to host cell surface was compromised in cells lacking LAMP, with no change in vacuole morphology, indicating the protein itself rather than only its sialic acid modifications as a determinant for decreased invasion. We here investigated the ability of LAMP-1/2-/- cells to perform membrane repair upon injury, the mechanism by which *T. cruzi* enters cells. We have observed that these cells do have the same lysosomal exocytic ability. However, their ability to perform comepensatory endocytic events after lysosomal secretion is compromised, without any defect in acid sphingomyelinase secretion, when compared to WT cells. As a consequence, cells are less efficient in repairing from injury, which also compromises *T. cruzi* host cell invasion. Moreover, we showed that LAMP-2 alone was enough to reproduce these phenotypes indicating that LAMP-2 plays a major role in this process.

Keywords:T. cruzi ; lamp ; membrane repair

HP023 - **EVALUATION OF PROTECTION AGAINST THE FORMATION OF CEREBRAL CYSTS IN IMMUNIZED MICE WITH IRRADIATED TACHYZOITES OF DIFFERENT STRAINS OF *TOXOPLASMA GONDII***

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Toxoplasma gondii is an obligate intracellular parasite that affects homothermous animals causing toxoplasmosis, a disease that does not present specific symptoms and rarely causes severe disease in its host, what corroborates to a significant number of affected individuals. Studies have shown that 255Gy irradiated tachyzoites do not cause infection in the host and induce immunity as natural infection, but currently there have been only studies with RH strain. In this study, mice were immunized by the parenterally route with three biweekly doses of irradiated 255Gy (Cobalt60) *T. gondii* tachyzoites RH, ME49 and both. Two weeks after last dose, we evaluated antibody responses (IgG, IgM and IgA), IgG subclasses (IgG1, IgG2a and IgG2b) and protection was measured by numbers of brain cysts, one month after challenge. By ELISA all immunized models presented antibodies production in their serum with higher IgG production in 255Gy RH and 255Gy RH+ME49 groups. IgM and IgA antibodies production was similar in both groups. The evaluation of IgG subclasses was characterized by a TH1 response by increased production of immunoglobulin of type IgG2a and IgG2b. All immunized groups presented significant protection when challenged with ME49, however, 255Gy RH+ME49 group showed higher protection, with three negative animals on brain microscopic analysis and one by real-time PCR. Our results show that irradiated parasites, in association with the different strains, presents an efficiency immune response with high protection and could help in the design of an efficient vaccine candidate to interrupt the transmission chain of toxoplasmosis.

Supported by:FAPESP **Keywords:***Toxoplasma gondii*; ionizing radiation; protection

HP024 - **TRYPANOSOMA CRUZI GENOTYPES IN CHILEAN CHRONIC CHAGASIC PATIENTS TREATED WITH NIFURTIMOX**

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Chagas disease (ChD), caused by the flagellated protozoan *Trypanosoma cruzi* is an endemic and important problem of public health in Latin America. Eventhough Chile has been declared free of transmission of *T. cruzi* by *Triatoma infestans* since 1999, it is estimated that there are over 150.000 individuals infected from Arica and Parinacota (XV Region, Latitude: -18° 28' 17.99" S) to the O'Higgins (VI Region, Latitude: -34° 10' 1.20" S). Human ChD has two clinical phases: acute and chronic, the latter is characterized by low parasitemia. According to the nomenclature used in recent years, *T. cruzi* is clasfied into six distinct discrete typing units (DTUs) named as TcI-TcVI, with a seventh one limited only to bats (TcBat). Each DTU has a variety of geographical distributions and ecoepidemiological associations. The distinction between the *T. cruzi*-DTUs can be made by diverse genetic markers. This study focused on aimed the molecular identification and genotyping of *T. cruzi* in 30 Chilean chronic chagasic patients (ECh) from Coquimbo (IV Region, Latitude: -29° 57' 11.95" S) that have been treated with nifurtimox (NFX). NFX was administered under Informed Consent during the years 2009-2010 for two months. DNA extractions (pre and post therapy) were prepared from blood samples received in Guanidine/EDTA. The presence of *T. cruzi* was determined by qualitative PCR using primers specific to amplify the minicircle variable region of the kinetoplastid DNA (kDNA) and the highly repetitive nuclear satellite DNA (satDNA). *T. cruzi* genotyping was performed by amplification of minixon and 18S and 24S α rRNA genes. In all the patients were observed *T. cruzi* PCR specific bands (kDNA and satDNA) in pre and post therapy conditions. The genotyping performed in 30 cases evidenced that infections with *T. cruzi*-DTUs corresponding to TcII/TcV/TcVI group. Genotyping methodology can be performed by conventional PCR in blood samples of patients with low parasitemia.

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Keywords:Genotyping; dtu; niurtimeox

HP025 - **PARASITOLOGICAL AND ELECTROCARDIOGRAFICAL RESPONSE IN CHRONIC CHAGAS PATIENTS 4 YEARS AFTER TREATMENT WITH NIFURTIMOX**

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Chagas disease (ChD) is a zoonosis caused by the flagellate protozoan *Trypanosoma cruzi* (*T. cruzi*). In Chile, the ChD is a prevalent infection, and it is estimated that 140.000 people are infected, mostly without tripanocidal treatment. Nifurtimox (NFX) and benznidazole (BNZ) are authorized for the specific treatment of the disease, being available in our country. The aim of this study was to evaluate the chemotherapeutic efficacy of NFX after 4 years of therapy performed in individuals with chronic ChD according to currently ministerial protocols, through the parasitic load of *T. cruzi* in blood, measured by Real Time PCR. On the other hand, the electrocardiographic condition pre and post-therapy was classified in 4 groups (A-C), according to results of electrocardiogram of 12 derivations. The population in study corresponded to 62 individuals with chronic ChD who were treated with NFX in 2009 (8-10 mg/kg/day for 60 days). The average parasite load in conditions of pre and post-therapy was 10,5 and 0,91 parasite equivalents/ml, respectively, with significant differences between the two averages ($p < 0,0001$). On the other hand, there were no differences ($p = 0,26$) of the parasite load between the diverse electrocardiographic condition after treatment. However, 63,4% of individuals did not progress to chronic Chagas cardiopathy. Currently it is estimated that the chemotherapeutic efficacy of chronic cases treated with NFX and BNZ does not exceed 30%. The results of this research are auspicious, since it was determined that there are differences between parasitic loads before and after treatment, and in 51,7% of the cases the parasitemia disappears. Such patients remain in prolonged follow-up to the establishment endpoint 9-10 years after treatment with NFX. **Supported by:** Fondecyt Projects 1120382-1161485

Keywords: Chagas disease; nifurtimox; real time pcr

HP026 - **QUANTIFICATION OF *TRYPANOSOMA CRUZI*'S PARASITEMIA IN RURAL AREAS: RODENTS AS SENTINELS.**

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Chagas disease, caused by the protozoan *Trypanosoma cruzi*, is maintained in sylvatic vectors and reservoirs as an endemic infection in part of Chile, even though the domestic vectorial transmission was interrupted in 1999. The aim of this study was to determine if the parasite circulates near rural houses by monitoring potential reservoirs as sentinels. Domestic and peridomestic areas of 8 localities of the endemic Coquimbo Region were prospected using live baited traps. Peripheral blood samples were collected from captured micro-mammals and immediately preserved with 1:1 volume of Guanidine-HCl/EDTA solution. DNA was extracted using a commercial kit and quantified using a fluorometer. The parasite loads were quantified by qPCR assays with *T. cruzi* nuclear satellite DNA primers cruzi 1 and cruzi 2. The standard curve for absolute quantification was performed with a ten-fold serial dilution using equal quantities of two DTUs: TcId (DM28c) and TcII (Y). Each sample was run in duplicate, along with a positive, negative and no template controls. We captured 57 rodents: 48 synanthropic (42 *Rattus rattus* and 6 *Mus musculus*) and 9 endemic species (5 *Phyllotis darwini* and 4 *Octodon degus*). From these, 42 (73.7%) were positive to *T. cruzi*: 66.7% of *M. musculus*; 73.8% of *R. rattus*; 50% of *O. degus*; and 100% of *P. darwini* were infected. The 28.6% of the infected rodents were captured indoors. Parasite loads fluctuated between $< 1 - 16,600$ par-eq/mL, with a median of 4.3 par-eq/mL. One *R. rattus* had the highest parasitemia, possibly due to an acute infection. This recent infection might have occurred in the vicinity of where it was captured. These results show that *T. cruzi* circulates very near to rural people in this Region. Considering that local sylvatic vectors are found near these houses and that sentinels were infected, we confirm an established peridomestic transmission, so rural rodent and vector control should be encouraged. **Supported by:** FONDECYT 1140650; 3140543. **Keywords:** Chagas disease; qpcr; reservoir

HP027 - EXPRESSION OF TIGHT JUNCTION PROTEINS IN EPITHELIAL INTESTINAL CELLS INFECTED WITH *GIARDIA LAMBLIA* UNDER LOW OXYGEN LEVELS.

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Giardia lamblia is a microaerophilic protozoa parasite and the ethiological agent of giardiasis, a diarrheic disease affecting animals and humans worldwide. *Giardia* is an extracellular parasite of the small intestine and causes the disease without penetrating the epithelia and adjacent tissues. Previous studies demonstrated that *Giardia*-induced epithelial dysfunction is associated with the loss (or delocalization) of tight junction proteins (TJs) and actin rearrangement in intestinal cells infected in vitro. Several cell lines have been successfully used as models of the small intestine in *Giardia* interaction experiments such as Caco-2 cells (human epithelial colorectal adenocarcinoma cells) under aerobic conditions. In this work, we evaluated the effect of *G. lamblia* infection on tight junction expression and transepithelial electrical resistance (TEER) on two different intestinal cell lineages: HT29 (human colorectal cancer cell) and Caco-2 cells under reduced oxygen levels (pO_2). Initially, we evaluated cell viability under low oxygen tension levels by flow cytometry analysis. Our data clearly shows that both HT29 and Caco-2 cells are viable in low O_2 up to 4h, indicating that parasite-host interaction experiments could be conducted under these conditions. Next, the expression of tight junctional proteins in both cell lines co-cultured with *Giardia* trophozoites under low pO_2 were analyzed by Western blot. Both Caco-2 and HT29 cells expressed increased levels of claudin-4 and ZO-1 at various time points (up to 4h) under low oxygen compared to control cells not infected. The *Giardia*-induced increase in intestinal TJs correlated with increased TEER values as a measure of tight junction integrity. **Supported by:** CNPq e FAPESP

Keywords: Intestinal parasite; cell barrier; permeability

HP028 - *GIARDIA LAMBLIA* TROPHOZOITES DISPLAY MUCINOLYTIC ACTIVITY AND PREFERENTIALLY BIND TO NON-MUCIN SECRETING HUMAN INTESTINAL CELLS

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The gastrointestinal tract (GI) represents a large surface area that functions as an interface between the human host and a diverse array of microorganisms like virus, bacteria, fungi, protozoa and multicellular parasites causing diseases in humans. It is covered by a continuous layer of mucus, mainly composed of glycoproteins from the mucin family. In *Giardia lamblia* infections, mucins are known to inhibit the in vitro attachment of trophozoites to substrates suggesting that the mucus may function as physical barrier against the parasite. In this work, we used cultured human colonic mucin-secreting intestinal LS174T cells and two human non-mucin-secreting intestinal cells HT29 (colonic) and HUTU80 (duodenal), to evaluate the effect of mucus on *Giardia* adhesion. The data clearly show that *Giardia* trophozoites bind preferentially and in higher numbers in HUTU80 cells, followed by HT29 and LS174T cells. Addition of commercially purified mucins (BSM, bovine submaxillary mucin and PGM, porcine gastric mucin) to the incubation medium significantly reduced the attachment of the trophozoite to HUTU80 and HT29 cells compared with controls. Finally, we evaluate trophozoites ability to enzymatically degrade mucins (mucolytic activity). At first, total trophozoites cell extracts were tested for protease activity by gelatin zymography confirming the activation status of the enzymes. Next, trophozoites were incubated in solution with BSM and PGM, aliquots were withdrawn at different time points and degraded mucins were analyzed by SDS-PAGE after Periodic acid-Schiff staining (PAS). Our data clearly show that trophozoites degrade BSM but not PGM in a time-dependent manner, suggesting that degradation of mucins may be a general mechanism exploited by *Giardia* to penetrate the mucus and adhere to the cell surface.

Supported by: CNPq and FAPESP

Keywords: Intestinal parasite; mucins; mucus degradation

HP029 - ANTIGENS CONTAINING AMINO ACIDS REPEATS ACT AS VIRULENCE FACTORS DURING TRYPANOSOMA CRUZI INFECTION AND ARE ALSO TARGETS FOR SERODIAGNOSIS OF CHAGAS DISEASE

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Proteins containing repetitive amino acid sequences are highly abundant in different intracellular protozoan parasites. It has been suggested that their repeat domains are part of mechanisms designed by these parasites to avoid an effective host immune response. To test this hypothesis, we are investigating two *T. cruzi* antigens containing amino acid repeats: TcL7a and trans-sialidase. TcL7a is a ribosomal protein identified after immunoscreening a cDNA library with sera from patients with Chagas disease that shows homology with other eukaryotic protein L7a. However, unlike the L7a from other eukaryotes, TcL7a contains an Ala-Pro-Lys-rich repeat domain at its N-terminal portion, which seems to contribute to promote parasitaemia by negatively modulating the host humoral immune response. Trans-sialidase (TS) is a surface *T. cruzi* antigen that contains a 12 amino acids repeat domain at its C-terminal portion, named SAPA repeats. TS is part of a polymorphic family of surface proteins encoded by a multigene family with more than 1000 copies in the *T. cruzi* genome. Some TS members possess the ability to transfer sialic acid residues from host glycoconjugates to mucins present on the surface of the parasite. Similar to TcL7a repeats, various studies have indicated that SAPA repeats may contribute to parasite virulence. To test their role as virulence factors, we generated recombinant versions of these two proteins, with and without their repeat domains as well as the proteins containing only the repetitive domain to be used in immunization experiments. Since these repeats are exclusive from *T. cruzi* proteins and are targets of the immune response, we have also tested both recombinant antigens containing TcL7a and SAPA repeats in ELISA using sera from patients with Chagas disease as well as other parasitic diseases. Using a gold nanoparticle-based assay, we aimed at developing a new serologic test with high sensibility and specificity to detect *T. cruzi* infection. **Supported by:** CNPQ

Keywords: Trans-sialidase; tcl7a; nanoparticles

HP030 - SINGLE-WELL DETECTION OF ANTI-*T. GONDII* IGG, IGM AND IGA ANTIBODIES FOR TOXOPLASMOSIS ANTENATAL CARE SCREENING USING FLUORESCENT CONJUGATES

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Toxoplasma gondii causes treatable central nervous system and eye diseases in the children of acutely infected mothers. Toxoplasmosis screening in pregnant women is mandatory to prevent those congenital infections and early therapy for the avoiding disease sequels. Recent studies show low (20%) toxoplasmosis prevalence in children, that will be adults and mothers, resulting in high proportion of seronegative women. For antenatal screening of a treatable infection, we need new quick and inexpensive assays. New Solid Phase Fluorescent Assays (FISA) allows direct antibody quantification in microplates as we reported simultaneous anti-*T. gondii* IgG and IgM detection high specificity and low cost due to improved fluorescent conjugates. Here, we standardized NHS-fluorescent conjugates in a single-well detection of anti-*T. gondii* IgG, IgM and IgA antibodies for toxoplasmosis antenatal care screening. Affinity-purified conjugates were prepared with amine-reactive Alexa Fluor[®] fluorescent dyes, tested with different excitation filters for IgG/IgM/IgA detection and compared with commercial peroxidase conjugates. We used 20 serum samples from adult volunteers at a large public hospital with external and in house isolated and conjunct (triplex FISA) anti-*T. gondii* immunoglobulin detection. There was excellent agreement (Kappa index=100%) between isolated and conjunct detection without false-positives and false-negatives results. This technique could have improved resolution with more adequate and suitable optic filters than commercially supplied. Using high throughput plates and assays, this technique will allowing the efficient detection of specific antibodies classes against *T. gondii*, especially in the future with the increase demand of monthly serology of seronegative women for antenatal care screening for toxoplasmosis.

Keywords: *Toxoplasma gondii*; fluorescent conjugates; solid phase serology

HP031 - LIPOPHOSPHOGLYCAN (LPG), THE MAJOR *LEISHMANIA* SURFACE GLYCOCONJUGATE, IS FOUND IN THE EXTRACELLULAR VESICLES (EVs) OF *LEISHMANIA INFANTUM* AND *LEISHMANIA BRASILIENSIS*.

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Glycoconjugates are highly expressed in the surface of *Leishmania* spp including lipophosphoglycan (LPG), glycoinositolphospholipids (GIPLs) and glycoprotein 63 (gp-63). The aim of this project is to determine if those major surface glycoconjugates are also expressed in extracellular vesicles (EVs) of New World species *Leishmania infantum* and *Leishmania Brasiliensis*, the causative agents of visceral and cutaneous forms, respectively. It is already known for *Leishmania* that gp63 is one of the molecules expressed in their EVs surface, especially in the Old World species such as *L. donovani*. However, the presence of LPG in the EV surface is still to be determined. Promastigote forms of *L. infantum* and *L. Brasiliensis* were incubated in serum-free media for 2 hours, 37 °C for vesiculation. Parasites were fixed for conventional scanning electronic microscopy (SEM) for vesicle release demonstration. A higher amount of EVs was qualitatively observed in *L. infantum* promastigotes by SEM. Culture supernatants were processed by differential centrifugation and vesicles were isolated by Size Exclusion Chromatography (SEC) and quantitated by nanoparticle tracking analysis (NTA). NTA successfully detected the presence of EVs in the expected size range of exosomes with modal sizes of 125.2 (±15.8) nm and 133.6 (±15.8) nm for *L. infantum* and *L. Brasiliensis*, respectively. Immunodetection of glycoconjugates was performed in ELISA and dot-blot assays, SEC fractions were probed with anti-LPG (CA7AE) and anti-gp63 mAbs (1:1000). LPG was detected in same the gp63-exosome-containing fractions. Those data strongly support that LPG is also present in the surface of promastigote-derived vesicles. The LPG of *L. Brasiliensis* is very-pro-inflammatory via TLR2, whereas that of *L. infantum* is very immunosuppressive. Since this molecule is present in the EVs, our next step is to investigate whether those properties will also reflect in the immunomodulation of murine macrophages upon EVs stimulation.

Supported by: CAPES, CNPQ AND FAPEMIG

Keywords: Leishmania; extracellular vesicles; lipophosphoglycan

HP032 - MICROBICIDAL EFFECT OF *TITYUS SERRULATUS* IN *TOXOPLASMA GONDII*-INFECTED MACROPHAGES: IDENTIFICATION OF THE KEY PEPTIDE

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INTRODUCTION: *Toxoplasma gondii* (Tg), the Toxoplasmosis etiological agent is an intracellular parasite capable of fool the immune response and persist in various hosts' organs. In immunosuppression cases, chronically infected individuals may suffer relapse, which leads to lesions mainly in the central nervous system. Inflammatory mediators (IM) produced in Tg infection are crucial to control parasite growth, avoiding disease reactivation. We show that venom of yellow scorpion *Tityus serrulatus* (TsV) induce production of IM by Tg-infected macrophages (MΦ), reducing parasite load. However, the characterization and pharmacodynamics of effector molecules need to be done.

METHODS AND RESULTS: TsV was separated in 7 fractions (Ts1-Ts7) by Gel Filtration Chromatography. Only fractions Ts6 and Ts7 presented immunomodulatory activity inducing nitric oxide (NO), IL-12, TNF, IL-6 and IFN-γ production and toxoplasmodicidal action by C57BL/6 MΦ. Aiming to isolate/purify effector molecules, the subfractions of Ts6 fraction was obtained using Cation Exchange Chromatography. MΦ were plated, infected 1:1(Tg:cell) ratio and stimulated with the subtractions of Ts6. Parasite replication assay was evaluated counting the number of intracellular Tg after 48h of infection/stimulus. The subfraction Sub6-C showed higher ability to stimulate MΦ, resulting in lower parasite load, compared with the non-stimulated MΦ and others subfraction. Sub6-C was sequenced, characterized, by bioinformatics generating conformational peptides from the original sequence of the Sub6-C :Sub6-C1. MΦ were infected and stimulated with Sub6-C1 [100,50,25 μg/ml]. Sub6-C1 was able to induce NO production by Tg-infected MΦ. It is known that this parasite strongly reduces levels of NO in activated MΦ, but the amount was still remained enough to contribute to the toxoplasmodicidal activity. **CONCLUSION:** Our data demonstrated a toxoplasmodicidal activity of TsV's components as promising drug against protozoan disease .

Supported by: CNPq

Keywords: Immunoparasitology; immunoregulation; toxinology

HP033 - TGF- β POLYMORPHISMS ARE A RISK FACTOR FOR CHAGAS DISEASE
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Transforming growth factor β 1 (TGF- β 1) is an important mediator in Chagas disease physiopathology. TGF- β 1 serum levels increase in patients with Chagas disease and are found active in the myocardium of patients with chronic Chagas disease. Furthermore, patients with higher TGF- β serum levels show a worse clinical outcome. Gene polymorphism may account for differences in cytokine production during infectious diseases. Here, we tested whether TGF- β 1 polymorphisms could be associated to Chagas disease susceptibility and severity in Brazilian population. We investigated five single nucleotide polymorphisms (-800 G>A, -509 C>T, +10 T>C, +25 G>C and +263 C>T). 152 patients with Chagas disease (53 with the indeterminate form and 99 with the cardiac form) and 37 non-infected controls were included. We found that genotypes C/T and T/T at position -509 of the TGF- β 1 gene were more frequent in Chagas disease patients than in non-infected controls ($p < 0.008$ and $p < 0.01$, respectively). Genotype C/T at position +10 of the TGF- β 1 gene was also more frequent in Chagas disease patients than in non-infected controls ($p = 0.001$). However, we could not find any significant differences in the distribution of any of the studied TGF- β 1 polymorphisms between patients with the indeterminate form or different stages of the cardiac form of Chagas disease. Interestingly, the T/T genotype at position -509 was associated with higher serum levels of TGF- β 1. Taken together these results show that -509 C/T and +10 T/C TGF- β 1 polymorphisms are associated with Chagas disease susceptibility in a Brazilian population. **Supported by:**DECIT / FAPERJ / IOC-FIOCRUZ / CAPES / INSERM

Keywords:Chagas disease; polymorphism; tgf

HP034 - ORAL ADMINISTRATION OF GW788388 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 important 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. GW788388 treatment also improved heart remodeling since restored the decreased LV ejection fraction. Furthermore, GW788388 treatment, three times a week, was able to reverse collagen expression in the heart of infected animals. We also observed that TGF- β circulating levels were increased due to *T. cruzi* infection and GW788388 treatment reverse these levels significantly. To date, the results are promising 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

HP035 - **LEISHMANIA AMAZONENSIS ALTERS MIRNA EXPRESSION TO CONTROLS NO/POLYAMINES PRODUCTION IN INFECTED MACROPHAGES**

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The non-coding microRNAs (miRNAs) 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. Production of Nitric Oxide (NO) versus polyamines during *Leishmania* infection plays a pivotal role in the survival of parasite. The *L. (L.) amazonensis* infection can subvert the miRNAs profile of mouse macrophages as well as the production of NO. Herein, the activity of arginase from parasite can be involved in macrophage miRNA modulation.

The WT-*L. (L.) amazonensis* infection of BALB/c mice Bone Marrow-Derived macrophage (BMDM) modulated 27% of the 84 miRNAs analyzed when compared to non-infected BMDM, from these, 78% were upregulated. The infection with arginase knockout-*L. (L.) amazonensis* (La_arg-) increased the percentage of miRNAs modulated to 43%, but only 22% of them were upregulated.

Moreover, the up-regulation of miR-294-3p and miR-721 was detected in WT-*L. (L.) amazonensis* infected BMDM, but a down-regulation was observed in BMDM infected with La_arg-. Also, infection with addback arginase addressed (La_arg+ARG) or not to the glycosome (La_arg+ΔSKL-ARG) showed an increased level of miR-294-3p and miR-721. Indeed, the Nitric oxide synthase 2 (Nos2) expression and NO production were higher in BMDM infected with La_arg- or La_arg+ΔSKL-ARG accompanied by an decrease of infectivity, when compared to WT. Blocking the binding regions of these miRNAs to Nos2 mRNA, caused an increase in NOS2 expression and NO production in BMDM infected with WT-*L. (L.) amazonensis*. 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; no production; post-transcriptional regulation

HP036 - **ARE GOATS INFECTED WITH TRYPANOSOMA CRUZI IN RURAL ENDEMIC AREAS OF CHILE?**

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Domestic vectorial transmission of Chagas disease was interrupted in Chile; however, *Trypanosoma cruzi* is maintained in sylvatic vectors and reservoirs in some areas of the country. Our aim was to determine if peridomestic transhumant goats were infected, and to determine their parasite loads. We sampled goats from 6 localities of the endemic Coquimbo Region, previous informed consent of their owners. Jugular blood samples were collected and immediately preserved with 1:1 volume of Guanidine-HCl/EDTA solution. DNA was extracted with a commercial kit and quantified using a fluorometer. We quantified *T. cruzi* DNA by qPCR assays using nuclear satellite primers cruzi 1 and cruzi 2. From 338 goat samples, 198 (58.6%) amplified *T. cruzi*'s DNA, ranging between 46.4% and 94.7% according to locality. Parasite loads fluctuated between <1 – 45.4 par-eq/mL with a median of 2.0 par-eq/mL. By locality, the frequency of infection (Fisher $p < 0.0001$) and the parasite loads of the infected individuals (Kruskal Wallis $p = 0.0001$) were different. Tulahuén had the highest percentage (%) of infected goats (94.7%), and the highest parasite load median (4.6 par-eq/mL). The % of infected goats and the parasite load median of the infected goats by locality were correlated (Spearman $\rho = 0.8857$; $p = 0.0188$), so in areas with high frequency of infection, caprine parasite loads would be higher; this could reflect a vectorial transmission risk for goatherds if they constantly share spaces with caprine herds in endemic areas. Goats are a food source for people, so they might transmit the parasite by contamination during slaughter, or when eating raw goat products. Caprine infection may be causing decreased production, reducing the income of rural people. Modifying goats' management might reduce their infection risk, such as converting rock piles' corrals into other materials that offer less resting places for triatomines; or avoiding unattended grazing, to ensure keeping goats away from vectors' foci.

Supported by:FONDECYT 1140650; 3140543. **Keywords:**Chagas disease; qpcr; peridomestic

HP037 - IMMUNOPROTEOME OF CHAGAS DISEASE: SEARCH FOR BIOMARKERS
CANDIDATES FOR CARDIAC DAMAGE PROGRESSION

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Chagas disease, caused by *Trypanosoma cruzi*, affects millions of people worldwide, especially in Latin America. Approximately 30% of the individuals in the chronic phase will eventually develop clinical manifestations. As the most severe and frequent symptoms are related to the heart, here, we focused on the search of potential protein biomarkers for detection of cardiomyopathy progression. After bloodstream trypomastigotes (Y strain) protein extraction, the *T. cruzi*'s immunoreactive proteins were obtained by immunoprecipitation through the interaction with IgG immobilized on magnetic beads. The IgG were previously purified by affinity chromatography from serum of patients in the indeterminate and cardiac (B1 and C) stages and from healthy donors. Through mass spectrometry analysis, the immunoreactive proteins were identified and quantified, and the pattern of recognition among the conditions was subsequently evaluated. The IgG derived from patients with diagnosed cardiac lesions led to the detection of ten differentially abundant proteins; four proteins were detected in higher amounts through the recognition by samples from B1 patients, while and other six different proteins had greater abundance in the fractions obtained by the reaction with samples from stage C patients. Thus, it was observed an alteration in the abundance and profile of bloodstream trypomastigotes' proteins recognition in the course of the disease. The significative variation in the abundance of the immunoreactive proteins, especially through the interaction with IgG from B1, suggests that these proteins are promising candidates of markers for the establishment of the cardiac form of the disease. However, these results need to be validated in a large number of patients.

Supported by:FAPERJ, CNPq and FIOCRUZ

Keywords:*Trypanosoma cruzi*; proteomics; immunoreactive protein

HP038 - THE ROLE OF HOST MICROBIOTA DURING CUTANEOUS LEISHMANIASIS

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Animals are colonized by their indigenous microbiota from the early days of life. The estimated number of associated bacterial cells is around 10^{13} per individual. Several studies have investigated the microbiota-host relationship and the use of germ-free (GF) animals has been an important tool in these studies. These animals, when infected with *Leishmania major* in the footpad, developed a typical Th1 immune response, but failed to heal lesions, while conventional (CV) mice developed the same response and controlled the infection. Macrophages from CV mice seems to respond better to IFN- γ stimuli comparing to macrophages from GF mice, which is important for parasite killing. Then, we evaluated the role of microbiota during an infection by a new world specie of this parasite, After infected with 10^4 metacyclic forms of *L. amazonensis* in the ear GF mice showed bigger lesions than CV mice until 8wks p.i. Afterwards GF mice showed smaller lesions than CV. CV group showed higher parasite load in the ear 6 wks p.i and higher arginase activity in the ear during the course of infection. In addition, GF mice produced more IL-1 β 4 wks p.i and both groups showed similar levels of IFN- γ production by lymph node cells. Using flow cytometry to characterize the inflammatory infiltrate in the ear during the course of infection, we observed that GF mice presented lesser recruitment of neutrophils and monocytes into the ears 6 wks p.i. Interesting, the GF mice infiltrate showed higher number of iNOS+ cells during the course of infection. The higher frequency of iNOS+ cells in the ear of GF mice could explain the lower parasite load in the GF ears. Our data suggest the role of host microbiota may vary between the cutaneous *Leishmania* species infection as GF mice are more resistant during *L. amazonensis* and more permissive for *L. major*. Next step will be identify why the absence of microbiota induce more iNOS in GF mice during *Leishmania amazonensis* infection.

Supported by:CNPq, CAPES, FAPEMIG

Keywords:*Leishmania amazonensis*; microbiota; germ free

HP039 - **MORPHOLOGICAL ASPECTS OF THE *TOXOPLASMA GONDII* AND CORTICAL ASTROCYTES INTERACTION, IN VITRO**

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Toxoplasmosis affects all warm-blooded animals, including humans and has a high prevalence worldwide. The ability to infect any nucleated cell allows *Toxoplasma gondii* to spread into any tissue. Once inside the host animal, the parasite is capable of crossing the Blood Brain Barrier and infect neurons and glial cells, disturbing cerebral functions, leading to behavioral and cognitive impairment. The parasite has a tropism for the Central Nervous System and, at the chronic phase, abundant cysts are found in the brain. Over the past decades, cortical astrocytes, recognized as auxiliary satellite cells have gained notoriety for their numerous functions in the CNS. Thus, the aim of this project is to study the kinetics of infection of primary cultures of cortical astrocytes and the establishment of cystogenesis. Cells were isolated from neonatal Swiss Webster mice and infected with tachyzoites of the ME-49 (type II) strain. Our results show a high susceptibility of astrocytes in harboring parasites. In the 1:1 ratio (parasite-host cell), with only 24 hours, 20% of the cells were infected, with an increase of infection of about 50% at 48 to 96 h interaction. Using a 5:1 ratio this infectivity was higher: 36% of the cells were infected in 24 hours of interaction, 64% in 48 hours and reaching a peak of 77% at 120 hours. Experimental maneuvers, such as short time parasite-host cell contact, about only 2 hours, were crucial for obtaining high rate of infection of astrocytes, and enable the establishment of *T. gondii* cystogenesis when analyzed by microscopy light, fluorescence and transmission electron. Ultrastructural analysis showed a close association of astrocytes organelles with parasitophorous vacuole membrane and the cyst wall. These data open up excellent prospects to explore the cell biology of astrocytes infection with *T. gondii*, particularly during cystogenesis. **Supported by:**FAPERJ, CNPq and IOC/Fiocruz PAPES VI

Keywords:Toxoplasma gondii; astrocytes; cystogenesis

HP040 - **LOW DEGREE OF VARIABILITY OF *TRYPANOSOMA CRUZI* KINETOPLAST DNA ON HUMAN BLOOD SAMPLES FROM TWO GENERATIONS OF A FAMILY WITH CHAGAS DISEASE**

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Chagas disease (CD), caused by the protozoon *Trypanosoma cruzi*, is characterized by different clinical forms, resultant of complex interactions among the environment, genetic factors of the parasite and host. Several methodologies have been used to evaluate the molecular epidemiology of *T. cruzi* and identify possible correlations between its clinical forms and the genetic variability of the parasite and host. In the present study, we used Low-Stringency Single Specific Primer-Polymerase Chain Reaction (LSSP-PCR) that allows evaluate the variability of *T. cruzi* populations in order to assess the similarity of the genetic profiles of circulating *T. cruzi* in two generations of a chagasic family (a mother (M), and four daughters: (F1); (F2); (F5) and (F6)) from the Alto Paranaíba region, MG state, BRA, an endemic area for CD. The infection was confirmed by serology using in direct hemagglutination method (IHA) and enzyme-linked immunosorbent assay (ELISA). For LSSP analysis the DNA was extracted from blood samples and amplified. Unweighted Pair Group Method with Arithmetic Mean (UPGMA) algorithm and Shannon index were used to construct a phenogram and indicate the genetic variability of the isolates of *T. cruzi*, respectively. The profiles presented a total of 8 bands and none of them was shared among all the samples. The UPGMA dendrogram distinguished the samples into three distinct clusters, in which the kDNA of *T. cruzi* obtained of the M showed a genetic profile distant of all the samples. F1 and F2 were more genetically closer and F5 and F6 more genetically related to M. The mean heterozygosity per locus (He) was 0.298, indicative of low genetic diversity among all samples and moreover, the Shannon diversity index was also considered low (0.466). Our data show that the genetic profiles of the *T. cruzi* samples in this study are similar. **Supported by:**CNPq Fapemig

Keywords:Trypanosoma cruzi; lssp; variability

HP041 - **DETERMINATION OF THE FUNCTIONAL ROLE OF A RNA BINDING PROTEIN ON THE GENE EXPRESSION REGULATION OF THE AMINO ACID TRANSPORTER AAP3 IN *LEISHMANIA (L.) AMAZONENSIS*.**

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The RNA binding-proteins (RBPs) are fundamental for processing control, transport, RNA stability and translation of proteins, regulating RNA metabolism and function by interaction with the 3'-untranslated regions (3'-UTR). Recent studies have shown the RBPs influence over the cell metabolism in trypanosomatids as in cellular cycle control, differentiation of promastigotes into metacyclics or amastigotes, in different hosts, as well as its involvement in post-transcriptional regulation of gene expression in *Leishmania spp.* L- arginine starvation led to an increase in the half-life of the AAP3 transporter transcripts (*La-aap3*), resulting in the increase of *La-AAP3* and a consequent increase in L-arginine uptake in stationary-promastigotes (Castilho-Martins et al 2011). The starvation also led to the increase of LinJ 04.0040 transcript, that codes for a putative RBP (Goldman-Pinkovich et al, 2016) in *L. (V.) donovani*. Here, we compared both *La-AAP3* transporter and *L. (L.) amazonensis* LinJ 04.0040 homolog expressions during log and stationary phase of *L. (L.) amazonensis* promastigotes, WT or knockout for arginase (*La-arg-*). We confirmed the increase of the levels of *La-aap3* transcript in stationary phase compared to log phase in WT cells, but higher levels of RBP were detected in stationary phase of WT and *La-arg-* parasite, compared to log phase. The levels of the *L. (L.)amazonensis* LinJ 04.0040 homolog transcript correlated with the increased levels of *La-aap3* transcripts in stationary phase WT promastigotes. These observations can implicate this RBP in the regulation of the expression of AAP3, through the stabilization of its mRNA, leading to an increase of L-arginine uptake in stationary promastigotes. **Supported by:**FAPESP

Keywords:Rna binding-protein; leishmania; transporter

HP042 - **MICROBIOTA INFLUENCES THE INFLAMMATORY RESPONSE AND THE PHAGOCYTIC CAPACITY OF MACROPHAGES DURING ORAL INFECTION WITH *TRYPANOSOMA CRUZI***

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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 related to the wide variety of indigenous bacteria. Thus, in conventional (microbiota-bearing) animals, the indigenous microbiota maintains a "physiological inflammatory state" which is 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*. To test our 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. So, we evaluated the immune response in both groups and we observed higher levels of IL-12p70 and IL-17 in the colon of infected GF and CV mice compared to the respective uninfected mice. The same trends were observed for IL-10 in the gut and for IL-12p70 and IL-17 in the mesenteric lymph node. Since macrophages play an important role in controlling parasite replication, we infected GF and CV bone marrow-derived macrophages. We observed that the CV macrophages have a high infection rate than GF macrophages. To test if this difference in infection rates was related to the phagocytic capacity of macrophages, we did a phagocytosis assay using *Saccharomyces boulardii*, finding the same result. We also observed that macrophages from CV mice showed increased production of IL-10 and NO, while GF had higher levels arginase. We concluded that the microbiota influences oral infection with *T. cruzi*, probably interfering in the parasite growth and promoting a pro-inflammatory state and by recruiting host cells. The lower parasite load in GF mice can be associated with lower phagocytic capacity of macrophages, considering the phagocytosis as an important regulator to the course of infection.

Supported by:CAPES, CNPq, FAPEMIG

Keywords:Trypanosoma cruzi; germ free mice; trypanosoma cruzi

HP043 - **EVALUATION OF PRODUCTION AND AVIDITY IGG, CIRCULATING ANTIGENS AND PRESENCE OF IMMUNE COMPLEXES IN EXPERIMENTAL VISCERAL LEISHMANIASIS**

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Visceral leishmaniasis (VL) is a neglected disease, an estimated 300,000 cases and 20,000 deaths annually. Hypergammaglobulinemia is found in VL but is not associated to effective control of the disease. Circulating immune complexes (CIC) were associated with hypergammaglobulinemia and we showed that CIC could interfere in the positivity of serology in infected hamsters. Widely used in epidemiology, serology does not distinguish active disease and may be inconclusive due to the presence of CIC. We study Leishmania CIC during experimental infection in hamsters and its impact in conventional serology. Serum samples from *L. (L.) infantum chagasi* infected hamsters were collected at 15, 30, 45, 60 and 90 days after infection. For IgG detecting and IgG avidity we used conventional ELISA (cELISA), an direct antigen capture (DAC) ELISA assay using rabbit IgG anti-*Leishmania* and a dissociative pH shock ELISA (dELISA), which is performing by diluted sample brought at pH 2,5, followed by neutralization. cELISA specific IgG showed gradual increase, with higher levels found after 30 days of infection. Detection of circulating antigen by DAC and the IgG increment in dELISA identified positive CIC samples in most periods of infection. Highest frequency of positive samples was found at 30 days of infection (28% and 54.1%, respectively). ELISA IgG avidity showed the same proportion of high-avidity antibodies in different samples dilutions (1/100, 1/200, 1/400 and 1/800) in all periods of infection, suggesting absence of avidity maturation of anti-*Leishmania* IgG antibodies. The association of these results indicates that during the experimental VL in hamsters, CIC were present and interfere with conventional serology, and could be also associated to immunopathology due to the absence of antibody avidity maturation. Antibodies and circulating immune complexes could be involved in the establishment of ineffective immune response associated to B cell activation in experimental VL. **Supported by:**CNPQ

Keywords:Visceral leishmaniasis; immune complexes; igg

HP044 - **TRANSCRIPTOMIC ANALYSIS OF STAGE TRANSITION IN TRYPANOSOMA CRUZI**

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American trypanosomiasis is a chronic and endemic disease, which affects millions of people. *Trypanosoma cruzi*, its causative agent, has a life cycle that involves complex morphological and functional transitions, as well as a variety of environmental conditions. This requires a tight regulation of gene expression, which is achieved mainly by post-transcriptional regulation. In this work we conducted the first RNAseq analysis of the three major life cycle stages of *T. cruzi*, amastigotes, epimastigotes and trypomastigotes. This analysis allowed us to delineate specific transcriptomic profiling for each stage, and also to identify those biological processes of major relevance in each state. Stage specific expression profiling evidenced the plasticity of *T. cruzi* to adapt quickly to the different conditions, with particular focus on membrane remodeling and metabolic shifts along the life cycle. Epimastigotes, which replicate in the gut of insect vector, showed higher expression on genes related to energy metabolism, mainly Krebs cycle, respiratory chain and oxidative phosphorylation related genes, and anabolism related genes associated to nucleotide and steroid biosynthesis; also a general down regulation of surface glycoproteins was seen at this stage. Trypomastigotes, living extracellularly in the bloodstream of mammals, express a plethora of surface proteins and signaling genes involved in invasion and evasion of immune response. Amastigotes mostly express membrane transporters and genes involved in regulation of cell cycle, an also express a specific subset of surface glycoproteins coding genes. In addition, these results allowed to improve the annotation of Dm28c genome, identifying new ORFs and set the stage for construction of networks of co-expression, which can give clues about coded proteins of unknown functions.

Keywords:Trypanosoma cruzi; surface proteins; rna seq

HP045 - **COMPARATIVE SECRETOME AND INTERACTOME ANALYSES OF PATHOGENIC AND NON-PATHOGENIC TRYPANOSOMES**

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Trypanosomes are parasitic flagellate protozoa that live in blood or other tissues of their hosts. Some of them can cause serious illness for humans such as Chagas disease (*Trypanosoma cruzi*) and Sleeping Sickness (*Trypanosoma brucei*). Conversely, *T. rangeli* and SECRETED P which share high genetic similarity with *T. cruzi* and *T. brucei*, respectively, are non-pathogenic to human. This intriguing fact has encouraged comparative studies between pathogenic and non-pathogenic species. Pathogenic species can secrete proteins that manipulate multiple host cell signaling pathways related to immune response and phagocytosis, favoring the parasite invasion, proliferation and survival. Bioinformatics tools can aid to predict proteins responsible for pathogenicity. Here, we present an integrated computational pipeline for analysis of secreted and transmembrane proteins in trypanosomes, and also preliminary findings on these proteins. The bioinformatics pipeline was mostly based on signal peptide and transmembrane detection, protein orthology clustering and protein-protein interaction. The softwares were settled at the Center for Biological Sequence Analysis. We separated proteins that are exclusively secreted from transmembrane proteins excluding those belonging to internal membranes and those addressed to internal compartments. We identified 192 and 202 potentially secreted proteins in *T. cruzi* and *T. rangeli* respectively. Among *T. cruzi* proteins, there are several sialidases, phospholipase A1 and Hsp70 which were previously characterized to be possibly related to *T. cruzi* virulence. Regarding *T. brucei* and *T. evansi* we found 68 and 217 secreted proteins, respectively. Secreted proteins that can modulate and manipulate host immune system were selected to build a Bayesian phylogeny and interactome analysis. These results will pave the way for a better understanding of their pathophysiology and ultimately leading to the identification of molecular targets for drug development.

Supported by: FAPESP AND FAPEMIG

Keywords: Secreted proteins; bioinformatics pipeline; chagas disease and sleeping sickness

HP046 - **TOXOPLASMA GONDII IMPAIRS NEURONAL DIFFERENTIATION IN NEURO2A CELLS**

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Toxoplasmosis is a worldwide spread zoonosis caused by *Toxoplasma gondii*, an obligate intracellular protozoan that affects all warm-blooded animals, including humans. The ability of translocate through the placenta barrier and to replicate in different fetal tissues, without being affected by the immune system of the fetus, makes *T. gondii* infection an important cause of prenatal complications. Vertical transmission may interfere on the development of the fetus, leading to abortions or several malformations, such as encephalitis, neurological defects, blindness and microcephaly. The parasite has a tropism for the Central Nervous System where tissue cysts are found. Due to the severity of the congenital transmission and its effects on the CNS, the aim of this project is to study the mechanisms involved in the pathogenesis of toxoplasmosis, focusing on neurogenesis. For this purpose, we used Neuro2a cells, a neuroblastoma cell line that have the potential to differentiate into neurons upon serum withdrawal. N2A cells were infected with tachyzoites of the ME-49 (type II) strain for 24h in the presence of Fetal Bovine Serum (FBS, 10%) and were induced to differentiate with the replacement of FBS for DMEM containing Bovine Serum Albumin. Uninfected cultures reached up to 60% of neuronal cells, as assessed by β -III-tubulin (TUJ-1) immunostaining. Our preliminary data indicate that *T. gondii*-infected cultures interfere in neurogenesis by decreasing in 28% the number of TUJ-1 positive cells. These results will contribute to a better understanding of the mechanisms involved in the pathogenesis of congenital toxoplasmosis.

Supported by: CIEE, CNPq (Edital Universal), PAPES VII/Fiocruz

Keywords: Neurogenesis; congenital toxoplasmosis; microcephaly

HP047 - **CLONING AND HETEROLOGOUS EXPRESSION OF ATP
DIPHOSPHOHYDROLASE 2 (NTPDASE2) FROM *LEISHMANIA (VIANNIA) BRASILIENSIS*
ET AND NSL STRAINS.**

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Leishmaniasis are diseases caused by Leishmania protozoa and *L. braziliensis* is the main causative agent of cutaneous leishmaniasis in New World. There is no human vaccines and only low number of drugs to treatments. In addition, these drugs present undesirable toxic effects and drug resistance treatments. Researches show that different strains of *L. Brasiliensis* display differentiated ectonucleotidase activity, who affects clinical outcome. Thus, it has been suggested that high extracellular nucleotides hydrolysis imply in higher parasite virulence and NTPDase2 enzyme has been thought to be involved in this process. Therefore, the aim of this study is cloning, sequencing and heterologous expression of NTPDase2 from *L. (Viannia) Brasiliensis* ET and NSL strains, in order to evaluate the presence of single nucleotide polymorphisms, which could justify the observed divergent ectonucleotidase activity. Furthermore, obtaining the recombinant protein would allow evaluate its potential for use in cutaneous leishmaniasis immunodiagnostic. The respective genes of both strains were amplified by PCR, using gene specific primers containing NdeI and HindIII enzyme restriction sequences. Amplicons were purified from 1% agarose gel followed by pJET 1.2/blunt blunt-end ligation and Escherichia coli DH5-Alpha transformation. As result, we obtained five ET clones and seven NSL clones. One clone of each strain was sub-cloned in pET21b expression vector and confirmation was made by PCR, digestion and sequencing. The results was assayed on Geneious 6.0.6 program and they confirm in frame vector cloning and hexa histidine carboxy-terminal tag. No difference in primary amino acids sequences was observed, however both sequences present two silent SNP's at 861 e 879 positions. In face of identical primary sequence of both enzymes, only heterologous expression of NSL NTPDase2 is under way. In addition we are expanding the number of single clones in order to find other non-silent SNP's.

Supported by:CAPES **Keywords:**Ntpdase2; cloning; heterologous expression

HP048 - **SECRETION OF CYP 19 BY MAMMALIAN STAGES OF TRYPANOSOMA CRUZI
INVOLVES MODIFICATIONS IN ITS UNIQUE N-TERMINUS**

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Cyclophilins are ubiquitous and evolutionarily conserved enzymes that have a peptide-prolyl-cis-trans-isomerase (Ppiase) activity. These enzymes are expressed from prokaryotes to eukaryotes. Normally cyclophilins are intracellular enzymes and their expression is increased under stress situations and in highly proliferative cells, such as tumor cells. In general, cyclophilins act as chaperones stabilizing other proteins and inducing changes in conformation that results in changes in signaling and protein-protein interactions. They are also found secreted in some organisms. In trypanosomes the most expressed cyclophilin have an additional N-terminal sequence that has been proposed to be involved in secretion out of the cells. We have previously shown that cyclophilin 19 (Cyp19) is involved in the interaction of the Trypanosoma cruzi, the protozoan parasite that causes Chagas disease, with the insect vector. Cyp19 was found secreted by the insect stage of T. cruzi, protecting the parasites from antimicrobial peptide concomitantly causing calcineurin signaling in parasites, and driving infectivity. Here we found that Cyp19 was expressed and released by all lifecycle stages of the parasite. Importantly, Cyp19 was found in the host cell cytoplasm. Therefore, we further investigated the mechanism of secretion. When using parasite extracts, Cyp19 migrated in SDS-PAGE at least as three molecular species. Only one of these forms was found in the parasite supernatant and this form was not recognized by an antibody directed to a peptide corresponding to the 10 amino acids of the protein N-terminus. We also found by mass spectrometry analysis that Cyp19 is acetylated at three lysine residues, including one present in this N-terminal domain. We conclude that either the acetylation in the N-terminal or its removal by proteases might be involved in the secretion process. As secreted cyclophilins control inflammatory responses, the release of Cyp19 might be relevant for the pathogenesis of Chagas disease. **Supported by:**CAPES/FAPESP **Keywords:**Trypanosoma cruzi; ciclophilin; secretion

HP049 - **LEISHMANIA SPECIES IDENTIFICATION BY HSP70 GENE BASED HIGH RESOLUTION MELTING ANALYSIS**

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Leishmania species are involved in human infections, with extensive distribution in BRA. The wide clinical spectrum of the disease is the main factor that make difficult an accurate diagnosis. The correct identification of the causative agent is important for the design of treatment strategies and eco-epidemiological studies. Recently, we showed that the use of two distinct amplicons of *hsp70* gene as target in High Resolution Melting technique (HRM) could identify Brazilian *Leishmania* species (Zampieri et al., 2016). However, *L. (L.) amazonensis*, *L. (L.) mexicana*, and *L. (V.) lainsoni* could only be distinguished by a combination of these two amplicons with the addition of another target to solve *L. (L.) amazonensis/L. (L.) mexicana* identity. In HRM methodology, polymorphic DNA regions can be used in identification if they are bounded by conserved regions. Here, we describe another sequence inside *hsp70* that presents polymorphisms that allow *L. (L.) amazonensis/L. (L.) mexicana* distinction. This way, the analysis of dissociation curves of three *hsp70* based amplicons resulted in the accurately identification of the reference strains of *L. (L.) infantum chagasi*, *L. (L.) amazonensis*, *L. (L.) mexicana*, *L. (V.) lainsoni*, *L. (V.) Brasiliensis*, *L. (V.) guyanensis*, *L. (V.) naiffi*, and *L. (V.) shawi*. DNA from *Trypanosoma cruzi* and *T. brucei* produced HRM profiles distinct from *Leishmania*. Non infected BALB/c mice and human DNA were negative for the three amplicons. DNA from naturally infected sandflies, experimentally infected mice and human biopsies were tested producing results compatible to other identification procedures. *hsp70*-HRM analysis is a low cost, reliable, easy to apply, potentially automated procedure that is a good alternative for the detection, quantification and identification of *Leishmania* species in biological and clinical samples, detecting less than one parasite per reaction, even in the presence of host DNA.

Supported by: FAPESP / CNPq **Keywords:** Diagnosis; leishmaniasis; dna melting analysis

HP050 - **ROLE OF ADENOSINE RECEPTORS ON NATURAL KILLER AND DENDRITIC CELLS RESPONSE DURING INFECTION WITH L. AMAZONENSIS**

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Natural Killer (NK) and dendritic cells (DC) are important effectors in innate immunity. These cells have receptors for adenosine and are subject to its effects.

ATP released by cells under stress, is capable of assisting in the establishment of inflammatory response, while adenosine, the product of ATP hydrolysis by ecto-nucleotidases, leads cells to an anti-inflammatory status.

NK cells, upon stimulation by IL-12 from macrophages and DC secrete IFN- γ . This cytokine is crucial to the elimination of *L. amazonensis* via the activation of infected macrophages and also for the development of subsequent adaptive responses, especially Th1.

L. amazonensis express enzymes able to degrade extracellular ATP to produce adenosine, thereby inducing anti-inflammatory response of various cells, including macrophages, DC and possibly NK cells.

The objective of this study is to evaluate the role of adenosine receptors on NK and DC cells during infection by *L. amazonensis*.

To this aim, bone marrow derived dendritic cells of C57BL/6 infected by *L. amazonensis* were co-cultured with freshly isolated NK cells from spleen of C57BL/6 mice in the presence or absence of antagonists for adenosine A2a and A2b receptors and the production of IFN - γ e IL-12 was measured by ELISA.

Furthermore, C57BL/6 mice were infected by *L. amazonensis* directly or DC infected by *L. amazonensis* in the presence or absence of antagonist A2 receptor and the production of IFN- γ by stimulated lymph node cells was measured by ELISA.

The results show that the presence of antagonists in co-cultures of DC and NK infected with *L. amazonensis* increase the production of IFN- γ in culture. However, the antagonists are not able to restore the IL-12 levels in the culture, after inhibition caused by *L. amazonensis*.

In addition, in vivo studies show that the presence of A2b antagonist can increase IFN- γ produced mice directly infected by *L. amazonensis* or animals that received *L. amazonensis*-infected DC.

Supported by: CAPES, FAPEMIG and CNPq

Keywords: Natural killer; leishmaniasis; adenosine

HP051 - **TREATMENT OF LEISHMANIA (LEISHMANIA) AMAZONENSIS-INFECTED BALB/C MICE WITH THE PALLADACYCLE COMPLEX DPPE 1.2 ASSOCIATED TO THE RECOMBINANT CYSTEINE PROTEINASE RLDCYS1.**

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Leishmaniasis affect 12 million people worldwide and 350 million are at risk of infection. The treatment is limited by toxicity and parasite resistance to the drugs, validating the development of new leishmanicidal compounds. Previous data from our laboratory showed the in vitro and in vivo activity of the palladacycle complex DPPE 1.2 against *L. (L.) amazonensis*. The decrease of parasite burden in *L. (L.) amazonensis*-infected BALB/c mice treated with DPPE 1.2 was followed by immune modulation characterized by increase of T CD4+ and T CD8+ lymphocytes. Our data also showed a significant protection mediated by Th1 lymphocytes against homologous infection in BALB/c mice immunized with a recombinant cysteine proteinase from *L. (L.) chagasi*, rLdcccys1. Cross reactivity between rLdcccys1 and a cysteine proteinase of 30 kDa from *L. (L.) amazonensis* was also demonstrated. Taken together, these findings led us to associate DPPE 1.2 to rLdcccys1 for the treatment of BALB/c mice infected with *L. (L.) amazonensis*. The treatment with 2.5 mg/Kg of DPPE 1.2 alone or associated to 10 mg/Kg of rLdcccys1 resulted in a parasite reduction of 35 and 260 fold, respectively, while this reduction was of 5 fold in animals treated with rLdcccys1. In all treated animals the leishmanicidal activity was followed by a significant increase of T CD4+ and T CD8+ lymphocytes in popliteal and inguinal lymph nodes. In addition, there was a significant reduction of active TGF- β in foot lesions from all treated animals. Evaluation of serum levels of TGO, TGP and urea showed no statistically significant alterations among groups, indicating that treatment with DPPE 1.2 alone or associated to rLdcccys1 did not induce hepato and nephrotoxicity. In conclusion, the treatment with DPPE 1.2 associated to rLdcccys1 showed an additive leishmanicidal effect, opening perspectives to explore the potential of this association as an alternative for the chemotherapy of cutaneous leishmaniasis caused by *L. (L.) amazonensis*.

Supported by: FAPESP **Keywords:** Leishmania (leishmania) amazonensis; dppe 1.2; rldcccys1

HP052 - **VESICLES PRODUCED BY ACANTHAMOEBA ISOLATES OF DIFFERENT GENOTYPES**

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Free-living amoeba of the genus *Acanthamoeba* are ubiquitous protozoa that occasionally cause severe infections in humans, such as granulomatous amoebic encephalitis (GAE), an opportunistic and usually fatal disorder and amoebic keratitis (AK), a potentially blinding corneal infection. Extracellular vesicles are potent intercellular communicators and are involved in several pathophysiological mechanisms and in host-parasite interaction as well. Pathogen-derived vesicles may carry pathogen associated molecular patterns (PAMPs) and are currently recognized as important triggers of immune response activation and pathogenesis. However, there are no reports describing vesicular production in the *Acanthamoeba* genus or their involvement in the pathophysiology of infections. The objective of this study is to isolate and characterize vesicles produced by *Acanthamoeba* of four genotypes (T1, T2, T4 and T11). Isolates obtained from dust (T1, T11), water (T2) and cornea of a patient with AK (T4) were cultured axenically in PYG medium. For vesiculation, parasites were cultured for 2 hours at 32°C in serum-free conditions. Vesicle isolation from culture supernatants was performed by differential centrifugation followed by ultracentrifugation (100.000 xg). Vesicle preparations were subjected to Nanoparticle Tracking Analysis (NTA) and protein content was quantified by Bradford assay. Preliminary data indicate variable concentrations of EV in supernatants (8.3×10^5 to 1.1×10^8 EVs/ μ L) with modal sizes ranging from $131.13 (\pm 13.2)$ nm to $141.8 (\pm 19.0)$ nm). Protein concentration varied from 43.4 to 72.5 μ g/mL. Additionally, trophozoites were analyzed by scanning electron microscopy, indicating differences in the production of vesicles among the genotypes. In conclusion, the production of vesicles by *Acanthamoeba* was described here for the first time and future studies will explore the role of these vesicles as possible mediators of innate immune response modulation. **Supported by:** FAPEMIG - CAPES **Keywords:** Acanthamoeba; extracellular vesicles; pamps

HP053 - **THE ROLE OF ADENOSINE A2B RECEPTOR IN VACCINATION OF C57BL/6J MICE WITH LEISHMANIA AMAZONENSIS-INFECTED DENDRITIC CELLS**

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Dendritic cells (DCs) are key members of immune system, important for the innate immune response and to connect it with adaptive immune response, making it more efficient to control infections, including those caused by Leishmania. DCs express on their surface ectonucleotidases, ATP receptors and adenosine receptors, that together regulate purinergic signaling pathways. Previously we showed an impairment of DC activation in Leishmania amazonensis infection, by a mechanism dependent of ectonucleotidases and adenosine receptors. This mechanism includes the parasite ability to induce ectonucleotidases on DCs that hydrolyze ATP to adenosine, which exert their anti-inflammatory effects by binding to A2B receptor present on the surface of these cells. This work aim to investigate the use of vaccines composed by L. amazonensis-infected dendritic cells treated with MRS1754, an antagonist of A2B receptor. MRS1754 treatment could reverse the inhibition caused by adenosine, stimulating the production of IL-12 by DCs and the differentiation of Th1 lymphocytes that will secrete IFN- γ , an important activator of macrophages making them more able to eliminate the parasites. C57BL/6J mice were inoculated with DCs infected with L. amazonensis in the absence or in the presence of MRS1754. After 3 or 12 weeks the animals were euthanized for evaluation of lesion development, tissue parasitism and the levels of IFN- γ produced by lymph node cells. Although lesion size and tissue parasitism were similar between control and MRS1754-treated groups, the levels of IFN- γ after 3 weeks of infection were higher in treated groups. After 12 weeks of infection, the production of IFN- γ was similar in both groups. In spite of the lack of protection, the fact that vaccination with MRS1754 induces a transient increase in the induction of IFN- γ production suggests an important role of purinergic signaling in L. amazonensis infection. **Supported by:** CNPq, FAPEMIG, CAPES

Keywords: Dendritic cells ; adenosine; leishmania amazonensis

HP054 - **ACANTHAMOEBA EXTRACELLULAR PROTEASES: HIGHER ACTIVITY OF AN ENVIRONMENTAL ISOLATE BELONGING TO GENOTYPE T1 COMPARED TO OTHER STRAINS**

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The amoebae of the genus *Acanthamoeba* are free-living protozoan that can eventually cause severe human infections, including granulomatous amoebic encephalitis (GAE) and *Acanthamoeba* keratitis (AK). Twenty genotypes have been described and T4 is the most frequently associated to both infections. Another factor involved in the ability of *Acanthamoeba* to act as a parasite is the production of proteases that can damage and kill the host cells. The aim of this study was to evaluate the secreted proteases profile of clinical and environmental *Acanthamoeba* isolates of different genotypes before and after interaction with MDCK cells. The secreted proteases of two keratitis isolates (genotype T4) and three environmental isolates (genotypes T1, T2 and T11) in axenic culture were characterized by zymography and quantified by azocasein assay. The isolates exhibited activity of three to four serine proteases varying from 40 to 123 kDa. The T1 isolate showed higher proteases activity than the others in both assays. The tests were repeated with conditioned medium obtained from trophozoites interacted with MDCK cells in a 2:1 proportion for three days, and the same proteases profiles were observed in all samples. T1 isolated exhibited a decreased activity compared to the condition before cell interaction, but still higher than other strains. The genotype T1 is considered rare, and most studies indicate its association with GAE cases. This fact associated with the higher proteases activity of the T1 isolate observed in this work suggest a possible virulence, hypothesis to be confirmed with experimental animal infection. **Supported by:** CAPES, PRPq-UFMG **Keywords:** Acanthamoeba; proteases; zymography

HP055 - EFFECT OF E-NTPDASE-2 ON THE ESTABLISHMENT OF THE INFECTION PROCESS BY LEISHMANIA (LEISHMANIA) AMAZONENSIS AND LEISHMANIA (LEISHMANIA) BRASILIENSIS IN MACROPHAGES

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Several studies have shown the importance of E-NTPDase in the process of cell adhesion, as in the studies of infection by *Trypanosoma cruzi*, and by our group, the uptake of *Leishmania amazonensis* promastigotes by macrophages. Based on these studies, we sought to evaluate the ability of E-NTPDase to modulate the immune response to *L. amazonensis*, PH8 strain and 111ld clone, and *L. Brasiliensis*, M2903 strain, in their different promastigote stages. The objective of this study was to evaluate the mechanisms by which *Leishmania* E-NTPDase modulates macrophage response. The ectonucleotidase activity was assessed by measuring the release of inorganic phosphate (Pi) by incubating promastigotes of *L. amazonensis* and *L. Brasiliensis* with ATP, ADP, or AMP. J774 macrophages were infected with *L. amazonensis* and *L. Brasiliensis* for 3 hours, 48 hours or 48 hours with IFN- γ /LPS to evaluate the adhesion and internalization rates by staining with Fast Panotic Kit and subsequent evaluation by light microscopy. The production of NO was determined by the Greiss method. Our results showed that promastigotes *L. Brasiliensis* and 111ld clone, grown in the presence of purines, showed less activity over all nucleotides compared to promastigotes of *L. amazonensis*. Regarding the adhesion process, it was found that both *L. Brasiliensis* promastigotes and the metacyclic forms of the 111ld clone showed lower adhesion to J774. The survival of promastigotes of *L. amazonensis*, especially the PH8 strain and clone 111ld grown in the absence of serum is maintained by a reduction in NO production. Our data demonstrate that the E-NTPDase is an important enzyme in the process of modulating the immune response in relation to the installation of *L. amazonensis* infection, but does not appear to be important for *L. Brasiliensis* infection. **Supported by:**CNPq, FAPEMIG and CAPES

Keywords:Leishmania; e-ntpdase; macrophages

HP056 - TREATMENT WITH PUTRESCINE REDUCES THE ABILITY OF TOXOPLASMA GONDII TO INHIBIT THE PRODUCTION OF NITRIC OXIDE OF ACTIVATED MACROPHAGES WITHOUT AFFECTING THE VIABILITY OF THE PARASITE

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Toxoplasmosis is one of the most frequent parasitic infections. It is caused by the protozoan *Toxoplasma gondii*. About 40% of the tachyzoite population of *T. gondii* exposes phosphatidylserine (PS) on the outer leaflet of the plasma membrane, mimicking apoptotic cells. PS is a phospholipid with anionic charge found in the inner leaflet of the plasma membrane of cells. Apoptotic cells expose PS inducing the release of transforming growth factor beta1 by macrophages and thus the reduction of nitric oxide (NO) production. NO is an important microbicidal agent of the immune system. The subpopulation that exposes PS (PS⁺) invades macrophages by active penetration and is able to inhibit NO production; the subpopulation that does not expose PS (PS⁻) is phagocytosed and do not reduce NO production. Polyamines are polycationic molecules involved in several biological reactions. Its polycationic profile gives affinity to anionic molecules, and the possible link with exposed PS. The aim of this work was to evaluate if putrescine can influences the ability of the PS⁺ subpopulation to reduce NO production after interaction with activated macrophages. Subpopulations were isolated and PS⁺ tachyzoites treated with putrescine (PUT). Macrophages were infected with the total population, and the subpopulations PS⁺, PS⁻ and PUT. Culture supernatants were collected after 24 and 48h and NO was quantified by the Griess reagent. PS⁺ tachyzoites treated with putrescine maintained its viability as assayed by flow cytometry. Macrophages infected with PUT produced more NO than macrophages infected with the PS⁺ subpopulation, similar to the level produced by macrophages infected with the PS⁻ subpopulation. These data suggest that putrescine reduces the capacity of the PS⁺ subpopulation to inhibit NO production of infected macrophages, possibly by blocking the exposed PS, suggesting the importance of this phospholipid in the evasion mechanism of this parasite. **Supported by:**FAPERJ, UENF, CAPES **Keywords:**Toxoplasma gondii; putrescine; nitric oxide

HP057 - INFECTION OF MOUSE NEUROSPHERES BY *TOXOPLASMA GONDII* INCREASES PANNEXIN1 EXPRESSION AND HOST CELL PROLIFERATION

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Congenital infection by *Toxoplasma gondii* affects the development of the fetus, causing, eventually, abortion and serious pathologies at birth to the later sequels. Most common sequels are microcephaly, encephalitis and damage to the Central Nervous System (CNS) that can lead to mental disabilities, cognitive impairment and even behavioral alterations. Little is known about the effect of infection with *T. gondii* on the differentiation of the brain cortex progenitors and what cellular and molecular mechanisms are involved. Thus, our aim is to investigate the impact of the infection in cortical neurogenesis, in an *in vitro* system. Among the various regulatory mechanisms of neurogenesis described so far, the expression of Gap Junction protein connexin43 (Cx43), has been described as a negative modulator of neuronal differentiation in the Subventricular Zone of the mouse embryo. Conversely, expression of pannexin1 (Panx1), former of membrane hemichannels, maintains the proliferation state and inhibits differentiation of neural precursor cells into neurons. We infected primary neural progenitor cells (NPC) isolated from Swiss Webster mice with tachyzoites of *T. gondii* (ME49 strain) and looked for parasite load and changes in host cell gene expression at different times. Quantitative RT-PCR showed increased expression of Panx1 at 96 hours post infection, with no changes in Cx43 levels. Nestin and GFAP, markers of progenitor cells were also increased in infected neurospheres. These observations suggested an increase in host cell proliferation induced by the infection. Morphometrical evaluations showed that *T. gondii* infected cultures had larger neurospheres, reinforcing the idea that proliferation is increased. The elucidation of the aspects involved in cortical malformations during vertical transmission of *T. gondii* is of great relevance and will help developing new strategies to reduce the burden caused by this parasite during pregnancy. **Supported by:**FAPERJ, CNPq (Edital Universal), PAPES VII/Fiocruz

Keywords:Congenital toxoplasmosis; neurogenesis; neurospheres

HP058 - THE SUMOYLATION SYSTEM IN TRYAPANOSOMA BRUCEI: PROTEOMIC STUDIES AND MOLECULAR TOOLS FOR TARGET PROTEINS

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Post-translational modification with the Small Ubiquitin-like Modifier (SUMO) is conserved in eukaryotic organisms and plays important regulatory roles in proteins affecting diverse cellular processes. In *Trypanosoma brucei*, member of one of the earliest branches in eukaryotic evolution, SUMO is essential for normal cell cycle progression and is likely involved in the epigenetic control of genes crucial for parasite survival, such as those encoding the variant surface glycoproteins. Molecular pathways modulated by SUMO have started to be discovered by proteomic studies; however, characterization of functional consequences is limited to a reduced number of targets. Here we present a proteomic strategy that allowed the identification of SUMOylated proteins in *T. brucei* together with their acceptor sites in an unambiguous manner. To further validate this targets we developed a bacterial strain engineered to produce SUMOylated proteins, by transferring SUMO from *T. brucei* together with the enzymes essential for its activation and conjugation. Due to the lack of background in *E. coli*, this system is useful to express and identify SUMOylated proteins directly in cell lysates by immunoblotting, and SUMOylated targets can be eventually purified for biochemical or structural studies. We applied this strategy to describe the ability of TbSUMO to form chains *in vitro* and to detect the lysine residues involved in this process. Furthermore, to validate targets, we applied an *in vitro* deconjugation assay using the *T. brucei* SUMO-specific protease capable of reverting the pattern of modification. This system represents a valuable tool for target validation, mutant generation and functional studies of SUMOylated proteins in trypanosomatids.

Supported by:CONICET, MINCYT

Keywords:Sumo; ubiquitin; ptm

HP059 - **AUTOPHAGY DURING *TRYPANOSOMA CRUZI* INFECTION: NEW INSIGHTS TO *IN VIVO* ACUTE DISEASE**

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Autophagy consists in eukaryotic constitutive process that degrades molecules and cellular structures to promote cell survival, but can also modulate other cell functions as infection, proliferation, antigen presentation and cell death. In Chagas disease, it has been proposed that the etiological agent *Trypanosoma cruzi* upregulates host autophagy *in vitro*, potentially affecting the outcome of infection. Among the clinical complications of this disease, cardiomyopathy is one the most severe, affecting 20-30% of chronic patients, and it has been intensely studied to understand variables in parasite persistence in the host. Thus, we hypothesized if autophagy influence *T. cruzi* acute infection *in vivo* modulating the cardiac manifestation in murine model. Swiss male mice were infected with different loads (1,000 or 10,000) of bloodstream trypomastigotes (Y strain) and the histopathological and parasitological analysis were performed during 15 days. In parallel, the presence of inflammatory cell populations were evaluated in heart and peritoneum, as well as the occurrence of autophagy and cell death markers (apoptosis and necrosis) by flow cytometry approach. *T. cruzi* infection, independently of parasitemia or amastigote nests, increases LC3-positive cells in peritoneal cavity, especially in macrophages. In infected heart, the number of CD8+ T cells increases in 15th dpi (day post infection), but no correlation with T cell autophagy was detected. In cardiac tissue, LC3 was intensely detected, recurrent in cardiomyocytes and angiogenic region, suggesting that infection also upregulates autophagy as reported in other heart diseases. These preliminary results corroborate *in vitro* data and reinforce the importance of autophagy in Chagas disease infection, especially in cardiomyopathy **Supported by:**FAPERJ, CNPq and FIOCRUZ **Keywords:**Autophagy; trypanosoma cruzi; chagas heart disease

HP060 - **TUBERCIDIN RESISTANCE PROTEIN IN *LEISHMANIA MAJOR* MUTANTS REVEALED A NOVEL ENDOPLASMIC RETICULUM PROTEIN**

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The identification of genes associated with drug resistance can contributed for the understanding of the mechanisms of action of compounds against *Leishmania*, as well as, for the identification of the resistance mechanisms mediated by the proteins encoded by these genes. Differently from the mammalian host, *Leishmania* is unable to synthesize purine nucleotides de novo and must rescue it from its host. Due to this metabolic difference, the purine metabolism can be considered a potential target for drug development. Tubercidin (TUB) is a toxic adenosine analog that is effective against *Leishmania*. Using a strategy of gene overexpression after cosmid genomic library transfection, we isolated, mapped, sequenced and identified two genes involved in TUB resistance in *L. major*. One cosmid presented the coding region of a NUPM1-like protein, an atypical multidrug resistance protein previously involved in TUB resistance in *L. amazonensis*. The other cosmid contained the coding region of a novel resistance marker involved in TUB resistance, described here as tubercidin resistance protein (TRP). Co-localization of TRP in the endoplasmic reticulum (ER) of *L. major* and *in silico* structural predictions indicated that TRP might be an ER lumen protein. Our findings may be useful to elucidate the purine pathway in the parasite and to understand the role of TRP in the mechanism of TUB resistance. In addition, we generated TUB resistant mutants of *L. major* by drug pressure that were 40-fold more resistant to TUB than *L. major* wild type. The resistance phenotype was stable after six months in the absence of the drug, suggesting that gene(s) mutation(s) and/or amplification(s) are involved in the mechanism of resistance. Interestingly, recent data have also demonstrated that TRP is overexpressed at mRNA and protein levels in these mutants compared to *L. major* wild type. **Supported by:**FAPESP, CAPES, CNPq and SiU

Keywords:Leishmania; tubercidin resistance protein; endoplasmic reticulum protein

HP061 - QUANTITATION AND ANTIGENICITY OF SUGAR CONTAINING MOIETIES IN WHOLE ANTIGENIC EXTRACT FROM *LEISHMANIA INFANTUM* PROMASTIGOTES.

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Visceral leishmaniasis is characterized by high amounts of serum IgG, inverting the normal globulin/albumin ratio. Circulating immune complexes interferes in the ELISA for IgG detection. We devised to study the sugar antigenic fractions in those extracts, assuming that those antigens are less specific due the number of moieties in antigen binding sites of antibodies. In order to identify and quantify total molecules present antigenic extract of promastigotes of *L. (L.) infantum*, we used and standardized several methods for quantification of proteins and sugars. Total extract was obtained from culture promastigotes with a denaturing detergent containing buffer. After clearing, the extract was submitted to molecular exclusion chromatography for obtaining large proteins or peptides and >10Kda peptides and other molecules. For the quantification and detection of proteins, we used several methods, as biuret, Bradford's and spectrophotometry. Sugar detection was accomplished by adapting Schiff reaction to microplate, using a standard curve of D(+) Galactose to detect sugar concentrations between 0.23 to 66.82 mg/ml. We found two major fractions, >10 kDa glycoproteins and proteins (GPP) with low sugar content, and a high sugar content (99%) <10 kDa peptides and small molecules (PSM) fraction. Both fractions were used as antigen in solid support for IgG ELISA against sera from experimentally infected hamsters, similar molecular reactivity in those samples. Both antigens presented showed complete distinction between positive and negative samples. PSM added to the reaction resulted in high ELISA blocking efficiency than GPP addition, without precipitation. Those small components of the extract could form soluble immune complexes due to their binding on isolated IgG molecules or receptors on B cells, resulting in an activation of antibody production, resulting in the VL serum high IgG content.

Keywords: Visceral leishmaniasis; sugar; elisa

HP062 - COMPARATIVE MOLECULAR EVALUATION OF ARGINASE ENZYME FROM DIFFERENT *LEISHMANIA INFANTUM* STRAINS

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Leishmaniasis is a public health problem in tropical and subtropical regions of the world. *Leishmania infantum* is one of the species that cause the visceral form of the disease and is present throughout the Mediterranean basin, but there are also found in the Middle East and South Asia. *L. chagasi* with strains circulating in the New World, is responsible for visceral leishmaniasis (VL) in Latin America and is considered identical to *L. infantum*, so it refers to it as *L. infantum* (syn *L. chagasi*). Up to now, this disease still remains with no vaccine and drugs are not ideal, with high toxicity. In this sense, many studies seek a better understanding of host-parasite relationship. Our group has studied this relationship with the main focus on the metabolism of L-arginine and mainly the nitric oxide synthase (NOS) and arginase (ARG) enzymes, that use this amino acid as a common substrate and are present in both parasite and host. In a previous work, we found out that *L. infantum* strains of the New and Old World present some differences in a biological point of view, relating with their infective capacity, that showed to be prominent in the Old World strain. *Leishmania* parasites contain a single ARG gene whose biochemical properties and expression has been studied in *L. amazonensis*, *L. mexicana* and *L. major*. In this work, a comparative molecular analysis of arginase between those *L. infantum* strains was carried out by gene sequencing and its expression in these parasites comparing with its *in vitro* infectivity. Our preliminary results showed an increase in the arginase expression in the *L. infantum* strain from the Old World, which can be associated with its *in vitro* infectivity, corroborating with our previous results. **Supported by:** CNPq

Keywords: *Leishmania infantum*; L-arginine metabolism; arginase

HP063 - **CHRONIC LESIONS OF TNFRP55-/- MICE INFECTED WITH LEISHMANIA BRASILIENSIS: DEVELOPMENT OF A MUCOCUTANEOUS LEISHMANIASIS MURINE MODEL**

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A chronic skin manifestation of leishmaniasis is caused by an exaggerated cellular immune response. Lesions in nasal mucosa and cartilage, months or years after an initial skin lesion, cause mutilation and morbidity in affected individuals. There is no murine model for the study of mucocutaneous leishmaniasis. However, data from our group showed that TNFRp55-/- mice, when infected with *L. major*, develop chronic lesions, which are not progressive as in the BALB/c classic susceptible strain, but there are no mucosal lesions, probably because *Leishmania major* is not associated with mucocutaneous leishmaniasis. The aim of this study is the characterization of chronic infection by *L. Brasiliensis* in TNFRp55-/- mice. WT mice (C57BL/6) and TNFRp55-/- mice were inoculated in the ear with *L. Brasiliensis* (1x10⁶ parasites) and lesions were followed for 15 weeks. In the chronic phase of infection (15 weeks), samples from the lesions were collected and processed for quantification of parasites and histological analysis of the inflammation. Despite the fact that we detected low and similar parasite loads in the ear of WT e KO, the cellular profile showed intense inflammatory infiltrate composed by TCD8+ cells and neutrophils in the lesions of TNFRp55-/. In addition, we found high levels of proinflammatory cytokines (IFN- γ and TNF) and IL-10 in ears, suggesting a mixed immune response, similar to mucosal lesions. Interestingly, we found more parasites in the spleen from the KO mice than in WT at eight weeks post infection. The fact that we were able to find parasites in the spleen seems to be specific for *L. Brasiliensis*, as we cannot find *L. major* parasites in this organ at this point of infection. These results suggest that the *L. Brasiliensis* can spread and survive in different environments in the mice **Supported by:**CNPq CAPES FAPEMIG **Keywords:**Leishmania Brasiliensis; mucocutaneous leishmaniasis; tnfrp55

HP064 - **EFFECT OF GLUTAMINE DEPRIVATION AND GLUTAMINE SYNTHETASE KNOCKDOWN ON CELL CYCLE PROGRESSION AND PROLIFERATION OF BLOODSTREAM FORMS FROM TRYPANOSOMA BRUCEI.**

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Trypanosoma (T) brucei is a causative agent of human African trypanosomiasis, also known as sleeping sickness, that threatens about three million people in Africa, and is lethal if it remains untreated. The chemotherapy is unsatisfactory, because only few drugs are available, moreover they cause serious side effects and have poor efficacy. The importance of amino acids metabolism has been described in trypanosomatids like *T. cruzi*, and procyclic forms of *T. brucei*, but little is known about bloodstream forms of *T. brucei*. The main goal of this work was to evaluate the role of glutamine (Gln) and the enzyme glutamine synthetase (GS) in the proliferation and cell cycle of *T. brucei* bloodstream forms. The growth pattern of bloodstream parasites (single marker bloodstream form, SMB) in medium containing a significant reduced amount of Gln remained unaffected for 72h, as compared with parasites growing in complete medium. Interestingly, induced GS-RNAi mutant parasites grew less in the Gln reduced medium than mutant parasites not induced, suggesting that GS activity is required for correct proliferation under Gln depletion. In addition, to better understand the effect observed, cell cycle was analyzed using propidium iodide by flow cytometry. The absence of Gln in the medium and/or GS-RNAi induction induced a cell cycle arrest on G2/M phase and increased the number of polyploid parasites. Compatible with these findings, cultures showed an increased number of parasites displaying abnormal cell and nucleus morphology as evidenced by DAPI staining. Altogether, these results demonstrate that Gln and GS play an important role in the correct cell cycle progression and proliferation in *T. brucei* bloodstream forms.

Supported by:FAPESP

Keywords:Glutamine; glutamine synthetase; cell cycle

HP065 - **COMPARISON OF THREE *TOXOPLASMA GONDII* STRAINS INTERACTION WITH CENTRAL NERVOUS SYSTEM CELLS**

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Toxoplasma gondii is an intracellular parasite and has the ability to form chronic and nonimmunogenic cysts in brain and muscle cells, persisting in the chronic phase. This infection can lead to damages in central nervous system of immunocompromised patients and congenitally infected foetus. *T. gondii* isolates have been grouped according to virulence in mice. Here, we compared the morphological aspects of the development of the highly virulent RH (type I), the avirulent Me-49 (type II) and the hybrid EGS (Types I/III) strains in rat glioma lineage C6. Infected C6 were monitored at 24, 48 and 96 h post infection. The cultures were analyzed by scanning and transmission electron microscopy. We compared the integrity of the glial cell and the ability of parasites to form rosettes in the parasitophorus vacuoles of the three strains. Analyses after 24 and 48 h showed more intense replication of RH and EGS strains and a great ability to form rosettes compared to Me49. 96 h post infection, tachyzoites of RH were no longer found inside cells, which were destroyed due to fast replicative cycle, characteristic of virulent strain. In C6 cultures infected with EGS we observed many ruptured cells and an increasing number of free parasites in the extracellular space, which corroborates the virulent behavior of this genetic hybrid strain found in other cell types. On the other hand, in the cultures infected with the non-virulent Me-49 strain, we observed infected glial cells with parasitophorus vacuoles containing rosettes of only 8 parasites suggesting slower replication comparing to more virulent strains. Our data support that proliferation capacity is associated to the genetic pattern and that virulent strains complete the lytic cycle faster in glial cells than the avirulent strain, including the genetic hybrid EGS, which showed a virulent proliferative behavior. The capacity to convert to bradyzoites and morphological characterization in shorter times are being performed. **Supported by:**FAPERJ e CNPq

Keywords:Glial cells; hibrid strains; toxoplasma

HP066 - **A ROUTE TO *TOXOPLASMA GONDII* NATURAL EGRESS**

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The cellular cycle of *Toxoplasma gondii* tachyzoites can be divided in several key steps, which can be summarized in invasion, development and egress. The latter is still the less studied, but it is crucial to new rounds of invasion, and thus, to the infection progress. So far, *T. gondii* egress has been studied with the aid of compounds capable of inducing the parasite release from the host cell, as calcium ionophore, which is quite toxic to the cell depending on the time of treatment, even in the lower concentrations needed to synchronize tachyzoites' motility end exit. The present study approaches the dynamics of *T. gondii* natural egress by super-resolution and electron microscopy, focusing in its determinant moments. We observed that after rosette disassembly parasites push the PV membrane before crossing it and move blindly in the cytoplasm of the host cell, finally crossing the plasma membrane. In this route, some tachyzoites incidentally penetrate the nucleus of the host cell that is lysed by the action of dozens of parasites on its membrane. Our results reinforced that the better understanding of the biology of *T. gondii* interactions with the host cell is necessary to pinpoint novel strategies to reduce the spread of the disease.

Supported by:CAPES

Keywords:Toxoplasma egress; calcium ionophore; electron microscopy

HP067 - CORRELATION OF NOS2 AND ARGINASE FROM PARASITE IN REGULATE MIRNA PROFILE AND MRNA EXPRESSION OF MURINE MACROPHAGE INFECTED LEISHMANIA AMAZONENSIS INFECTION

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Leishmania amazonensis can modulate the expression of host inducible Nitric Oxide Sintase (NOS 2) and Arginase 1 (ARG 1). While NOS2 metabolizes L-arginine to produce NO to kill the parasite, ARG1 supplies the polyamines pathway allowing its survival. The *L. amazonensis* arginase compete with NOS2 for L-arginine during macrophages infection and its absence proved to be an attenuating factor of infectivity of BALB/c macrophages. Parasite can also modulate microRNAs (miRNAs) to evade host inflammatory responses, modulating NOS2 and ARG1.

Here, we showed that *L. amazonensis* infection can subvert the miRNAs profile and mRNA expression of C57BL6 murine macrophages wild type (WT) or knockout of NOS2 (NOS2-) and determined the implications of miRNA action in the infectivity. The bone marrow derived macrophages (BMDM) of C57BL/6 NOS2 knockout were more susceptible to wild type WT-*L. amazonensis* (La-WT). The La-arg- parasite showed to be less infective than the WT parasite, in both models of macrophages. The amount of Nos2 mRNA was higher in La-WT infection of WT-macrophages after 4-24h compared to the non-infected control, and was reduced in La-arg- infection. The expression of arginase (La-ARG) and L-arginine transporter (La-AAP3) were higher in La-WT-infection of both macrophages. The absence of arginase reduced the expression of La-AAP3 in both macrophages.

La-WT infection of WT-macrophages modulated 32% of 84 miRNA analyzed, from these, 50% were upregulated, after 4-24h of infection. On the other hand, the La-arg- infection modulated about 25% of miRNAs tested, and from, 60% were upregulated. In silico search for mRNA targets of regulated miRNAs indicated pathways involved in activation and polarization of macrophage, cells proliferation and differentiation, formation of phagolysosome, pro-inflammatory cytokines and immune response. In conclusion miRNA profile of macrophage can be altered during *L. (L.) amazonensis* infection and subvert the host immune response.

Supported by:FAPESP **Keywords:**Leishmania; microrna; nos 2

HP068 - DOES SPLENOCYTE APOPTOSIS IS AFFECTED BY GHRELIN ADMINISTRATION DURING THE ACUTE PHASE OF CHAGAS' DISEASE?

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CHAGAS' DISEASE IS ONE OF THE MOST IMPORTANT PARASITIC DISEASES IN AMERICAS DISPLAYING UNTIL NOW INEFFECTIVE TREATMENT. GHRELIN IS A PEPTIDE HORMONE PREDOMINANTLY PRODUCED BY THE X/A-LIKE CELLS OF THE STOMACH AND EVIDENCES HAVE SUPPORTED ITS ROLE IN SEVERAL PHYSIOLOGICAL FUNCTIONS INCLUDING GROWTH HORMONE SECRETION, ANTI-INFLAMMATORY AND ANTI-OXIDATIVE ACTIVITY. THE AIM OF THIS STUDY WAS TO EVALUATE SPLENIC APOPTOSIS PROCESS IN *TRYPANOSOMA CRUZI* INFECTED RATS AND TREATED WITH GHRELIN. TWENTY MALE WISTAR RATS (100-110 g) WERE GROUPED IN: CONTROL (C), CONTROL/GHRELIN TREATED (CG), INFECTED (I) AND INFECTED/GHRELIN TREATED (IG). ANIMALS WERE INFECTED WITH 2×10^5 TRYPOMASTIGOTES (Y STRAIN) AND SUBCUTANEOUSLY SUPPLIED WITH 100 µg OF GHRELIN/KG/DAY DURING 14 DAYS. THE GROUPS WERE EUTHANIZED ON THE 15th DAY AFTER INFECTION AND SPLENIC CELLS WERE COLLECTED. APOPTOSIS PATTERNS WERE EVALUATED USING PROPIDIUM IODIDE-PI/ANNEXIN V-FITC DOUBLE-STAINING METHOD. CELLS PERCENTAGE WAS IDENTIFIED AS EARLIEST STAGE (ANNEXIN-V⁺PI⁻), LATER STAGE (ANNEXIN-V⁺PI⁺) AND VIABLE CELLS (ANNEXIN-V⁻PI⁻). THESE PARAMETERS WERE ACCESSED USING A FACSCAN FLOW CYTOMETRY AND A FACSDIVA SOFTWARE. OUR RESULTS DEMONSTRATED A STATISTICALLY REDUCED APOPTOSIS IN INFECTED AND TREATED RATS (IG) WHEN COMPARED TO INFECTED (I) COUNTERPARTS. VIABLE CELLS PERCENTAGES (ANNEXIN-V⁻PI⁻) WERE STATISTICALLY ENHANCED WITH GHRELIN TREATMENT. THESE RESULTS ARE IMPORTANT CLUES FOR AN ALTERNATIVE IMMUNOMODULATORY TREATMENT, SINCE GHRELIN ADMINISTRATION SIGNIFICANTLY ENHANCED CELL VIABILITY AND CONCOMITANTLY REDUCED SPLENOCYTE APOPTOSIS DURING THE ACUTE PHASE OF CHAGAS' DISEASE.

Supported by: FAPESP (2014/18682-3); CAPES **Keywords:**Acute chagas' disease; ghrelin; apoptosis

HP069 - **JUNCTION COMMUNICATION IN THE IMMUNE SYSTEM: MODULATION OF THE GAP JUNCTION BY INFECTION WITH TOXOPLASMA GONDII AND INFLAMMATION CONDICTION.**

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Toxoplasma gondii is a protozoan parasite responsible for toxoplasmosis, and it's believed that this parasite has infected one-third of the world population. In immunocompromised individuals toxoplasmosis may be cause problems in the central nervous and visual systems. Some of these complications are associated with the change of intercellular communication mediated by Junctions Communicators. However, there are still systems that aren't fully characterized regarding the junctional communication, including the innate immune system, represented by Macrophages. In view of this, the aim of this study is to evaluate the structural and functional modulation of gap junctions formed by Connexin 43 (Cx43) in macrophage lines and peritoneal macrophages after infection with *Toxoplasma gondii*, and treatments with pro-immune-inflammatory factors. The methodology used is: (1) J774-G8 macrophage cell line culture; (2) Primary culture of peritoneal macrophages of Swiss mice; (3) Western Blot Assays; (4) Immunofluorescence assays and analysis by confocal microscopy; and (5) Intracellular dye microinjection (functional assessment of gap junctions). The cell cultures are activated with pro-inflammatory immune factors (lipopolysaccharide (LPS), Tumor Necrosis Factor- α (TNF- α) and interferon- γ (IFN- γ) or infected with the RH strain of *Toxoplasma* in its tachyzoite form. Preliminary results revealed that J774-G8 cells showed significant changes in their profile junctional communication dye injection experiments, when subjected to microenvironments with inflammatory pro-immune factors combined (LPS + IFN- γ and IFN- γ + TNF- α) in incubations 48 hours. **Supported by:** CAPES, CNPq and FAPERJ

Keywords: Gap junction; macrophages; *Toxoplasma gondii*

HP070 - **ANALYSIS OF GENE EXPRESSION IN A LUTZOMYIA LONGIPALPIS-DERIVED CELL LINE**

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Preventing the development of the parasites in the insect vector is a feasible strategy to control the spreading of this infection. The success of this strategy will only be achieved with the full knowledge of the interaction events that occur between the parasite and its insect vector. In vitro studies with insect cell lines can be useful to shed light on some of these interaction events, as they provide a more controlled environment. In this context, it was previously demonstrated that a cells line obtained from embryonic tissue of *Lutzomyia longipalpis* (Lulo cells) can be an appropriate model for such studies. Therefore, we aimed to develop molecular and biochemical studies about Lulo cells to further establish this cell line features. In a recent proteomic approached of Lulo cells, we have observed that promastigotes of *Leishmania (Viannia) Brasiliensis* are able to bind to some proteins of the Lulo cells (data submitted to publication). Based on this previous study, we analysed, by RT-Real-Time PCR assays, the expression profile of some Lulo cells genes, which we observed to be relevant for host-parasite interactions, before and after incubation of Lulo cells with *L. (V.) Brasiliensis* during different periods. The gene targets are based on the following *Lu. longipalpis* protein sequences: enolase, zinc ion bind protein, heat shock protein, oxidoreductase, peroxiredoxin and putative Cu/Zn superoxide dismutase. As housekeeping reference genes, we used the *Lu. longipalpis* GAPDH and RD 49 genes. The relative expression of the target genes was assessed using the $\Delta\Delta C_t$ methodology. Trials in different times of interaction with the parasite *Leishmania* spp will be perform to simulate what happens in the phlebotomine gut and can evaluate the possible role of these molecules of the parasite during the process of access to vector. This study represents a step towards the establishment of a new model in vitro of the interaction between *Leishmania* and the Lulo cells. **Keywords:** Insect cells *lutzomyia longipalpis* (lulo); new model in vitro; gene expression

HP071 - HYPOSIALYLATED N-GLYCANS PREDOMINATE IN HUMAN SERUM GLYCOPROTEINS DURING THE CHRONIC PHASE OF CHAGAS DISEASE
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Trypanosoma cruzi, the etiological agent of Chagas disease, infects around 9 million individuals in the world, most of them in Latin America where the disease is still endemic. Chagas' disease is responsible for the largest number of cardiomyopathies in Latin America. In 2012, government agencies, non-governmental organizations, and pharmaceutical companies agreed to support WHO's main objective of eliminating ten neglected tropical diseases, including Chagas disease, by 2020. One major obstacle to fulfilling those goals lies in the lack of readily available, easy-to-use, reliable, and low-cost diagnostic tools to identify infected patients, monitor the impact of drug programs, and watch for disease re-emergence.

Glycosylation is the most common protein modification and profoundly regulates many biological processes. Aberrant glycosylation of serum proteins is described in chronic and infectious diseases, but few glycomic analysis are described in infectious disease, especially diseases caused by protozoa.

The aim of our study is to compare the structures of N-glycans isolated from serum glycoproteins of patients with Chronic Chagas Cardiomyopathy (CCC) with those of healthy donors. N-glycans were enzymatically released from total serum glycoproteins by N-Glycosidase F, purified by chromatography, permethylated and analyzed by mass spectrometry/MALDI-TOF. N-glycans isolated from the serum of CCC patients showed a significant decrease in sialic acid content. Although, N-glycans from healthy individuals or patients with CCC are predominantly bi-antennary glycans, the chemical structure of N-glycans from both groups of individuals are different. Interestingly, N-glycans isolated from patients with the indeterminate form of Chagas disease are similar to those found in the serum of healthy individuals. **Supported by:** FAPERJ, CAPES, CNPq.

Keywords: *T. cruzi*; n-glycans; chagas disease

HP072 - ABSENCE OF LAMP IN HOST CELLS CHANGES INFECTIVITY AND CELLULAR ADHESION OF NEWLY RELEASED *TRYPANOSOMA CRUZI*

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Trypanosoma cruzi invade cells by interacting and adhering to host cell plasma membrane, which then leads to intracellular calcium increase and lysosomal exocytosis at parasite attachment site. Release of acid sphingomyelinase from lysosomes will then induce a compensatory endocytosis event that will drive parasite into the host cell, with continuous accumulation of lysosomal membrane for the formation of a mature parasitophorous vacuole. It has been shown that host cell sialic acid has an important role in cellular invasion by *Trypanosoma cruzi*, mainly in the early stages of infection, due to parasite trans-sialidase enzyme, whose activity is believed to facilitate adhesion and host cell-parasite recognition. This enzyme is responsible for transferring sialic acid residues from the host cell to parasite surface proteins enhancing infection ability. We have previously shown that LAMPs (Lysosome Associated Membrane Proteins), which are rich in sialic acid residues, are important for *T. cruzi* host cell infection. We showed that mouse embryonic fibroblast (MEFs) cell line lacking LAMP are less permissive to *T. cruzi* invasion. On the other hand, internalized parasites multiply faster in these cells. In the present study, we investigated whether parasites derived from wild type (WT) or LAMP knock out cells (LAMP-KO) present the same cell infection ability. For this we harvested parasites from WT and LAMP-KO MEFs infected with Y strain and used these parasites to evaluate adhesion and invasion rates in WT MEFs or LLC-MK2 cell lines. Preliminary results showed that parasites derived from LAMP-KO MEFs have a lower invasion and adhesion rate in WT MEFs compared with parasites grown in WT MEFs, suggesting that absence of LAMP promotes changes in parasite surface proteins, resulting in a lower infectivity rate in WT MEFs. We will next investigate if these differences will also influence invasion of other cell types. **Supported by:** CAPES, CNPq, FAPEMIG

Keywords: Lamp; *t. cruzi*; cell invasion

HP073 - CUTANEOUS LEISHMANIASIS ASSOCIATED WITH DIFFERENT CLINICAL FORMS AND DEGREES OF PARASITISM IN NATURALLY INFECTED DOGS BY L. INFANTUM

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Canine visceral leishmaniosis (CVL) is a zoonosis of major public health impact caused by organisms of the genus *Leishmania* and transmitted to man and animals by infected blood-sucking sandflies of the genus *Lutzomyia*. The skin is the first point of contact with organisms of the genus *Leishmania* for sandy fly vectors and was considered an important reservoir compartment for parasites in infected dogs. The aim of this study was to determine the main histopathologic alterations in ear skin of dogs naturally infected by *Leishmania infantum* with different clinical status and different degrees of parasitism. For this, thirty-four dogs naturally infected with *L. infantum* were divided by clinical form in: asymptomatic (AD, n=11), oligosymptomatic (OD, n=11) and symptomatic dogs (SD, n=12); and in different degrees of parasite load in skin: low (LP, n=11), median (MP, n=11) and high (HP, n=12) parasitism. In addition, ten dogs were used as controls (CD, n=10). After necropsy, skin samples were collected for further histological and parasitological analysis. The OD and SD groups presented higher parasite burden than AD group. The inflammation was higher in SD group when compared to other groups (OD and AD). LP, MP and HP groups showed higher inflammatory process than CD group, showing that greater the parasite load greater the inflammatory process in the skin. The number of mast cells was larger in the OD and LP groups than CD group suggesting that these cells may be involved in tissue remodeling, since there was an increase of type III collagen fibers and decrease type I collagen fibers in these groups. In this sense, these results add new insights about the pathogenesis of CVL, being a reliable indicator for the severity of clinical disease in dogs.

Keywords:L. infantum; dogs; skin

HP074 - EFFECT OF BLOCKERS AND AGONISTS OF TLR-2 AND TLR-6 IN THE INFECTION OF DERMAL FIBROBLASTS WITH BY LEISHMANIA AMAZONENSIS
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In infection by protozoa *Leishmania* genus, the early stages of infection are crucial for the evolution of disease, being involved several factors, including the interaction of molecules at the parasites surface in the initial response of the host cells. To elucidate the involvement of TLR-2/1 and TLR-2/6 were performed activation assays and blocking the receptors in infection by *L. amazonensis* in dermal fibroblasts (DF). Obtained from primary cultures of skin of C57BL/6 mice embryos. DF were plated in DMEM at 37 °C with 5% CO₂/24 h. After, cells were incubated for 30 min/37 °C with 10mg/ml anti-TLR-2 or 10mg/ml anti-TLR-6 in DMEM with 2% BSA. For activation with agonists cells were incubated with 0.1 µg/ml Pam3Cys-Ser- (Lys) 4 (Alexis) or 0.1µg/ml MALP-2 (Alexis) for 12 h/37 °C. After, cells were infected with *L. amazonensis* promastigotes by 2, 6, 24 and 48 hours of interaction. For morphological and phenotypic analysis used light microscopy and electronics, immunofluorescence and flow cytometry. Cultures treatment with agonists and blockers revealed that modulation of TLR-2 can reverse the susceptibility of FD, which may be a determining factor for the development of an immune response more effective in controlling infection by *L. amazonensis*, and a way in the search for alternatives in the development of new therapies for the leishmaniasis treatment.

Supported by:Faperj

Keywords:Leishmania amazonensis; dermal fibroblasts; tlr-2 and tlr-6

HP075 - PRODUCTION AND EVALUATION OF CHIMERIC PROTEINS WITH POTENTIAL FOR THE DIAGNOSIS OF HUMAN AND CANINE FORMS OF VISCERAL LEISHMANIASIS

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Current serological assays used for the diagnosis of Visceral Leishmaniasis (VL) are generally based on recombinant proteins, since they have high sensitivity and specificity associated with low cost and easy implementation. However, no single assay has been shown to be effective for the diagnosis of both the human and canine forms of VL. To solve that, mixtures of antigens have shown the most promising results, but they increase the production costs and impair standardization. An alternative is the use of chimeric proteins which can potentially be capable of generating results with good sensitivity and specificity for both forms of the disease. This study aimed to assess the potential for novel chimeric proteins composed of antigenic regions from preselected *L. infantum* proteins which were efficient for the diagnosis of either human or canine VL. First, the antigenic regions from known immunogenic polypeptides were selected through software prediction tools and their coding sequences joined into single synthetic sequences optimized for bacterial expression. Three combinations were thus generated and, after commercial synthesis, the chimeric genes were cloned into bacterial expression vectors, expressed in *Escherichia coli* as His-tagged proteins and purified by affinity chromatography. The diagnostic potential of the chimeric proteins for VL was evaluated through ELISA assays with sera from humans and dogs affected with this disease. The chimeric constructs displayed 72%, 71% and 15% sensitivities for the human sera and 100%, 97% and 62% for the canine sera, respectively. The results showed that the chimeric proteins were efficient in detecting dogs infected with VL, however changes are required in order to improve performance with sera from infected human individuals. **Supported by:**CNPq and CAPES

Keywords:Antigenic proteins; visceral leishmaniasis; serological diagnosis

HP076 - INDIVIDUALS LIVING IN LOW ENDEMICITY AREAS TO MALARIA IN THE BRAZILIAN AMAZON EXHIBIT LONG-LIVED ANTIBODIES AND SPECIFIC MEMORY B-CELLS TO *PLASMODIUM VIVAX* STAGE ANTIGENS (AMA-1 AND MSP-9)

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The development of an effective and safety antimalarial vaccine for humans has been, a long time ago, one of the greatest hope for malaria combat in the World. Although specific antibodies have a significant role for protection against the plasmodial infection as observed in passive transfer studies, little is known about the mechanisms/factors driving humoral immunity during infection. Then, we evaluated by ELISA the profile of antibody responses against two synthetic peptides from *P. vivax* (AMA-1_(S290-K307) and MSP-9_(E795-A808)), which are important candidates to compose an antimalarial vaccine, in five groups of subjects living in the Brazilian Amazon (exposed but not infected individuals; acute cases and convalescent individuals 30, 60 and 180 days post-infection). We also investigated the magnitude of antigen-specific memory B-cells (MBC) using the ELISpot assay. We observed that acute individuals living in areas of low malaria transmission presented antigen-specific MBCs and circulating antibodies against both tested antigens, which were maintained for >180 days in the absence of infection. However, no positive association was observed between prevalence or levels of malaria-specific antibodies and frequency of MBCs over time. Taken together, these results suggest that *P. vivax* infections can induce long-lived antibodies and antigen-specific MBC responses even in low-transmission regions.

Supported by:UFJF, CAPES, CNPq, FAPEMIG, FAPEMIG-Rede Mineira and IOC/FIOCRUZ

Keywords:Vivax malaria; antibodies; memory b-cells (mbc)

HP077 - **CHARACTERIZATION OF THE EFFECTS OF GAMMA RADIATION ON LABELED COMPOUNDS USED IN EXPERIMENTAL DEVELOPMENT OF *T.GONDII* VACCINES.**

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Toxoplasma vaccines will be extremely important for safe meat production, by avoiding *T.gondii* cysts contamination in meat cuts. Attenuated vaccines as Toxovax[®] blocks the use of meat from vaccinated animals due remaining meat cysts. Irradiated tachyzoites or irradiated *T.gondii* extracts promote immunization of mice without adding meat cysts in the host. The irradiation process is a physical process that affect targets as DNA a not proteins directly or indirectly by water radiolysis. We devised to study the effects of ionizing radiation on labeled soluble tachyzoites antigen (STAG) of RH tachyzoites, using isolated protein (BSA) as control, looking for changes induced by water radiolysis. We labeled those antigens with NHS-biotin and exposed those labeled proteins to Cobalt-60 (Co-60) gamma radiation at 0.25kGy and 1.5kGy. To study the effects of isolated free radicals, we use specific scavenger radicals that compete with each specific reactive species during irradiation. Our data suggest that STAG or BSA labelled with a high (20-80) biotin protein ration were not affected after radiation at 0.25kGy and 1.5kGy. We characterized those changes both by molecular weight effects after SDS PAGE and blotting, looking for biotin moieties with avidin-peroxidase. In presence of hydroxyl radical scavengers as butanol, there was observed a potentiation of the radiation effect of aggregation on proteins, while a sulfhydryl protector, dithiothreitol (DTT) protects from this effect. Our results show that the effect of hydroxyl radical on proteins is related to their primary structure that could be affects its immune response. As reported in irradiated tachyzoites or STAG, a better immune response to irradiated product must be due either to the aggregation of proteins inductive of inflammatory focus or to oxidative changes in protein primary structure that induces its uptake by antigen presenting cells. Free radical scavengers or competitors would help in solving those questions.

Supported by:FAPESP

Keywords:Toxoplasma gondii; protein; gamma radiation

HP078 - **OCURRENCE OF PREVIOUS INFECTIONS AND LEVELS OF PARASITAEMIA AND CYTOKINE DURING VIVAX MALARIA**

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A delicate balance between inflammatory and regulatory cytokine responses is crucial to determine the outcome of disease in infected individuals. We evaluated 30 individuals living in the Brazilian Amazon for parasite density and cytokine levels: 20 (67%) reporting one or more previous malaria (mean: 4.5) and 10 (33%) were primoinfected. All acute episodes were associated to *P. vivax* infection as detected by microscopy (thick film) and PCR. Primoinfected individuals showed a greater parasite density when compared to those reporting one or more previous episodes of infection (5322 and 1223 parasites/mm³ of blood, respectively; $p < 0.05$). When the plasmatic cytokines were evaluated by flow cytometry, primoinfected individuals exhibited slightly increased levels of IL-10 in comparison with levels detected in individuals who reported previous cases of malaria. IL-10 and IL-6 levels were positively correlated regardless of the number of previous episodes of malaria ($r = 0.81$; $p < 0.0001$), while no correlation was found between IFN- γ levels and IL-10/IL-6 levels. Primoinfected individuals also exhibit a higher IL-10/IL-6 ratio compared to individuals reporting previous infections (6.8 and 2.6; respectively). The predominance of IL-10 can contributes to the greater parasite density observed in primoinfected individuals. Thus, the balance between pro- and anti-inflammatory cytokines appears to be critical in antimalarial immunity, controlling circulating parasitaemia and morbidity of disease.

Supported by:UFJF, CAPES, CNPq, FAPEMIG, FAPEMIG-Rede Mineira and IOC/FIOCRUZ

Keywords:Vivax malaria; parasitaemia; cytokine levels

HP079 - **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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Human African Trypanosomiasis (HAT), also known as sleeping sickness, is responsible for thousands of deaths in Africa, and a disease that requires treatment with better efficacy and less toxicity. HAT is caused by the kinetoplastid protozoan parasites *Trypanosoma brucei rhodesiense* and *Trypanosoma brucei gambiense*. Our group has previously shown that an ecotin-like inhibitor of serine peptidase 2 (ISP2) is required for the virulence of another trypanosomatid parasite, *Leishmania major*. To investigate the role of ISP2 in *T. brucei rhodesiense*, we generated ISP2-null parasites (Δ isp2) and compared infection profiles and immune responses to that of a wild-type (WT) infection. The Δ isp2 bloodstream form *T. rhodesiense* grew normally in vitro when compared with WT parasites. Using the C57B/6 mouse model, we observed that mice infected with the Δ isp2 mutant displayed lower waves of blood parasitemia than the mice infected with WT *T. rhodesiense*, controlling the infection more efficiently. All infected mice showed splenomegaly and hepatomegaly. Cytokines levels of splenocytes ex vivo were measured by ELISA, revealing higher concentrations of INF- γ and IL-6 in mice infected with Δ isp2 as compared to those infected with WT, whilst the concentration of KC was higher in the WT-infected mice. However, in the supernatant of hepatocytes ex vivo, the levels of the INF- γ , MCP1 and IL-12 were higher in WT-infected mice compared to those infected with Δ isp2. Flow cytometry analyses showed decreased numbers of CD4+ and CD8+ T cells in both groups of infected mice compared with the spleen of non-infected control mice. Taken together, our observations suggest that lack of ISP2 provokes alterations in the immune response of infected mice, leading to a better control of parasite burden. **Supported by:** FAPERJ, CNPq e CAPES

Keywords: *Trypanosoma brucei*; isp2; cytokines

HP080 - **TRYPANOSOMA CRUZI INTRACELLULAR DEVELOPMENT IN HUMAN CARDIOMYOCYTE CULTURES: INFLUENCE OF ROS**

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We have previously shown that the differential distribution of *Trypanosoma cruzi* populations is primarily the result of the direct interaction between parasite and host cell. In this work we have infected primary BALB/c cardiomyocyte (bCM) cultures with two *T. cruzi* clonal populations, Col1.7G2 and JG (DTU I and II, respectively), and showed a higher multiplication rate of JG in these cultures, when compared to Col, reproducing the selection of JG in the hearts of BALB/c doubly infected mice (JG + Col1.7). More recently, we have shown that JG infection of bCM cultures induces higher amounts of ROS when compared to those infected with Col1.7. We have also shown that without ROS stimuli Col1.7 produces more antioxidant enzymes, suggesting that Col1.7 is less affected by an oxidative environment. These results together suggested that, as observed before for macrophages, ROS production might be signaling to the parasite and contributing to increasing its intracellular multiplication rate. Here we investigated whether we were able to reproduce these results in human cardiomyocyte (hCM) cultures derived from human induced pluripotent stem cells. For the hCM culture, Col1.7 invasion rate was higher than that observed for JG strain. Nevertheless, JG intracellular multiplication rate was higher when compared to Col1.7, as observed for the bCM cultures. We have also observed that hCM cultures infected with JG strain were able to produce higher amounts of ROS in comparison to those infected with Col1.7, as found for bCM cultures. Additionally, we tested whether exposure to ROS, condition found in the cytoplasm of infected cells, would change the profile of production of antioxidant enzymes by the two parasite populations. Even under stress conditions, Col1.7 produced more antioxidant enzymes when compared to JG. These results reinforce data obtained with the mouse CM model suggesting that the oxidative stress may be able to boost parasite *T. cruzi* intracellular development. **Supported by:** CNPq, FAPEMIG, CAPES, Pluricell

Keywords: *Trypanosoma cruzi*; cardiomyocytes; reactive oxygen species

HP081 - **CD39 AND CD73 EXPRESSION IN DRAINING LYMPH NODES CELLS OF L. AMAZONENSIS INFECTED C57BL/6 MICE**

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Extracellular ATP induces an inflammatory response that is regulated by its hydrolysis to adenosine by the sequential action of CD39 and CD73. Adenosine, on the other hand, induces an anti-inflammatory response. In vitro tests performed in our laboratory have shown that dendritic cells infected by *L. amazonensis* display increased expression of CD39 and CD73 as a potential mechanism to regulate the immune response. The aim of this study is to evaluate the kinetics of cell migration, the expression of CD39 and CD73 and cytokine production at lymph nodes draining an ear infection, in the murine model of the disease. C57BL/6 mice were infected in the ear with 10⁵ metacyclic *L. amazonensis* promastigotes. Flow cytometric analysis showed an increase in macrophages in infected animals at 3 weeks of infection. These cells also show increased expression of CD39 and CD73. Dendritic cells show a similar pattern, with increase in numbers and expression of CD39 and CD73. The population of NK cells is also increased with infection. However, the population of T and B lymphocytes shows no change, as does the production of IFN- γ at this time point. We propose that the lack of T cell proliferation and cytokine production may be explained by the increased expression of ecto-nucleotidases (CD39 and CD73) that could interfere with proper antigen presentation and inhibit an appropriate clonal expansion of lymphocytes due to increased adenosine production at the lymph node. **Supported by:**CNPq/FAPEMIG

Keywords:Leishmania; cd39; cd73

HP082 - **LEISHMANIA PROMASTIGOTES INDUCE MAST CELL DNA EXTRACELLULAR TRAPS**

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Leishmania infection is initiated when promastigotes are inoculated by the insect vector into the skin and local inflammation begins. The interaction with the innate cells includes resident mast cells. An increasing number of studies have investigated the role of mast cell in the immune response to *Leishmania*. It has been shown that *Leishmania* is phagocytized and replicates within mast cells, induces degranulation and cytokine release. Mast cells also discharge MCETs (mast cell extracellular traps), which are scaffolds of chromatin decorated with granular proteins that snare and kill bacteria. Although mast cell-*Leishmania* interaction has been reported in the literature, MCETs induction by these parasites has not yet been evaluated, as well as its impact on *Leishmania* survival. In this study we evaluate the release of MCETs induced by *L. amazonensis* and *L. major* in susceptible (BALB/c) and resistant (C57BL/6) mice. We also evaluate the MCET release by human mast cell lineage (HMC-1). Murine mast cells were obtained from bone marrow cells cultured in RPMI with 20% fetal bovine serum, IL-3 to differentiation monitored by toluidine blue staining. For the interaction assays mast cells were resuspended in RPMI without serum and incubated with stationary phase promastigotes. The supernatant were analyzed by MCETs double stranded DNA content measured by Picogreen kit and by fluorescence microscopy. Our results showed a dose-dependent induction of MCETs obtained in both mice strains when stimulated with *L. amazonensis* and *L. major* after 10 and 90 minutes of promastigotes-mast cells interaction. The human lineage released MCETs only after 90 minutes interaction. These preliminary results demonstrate for the first time that *L. amazonensis* and *L. major* promastigotes induce the release of MCETs.

Supported by:CNPq e FAPERJ

Keywords:Mast cell; leishmania; extracellular traps

HP083 - **SPLenic IMMUNOPATHOLOGICAL STUDY OF VISCERAL LEISHMANIASIS
NATURALLY INFECTED DOGS**

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Spleen is one of the main affected organs in canine visceral leishmaniasis (CVL). Disorganization of splenic white pulp (SWP) has been associated with immunosuppression and disease progression. This study aims to assess structural and cellular changes in the splenic extracellular matrix of dogs with CVL correlating with the parasite load and symptoms. 41 animals were grouped according to SWP organization as: 1- Organized to slightly disorganized (OR-SD, n = 11); 2- Moderate to intense disorganization (MD-ID, n = 30). Splenic fragments were collected for the parasitic load quantification through qPCR, histopathological analysis and immunohistochemistry. CD3+, CD4+, CD8+, CD21+, IFN- γ +, IL-10+ cells, MMP-9, ADAM-10, as well as, laminin and fibronectin expression were evaluated. The disorganization was accompanied by reduction in the quantity of lymphoid follicles ($p > 0.0001$). There was no correlation between SWP organization and parasite load nor clinical signs. MD-ID animals showed high levels of clinical signs ($p = 0.021$), high laminin ($p = 0.045$) and collagen deposition ($p = 0.036$), reduction in CD4+ ($p = 0.027$) and IFN- γ + cells ($p = 0.048$). ID animals presented higher labeled for ADAM-10 expression ($p = 0.013$) and low label for MMP-9 expression ($p = 0.004$). Positive correlations between CD8 and parasite load ($p = 0.042$, $r = 0.320$), CD8 and CD4 ($p = 0.028$, $r = 0.343$), IFN- γ and IL-10 ($p = 0.001$, $r = 0.497$), laminin and fibronectin expression ($p = 0.043$ and $r^2 = 0.393$) were observed. Inverse correlations between laminin and TCD3+ cells ($p = 0.019$ and $r^2 = -0.637$), ADAM-10 and follicles/mm² ($p = 0.022$, $r = -0.486$) were observed. The data suggested that the splenic disorganization in CVL involves a higher laminin and fibronectin deposition and ADAM-10 expression. These alteration on matrix compounds induced by proteases activity could lead to reduction of CD3+ (mostly CD4+ cells), but not CD8+ cells, and consequently to immunosuppression and disease progression.

Keywords: Lvc; spleen; mec

HP084 - **THE ACTIVITY OF HOST NEUTROPHIL ELASTASE IS REQUIRED FOR THE
INTRACELLULAR GROWTH OF *LEISHMANIA DONOVANI* AND VISCERAL PARASITEMIA
VIA TOLL-RELATED INDUCTION OF INTERFERON β**

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Visceral leishmaniasis is deadly as the parasites disseminate to the liver and spleen causing inflammation and tissue destruction. *Leishmania* sp. have three genes similar to bacterial ecotin: ISP1, ISP2 and ISP3. Ecotin is an inhibitor of S1A family proteases, such as neutrophil elastase (NE). In *L. major*, ISP2 inactivates host NE and prevents triggering of TLR4 and TLR2 during parasite phagocytosis, which is important for parasite survival and growth in macrophages. Here, we describe that the expression of ISP2 is not detected in *L. donovani*. We show that NE activity abrogation by a synthetic inhibitor or infection of macrophages from *ela*^{-/-}, *tlr4*^{-/-} or *tlr2*^{-/-} mice prevented the intracellular growth of parasites. Addition of exogenous NE recovered the intracellular growth of *L. donovani* in *ela*^{-/-} macrophages. NE and TLR4 colocalized with the parasite in the parasitophorous vacuole. Parasite loads in the liver and spleens of *ela*^{-/-} mice at 7 days post-infection were reduced as compared with C57BL/6 mice, and was accompanied by increased nitric oxide (NO) and decreased transforming growth factor β (TGF β) production. Transgenic *L. donovani* lines expressing ISP2 of *L. major* (*L. donovani*:ISP2) were generated and transgenic parasites displayed impaired intracellular growth in vitro and decreased parasite burden in mice. Inhibition of NO or superoxide generation in macrophages enhanced survival of wild type parasites but failed to recover the growth of *L. donovani*:ISP2 at 24h. Macrophages infected with *L. donovani*, but not with *L. donovani*:ISP2, showed increased levels of the transcription factor IRF3 in the nucleus at 6h up to 24h. Intracellular growth of *L. donovani* in *ela*^{-/-} macrophages or of transgenic ISP2-parasites was fully recovered by addition of exogenous IFN β , but not by IFN α . We propose that *L. donovani* utilizes the host NE-TLR molecular machinery to generate IFN β and fuel parasite growth. **Supported by:** CNPq **Keywords:** *L. donovani*; infection; macrophages

HP085 - INFECTION OF HUMAN GLIOMA AND MURINE NEUROBLASTOMA CELL LINES WITH *TRYPANOSOMA CRUZI* COLOMBIAN STRAIN

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Chagas disease (CD), caused by the protozoa *Trypanosoma cruzi* presents an acute phase asymptomatic or symptomatic sometimes with severe myocarditis and encephalitis, followed by a chronic phase including the indeterminate, cardiac, gastrointestinal, mixed, rarely accompanied nervous manifestations. The meningoencephalitis in CD is observed mainly in children and immunocompromised patients, the latter can also occur as a reactivation of the disease during the chronic phase. Studies using animal model demonstrated that the glial cells, astrocytes and rarely neurons can be infected with *T. cruzi*. Thus, it is extremely important to elucidate how glial cells and neurons are activated by and respond to this pathogens. The goal of the work was assess comparison of the infectivity by *T. cruzi* Colombian strain in different cell lines of the CNS. Human glioma U373 and murine neuroblastoma Neuro-2A cell lines (3x10⁴cells/well) were infected with trypomastigotes maintained in Vero cells previously infected by metacyclics trypomastigotes. Glial cells and neurons were cultivated and infected after 24 hours. The infection was evaluated after 24, 48, 72, 96 and 120 hours. For this the cells were fixed with methanol- and stained with Giemsa. Stained cultures were observed under light microscope and the percentage of infected cells and quantity of amastigotes per cell were determined. Our preliminary results showed that the percentage of infected glial cells is time-dependent and correspondent to 10-15%. The amastigotes number increased at 96 and 120h pi. The percentage of the infected neurons was bigger than glial cells at 24h pi. However, the amastigotes number in neurons was lower. This study suggest that neurons and glial cell are susceptible to *T. cruzi* Colombian strain infection and indicate that neurons of the CNS can be infected in nervous alterations in vitro assays. Our propose is to investigate this fact using the murine model infected with Colombian and other strains. **Supported by:**CAPES

Keywords:Chagas disease; colombiana strain ; central nervous system

HP086 - INVOLVEMENT OF MACROPHAGE MIGRATION INHIBITORY FACTOR (MIF) IN THE IMMUNE RESPONSE INDUCED BY NEOSPORA CANINUM

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Neospora caninum is a protozoan of phylum apicomplexan spread worldwide, being relevant due to induction of abortion in bovine leading to great economic impact in beef and milk industries. MIF is a cytokine involved in response against intracellular protozoans, which is produced for several immune cells, and it is find intracellularly preformed turning this cytokine into an important factor in acute inflammatory response, exhibiting a central role in innate immunity. In this sense, research is necessary to understand the host-parasite relationship and elucidate the role of MIF in the immune response during infection by *N. caninum* . Thus, the present study aimed to evaluate the role of MIF cytokine in modulating the immune response by *N. caninum*. Therefore, we analyzed the cytokine production and its induced phenotype using C57BL/6 wild type (WT) and genetically engineered MIF deficient (MIF^{-/-}) mice, infected with tachyzoites of Nc-Liv parenterally. Based on this infection it was possible to observe an acute production of MIF in cells and peritoneal cavity fluids, spleen and lungs. Additionally, it was demonstrated that MIF^{-/-} mice possess a decreased parasitism during acute and chronic phase and that MIF secretion is independent of TLRs. Moreover, it was observed an elevated production of TNF- α e NO and an increased pulmonary inflammation in those animals as well as a higher survival and recovery of body weight in comparison with WT mice. In addition, results in vitro indicated no difference in IL-12 and IFN- γ production between WT e MIF^{-/-}. However, it was observed a high production of TNF by MIF^{-/-} animals. In sum, it is possible to conclude that MIF presents a relevant modulatory role during the establishment of infection by *N. caninum*. **Supported by:**CAPES, CNPq and FAPEMIG

Keywords:Neospora caninum; mif; immune evasion

HP087 - THE NATURAL CYSTEINE PEPTIDASE INHIBITOR OF THE *T. BRUCEI RHODESIENSE* PLAYS A ROLE IN PARASITE VIRULENCE AND BRAIN ENDOTHELIAL ACTIVATION.

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Human African Trypanosomiasis, also known as sleeping sickness, is caused by *T. brucei gambiense* (chronic disease) and *T. brucei rhodesiense* (acute disease). In HAT, *T. b. rhodesiense* and *T. b. gambiense* penetrate the central nervous system by unknown mechanisms, leading to meningoencephalitis. The *T. brucei* L-like cysteine peptidase (CP) was implicated in parasite penetration of the blood brain barrier. CP activity is modulated by the endogenous inhibitor of cysteine peptidases (ICP) that belongs to the chagasin family. To investigate the role of ICP in *T. b. rhodesiense* we generated ICP null mutants (Δicp). Δicp were shown to have 1.5x more CP activity and traverse brain microvascular endothelial cell (BMEC) monolayers in vitro more efficiently than WT parasites. FACS analysis of BMECs showed that Δicp induce the surface expression of VCAM-1 and E-selectin but reduce surface ICAM-1. Human isolated neutrophils also adhered at higher numbers to BMECs previously exposed to Δicp compared to WT. However, T Lymphocyte CD4⁺ and CD8⁺ adhered less to BMECs pre-incubated with Δicp . Immunohistochemistry on cryosectioned brain tissue showed higher amounts of stained microglia at day 5, suggesting that infection affects the central nervous system before parasite penetration. Parasite levels in blood of BALB/c mice were more pronounced in mice infected with WT parasites than Δicp . All BALB/c mice infected with parasites displayed splenomegaly, although those infected with Δicp showed higher cellularity. At day 5 of infection, TNF- α , IFN- γ and IL-6 were increased in the blood serum of mice infected with WT parasites compared to Δicp . Cytokine levels of splenocytes ex vivo were also detected by ELISA, which showed increased levels of IFN- γ and IL-6 in the splenocytes supernatant cultures of mice infected with Δicp . We propose that in the absence of ICP, infected mice develop a more prominent inflammatory immune response in the spleen that might be associated to parasite control. **Supported by:**CAPES **Keywords:**lcp; t. brucei; cysteine peptidase

HP088 - CERAMIDE PROFILING OF *LEISHMANIA* UNDER STRESS CONDITIONS

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Leishmania are digenetic parasites with a life cycle alternating between extracellular promastigotes, which live in the gut of sand fly vector, and intracellular amastigotes, which live in an acidic pH environment inside phagolysosomes of mammalian phagocytic cells. In the last two decades, our lab and others have demonstrated the importance of sphingolipids for the parasite development and infectivity. In this work, we aimed to evaluate the expression of free ceramide in *Leishmania (Viannia) Brasiliensis*, etiologic agent of cutaneous and mucocutaneous leishmaniasis in BRA, under pH and temperature stress, mimicking the transition of parasite from invertebrate to vertebrate hosts. Profiling of free ceramide was performed by electrospray ionization mass spectrometry. Their structures were confirmed by collision induced dissociation, a method based in molecule fragmentation. Ceramides were purified from parasite lipid extracts followed by alkaline hydrolysis, to remove glicerophospholipids, and silicic acid chromatography. It was demonstrated for the first time that free ceramides are expressed in *Leishmania*. In control promastigotes (cultured at 23 °C, pH 7.2), it were identified six free ceramide ions, while under stress (cultured at 35 °C, pH 5.5), it were detected 11 free ceramide ions. Some interesting differences in ceramide composition were detected for promastigotes under stress conditions. We observed a prevalence of hydroxylated fatty acids (16:0:OH) and methylated and hydroxylated fatty acids (13:0:OH; 15:0:OH) in ceramides from promastigotes cultured under stress condition. Conversely, in the free ceramide fraction of control promastigotes, the only hydroxylated fatty acid detected was 15:0:OH, besides predominant 14:0 and 16:0-fatty acids. Differences in ceramide composition may help to clarify the sphingolipid metabolism in amastigotes and promastigotes and the role of these molecules during infection. **Supported by:**CNPq/CAPES/FAPESP **Keywords:**Leishmania; ceramide; mass spectrometry

HP089 - BOTH TRYPOMASTIGOTE AND AMASTIGOTE FORMS OF *T. CRUZI* ANSWER TO ROS SIGNALING AND PROLIFERATES BETTER INSIDE PHOX KO MACROPHAGES

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Background: Macrophages are cells that have a broad spectrum of activation depending on external stimulation. When activated, they produce effector molecules responsible for elimination of intracellular parasites, such as NO and ROS. In *Trypanosoma cruzi* infection, macrophages produce ROS in a process called respiratory burst. We have shown that the parasite needs a signal provided by ROS to multiply. In Phox KO macrophages (from mice deficient in the gp91phox) *T. cruzi* multiplies less compared to C57BL/6 WT macrophages. When we add H₂O₂, we observe that multiplication is recovered in Phox KO cells. Objective: In order to understand why *T. cruzi* multiplication is impaired in Phox KO macrophages, we evaluated the profile of activation of these cells and investigated in which stage of *T. cruzi* life cycle ROS is important to trigger proliferation in macrophages. Methods and Results: Macrophages from Phox KO and WT mice were stimulated with IL-4 or IFN- γ /LPS before *T. cruzi* infection and arginase production was evaluated. We showed that arginase activity in Phox KO macrophages is higher than in cells from C57BL/6 WT mice infected with *T. cruzi*. In parallel, parasites were treated with H₂O₂ before, 2 h and 24 h after infection of macrophages. Our results showed that after receiving ROS signal, the parasites multiply efficiently in Phox KO macrophages, independently of when this signal is provided (to trypomastigotes before or after infection or to amastigotes). Conclusions: The profile of activation of Phox KO macrophages suggest that these cells are subjected to infection once elevated levels of arginase are detected. However, without a signal provided by ROS, *T. cruzi* did not multiply inside these cells. Our data demonstrated that this signal could be given in any time of development of the parasite (trypomastigotes or amastigotes forms), inducing their multiplication even in cells in which this molecule is absent. **Supported by:**INCT Redoxoma, FAPEMIG, CAPES and CNPq
Keywords:T. cruzi; phox ko; ros

HP090 - THE ROUTE OF INOCULATION INFLUENCES THE OUTCOME OF INFECTION BY *TRYPANOSOMA CRUZI*

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The route or site of inoculation has been shown to influence disease outcome in numerous models of infection. However, the factors that determine route-specific influence on *T. cruzi* infection remain poorly defined. While it may seem obvious that intradermal inoculation of the skin would best replicate the natural infection, intraperitoneal inoculation remains a favored route of infection in *T. cruzi* studies. In recent studies our group demonstrated that Phox KO macrophages (from mice deficient in the gp91phox) showed reduced parasitism, when infected with *T. cruzi*, as compared to C57BL/6 WT macrophages. However, in vivo, we had a different result. C57BL/6 WT and Phox KO mice infected by intraperitoneal route displayed similar parasitemia, which peaked around 9 days post-infection and was subsequently controlled. Phox KO animals exhibited higher mortality when compared to WT controls, starting at day 15 and reaching 100% mortality by 21 days of infection. In order to do that we infected C57BL/6 WT and Phox KO mice with blood tripomastigotes and observed parasitaemia and mortality. Here we found that initiation of *T. cruzi* infection by intradermal inoculation of the ear, compared to inoculation via the intraperitoneal route, results in differences in parasitemia and mortality profile of Phox KO and C57BL/6 WT mice. Intradermal infection induced increased levels of parasitemia in C57BL/6 WT mice compared to Phox KO mice, which peaked around 8 days post-infection. This result is in agreement with what we observed in vitro after macrophages infection. By intradermal route infection, the mortality of Phox KO mice was also elevated compared to C57BL/6 WT. Our results suggested that the route of infection influences the development of the disease, although the mechanism by which this occurs remains to be elucidated. **Supported by:**INCT Redoxoma, FAPEMIG, CAPES and CNPQ
Keywords:Trypanosoma cruzi; route inoculation; ntradermal inoculation

HP091 - PEPTIDES PREDICTED IN SILICO AND CHEMICALLY SYNTHETIZED INDUCE PARTIAL PROTECTION AGAINST TOXOPLASMA GONDII EXPERIMENTAL INFECTION IN MICE

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The toxoplasmosis, disease caused by the protozoan *Toxoplasma gondii*, is highly severe in individuals with impaired immune system and fetuses of pregnant women and animals. The current treatment is efficient against the acute phase, but unable to eradicate the chronic toxoplasmosis. The lack of alternative treatments, or even other measures to prevent the infection or its reactivation, led several research groups to investigate different antigens and adjuvants as potential vaccines, but still there are no commercially available vaccines. Thus, in this work we predicted 22 peptides based on proteins of *T. gondii*: micronemes (MIC), surface related sequences (SRS), dense granules (GRA) and roptries (ROP). They were chemically synthesized and submitted them to a serological screening. The peptides more reactive were divided according to the original organelle and, along with aluminum hydroxide, used to immunize groups of C57Bl/6 mice. Then they were infected with a sublethal dose of *T. gondii*. Blood samples were collected and used in ELISA assays to assess the titers of antibody anti-STAg (soluble *T. gondii* antigen) and against the peptides used in the immunization. These experiments showed that the animals were successfully immunized. Blood samples collected 48 hours after the infection were used to assess IL-10, IL-4, IL-6, IL-2, IL-17, interferon- γ and tumoral necrosis factor cytokines. The results showed that all cytokines concentrations were lower than PBS control, while maintaining the predominance of pro-inflammatory (Th1) cytokines. The SRS and MIC groups showed cytokines titers similar to the group immunized with STAg. The DNA present in brain samples were collected and submitted to quantitative PCR. The groups MIC and SRS had significantly less parasite burden than PBS group. These results suggests that the peptides from MIC and SRS groups should be used in further studies to design vaccines against *Toxoplasma gondii*. **Supported by:** CAPES, CNPq

Keywords: Toxoplasmosis; *toxoplasma gondii*; peptides

HP092 - UTR-BASED GENE EXPRESSION OF PROTEINASES ON PROMASTIGOTES AND AMASTIGOTES OF LEISHMANIA (V) BRASILIENSIS

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Leishmania is a digenetic protozoan parasite causing leishmaniasis in humans. The available therapy is based on compounds developed more than eight decades ago. They are far from ideal due to substantial side effects, justifying the urgent need to identify better compounds. To achieve these goals, a thorough understanding of the host-pathogen-vector triangle, and particularly of their intimate interactions at the molecular level, is imperative. *Leishmania* express many peptidases, metallo and cysteine are the most abundant protease from this parasite and a pivotal in virulence factor in mammals. However was observed significant distinctions in many protease features, such as occurrence, conservation and quantity, an aspect that may be related to their adaptations to the peculiarities of each microenvironment they inhabit. *Leishmania* sp exhibit a number of remarkable biological phenomena, especially at the RNA level that often deviate dramatically from eukaryotic paradigms, the regulation of gene expression occurs almost exclusively at the post-transcriptional level and could involve mRNA stability and translational efficiency. Here we accessed the factors involved in proteinase gene expression, such as conserved signaling sequences in UTR and their influence on expression of this enzyme group intrinsically linked to the virulence of the parasite. By using bioinformatics and real-time PCR approaches, was observed that the amastigotes and promastigotes of *Leishmania Brasiliensis* expressed differentially in vitro genes of the three main protease classes: cysteine, serine and metallo. Furthermore, it was observed some proteases have UTR sequence motifs that could be involved on regulation of gene expression associated life stage of *Leishmania*. This study is first step to try understanding the factors involved on regulation of proteinases gene expression and maybe, indicate promising targets for the development of new therapeutic strategies against this disease. **Supported by:** CNPQ, FIOCRUZ, FAPERJ

Keywords: *Leishmania*; proteases; gene expression

HP093 - A NEW PEPTIDE PREDICTED FROM THE MICRONEME 8 PROTEIN OF TOXOPLASMA GONDII IS CAPABLE OF IDENTIFYING THE INFECTIVE FORM THROUGH A BLOOD SAMPLE.

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The correct diagnosis of an acute infection in toxoplasmosis is of great importance for effective therapy and reduction of severe consequences. The commonly diagnostic tests are not able to distinguish acute from chronic profiles accurately. To address this problem, in this work we evaluated a Microneme 8 peptide (pMIC8) in comparison with soluble Toxoplasma antigen (STAg). Initially, we evaluated the kinetic of IgG antibodies in mice infected with *T. gondii* against pMIC8 and STAg. Moreover, we evaluated 124 human serum samples divided into five groups: group I (up to 4 months of infection); group II (5 to 8 months of infection); group III (8 to 12 months of infection); group IV (over 12 months); group V (seronegative). In murine model, pMIC8 showed as a potential marker of recent infection with strong detection in early phase of infection. In humans, IgM and IgA to pMIC8 shown a better characterization of the time of *T. gondii* infection in serum samples up to 12 months (groups I, II and III) when compared with STAg. To IgG, the detection of pMIC8 was higher in sera from group I and lowest detection in sera from group IV. This serological pattern was the opposite of those observed to STAg (lowest detection in group I). In order to underline this difference we used a ratio of values of IgG to STAg and pMIC8. This ratio presented an accurate value in serological differentiation. These findings suggest that pMIC8 could be a tool in serodiagnosis of toxoplasmosis.

Supported by: CNPq, CAPES

Keywords: Microneme 8; differential diagnosis; acute toxoplasmosis

HP094 - STUDIES ON LEISHMANIA (VIANNIA) BRASILIENSIS AND L. (V.) NAIFFI MIXED INFECTIONS

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1.LABORATÓRIO DE PESQUISAS EM LEISHMANIOSE - IOC- FIOCRUZ, Rio de Janeiro, RJ, BRA.

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In some endemic regions more than one species of *Leishmania* affect humans. The mixed infection in an individual may represent a rare event, however, this information might be underestimated due to lack of sensitive diagnostic methods available. Golden hamster (*Mesocricetus auratus*) is the choice model for *Leishmania (Viannia)* species infection, since mice are resistant to some strains. We developed studies aiming to elucidate clinical and immunopathological aspects of mixed infection with two sympatric species from *L. (Viannia)* subgenus. We detected and analyzed changes in susceptibility, virulence and disease progression. Golden hamsters were infected with *Leishmania Brasiliensis* and *L. naiffi* strains individually or as mixed infection. The infection course was followed up to ten weeks and there-isolated parasites present in fragments of skin lesions were characterized by performing isoenzyme assay (MLEE). Also DNA from lesion' fragments were used for PCR-RFLP hsp70. The parasite load was assessed by qPCR, and histopathological analysis were performed by H&E staining. We observed changes in the course of experimental infections with animals mixed infected showing larger and more severe lesions in relation to single infections. It was also possible to characterize by MLEE the presence of *L. Brasiliensis* and *L. naiffi* in cultures derived from mixed infected animals. But, after several passages we could not observe the electromorph relative to *L. naiffi*. PCR-RFLP hsp70 gene analysis was able to detect only *L. Brasiliensis* in lesion of mixed infected animals. In conclusion, the mixed infection of *L. Brasiliensis* and *L. naiffi* seems to induce more severe lesions than the single infections. Aspects of the immune response observed by the mRNA expression for cytokines may clarify the mechanism involved in this phenomenon. **Supported by:** CNPq; Faperj, PAEF/IOC/FIOTEC, FIOCRUZ

Keywords: Mixed infection; leishmania (viannia) Brasiliensis; leishmania (viannia) naiffi

HP095 - **EVALUATION OF CARDIAC REGENERATION AFTER INHIBITION OF TGF- β PATHWAY IN EXPERIMENTAL MODEL OF CHRONIC CHAGAS DISEASE**

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Studies published by our group demonstrated the involvement of TGF- β in the development of Chagas disease cardiomyopathy. The activation of the TGF- β signaling pathway was observed in cardiac tissue of *T. cruzi* infected animals followed by increased expression of extracellular matrix proteins. This fibrotic process results in functional commitment of the heart and TGF- β is the most important protein involved in fibrosis process. Recently, we showed that *T. cruzi*-infected animals during the experimental chronic phase of Chagas disease presented cardiac damage with increased collagen deposition, and increased levels of circulating TGF- β . Treatment of animals at 120 days post infection (dpi) with GW788388, an inhibitor of TGF- β pathway, was able to decrease TGF- β circulating levels, improve the electrocardiographic state of infected animals and revert heart fibrosis. The aim of the present study is to understand the mechanisms involved on the GW788388 activity to revert cardiac fibrosis observed in *T. cruzi* infected mice and evaluate possible candidates involved on heart regeneration. To this end, C57Bl/6 animals were infected with *T. cruzi* (Colombiana strain- 10²) and treated orally with 3mg/kg of GW788388 120 dpi in two schemes: once a week or three times a week. Hearts obtained from infected animals treated or not with GW788388 were collected and protein or mRNA expression were investigated for MMP-2 and MMP-9 by Western blot methods, and for targets of cardiac regeneration: Gata-4, Gata-6, T-box5, Nkx2-5, troponin T, desmin and titin by RT-qPCR. Our data showed that MMP-2 and MMP-9 expression were not altered in the heart of animals during the chronic phase of *T. cruzi* infection, however, its activity were decreased. On the other hand, GW788388 treatment increased both MMP-2 and MMP-9 activities. Preliminary data indicates that GW788388 treatment induced cardiac regeneration, observed by an increase of Gata-6 expression in the heart of infected animals. **Keywords:** Chagas disease; tgf-beta; fibrosis

HP096 - **EFFECT OF ANGIOTENSIN CONVERTING ENZYME (ACE) INHIBITOR AND AT1 RECEPTOR ANTAGONIST IN HOST INFECTION BY *PLASMODIUM CHABAUDI***

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Malaria represents a medical emergency because it may progress to death. The main pathological changes of malaria are severe anemia, coagulation disorders, splenomegaly, hepatomegaly and obstruction of microvasculature. Malaria infection in hypertension state is poorly studied and could represent a severe condition in these patients. We evaluate the blood parasitemia and survival of rodents infected with *Plasmodium chabaudi* and treated with ACE inhibitor (captopril), AT1 receptor antagonist (losartan) and antimalarial (chloroquine). Western blotting and immunofluorescence microscopy of kinin receptors B1 and B2 was performed in liver of infected mice, which indicate modulation in liver blood pressure. Survival curves and parasitemia in Balb/C mice were performed under treatment with chloroquine (CQ, 20 mg/kg) in combination with captopril (CP, 45 mg/kg) and losartan (LT, 10 mg/kg) oral administered at a daily dose for 15 days. The intracellular parasite proteolysis (isolated cells) was not changed after treatment with CP using the substrate Abz-FR-(Dnp) P-OH, which indicated that the parasite has no ACE-like protease. The effect of antimalarial CQ (25 μ M) was observed on cysteine-protease activity using the substrate Z-FR-AMC, with reduction of 29% (p<0.05) compared to controls. Addition of CQ (10 μ M) together with CP (10 μ M) reduced the intracellular proteolysis in 32%. In vivo studies observing the infection of Balb/C mice with non-lethal *P. chabaudi* demonstrated that groups (CP, LT and CP + LT) did not survive after reaching the parasitemia peak that occurs between the 9th and 13th day.

The effect of vasodilatation worsened the parasitemia profile in non-lethal murine malaria model compared to control resulting in premature death. In addition, intracellular proteolysis in malaria parasites did not present ACE-like activity. Our data are relevant to concern the situations of patients infected by *Plasmodium sp* that are under hypertension treatment. **Supported by:** FAPESP **Keywords:** Malaria; liver; kinin

HP097 - DIFFERENTIAL TRANSCRIPTOME OF HUMAN DENDRITIC CELLS AT FIRST CONTACT WITH *TRYPANOSOMA CRUZI*: PRELIMINARY ANALYSIS
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Dendritic cells (DCs) are important components of human immunologic system and are able to capture antigens at the infection site. Antigen internalization and presentation are accomplished with high efficiency by these cells, giving them a central role in linking natural and acquired immune response against pathogens, including etiologic agent of Chagas disease: *Trypanosoma cruzi*. DCs can modulate host immunologic response depending on multiple factors, which may influence the development of the disease clinic forms. In this context, this work simulates the initial contact parasite-DCs in a natural infection in order to study DCs functional genic modulation in such condition. Here, we present the standardization of DCs derived from monocyte obtained from three healthy voluntaries and the optimization of in vitro metacyclic trypomastigote differentiation to accomplish parasite-DCs and, subsequently, proceed to total RNA extraction for sequencing and differential transcriptome assembly. After a 12 h interaction, infection rates were about 39%, an average of 2.4 amastigotes/infected DC was obtained and 80% of DCs from infected assays were activated. Total RNA from control and infected samples was extracted for sequencing and assembly of the reads accordingly to human reference genome. The results showed good reliability with ~23,000,000 paired-end reads per sample, and a correlation coefficient of ~1 between duplicates, solid indications of data quality. Different gene expression was observed among donors and between control and infected samples. A detailed vision of antigen presenting cells biology after initial contacts with *T. cruzi* could provide new targets for the disease treatment and will help understanding the differential evolutions of Chagas disease in patients and in the resistance and tolerance to parasite. **Supported by:**CAPES **Keywords:**Differential transcriptome; dendritic cell; trypanosoma cruzi

HP098 - THE TRANSCRIPTOME OF *TRYPANOSOMA CRUZI* EXTRACELLULAR AMASTIGOTES REVEALS DIFFERENCES IN TRANSCRIPT LEVELS BETWEEN G AND CL STRAINS REGARDING MOLECULES POTENTIALLY INVOLVED IN INVASION TO HOST CELLS.

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Amastigotes of *Trypanosoma cruzi* generated by the extracellular differentiation of bloodstream or the corresponding tissue culture derived from trypomastigotes are named extracellular amastigotes (EAs). EAs are alternative infective forms of *T. cruzi* and together with bloodstream trypomastigotes sustain the parasite cycle in mammalian hosts and cells. Differences in the infectivity of EAs from two strains, G (derived from sylvatic, type I) and CL clone (derived from the vector *Triatoma infestans*, type VI) have been widely acknowledged: G strain EAs are much more infective *in vitro* than CL parasites. There is still little information regarding the elements involved in EA entry into mammalian cells. With the aim of verifying the transcripts differentially expressed by both strains polysomal RNA was extracted from EAs of both G strain and CL clone, converted to cDNA and hybridized in a biochip (TcBiochip v.3.1 Laboratório de Genômica Funcional, ICC/FIOCRUZ, Paraná) with up to 6,000 probes representing 5,000 different genes of *T. cruzi* (<http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GPL1061>), approximately. Among molecules highly expressed in G strains are P21 and mevalonate kinase which have been acknowledged to positively modulated internalization of *T. cruzi* EAs into cells. Conversely amastin is highly expressed in CL parasite and has showed to negatively modulate EAs invasion. In addition, from the 108 transcripts selectively augmented in CL, 26 are transmembrane, including sialidases and phospholipase B, and two display signal peptide. Regarding G transcripts, 202 were selectively augmented, among them 56 transmembrane proteins, such as cysteine peptidase, and mucin-associated surface protein (MASP). These results can contribute to a better knowledge on molecular mechanisms underlying *T. cruzi* cellular invasion. **Supported by:**FAPESP AND FAPEMIG
Keywords:Extracellular amastigotes; transcriptome; g and cl strains

HP099 - **IDENTIFICATION AND MOLECULAR CHARACTERIZATION OF BECLIN1/VPS30 PROTEIN IN *TRYPANOSOMA CRUZI***

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Autophagy is an essential process that consists of selective degradation of cellular components, and is usually regarded as a survival mechanism. In recent years, autophagy has been implicated in several other biological processes, such as host defense, innate and adaptive immunity, cellular remodeling and even in clinical settings, such as neurodegenerative disorders, infectious diseases and neoplasms. The mammalian autophagy gene Beclin1 is important for localization of autophagic proteins to a pre-autophagosomal structure (PAS), depending on the interaction with the phosphatidylinositol 3-kinase class III, Vps34. Beclin1 is the ortholog of the Atg6/Vps30 protein in yeast, where it forms two distinct complexes: in complex I, Atg14 links Vps30 to Vps34 and plays a specific role in autophagy, while in complex II, Vps38 links Vps30 to Vps34 to play roles in vacuolar protein sorting. Beclin1 coordinately regulates the autophagy and membrane trafficking involved in several physiological and pathological processes. In *Trypanosoma cruzi*, these molecules have not been characterized yet. Our group has previously characterized TcVps34 and showed that its overexpression modulates *T. cruzi* cellular invasion. This study aimed to identify the Beclin1 gene in *T. cruzi* and characterize its possible interaction with other proteins, such as TcVps34. Using bioinformatics tools, yeast Vps30 as query and the greatest similarity degree as a criterion of recognition we found a hypothetical gene in *T. cruzi*, with two Apg6 conserved domains at C terminal which is shared with Beclin1/Atg6/Vps30 in human and yeast. To establish its biological function, TcBeclin was cloned into bacterial vector pET21, in addition to *T. cruzi* expression vectors pTREX_RFP and pROCK_HA. Experiments to determine subcellular localization, protein interaction (TcVps34-TcBeclin1 and others), and the effect of overexpressing this protein on the parasite capacity of invading host cells are underway. **Supported by:**FAPEMIG AND FAPESP

Keywords:Beclin1/vps30; vps34; protein interaction

HP100 - **CAN TOXOPLASMA GONDII BE IN VIVO CONTROLLED BY INHIBITION OF THE PROTEASOME?**

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Toxoplasma gondii is an obligate intracellular parasite that causes toxoplasmosis. During infection of host cells, *T. gondii* uses many mechanisms for evasion of the immune system, such as the inhibition of nitric oxide (NO) which acts as a microbicidal agent. Macrophages activated with interferon-gamma and lipopolysaccharide produce NO via inducible NO synthase (iNOS), however, *T. gondii* infection inhibits NO production by iNOS degradation. Previous studies have shown that the proteasome pathway is responsible for iNOS degradation and its pharmacological inhibition prevents the degradation of the enzyme. Therefore, it is possible that with the systemic inhibition of the proteasome, mice may become more resistant to *T. gondii* infection. This study aims to verify the relevance of the proteasome pathway in the in vivo *T. gondii* infection, confirming its participation in iNOS degradation. For this, C57BL/6 mice were orally infected with the ME-49 strain of *T. gondii* and treated with the proteasome inhibitor MG132. No differences in survival during the acute phase of the infection and no variances in cyst number and size were found. In vitro assays with activated macrophages were performed to verify if MG132 was able to reverse the inhibition of NO production after *T. gondii* infection, but no differences were detected, indicating that MG132 may not be efficient in the inhibition of the proteasome. Bortezomib, a proteasome inhibitor used on cancer treatment, was also used in the in vitro assays and it was capable to reverse the inhibition of NO production after macrophage infection by *T. gondii*. At the moment we are testing if bortezomib is able to increase survival of *T. gondii* infected mice. The possible increase in survival will indicate that iNOS degradation by the proteasome pathway is important in infections, allowing the use of pharmacological inhibition of the proteasome as an experimental treatment against toxoplasmosis. **Supported by:**CAPES

Keywords:*Toxoplasma gondii*; proteasome; inos

HP101 - AHR SHAPING THE IFN-GAMMA-MEDIATED TRYPANOCIDAL ACTIVITY BY MACROPHAGES MODULATING THE AVAILABILITY OF ARGINASE AND IDO LEVELS
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Trypanosoma cruzi is a protozoan parasite that causes Chagas disease, an important neglected illness. The aryl hydrocarbon receptor (AhR) is a transcription factor involved in regulation of *T. cruzi* infection. Nevertheless, the molecular mechanism by which it occurs is unknown. Herein, we use macrophages (MO) to clarify how AhR influence effector molecules as nitric oxide (NO) and peroxynitrite (ONOO-), and important enzymes as arginase and IDO. To unveil the relation between AhR and *T. cruzi* we stimulated WT MO with *T. cruzi* antigens (AgTc) and we found that AhR is upregulate. Next, we infected WT and AhR KO MO and/or treated the cells with IFN- γ . The growth was evaluated at 4 and 48h post infection (PI) and released trypomastigotes on days 4-7 PI. We did not find difference in the uptake of parasite. However, the amount of amastigotes 48h PI and trypomastigotes were lower in AhR KO MO. Interestingly, when the *T. cruzi*-infected AhR KO MO were stimulated with IFN- γ the cells lost partially the microbicidal ability. This control is not related with NO but with the higher levels of arginase and IDO. As the arginase activity is the same between *T. cruzi*-infected WT and AhR KO MO it still not explain the higher ability of AhR KO MO in absence of IFN- γ stimulation in the control of *T. cruzi* replication. To elucidate if ONOO- plays a role in this aspect, we treated MO with FeTPPS, an ONOO- scavenger. We found that ONOO- is important to control parasite replication in *T. cruzi*-infected WT MO, however *T. cruzi*-infected and treated AhR KO MO still control parasite replication. Our results suggest that *T. cruzi* induces AhR expression in macrophages that, in turn, regulates the host cells signaling pathways induced by IFN- γ , which determines availability of arginase and tryptophan levels for parasite replication and host production of ROS. By manipulating the levels of these molecules, AhR may act as a central regulator of parasite replication and dissemination. **Supported by:**CNPq, CAPES and FAPEMIG

Keywords:Trypanosoma cruzi; aryl hydrocarbon receptor; arginase

HP102 - HOW TWO DIFFERENT STRAINS OF TRYPANOSOMA CRUZI (CL BRENER AND Y) RESPOND TO HYPOXIC ENVIRONMENT IN A BIOENERGETICS APPROACH?

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Trypanosoma cruzi faces different environments during its biological cycle such as changes in oxygen levels that challenge parasite to divert its metabolic activity. It is well recognized that this parasite presents multiplicity of its genotypes and phenotypes, thus metabolic responses may vary along parasite strains. Once it is unclear the influence of oxygen fluctuation upon *T. cruzi*, we compared the response to hypoxic conditions between two strains (CL Brener and Y strain) of *T. cruzi* epimastigotes and measured mitochondrial respiration differences between these parasites. Hypoxic condition was induced by N₂ saturation. After 5 days on hypoxia, parasites were quantified by counting. Our results showed that hypoxia induced epimastigotes proliferation and CL Brener cells proliferates more than Y parasites suggesting CL Brener cells are more adapted to hypoxic events. We also evaluated ROS production using the probe DCFDA. The results showed hypoxia increased the levels of ROS in both strains. In order to investigate energetic metabolism variances between both strains, we performed a high resolution respirometry assay using a substrate-uncoupler-inhibitor-titration protocol in digitonin-permeabilized cells. Oxygen consumption measurements indicated that RCR values were higher in Y cells than CL Brener parasites. Moreover, proton leak values were higher in CL Brener parasites, indicating that respiration in these cells is more uncoupled than in Y strains. In agreement with this result, Y cells also demonstrated an increase in ETS capacity in relation to CL Brener. Then, respirometry results suggest that Y parasites uses respiration process to ATP production more than CL Brener cells, that probably produce more ATP out of mitochondria than Y strain. Taken together, our results suggest the variances between Y strain and CL Brener *T. cruzi* epimastigotes on bioenergetics metabolism and it may support the mild variances on response of these parasites to hypoxia challenge. **Supported by:**CNPq, FAPERJ, INCT-EM **Keywords:**Trypanosoma cruzi ; bioenergetics; different strains

HP103 - **PLATELET-ACTIVATING FACTOR (PAF) AND LYSOPHOSPHATIDYLCHOLINE (LPC) MODULATES THE INFECTIVITY OF *LEISHMANIA INFANTUM***

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Visceral leishmaniasis is caused by *L. infantum* in the New World. Platelet activating factor (PAF) is a phospholipid mediator of many biological and pathophysiological processes, including cell differentiation. Lysophosphatidylcholine (LPC) is the most important bioactive component of membrane lipoproteins and is involved in diseases such as atherosclerosis and inflammatory diseases, being produced in physiological and pathological conditions. In previous studies we demonstrated that PAF stimulates cell differentiation of *Herpetomonas muscarum muscarum* and *Trypanosoma cruzi*, in addition to modulate infection of mouse peritoneal macrophages by *L. amazonensis*, and that *T. cruzi* synthesizes a C18:1-LPC, with the ability to aggregate platelets, similarly to PAF. In the present study, we demonstrate the effects of PAF and C18:1-LPC on the infection in mouse peritoneal macrophages by *L. infantum*, when the parasites were treated for 4 hours with 10^{-5} M PAF, C18:1-LPC and/or 10^{-5} M WEB 2086 (PAF receptor antagonist). An increase of 96% and 75% was observed in the interaction when the parasites were treated with PAF or LPC, respectively. However, when the parasites were treated with PAF + WEB or C18:1-LPC + WEB, there was a reduction of the effects caused by these phospholipids of about 40% and 30%, respectively. We also tested the effects of PAF or C18:1-LPC in the survival of *L. infantum* in mouse peritoneal macrophages. An increase of 91% and 84% in the survival of these parasites was observed when they were treated with PAF or C18:1-LPC, respectively, as compared to the control. The treatment with PAF + WEB and C18:1-LPC + WEB were also able to reverse these effects in 45% and 43%, respectively. These results suggest a modulation of infection of mouse peritoneal macrophages by *L. infantum* by PAF and C18:1-LPC, and that these effects probably occur via a putative PAF receptor in these parasites, as WEB 2086 was able to reverse the effects promoted by these phospholipids

Supported by: CNPq, FAPERJ, CAPES and INCT-EM.

Keywords: *L. infantum*; paf; lpc

HP104 - **HAEMATOLOGICAL FINDINGS IN DAIRY CATTLE INFECTED WITH *TRYPANOSOMA SP.* IN RIO DE JANEIRO STATE**

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Trypanosomiasis is a serious health problem in cattle, where the disease is endemic. The protozoan is native of Asia and Africa where the tsetse fly is the biological vector. In BRA, blood-sucking insects act as mechanical vectors and needle-sharing transmission is also important in herds which uses oxytocin to induce lactation. The economic impacts of trypanosomiasis are related to the decrease of production and mortality. In early 2016, some cows from a dairy farm, in the South of the state, developed an illness with prostration, weight loss, decreased milk production, conjunctivitis and pale mucous membranes. The recent introduction of animals infected with *T. vivax* from Minas Gerais state to Rio de Janeiro, possibly caused the death of two cows in this state. Blood samples were collected from 87 cows in milk production, with an average age of 5.7 years, 80% belonging to Girolando breed. Blood smears were made by buffy coat. Trypomastigotes of *Trypanosoma sp.* were visualized in blood smears in 45 (51.7%) of the samples. From positive samples, haemograms were performed and the results were: 21 (46.7%) had normocytic normochromic anemia and 4 (8.9%) showed borderline results; 19 (42,2%) had absolute neutrophilia and 4 (8.9%) presented monocytosis, common in protozoan diseases. Thrombocytopenia, another usual finding associated to protozoan infection, was observed in 7 (15.6%) of the positive animals. Normocytic normochromic anemia has been reported in acute infections caused by *T. vivax*. The results confirm the *Trypanosoma sp* infection. Clinical findings and the origin of some animals lead to suspicion of *T. vivax*. Biometric and molecular studies are being conducted to confirm the species. *Trypanosoma sp* in cattle in Rio de Janeiro State had never been recorded. Woo technique and blood tests can be used for identification of positive animals in the acute phase of the disease. **Supported by:** Cnpq **Keywords:** *Trypanosoma sp.*; haematology; bovine

HP105 - LAMININ ISOFORMS 411 AND 511 INDUCES NEUTROPHILS EXTRACELLULAR TRAPS (NETS) AND MODULATE NETOSIS INDUCED BY LEISHMANIA AMAZONENSIS

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Neutrophils are the first cells to migrate to infected sites, where they can kill pathogens by an array of mechanisms. They can also release structures, known as neutrophils extracellular traps (NETs), which are formed as scaffolds of decondensed chromatin associated with granular and cytosolic proteins. *Leishmania* promastigotes are inoculated by the insect vector in a pool of blood, in close contact with neutrophils and proteins of the extracellular matrix (ECM). Here, we aim to study the interaction of neutrophils with laminins and *Leishmania amazonensis* promastigotes (La), either isolated or in association, analyzing the induction of NETs formation. Initially, we evaluate if laminin isoforms (111, 211, 332, 411, 421 and 511) in suspension or adsorbed to plates would influence NETs release. We observed that neutrophils in either case released NETs. We also analyzed in neutrophils the expression of $\alpha 6$ integrin, a major laminin receptor (VLA-6/CD49f). Our results showed that about 70% of neutrophils express this receptor. Next, we evaluate if $\alpha 6$ integrin participates in NET generation induced by laminins isoforms and we observed that pretreated of neutrophils with anti- $\alpha 6$ integrin antibody (GOH3) decreased 32% and 35% NETs release after interaction with laminins 411 and 511 respectively, compared with neutrophils stimulated only with these laminins. We characterize that the mechanism of NETs release induced by laminins 411 and 511 is dependent neutrophil elastase and peptidylarginine deiminase 4 (PAD4). Interestingly, NET release by laminin 511, but not by 411 is dependent on ROS generation. Finally, we evidenced that $\alpha 1$, $\alpha 4$ and $\alpha 5$ laminin chains colocalize with NETs induced by La, and that laminins 411 and 511 modulate NETs induced by La.

We thank the Hemotherapy Service of Hospital Universitário Clementino Fraga Filho, UFRJ Supported by FAPERJ, CAPES and CNPq.

Keywords: Neutrophil extracellular traps; laminin; *Leishmania amazonensis*

HP106 - MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF HAEMOPROTEUS PARAMULTIPIGMENTATUS VALKIŪNAS, 2013 (APICOMPLEXA: HAEMOSPORIDA) FROM COLUMBIFORMS BIRDS IN ATLANTIC FOREST

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The aim of this study was to evaluate the prevalence of *Haemoproteus* spp. in Brazilian Columbiforms birds and to realize a morphological, morphometric and molecular characterization of the species *Haemoproteus (Haemoproteus) paramultipigmentatus*. Were captured 76 birds, 63 of the species *Columbina talpacoti* and 13 *Leptotila verreauxi*. The blood samples were collected by puncturing the brachial vein, stored at -20°C for molecular analyses and blood smears were prepared, fixed in methanol and stained with Giemsa, for morphological analyses. The prevalence and the parasitemia were estimated by light microscopy. For molecular analyses, the Cythochrome b gene was amplified in a nested-PCR, and posteriorly sequenced to perform the phylogenetic analyses (maximum likelihood and bayesian inference). The prevalence of *Haemoproteus* spp. was 65.78%. Among the birds analyzed, 26 were infected with specimens morphologically identified as *H. paramultipigmentatus* (prevalence, 37.14%; parasitemia, 0.12%), since the specimens presented all the taxonomic characters of this species, such as: macrogametocytes with absence of volutin granules; with elongated and slender body, with irregular, or slightly ameboid outline; fully grown gamotocytes giving a horn-like appearance and that push the nuclei in their middle part. The *H. paramultipigmentatus* lineages, characterized in this study, emerging, with a strong branch support value (98/1), in a monophyletic clade with other lineages of the same species previously characterized. This found corroborates the morphological identification. The present study is the first record of the species *H. paramultipigmentatus* in BRA and in the species *C. talpacoti* and *L. verreauxi*, since this species were reported only in *Columbina passerina socorroensis* in Mexico. **Supported by:** CAPES - COORDENAÇÃO DE APERFEIÇOAMENTO DE PESSOAL DE NÍVEL SUPERIOR **Keywords:** Avian parasites; columbidae

HP107 - LAMININ ISOFORMS 411 AND 511 INDUCES NEUTROPHILS EXTRACELLULAR TRAPS (NETS) AND MODULATE NETOSIS INDUCED BY LEISHMANIA AMAZONENSIS

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Neutrophils are the first cells to migrate to infected sites, where they can kill pathogens by an array of mechanisms. They can also release structures, known as neutrophils extracellular traps (NETs), which are formed as scaffolds of decondensed chromatin associated with granular and cytosolic proteins. *Leishmania* promastigotes are inoculated by the insect vector in a pool of blood, in close contact with neutrophils and proteins of the extracellular matrix (ECM). Here, we aim to study the interaction of neutrophils with laminins and *Leishmania amazonensis* promastigotes (La), either isolated or in association, analyzing the induction of NETs formation. Initially, we evaluate if laminin isoforms (111, 211, 332, 411, 421 and 511) in suspension or adsorbed to plates would influence NETs release. We observed that neutrophils in either case released NETs. We also analyzed in neutrophils the expression of $\alpha 6$ integrin, a major laminin receptor (VLA-6/CD49f). Our results showed that about 70% of neutrophils express this receptor. Next, we evaluate if $\alpha 6$ integrin participates in NET generation induced by laminins isoforms and we observed that pretreated of neutrophils with anti- $\alpha 6$ integrin antibody (GOH3) decreased 32% and 35% NETs release after interaction with laminins 411 and 511 respectively, compared with neutrophils stimulated only with these laminins. We characterize that the mechanism of NETs release induced by laminins 411 and 511 is dependent neutrophil elastase and peptidylarginine deiminase 4 (PAD4). Interestingly, NET release by laminin 511, but not by 411 is dependent on ROS generation. Finally, we evidenced that $\alpha 1$, $\alpha 4$ and $\alpha 5$ laminin chains colocalize with NETs induced by La, and that laminins 411 and 511 modulate NETs induced by La.

We thank the Hemotherapy Service of Hospital Universitário Clementino Fraga Filho, UFRJ Supported by FAPERJ, CAPES and CNPq.

Keywords:Neutrophil extracellular traps; laminin; leishmania amazonensis

HP108 - EFFECTS OF INHIBITION OF MIGRATION OF MACROFAGES (MIF) IN MIGRATION OF EXTRAVILLOUS TROPHOBLASTIC CELLS (HTR-8/SVNEO) AND IN INTRACELULAR PROLIFERATION OF TOXOPLASMA GONDII

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The process of migration and invasion of extravillous trophoblastic cells in maternal tissues play a critical role for success of embryo implantation. These cells release MIF and other important cytokines for regulation of immunological response. MIF is involved in pathogenesis of disease caused by some protozoa, including *Toxoplasma gondii*. Then, the aim of this study was evaluate the MIF functional role on mechanisms of migration and invasion of extravillous trophoblastic cells (HTR8/SVneo) infected by *T. gondii*. For this purpose, HTR8/SVneo cells were infected and treated with MIF or MIF inhibitor (ISO-1). Therefore, experimental groups were: control (uninfected and untreated cells); MIF (uninfected cells and treated with MIF); ISO-1 (uninfected cells and treated with ISO-1); Tg (infected and untreated cells); Tg+MIF (infected cells and treated with MIF); Tg+ISO-1 (infected cells and treated with ISO-1). In other, to verify the cell viability by MTT, *T. gondii* intracellular proliferation by beta-galactosidase, and migration assay (Scratch), HTR8/SVneo cells were infected with RH strain (2F1) tachyzoites of *T. gondii* and treated with MIF or ISO-1. Our results show that cellular viability were not changed in infected and/or treated with MIF or ISO-1. Cells treated with MIF demonstrated high parasite burden, when compared to control. In relation to cellular migration assay, cells treated with ISO-1 migrated less than uninfected cells or cells treated with MIF. When cells were infected, there was less migration in cells treated with MIF (Tg+MIF) or ISO-1 (Tg+ISO-1) in relation to control. We also observed less migration of infected cells and treated with MIF (Tg+MIF), when compared to infected cells (Tg). In conclusion, treatments with MIF or ISO-1 did not change cell viability, MIF treatment increased *T. gondii* intracellular proliferation and the MIF absence decrease cells migration. **Supported by:**FAPEMIG, CAPES e CNPq **Keywords:**Mif ; toxoplasma gondii; cellular migration

HP109 - IN VITRO EVALUATION OF THE EUPHORBIA TIRUCALLI (EUTIRUCALLIN) AND SYNADENIUM CARINATUM (SCLL) LATEX LECTINS AND AQUEOUS EXTRACTS ON TOXOPLASMA GONDII INFECTION.

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Toxoplasma gondii is a cosmopolitan intracellular protozoan that has been found to infect all warm-blooded animals tested, including humans. The present study investigated the *Euphorbia tirucalli* and *Synadenium carinatum* aqueous extracts and lectins effect on *Toxoplasma gondii* infection. Firstly, aqueous extracts were obtained from the latex of the *E. tirucalli* and *S. carinatum* and they were purified on immobilised D-galactose-agarose to obtain lectins. *E. tirucalli* and *S. carinatum* lectins exhibited two subunits with apparent molecular weights of 32 and 64 kDa for Eutirucallin and 28 and 30 kDa for ScLL. Additionally, the cytotoxicity effect was observed by Trypan blue assay for aqueous extracts. Anti-*Toxoplasma* activity as infection and replication of parasites against Human Foreskin Fibroblast (HFF) cells, was tested using parasites pre-treated with fractions and extracts and was more than 50% using both *E. tirucalli* and *S. carinatum* products. In that sense, this study demonstrated that *E. tirucalli* and *S. carinatum* lectins can be effective for treatment and control of *Toxoplasma gondii* infection.

Supported by: CAPES, FAPEMIG, CNPQ

Keywords: *Euphorbia tirucalli* - *synadenium carinatum* ; lectins; *toxoplasma gondii*

HP110 - LEISHMANIA TARENTOLAE AS A PLATFORM FOR THE STUDY OF GENES POSSIBLE REQUIRED FOR LEISHMANIA VISCERAL INFECTION

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Parasites of the genus *Leishmania* cause a variety of human diseases that range from self-healing cutaneous lesions to visceral infections that could result in death. Although host health status and genetic background can influence the outcome of infection the major factor that determines the tropism and pathology of *Leishmania* infection is the species of *Leishmania*. However, the *Leishmania* genes and mechanisms responsible for these different pathologies are not known. In this regard, we transfected separately two different *L. infantum* genes in *L. tarentolae*, a parasite that are nonpathogenic to humans. These recombinant parasites were analyzed by PCR to confirm the integration of the genes in the genome of the parasite and the expression of the genes in *L. tarentolae* were confirmed by Western blot and RT-PCR. As a control we generated a third recombinant parasite transfected with the plasmid vector with no gene inside (*L. tarentolae*-pLEXSYØ). These parasites are then been used in in vitro analysis to access the impact of the expression of these genes in the biology of the parasite. Transfection and expression of the two genes were confirmed, indicating the presence of transcripts and protein adequately. The analysis of the growing curves suggests that the growth fitness of the transfected *Leishmania* strains were not significantly altered. Ongoing experiments were also designed to access if the transgene expression modified the other aspects of the parasite biology such macrophage infections and ability to visceralize in a mouse model of infection.

Supported by: FAPEMIG, CNPq, CAPES

Keywords: Leishmaniasis; *leishmania tarentolae*; visceral infection

HP111 - POLYMORPHISM EVIDENCE IN *PLASMODIUM (HAEMAMOEBIA) LUTZI* (LUCENA, 1939) (APICOMPLEXA, HAEMOSPORIDA) ISOLATED FROM BRAZILIAN WILD BIRDS
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The aim of this study was to characterize the species *Plasmodium (Haemamoeba) lutzi* found in some birds (one *Arremon semitorquatus*, two *Turdus leucomelas* and three *Turdus rufiventris*) of Atlantic Forest of Minas Gerais. Blood smears were analyzed under light microscopy for morphological characterization; and the mitochondrial cytochrome b gene (cyt b) were sequenced for molecular characterization. Morphological variations were observed between the found parasite and its original description, such as: the absence of the "ring" stage and small vacuoles in trophozoites; absence of meronts and gametocytes in polychromatic erythrocytes; larger size pigments granules in meronts and gametocytes (> 0.5 µm) and the presence of small vacuoles between pigment accumulation in fully grown macrogametocytes. The cyt b of two isolates were sequenced in this work, ASJB433, from *A. semitorquatus* and TRJB16, from *T. rufiventris*. These sequences emerged in a monophyletic clade with previously characterized *P. lutzi* sequences isolated from *Turdus fuscater* in Colombia and several other bird species from Peru; corroborating our morphological identification. Moreover, as noticed for morphological features, a certain degree of the genetic diversity was also observed across the different lineages of *P. lutzi* indicating the existence of polymorphisms within this taxon, which is not common in Haemosporida. These results allow discussions about borders for species delimitation within avian hemosporidians and highlight the importance of multidisciplinary approaches for a more efficient species identification. **Supported by:** CAPES; CNPq
Keywords: Plasmodium; polymorphism; avian malaria

HP112 - EVOLUTIONARY ANALYSIS AND FUNCTIONAL CHARACTERIZATION OF PROSTAGLANDIN F_{2α} SYNTHASES FROM *TRYPANOSOMA CRUZI*
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Recently, it was determined that trypanosomatids are capable of synthesizing prostaglandins, which raised questions about their role during parasitic infections. This work aims to study the relevance of prostaglandin F_{2α} (PGF_{2α}) synthases in the establishment of *Trypanosoma cruzi* infection. The vast majority of characterized PGF_{2α} synthases belong to the aldo-keto reductase (AKR) protein family. Interestingly, in *T. cruzi* the PGF_{2α} production is catalyzed by TcOYE which belongs to the Old Yellow Enzyme family. This enzyme does not present homologs in mammals and other trypanosomatids. Moreover, there is a protein belonging to the AKR family (TcAKR) whose PGF_{2α} synthase activity could not be determined in vitro and its biological function is unknown. In this sense, we performed an evolutionary and functional comparison of TcAKR and TcOYE to clarify the differences between them and whether they are essential for infectivity.

Since TcOYE presents structural and sequence homology with bacterial OYEs, we propose that it may be acquired by horizontal transfer. The expression during the life cycle and the expression in strains with different virulence was analyzed by Western blot. They are expressed throughout the whole life cycle and surprisingly they exhibit higher expression in the most virulent strains. Infection assays of HeLa cells with *T. cruzi* over-expressing TcOYE and TcAKR evidenced that over-expression of TcAKR enhances *T. cruzi* infectivity, suggesting an important role in mammalian host cell infection. In conclusion, our results indicated that TcOYE and TcAKR have different evolutionary histories but both seem to be involved in *T. cruzi* infection.

Keywords: Prostaglandin f_{2α} synthases; trypanosoma cruzi

HP113 - **BEHAVIOUR OF TWO LEISHMANIA INFANTUM STRAINS IN EXPERIMENTALLY INFECTED BALB/C MICE**

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Leishmania infantum is the agente responsible for causing the zoonotic visceral leishmaniasis (VL). This neglected disease is severe and fatal if untreated. The species of the parasite, immunity, genetic characteristics and status of the host play a crucial role in the development of different clinical manifestations of the disease. Knowledge of *Leishmania* infectivity is essential for understanding how the contact between the pathogen end host cells can lead to pathogenesis. Different *L.infantum* strains may have different behaviors in infected animals and infect the target organs in different ways. In this respect, a comparative study was performed using BALB/c mice infected with two *L. infantum* strains, MHOM/BR/1972/BH46, isolated from a human case, and MCAM/BR/2008/OP46, isolated from a symptomatic infected dog and a non-infected control group. Thus, in this work, the parasite burden was assessed in liver, spleen and bone marrow using the serial limiting dilution technique. Liver, spleen and mice weight was also assessed and compared between the animals groups. Moreover, biochemical analyses using serum of mice were performed to quantify level of glucose, triglyceride and cholesterol and its fractions, HDL and LDL. The results showed a substantial difference between parasite burden in spleen and bone marrow in the animals infected with *L. infantum* OP46 strain compared to BH46 strain. Furthermore, an increase in spleen weight was observed in the animals infected with OP46 strain compared to non-infected control group. However the infection both strains did not change the biochemical analysis. These data show a different pattern of infection between the two strains used for the study. Additional studies are in progress to evaluate molecular factors that may be associated with infection in mice. **Supported by:** CAPES/FAPEMIG/UFOP

Keywords: Bh46; op46; parasite quantification

HP114 - **THE ROLE OF ANNEXIN A1 IN THE CONTEXT OF LEISHMANIA AMAZONENSIS INFECTION**

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Annexin A1 (AnxA1, a 37-kDa calcium-dependent phospholipid-binding protein) has important roles in the modulation of inflammatory responses. However, there are no investigations on its role in *Leishmania amazonensis* in infections, which may provide new insights for the development of effective therapies against leishmaniasis. This study has evaluated the differences in the inflammatory responses' profiles of BALB/c wild type (WT) and AnxA1 Knockout (KO) mice infected by *L. amazonensis*. Animals were infected with parasites in the stationary promastigote phase and the course of infection was evaluated weekly. Cytokine levels (IL-4 and IL-10) were assessed in lymph node cells and tissue damage was evaluated by histological analysis. Parasite loads were evaluated by a limiting dilution assay. The WT and KO mice cell infection were analyzed by in vitro assays utilizing bone marrow-derived macrophages (BMDM), and the rate of metacyclogenesis was evaluated by Ficoll density gradient. The results showed that after eight weeks of infection the footpad lesions were significantly larger in the KO group and during the first five weeks of infection, IL-4 and IL-10 cytokine levels were higher in the KO animals and histological analysis demonstrated an increased damage to the epidermis of Anx- / - animals, with the presence of a mixed inflammatory cell infiltrate, even though the parasite load was the same for both groups. During the first 24 hours of infection, the in vitro assays showed an increased number of infected BMDM in KO mice cells, as well as a higher number of parasites per cell, when compared to WT group. These preliminary results indicate that in vitro Anx- / - BMDM are more susceptible to infection. Already In vivo, the lack of AnxA1 results in more intense inflammatory responses, which are not controlled by increasing IL-4 and IL-10 levels. Thus, AnxA1 seems to contribute for the control of inflammatory responses during infection with *L. amazonensis*.

Supported by: CNPq, CAPES, FAPEMIG

Keywords: Leishmania amazonensis; inflammation; annexin a1

HP115 - JUNCTIONAL COMMUNICATION IN THE IMMUNE SYSTEM: MODULATION OF THE GAP JUNCTION BY INFECTION WITH *TRYPANOSOMA CRUZI* AND INFLAMMATION CONDICTION.

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Trypanosoma cruzi is the causative agent of Chagas' disease that affects 6 million people in BRA, 14 million in Latin America, keeping at risk of 100 million people. In Chagas' disease, various physiological systems undergo changes and of these complications are associated with functional impairment of the gap junction. However, these junctions are not fully characterized in some systems. Among these, we can highlight the immune system, and particularly the macrophages that participate in the innate response process. Morphological and functional characterization of gap junctions in macrophages has been the subject of study of various groups. However their regulatory mechanisms still deserve clarification, mainly before pathological changes, such as in infectious and inflammatory processes caused by *T. cruzi*. Thus, the main objective of this study is to evaluate the structural and functional modulation of gap junctions formed by Connexin43 in macrophage lines J774-G8 and peritoneal macrophages following activation with pro-inflammatory immune factors and infection with *T. cruzi*. tests will be performed: (1) Immunoelectrophoresis (Western Blot); (2) Immunofluorescence; and (3) Intracellular dye microinjection (functional assessment of gap junctions) in normal cells, activated with factors (lipopolysaccharide (LPS), tumor necrosis factor- α (TNF- α) and Interferon- γ (IFN- γ)), and infected with the parasite in his trypomastigote form (strain Y). Preliminaries results showed that J774-G8 macrophage cell line present significant changes in their intercellular communication profile by gap junctions, when submitted to the microenvironment stimulated with pro-inflammatory immune factors (LPS + IFN- γ and IFN- γ + TNF- α) in incubations 48 hours. Experiments demonstrated a significantly increased of communication mediated by gap junctions. **Supported by:**FAPERJ, CNPq and CAPES.

Keywords:Gap junction; macrophages; trypanosoma cruzi

HP116 - MICE UNDER DEFICIENT DIET IN VITAMIN D PROMOTES INCREASE OF THE RESISTANCE TO *L. AMAZONENSIS* INFECTION THROUGH INCREASE OF THE TCD4+ IFN-GAMMA+ CELLS

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Leishmaniasis comprises a group of neglected diseases, which may have different clinical manifestations; may in some cases lead to death. A science that has taken prominence is Immunonutrition, where we can see the evaluation of pharmacological benefits and role in immunity that some nutrients in food can bring. It is known that vitamin D is a key hormone to various physiological activities and tests in vitro have suggested that this vitamin have an inhibitory effect on the cells of the immune system. Thus, the objective of this study is to evaluate the development of cutaneous leishmaniasis in mice under diet deficient in vitamin D. In our study, we used C57BL/6 and BALB/c mice, where a group maintained a normal diet without any nutritional restriction and another group underwent particular diet with vitamin D deficient diet for a period of 45 days before the infection, and then, these animals were challenged with 2.0×10^5 promastigotes of *L. amazonensis* Josefa strain. Until the end of the experiment, mice were submitted to normal or deficient diet in vitamin D. The development of lesion was assessed by pachymetry and the parasite load of the infected footpad and the spleens were determined at 2 months for BALB/c mice and at 3 months for C57BL/6 mice of infection by limiting dilution. In addition, flow cytometry was performed in the end of the experiment. Mice (C57BL/6 or BALB/c) under deficient diet in Vitamin D displayed smaller lesions in comparison to mice under normal diet. When evaluated the parasite load in infected footpad, there was no statistical difference between the groups. However, when analyzing the cytometry data, it was observed that the percentage of TCD4+ lymphocytes, TCD4+IFN- γ + lymphocytes was significantly higher in animals under diet deficient in vitamin D. All together, Vitamin D contributes for host susceptibility to murine cutaneous leishmaniasis. **Supported by:**FAPERJ

Keywords:Leishmaniasis; immunonutrition; diet deficient in vitamin d

HP117 - **THE ROLE OF EOSINOPHILS IN THE CONTEXT OF *LEISHMANIA BRASILIENSIS* INFECTION**

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Eosinophils are recruited to the site of injury in different models of parasite infection, including Leishmania. In agreement, we found that eosinophils were present in increased numbers of in the inflammatory infiltrate induced by infection with *Leishmania Brasiliensis* in BALB/c mice, which is a naturally resistant model to this species infection. To better understand the role of those immune cells, injury and inflammatory responses in *L. Brasiliensis*-infected BALB/c wild type (WT) and GATA-1 knockout (KO) mice were comparatively evaluated. Animals were infected with parasites in the stationary promastigote phase and the course of infection was evaluated weekly. The rate of metacyclogenesis was evaluated by Ficoll-Paque density gradient and flow cytometry. Parasite loads were evaluated by a limiting dilution assay. Cytokine levels (IL-4 and IL-10) were assessed in lymph node cells and tissue damage was evaluated by histological analysis. The results demonstrated that, in the first week of infection, KO mice had on average bigger footpad lesions, even though the parasite load was similar for both groups. The percentage of metacyclics was very low (0.6-1.1%) in the cultures used to infect mice. In the second week of infection, there was a significant difference in the levels of IL-4 and IL-10 between WT and KO mice, which were higher in the GATA1 KO mice. A similar score for skin inflammatory responses were found between the groups upon histological analysis. These data suggest that lack of eosinophils may not disturb significantly the course of infection in *L. Brasiliensis*-infected BALB/c and that its potential role in anti-inflammatory responses may be compensated by other cells and mechanisms, by increasing IL-4 and IL-10 cytokines levels. Nonetheless, these are preliminary results and additional studies may help to better understand the mechanisms involved in the modulation of the inflammatory process and the role of eosinophils in the context of leishmaniasis. **Supported by:**CNPq, CAPES, FAPEMIG
Keywords: leishmania Brasiliensis; eosinophils and leishmaniasis; inflammation response

HP118 - **COMBINATION THERAPIES WITH ENALAPRIL AND BENZNIDAZOLE REDUCES HEART INFLAMMATION IN THE EXPERIMENTAL INFECTION WITH *TRYPANOSOMA CRUZI***

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Trypanosoma cruzi infection triggers an inflammatory process capable to develop functional and morphometrical alterations in cardiac tissue. In an attempt to reduce this cardiac alteration, several pharmacological therapies (eg. inhibitors of angiotensin converting enzyme - ACE) have been proposed due their potential anti-inflammatory effects. In this study, we evaluated treatment with Enalapril (ACE inhibitor) and Benznidazole (Bz) in a single or in a combinatory therapies (CT) during acute and chronic phases of experimental T.cruzi infection. C57BL/6 mice were infected with VL-10 strain of T.cruzi and treated during 20 days with different dosages of Enalapril (15, 20, 25mg/kg), Bz (60, 80, 100mg/kg) and combinations of both (15+60;20+80;25+100mg/Kg). Serum and heart samples were processed to immunoassay (TNF, IL-10, CCL2/5), enzymatic activities of metalloproteinases (MMP-2/-9) and histopathology at the 30^o and 120o days post infection. We observed that CTs reduced plasma levels of TNF, IL-10, CCL2 and CCL5 in the acute phase of infection and, this same profile was maintained in the chronic one. CT does not alter the activity of MMP-2 and -9 in both phases of infection, exception to the MMP2 whose activity was elevated with the 100 + 25mg/kg therapy during the chronic phase. In addition, CT has reduced the inflammatory infiltration in the cardiac tissue in acute and chronic phases been effective in reducing the collagen's neogenesis in the latter phase of infection. In conclusion, we assume that treatment in combination using Enalapril and Bz may regulate the inflammatory process suggesting a potential protective effect against cardiac damages caused by experimental *T. cruzi* infection.
Supported by:UFOP, CNPq, FAPEMIG, CAPES
Keywords:Trypanosoma cruzi; inflammation; ace inhibitor

**HP119 - A ROLE FOR THE GUANINE EXCHANGE FACTOR RASGEF1B IN
CARDIOMYOCYTES DURING T. CRUZI INFECTION IN MICE**

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Chronic myocarditis is a major cause of morbidity among chagasic patients. During cardiac inflammation elicited by the parasite, different cell types such as macrophages and lymphocytes are responsible for the host response to *T. cruzi* infection by producing inflammatory mediators. In addition, cardiomyocytes are also capable of expressing inflammatory mediators but also intracellular molecules that can regulate parasite load in the heart. However, it remains to be fully elucidated how molecules that are expressed by cardiomyocytes participate in the host response to *T. cruzi*. RasGEF1b is a Toll-like receptor (TLR)-inducible Ras guanine exchange factor whose expression is induced in the heart of *T. cruzi* infected mice. To examine the role of RasGEF1b in the heart during experimental Chagas disease, we have generated cardiomyocyte-specific conditional deletion of Rasgef1b in mice by breeding Rasgef1bFlox/Flox mice with Myh6Cre+/Cre- mice. Gene targeted recombination in cardiomyocytes was confirmed by RT-qPCR analysis that revealed a significant reduction of steady-state mRNA levels of Rasgef1b. Mice are fertile and viable, and show no gross abnormalities. Analysis of cardiac parameters in the hearts from healthy cardiomyocyte-specific conditional Rasgef1b knockout mice revealed decreased stroke volume, cardiac output, LV mass and cardiomyocytes diameter. To evaluate parasitemia and survival upon infection with *T. cruzi*, wild-type and mutant mice were intraperitoneally infected with 100 blood-trypomastigote forms of the Y strain. The survival percentage of mutant mice was as similar as to wild-type mice. However, parasitemia was three-day delayed and significantly increased in the mutant mice compared with the wild-type. This result indicates that RasGEF1b in cardiomyocytes may play a dual role in the setting of Chagas disease by early contributing but later controlling the parasitemia in mice.

Supported by: CAPES, FAPEMIG, INCTV

Keywords: Rasgef1b; cardiomiopathy; trypanosoma cruzi

**HP120 - THE EXPRESSION AND PRODUCTION OF CARDIAC ANGIOGENIC MEDIATORS
DEPEND ON THE *TRYPANOSOMA CRUZI*-GENETIC POPULATION IN EXPERIMENTAL OF
C57BL/6 MICE INFECTION**

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Mammalian cardiac cells are important targets to the protozoan *Trypanosoma cruzi*. The inflammatory reaction in the host aims at eliminating this parasite, which can lead to cell destruction, fibrosis and hypoxia. Local hypoxia is a well-defined stimulus to the production of angiogenesis mediators. Assuming that different genetic *T. cruzi* populations induce distinct inflammation and disease patterns, the current study aims to investigate whether the production of inflammatory and angiogenic mediators is a parasite strain-dependent condition. The C57BL/6 mice were infected with the Y and Colombian strains of *T. cruzi* and euthanized at the 12th and 32nd days, respectively. The blood and heart tissue were processed in immune assays and/or qPCR (TNF, IL-17, IL-10, CCL2, CCL3, CCL5, CCR2, CCR5 and angiogenic factors VEGF, Ang-1, Ang-2) and in histological assays. The *T. cruzi* increased the inflammatory and angiogenic mediators in the infected mice when they were compared to non-infected animals. However, the Colombian strain has led to higher (i) leukocyte infiltration, (ii) cardiac TNF and CCL5 production/expression, (iii) cardiac tissue parasitism, and to higher (iv) ratio between heart/body weights. On the other hand, the Colombian strain has caused lower production and expression VEGF, Ang-1 and Ang-2, when it was compared to the Y strain of the parasite. In summary, the present study highlights that the *T. cruzi*-genetic population defines the pattern of angiogenic/inflammatory mediators in the heart tissue, and that it may contribute to the magnitude of the cardiac pathogenesis. Besides, such assumption opens windows to the understanding of the angiogenic mediator's role in association with the experimental *T. cruzi* infection. **Supported by:** UFOP, CNPq, FAPEMIG, CAPES

Keywords: Trypanosoma cruzi; angiogenesis; inflammation

HP121 - IMMUNOPATHOLOGICAL STUDY OF LAMININ, FIBRONECTIN AND TCD3+ CELLS OF LIVER IN NATURALLY INFECTED DOGS WITH VISCERAL LEISHMANIASIS

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Canine visceral leishmaniasis is a life-threatening vector-borne zoonotic disease caused by *Leishmania infantum*. In general, an intense chronic inflammatory reaction is observed in liver. Chronic inflammation, a common consequence of parasitic infections, is a potent promoter of extracellular matrix (ECM) formation. Laminin (LN) and fibronectin (FN) are ECM components that are important in the development and maintenance of cellular organization. Studies with *Leishmania* spp. have provided strong evidence that the parasite uses host FN and LN to bridge their association with host monocytes and macrophages. The aim of this work was to study alterations in LN, FN and TCD3+ in liver from naturally infected dogs and correlate with symptomatology and parasitological findings. Liver fragments were collected for the parasitic load quantification by qPCR. Frozen sections were stained by immunohistochemistry for FN (n=38), LN (n=35) and TCD3+ (n=11). The percentual area occupied by the stained LN and FN was measured using ImageJ. TCD3+ cells were counted in granulomas, portal space and hepatic parenchyma. FN deposition was significantly greater in the polisymptomatic (n=15) than asymptomatic (n=13) animals (p=0.048). No significantly correlation between LN and symptomatology or parasite load was observed. There was a positive correlation between the parasite load and FN deposition (p=0.053 r=0.330). Six dogs with low parasite load had number TCD3+/granuloma (Median=22.7%) greater than the dogs with high parasite load (Median=9.4%). Inverse correlation between parasite load and TCD3+/granuloma (p=0.018 and r²= -0.842) was observed. Our work corroborates the studies that report canine visceral leishmaniasis is a cause of fibrogenesis in liver. High levels FN expression associated with parasite load in symptomatic animal reflects the inflammatory and degenerative processes. The number of granuloma TCD3+ cells appears not accompanied the increasing of parasite load.

Supported by: Auxílio Instalação-Faperj; Universal - CNPq; PAEF-Fiocruz; CAPES; IOC-FIOCRUZ
Keywords: Canine visceral leishmaniasis; liver; pathology

HP122 - DOWN MODULATION OF ARGINASE 1 ACTIVITY AND GENE EXPRESSION OF INFECTED MACROPHAGES DEPENDS ON THE *TOXOPLASMA GONDII* STRAIN

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Toxoplasma gondii is an obligate intracellular parasite that can infect virtually any nucleated cells, including macrophages. Macrophages are important cells of the immune system and control parasite replication. However, along its evolutionary history, *T. gondii* developed many evasion mechanisms from the immune system such as increased expression of arginase 1 (ARG1) in infected macrophages. ARG1 is important for the synthesis of polyamine needed for parasite cell division and due to its cationic nature may be involved in the control of free radicals such as nitric oxide. However, the role of ARG1 in macrophages infected with different strains of *T. gondii* is still controversial, and may also depend on the infected macrophage lineage. The aim of this work was to evaluate the gene expression and activity of ARG1 in macrophages infected with different strains of *T. gondii*. Peritoneal macrophages of C57BL/6 mice cultivated with DMEM supplemented with FBS and activated for 24h with LPS and IFN- γ were infected with *T. gondii* of either the RH or ME-49 strains. ARG1 activity was measured based on α -isonitrosopropiophenon reaction and RT-PCR was used to determine mRNA expression of ARG1. Activated macrophages infected with the RH or the ME-49 strain of *T. gondii* exhibited less or similar ARG1 activity, respectively, when compared with uninfected macrophages. Gene expression analysis by RT-PCR of macrophages infected with the RH strain of *T. gondii* indicated a lower ARG1 mRNA expression when compared with uninfected macrophages. Curiously, macrophages infected with the ME-49 strain of *T. gondii* presented an increased ARG1 mRNA expression in 6 and 12h of infection when compared with uninfected macrophages. These results better demonstrates the evasion mechanisms used by *T. gondii* to persist in classically activated macrophages and show strain heterogeneity. **Supported by:** UENF, CAPES, CNPq, FAPERJ

Keywords: *Toxoplasma gondii*; arginase 1; evasion mechanisms

HP123 - **FAILURE IN THE INCREASE OF PROTECTION BY ASSOCIATION OF LAAG WITH ADJUVANTS BY INTRANASAL ROUTE AGAINST L. AMAZONENSIS**
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Leishmaniasis is an infectious disease caused by parasites from genus *Leishmania* and transmitted by female of sandfly. Lesions in the host's cutaneous region is one of the clinical manifestations, which could increase in severe manifestations such as deformities in the skin. The existing therapy is associated with the use of chemotherapy, which is extremely toxic to the individuals and often refractory. Thus, it shows the need of a search for alternative therapies as vaccines. Adjuvants are substances added to vaccines to enhance or modulate the immunogenicity of the antigen present in the formulation. Our focus is to evaluate the ability of adjuvants (CAF01, CAF09 and CAF01 plus MLA) associated with total antigens of *Leishmania amazonensis* (LaAg) by intranasal route to improve immunogenic and protective responses against infection caused by *L. amazonensis*. The animals C57BL/6 were vaccinated twice by intranasal route prior to the infection. The animals were divided into groups treated with the respective vaccines (LaAg alone, LaAg+CAF01, LaAg+CAF09, LaAg+CAF01+MPLA) and control group (PBS). After, metacyclic promastigotes of the parasite were inoculated in the right footpad of mice. Initially, it performed a challenge with low parasitic load, which constitutes infection using 2×10^5 parasites. With the objective of analyzing the damage to the infected region was used a caliper to measure the size of the lesion. The parasitic load's determination was performed by the limiting dilution technique (LDA) with a ratio of 1: 50 in 96-well plates containing RPMI supplemented with hemin to as leg regions spleen and draining lymph node. At this stage, we observe that animals vaccinated with LaAg managed to solve the injury, while those who possessed an association with adjuvants was not an improvement compared to the LaAg. Subsequently, we performed a challenge using a high dose, with the largest number of parasitic load, which was 5×10^6 parasite. **Supported by:**FAPERJ and CNPQ
Keywords:Vaccine; parasitology; biotechnology

HP124 - **EFFECT OF SECOND GONOTROPHIC CYCLE ON LEISHMANIA MEXICANA DEVELOPMENT IN LUTZOMYIA LONGIPALPIS**
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Lutzomyia longipalpis is the main vector of Visceral Leishmaniasis (VL) in Latin America. Phlebotomine infection occurs during blood meal where insects ingest macrophages containing amastigote forms of *Leishmania* parasites. Despite several works studying different physiological and molecular mechanisms that are important to parasite infection in sandflies, there are no studies about these interactions during the second gonotrophic cycle. The most important reason for this limited number of works in this subject is the difficulty in obtaining engorged females after a second blood feeding. Our work has standardized conditions to carry out the second gonotrophic cycle and our results show that oviposition is an essential factor for the success of multiple feedings. Considering the important role of the second blood meal for leishmaniasis transmission, we have evaluated the impact of the second gonotrophic cycle on longevity, digestion, trypsin activity and *L. mexicana* development within the gut of *L. longipalpis* females. Our results indicate that blood feeding increases the mortality of females after first and second feedings comparing with sugar feeding only. There was no difference in protein ingestion between first and second blood feedings in infected or uninfected groups. However, the trypsin activity was lower during the second gonotrophic cycle. Hemocytometer counts showed that there was no difference in parasitic load at 3 and 6 days between the first and second blood meal. Finally, our results agree with previous works where light microscopy has shown a huge dilatation on cardia in infected flies. This is the first description of an optimized protocol for obtaining a massive number of sand fly females fed on a second blood meal and the first description of some biological and parasitological aspects on the second gonotrophic cycle of sand flies. **Supported by:**FIOCRUZ, CNPq, CAPES, FAPERJ, CNPq (Ciência sem Fronteiras), INCT-EM
Keywords:Lutzomyia longipalpis; second gonotrophic cycle; trypsin activity

HP125 - FLOW CITOMETRY ANALYSIS OF *IN VITRO* LEISHMANICIDAL AND CYTOTOXIC ACTIVITY OF SYNTHETIC DERIVATES OF ALDIMINES AND HANTZSCH ADDUCTS AGAINST *L. INFANTUM*

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Visceral leishmaniasis (VL) is a fatal infectious disease if non treated. Ninety percent of VL cases occur in India, Bangladesh, Sudan, Nepal, and BRA. The conventional drugs for treatment of VL have limitations, including unresponsiveness, relapse, high toxicity, parenteral administration lasting for long periods of time and lack of effectiveness in HIV-VL patients. There are also issues related with the crescent appearance of resistant parasites to all drugs available, making it essential the execution of drug screening studies for new leishmaniasis treatment alternatives. In this study we tested the leishmanicidal and cytotoxic activity of synthetic derivatives of Aldimines and Hantzsch Adducts (HA) against *L. infantum*. For this purpose, we synthesized 7 Aldimines derivatives and 5 HA derivatives and analyzed these compounds for cytotoxicity against DH82 canine macrophages. This cytotoxicity tests were performed with different concentrations of drugs, using MTT assay to calculate the IC50% (ug/mL) of each drug, using this specific concentration to perform a leishmanicidal assay after. The leishmanicidal activity of each drug against the amastigote form of *L. infantum* GFP OP46 strain was evaluated at 24, 48 and 72h after infection using flow cytometry. In the assay we compare the rate of infection in control groups (negative control: DH82 macropages plus *L. infantum* GFP OP46 strain; amphotericin control: DH82 macropages plus *L. infantum* GFP OP46 strain with amphotericin drug) with the different drug groups (DH82 macropages, *L. infantum* GFP OP46 strain and the respective IC50% of each drug). We observed that the Aldimines derivatives 3H7, 3H8 e 3H9 decrease infection rate in a similar manner to the amphotericin control group at 24, 48 and 72 hours after infection. These results demonstrated the *in vitro* potential of Aldimine derivatives as leishmanicidal drugs and open the perspective to study these derivatives using *in vivo*. **Supported by:**UFOP, FAPEMIG, CNPq, PPSUS/MS and DECIT/MS **Keywords:**Visceral leishmaniasis; leishmania infantum; drug screening

HP126 - IMMUNOPHENOTYPING OF PERIPHERAL BLOOD FROM MICE INFECTED WITH BERENICE-78 *TRYPANOSOMA CRUZI* STRAIN

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After controlling vectorial and transfusional infection of Chagas disease in BRA, the oral contamination became the main mechanism of transmission in different regions, and, paradoxically, there are few studies that address this route of infection. Based on this, the aim of this study was to evaluate the systemic immune response in Swiss mice orally (OR) or intraperitoneally (IR) infected with trypomastigotes metacyclic from Berenice-78 *Trypanosoma cruzi* strain. Thus, twenty four animals from each group were euthanized at 14, 21, 28, 35, 42 and 180 days after infection (DAI) and blood was collected for quantification of T and B lymphocytes and the parasitemia curve was analyzed over time. Infection by OR showed higher parasitemics levels, and the highest peak of parasitemia was 1.151.000 trypomastigotes/0.1 mL of blood. Conversely, infection with IR showed lower parasitemics levels, being the highest peak of parasitemia 956.825 trypomastigotes/0.1 mL of blood, both on 18th DAI. In the analysis of peripheral blood mononuclear cells an early reduction in the percentage of CD4⁺ T lymphocytes was observed on animals of OR group on day 28 after infection, whereas on animals of IR group this reduction occurred only on 35th DAI. On the other hand, it was observed an increase in CD8⁺ T lymphocytes in both routes of infection from day 21 after infection, however, on IR this increase persists up to 180 days after infection, whereas on OR on 35th DAI the percentage of CD8⁺ begins to decay. In relation to B lymphocytes, was observed a reduction in the percentage of that cell already on 28th DAI on OR, whereas in the IR that reduction only occurs on 42th DAI. Thus, it is observed that, regardless of the route of infection, CD8⁺ is the main cell to increase in peripheral blood, but with different profiles between the routes. Furthermore, these data suggest that oral infection has a distinct parasitological/immune response profile compared to the intraperitoneal route. **Supported by:**UFOP, CAPES, FAPEMIG **Keywords:**Chagas disease; oral infection; immunophenotyping

HP127 - SCREENING OF RECOMBINANT PROTEINS OF LEISHMANIA (LEISHMANIA) INFANTUM CHAGASI FOR THE SEROLOGICAL DIAGNOSIS OF CANINE VISCERAL LEISHMANIOSE

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The control of visceral leishmaniasis (VL) in BRA has proven ineffective and highly dependent on sensitive serologic tests for detection of infected dogs, the main reservoir for the transmission of parasites. However, the available diagnostic methods have important drawbacks, particularly in the identification of asymptomatic dogs that also transmit disease, and a high chance of cross reactivity. In this context, there is a need for more sensitive and specific diagnostic tests. This study evaluated the potential for serological diagnosis of canine VL (LVC), using an ELISA platform, of four recombinant proteins, named Cit, Cin, Cyclo, and Calk from *Leishmania (Leishmania) infantum chagasi*, which display mapped B-cell epitopes. Initially, the coding region the selected antigens have been cloned, expressed in *Escherichia coli* and purified on affinity column. Once purified and characterized by SDS PAGE and Western blot, these proteins were evaluated in ELISA. Sera from previously characterized dogs as infected (n = 30) and negative (n = 25) were employed. The evaluation of the proteins Cyt, Cin, Cyclo, and Calk resulted in general sensitivities of 90% for Cyt; 93% for Cin; 83% for Cyclo and 85% for Calk and specificities of 92%; 52%; 96% and 76%, respectively. Our findings suggest that the antigens tested have high potential for the serodiagnosis of CVL.

Supported by:Fapemig, CNPq, MS/DECIT, INCTV

Keywords:Visceral leishmaniasis; recombinant protein

HP128 - ORAL INFECTION WITH VL-10 *TRYPANOSOMA CRUZI* STRAIN IN MICE

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Even with the intense control of epidemiological surveillance and to the elimination of *Triatoma infestans*, considered the main transmission vector of Chagas disease in BRA, more than a thousand cases of the disease have been reported in the last 10 years. Of these, about 70% are related to oral infection, and yet, studies involving this route of infection are still scarce in the literature. Based on that, this study evaluated the systemic immune response in Swiss mice orally (OR) or intraperitoneally (IR) infected with trypomastigotes metacyclic from VL-10 *Trypanosoma cruzi* strain. Thus, mice were euthanized at 14, 21, and 28 days after infection (DAI) and blood was collected for quantification of T and B lymphocytes and the parasitemia over time was analyzed. Infection by OR showed higher parasitemics levels ($2,3 \times 10^6$ parasites/0,1mL of blood), presenting the maximum peak of parasitemia at day 17 after infection. Beyond that, infection with IR showed lower parasitemics levels, being the highest peak of parasitemia was $2,17 \times 10^6$ parasites/0.1 mL of blood, also at 17th DAI. In the analysis of peripheral blood mononuclear cells it was observed an increase in CD8⁺ T lymphocytes in both routes of infection from day 21 after infection. In relation to B lymphocytes, was observed a reduction in the percentage of that cell on 21th DAI on IR, whereas on OR it was not observed variation. Therefore, it is suggested that the increase observed of CD8⁺ T lymphocytes in the peripheral blood of infected mice started soon after the parasitic burst in order to try to combat intracellular parasite, regardless of the route of infection.

Supported by:UFOP, CAPES, FAPEMIG

Keywords:Chagas disease; oral infection; vl-10

HP129 - δ -AMASTINS: A SURFACE TETRASPANIN-PROTEIN AFFECTING CELL DIFFERENTIATION OF TRYPANOSOMA CRUZI

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A better understanding of the molecular mechanisms of host cell invasion by *T.cruzi* is critically for development of novel means for molecular intervention. Delta-amastins are surface proteins encoded by multigene families and highly expressed in the intracellular amastigote stages of *T.cruzi*. In this study, we investigated the role of δ -amastin in parasite biology. In order to determine δ -amastin function, we have accomplished transcriptomic profile, protein-protein interaction assay, and phenotypic analysis. Initially, full-length δ -amastin (AF), and mutated forms 5TMF (five Thr replaced by Ala) and 1CMF (C41 to Ala41) were overexpressed fused to GFP in *T.cruzi*. Confocal microscopy images exhibited a similar plasma membrane localization for AF::GFP and 1CMF::GFP, however, parasites expressing 5TMF::GFP showed plasma membrane, cytoplasm, and perinuclear localizations. In spite of phenotypic assays showed that ectopic expression of both wild and mutant amastins does not interfere with extracellular amastigote (EA) infectivity, AF::GFP expression favored epimastigote (EPI) to metacyclic trypomastigote (MT) differentiation and reduced the amastigogenesis rate. Additionally, parasites expressing 5TMF::GFP show stronger reduction on EPI to MT differentiation, and acceleration on TCT to EA. Another remarkable difference of WT, AF::GFP, and 5TMF::GFP parasites was the TCT invasion capacity, where 5TMF::GFP showed a pronounced invasion capacity compared to WT, and AF::GFP (lowest invasion rate). Preliminary results of protein-protein interactions using BioID approach and transcriptomic analyzes (WT versus AF::GFP) suggest that δ -amastins is somehow associated with differentially expressed proteins during its life cycle. These results must be refined to have a better view towards amastin function. **Supported by:** CAPES; CNPq; Fundação Araucária **Keywords:** Delta-amastin; amastigote; surface protein

HP130 - ANALYSIS OF THE VIRULENCE VARIATION AMONG TRYPANOSOMA CRUZI STRAINS, AFTER THE PASSAGE IN VERTEBRATES AND INVERTEBRATES HOSTS.

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Chagas disease is caused by the Protozoan *Trypanosoma cruzi*, which presents a heteroxen cycle: the hosts are triatomine bugs (invertebrate) and mammals (vertebrate). The common infective form to vertebrates is the infective metacyclic trypomastigote form (IMT), eliminated in the triatomine bug faeces. Although studies demonstrating a significant increase in the virulence and pathogenicity of the parasite after passage in the vector, little is known about this process. This work studied the interaction between the parasite and the vertebrate and invertebrate hosts. Experimentally, *Rhodnius prolixus* was utilized as a vector and Swiss mice as vertebrate host. Swiss mice were divided into groups (I, II and Negative Controls). In Group I, 40 Swiss were infected with Trypomastigote Cell Culture (Colombiana and Y). After 20 days of infection, the animals of this Group were anesthetized and used for feeding of Triatomine bugs. Triatomine bugs were dissected after 30 days when the gastrointestinal tract was macerated to obtain a parasitic pool, which was used for the mice infection from Group II. Group II consisted of 2 subgroups, 20 animals infected with Colombian strain and 20 with Y strain. The three subgroups were inoculated with saline, macerated gastrointestinal tract and saliva of the vector. The confirmation of infection was performed by monitoring the parasitaemia using the Brenner's method. After the comparison of the different curves, Colombian strain demonstrated the highest patent period, confirming that this strain is more pathogenic and virulent than Y strain. On the other hand, Y strain presented an earlier peak of parasitaemia (12 days). After passing by the invertebrate host, the patent period was expected to last longer than before, but this result was not observed due to the death of the animals, but that also allows us to infer that parasites isolated from *R. prolixus* became more virulent than those from tissue cell culture. **Supported by:** CAPES, CNPq, FAPEMIG **Keywords:** Chagas; triatomine bug; infective metacyclic trypomastigote

HP131 - **IMPACT OF MESENCHYMAL STEM CELLS (MSC) THERAPY IN LEISHMANIA SSP. INFECTION**

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Cell therapy aims at restoring tissue structure and functionality through the use of a cell. For leishmaniasis, available chemotherapy is inadequate as there are side effects. In this study, we evaluated the potential application of mesenchymal stem cells (MSC) treatment against murine cutaneous leishmaniasis. In vitro, co-culture of infected macrophages with MSC increased parasite load on macrophages in comparison to controls (macrophage without MSC). In vivo, BALB/c mice were infected with 2x10⁶ *L. amazonensis* (strain Josefa) promastigotes in the footpad of the right paw. Seven days after infection, animals were treated with 1x10⁵ MSCs, either intralesional (i.l.), in the same site of infection, or intravenously (i.v.), through the external jugular vein. Control received the same volume (50 µl) of PBS by routes i.l. or i.v. Thirty days after the first treatment, animals received a second administration of MSC. The clinical profile of the disease was checked by lesion development through the thickness measured by pachymetry. Forty-two days after infection, animals were euthanized. Parasite burden was quantified by limiting dilution technique (LDA). Lymph node and spleen cells were phenotyped by flow cytometry. Intravenous treatment with MSC resulted in a small worsening of the injury. The animals treated with MSC i.v. presented a significant difference in parasite load in comparison with your controls and, the cellular profile analysis of lymph nodes showed an increase in effector and regulatory T cells in animals. Increased number of regulatory T cell and IL-10 producing T CD4+ and TCD8+ cells in the spleen, and upregulation of IL-10 in the footpad. The excessive production of IL-10 could be associated to the disease-aggravating effects of MSC therapy by the i.v. route. On the other hand, it was not observed any harmful effect on i.l. treatment. Our results showed that stem cells do not contribute to the infection control in cutaneous leishmaniasis. **Supported by:**FAPERJ, CAPES, CNPQ

Keywords:Leishmania amazonensis; mesenchymal stem cell; cell therapy

HP132 - **ANTILEISHMANIAL EFFECT AND CYTOTOXIC ACTIVITY OF ESSENTIAL OILS ON *L. BRASILIENSIS***

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Among the plant-derived molecules described as potential antiparasitic drugs, the essential oils (EOs) stand out. The objective of this study was to evaluate the antileishmanial effect on *Leishmania Brasiliensis*, and cytotoxicity on mammalian cells of four EOs together with their chemical characterization. EOs of *Myroxylon peruiferum*, *Bulnesia sarmientoi*, *Salvia sclarea*, and *Melissa officinalis* were purchased from QUINARI® Company. Chemical characterization was performed by gas chromatography-mass spectrometry (GC-MS). In vitro activity tests were conducted on *L. Brasiliensis* promastigotes (antileishmanial) and L6 cells (cytotoxicity), both assessed by fluorometric method (Resazurin). The GC-MS of EOs identified α-copaene (13.4%) and guaiol (9.3%) for *M. peruiferum* as majority compounds; linalool (28.8%) and linalyl acetate (60.1%) for *S. sclarea*; guaiol (48.3%) and 2-undecanone (19.5%) for *B. sarmientoi* and neral (37.2%), and geranial (5.5%) for *M. officinalis*. For promastigotes, all tested EOs showed antileishmanial effect (*B. sarmientoi* IC₅₀/24h = 196.9 µg/mL; *M. peruiferum* 210.5 µg/mL; *M. officinale* 212.3 µg/mL and *S. sclarea* 338.0 µg/mL). The ascending order of cytotoxicity was *S. sclarea* (CC₅₀/24h = 375.4µg/mL), *M. officinalis* (297.5 µg/mL), *B. sarmientoi* (163.5 µg/mL) e *M. peruiferum* (160. 8 µg/mL). Cytotoxicity and antileishmanial activity were compared using the selectivity index (SI), and the values were 1.40 (*M. officinalis*), 1.11 (*S. sclarea*), 0.83 (*B. sarmientoi*) and 0.76 (*M. peruiferum*). These results indicate that *M. officinalis* EO and *S. sclarea* EO were selective against *L. Brasiliensis*. The results of this study offer prospects for future research contributing to the discovery of effective drugs for the treatment of parasitic diseases with less side effects than the drugs commonly used. **Supported by:**FAPDF, CAPES, CNPq **Keywords:**Salvia sclarea; melissa officinalis; leishmania Brasiliensis

HP133 - AVIAN MALARIA IN ICTERIDAE (PASSERIFORMES) BIRDS OF THE BRAZILIAN ATLANTIC FOREST

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With a distribution restricted to the American continent, from Alaska to Tierra del Fuego and with ~110 described, the family Icteridae (Passeriforme) is one of the most well studied families with respect to ecology and behavior of birds. However, scarce data on the diversity of parasites are available on literature for this family. Here, blood samples of 12 specimens of the family Icteridae (*Cacicus haemorrhous*, *Gnorimopsar chopi*, *Psarocolius decumanus*) sampled from fragments of rain forests located in the southeastern of Minas Gerais were analyzed for the presence of *Plasmodium* spp. by microscopy and by using a molecular approach based on the amplification of the parasite cytochrome b. From the total, 9 individuals were positive (75%) for *Plasmodium* spp. with an average parasitemy of 0.10%. In *Psarocolius decumanus* (n=3) the parasitemy was 0.41%, while in *Cacicus haemorrhous* (n=6) and in *Gnorimopsar chopi* (n=2) was of 0.03% each. All evolutive forms of the parasites (trophozoite, meront, microgametocyte and macrogametocyte) could be observed during our microscopic analysis; while with the molecular approach one *G. chopi* and two *P. decumanus* were identified as positives. BRA holds a great diversity of birds of which, many are considered in danger or in process of extinction. Considering that in the last few years avian malaria has been associated with reduction of population sizes, works like this focused on the identification of haemoparasites infecting wild population of birds are of great importance and should be incorporated on strategies and programs of management of animals in risk of extinction.

Supported by: CAPES-Coordenação de Aperfeiçoamento de Pessoal de Nível Superior

Keywords: Hemosporida; plasmodium spp; icterids

HP134 - IDENTIFICATION OF NEW ANTIGENS OF GENUS LEISHMANIA SPECIES WITH POTENTIAL USE IN THE SEROLOGICAL DIAGNOSIS OF LEISHMANIASIS

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Leishmaniasis is a complex group of diseases caused by intracellular protozoan of the genus *Leishmania* that infect macrophages of a variety of mammals. The progress in the disease control still requires more reliable technologies and evaluations. The most used diagnostic tests for Leishmaniasis are based on ELISA, using *Leishmania* raw extract or recombinant proteins as antigens. Over the past years, our group developed immunogenomic studies in the search for new antigens that are more sensitive and specific and that can detect both cutaneous and visceral leishmaniasis. In this study, we have identified and tested *L. brasiliensis* proteins, which have similarity to *Homo sapiens* proteins present in ImmunomeBase database. Two parasite genes were selected and recombinantly expressed in a heterologous system. After purification by affinity chromatography, the proteins were used as antigens in serological testing by ELISA for cutaneous and visceral leishmaniasis diagnosis. In ELISA with canine and human sera, antigen Li showed sensitivity of 75% and specificity of 76% and sensitivity of 68% and specificity of 53%, respectively. Similar results were obtained with the antigen Lm that had a sensitivity of 81% and specificity of 87% and sensitivity of 80% and specificity of 78%, respectively with canine and human sera, being also able to differentiate *Leishmania* species. To improve our results, we are currently evaluating the antigenic properties of proteins identified in this study, based on linear B cell epitope predictions. We are aiming to identify more specific peptides to be used to improve our antigens in serological diagnosis. The use of genomic approaches opens the prospect of an intervention and identification of new and efficient targets for diagnosis of leishmaniasis.

Supported by: CAPES/FAPEMIG/CNPq

Keywords: Leishmania; antigen; serodiagnosis

HP135 - PRESENCE OF THE SNP IN THE PROMOTER REGION OF THE IL-17A GENE IN PATIENTS WITH AMERICAN CUTANEOUS LEISHMANIASIS

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This study aims to evaluate the immune response to American cutaneous leishmaniasis (ACL) in the presence of the SNP IL-17A -197G/A (rs2275913), associating it with clinical diagnostic and parasite loads in individuals from endemic areas in Pernambuco State. 145 individuals were clinically evaluated and diagnosed by real-time PCR and grouped as symptomatic group (Sg), asymptomatic (Ag) and without infection (Wlg). Genotypes were determined by PCR-RFLP and sequencing. Allele and genotype frequencies were determined and evaluated for Hardy-Weinberg equilibrium (EqHW). The proportion of allele A carriers in the groups was compared using the X2 test, and the association of genetic variation with the clinical outcome of infection was measured by Odds ratio (OR). The parasite load present in each group was compared, and also between genotypes. 55 individuals were classified in Sg, 58 in Ag and 30 in Wlg. 6.71% of individuals are homozygous for allele A; 41.79% heterozygous for AG; and 52.98% homozygous for GG. The frequencies of genotypes distributed between the groups are EqHW (Sg: p= 0.5694; Ag: p= 0.9522; Wlg: p= 0.2733). The number of A carriers in Sg and Ag did not increase significantly (AG: p= 0.8897; AA: p= 0.4454; AG/AA: p= 0.7236), and A carriers had the lowest chance of developing the disease facing infection (AG: OR= 0.56; AA: OR= 0.97; AG/AA: OR= 0.87). Lower parasite loads among Sg compared to Ag (p= 0.001) was found, as well as between the AA compared to the AG and GG. The initial results indicate association of the allele A with reduced parasite loads, and lower susceptibility to the development of lesions in patients with ACL, however, to reach significant conclusions, the number of individuals will be expanded and the panel of cytokines by the standardized cell stimulation conditions will be analyzed, allowing greater understanding of the role of IL-17A in the pathogenesis of ACL.

Supported by:Centro de Pesquisas Aggeu Magalhães, Fundação Oswaldo Cruz – PE

Keywords:Single nucleotide polymorphisms; il17; american cutaneous leishmaniasis

HP136 - MODULATION OF ARGINASE 1 ACTIVITY AFTER INFECTION WITH TOXOPLASMA GONDII DEPENDS ON THE MACROPHAGE LINEAGE BUT NITRIC OXIDE PRODUCTION IS ALWAYS INHIBITED

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Resident macrophages are a population of immune cells with low microbicidal capacity. Classically activated macrophages with interferon-gamma and lipopolysaccharide express inducible nitric oxide (NO) synthase and produce NO, a microbicide molecule able to control *Toxoplasma gondii* replication. However, the parasite subverts the cell-autonomous immunity of these cells by degrading inducible NO synthase, reducing NO production, persisting in activated macrophages. Alternatively activated macrophages with interleukin-4 express arginase 1 (ARG1). ARG1 induction in macrophages infected with *T. gondii* is considered an evasion mechanism because polyamines are produced being crucial for parasite replication. However, the induction of ARG1 in resident or activated macrophages infected with *T. gondii* is controversial. Thus, we evaluated NO production and ARG1 activity in two macrophage lineages (mice peritoneal and RAW 264.7) infected with *T. gondii* of the RH strain. NO production was evaluated by the Griess reagent and ARG1 activity was measured with the use of α -isonitrosopropiophenon. Classically activated peritoneal macrophages and RAW 264.7 infected with *T. gondii* showed a significant decrease in NO production as compared to uninfected cells. As expected, NO was not produced by infected or uninfected resident macrophages of both lineages. Both resident and activated peritoneal macrophages showed a reduction in ARG1 activity after *T. gondii* infection when compared to uninfected cells. However, resident and activated RAW 264.7 infected with *T. gondii* showed a higher ARG1 activity when compared to uninfected cells. Thus, the modulation of ARG1 by *T. gondii* infection depends on the macrophage lineage. Further studies are necessary to understand the importance of the distinct modulation of ARG1 in both activated macrophage lineages after *T. gondii* infection.

Supported by:UENF, CAPES, CNPq, FAPERJ.

Keywords:Toxoplasma gondii; inducible nitric oxide synthase; arginase 1

HP137 - **MODULATION OF 5-LIPOXYGENASE ALTER THE CLINICAL SIGNS AND COGNITIVE BEHAVIOR DURING CEREBRAL MALARIA IN MICE**

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Introduction: Cerebral malaria (CM) is a major complication in the health human caused through the *Plasmodium falciparum* infection. The *Plasmodium berghei* ANKA (PbA) infection is able of resemble many aspects of CM; thus, this model has been used to investigate the pathogenesis of CM. CM is a complex and multifactorial syndrome, and the mechanisms underlying the cerebral damage are not fully understood. It has been suggested that the sequestration of parasitized erythrocytes to the cerebral vasculature, an overly vigorous immune response to parasite products and increased permeability of the blood-brain barrier contribute to the development of CM. Lipoxin (LX) A4 production by the activation of the enzyme 5-lipoxygenase (5-LO) down-modulate and promote the modulation of inflammatory processes. The beneficial effects of LXA administration in models of inflammatory pathology, along with the fact that administration of Zileuton leads to the inhibition of 5-LO activity, has suggested therapeutic promise for specific harnessing of the biological activities of LXA. Here in, we investigated if inhibition of 5-LO activity by Zileuton during PbA infection could be a potential therapeutic toll. Methods and Results: WT (C57Bl/6) mice were pre-treated with Zileuton (50mg/ kg) and infected with PbA and then followed by daily administration of Zileuton twice a day (each 12h). Parasitemia, body weight, survival and clinical signs of the disease were evaluated periodically. Our results showed that mice treated with Zileuton displayed higher parasitemia significantly, increased of weight loss when compared to untreated PbA-infected mice. Moreover, the results showed that at 5 dpi, PbA-infected mice treated with Zileuton exhibited a deficit of recognition behavioral, without locomotor changes. Conclusions: The results of this study indicate that the early administration of Zileuton exacerbates the severe clinical signs associated with CM in mice infected with PbA. Financial support: Capes, CNPq, FAPEMIG. **Supported by:** CNPQ / FAPEMIG **Keywords:** *Plasmodium berghei* anka; 5-lipoxygenase; lipoxin

HP138 - **EFFECT OF ALUMINUM HYDROXIDE ON MACROPHAGES INFECTED WITH TRYPANOSOMA CRUZI**

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Chagas disease, caused by *Trypanosoma cruzi*, is a disease for which the available drugs are not sufficiently safe or effective and no vaccines are available. Activation of the immune response is important to determine the outcome of infection. Aluminum hydroxide (HA) phagocytosis by effector cells, followed by the rupture of phagolysosomes, has been shown to activate inflammasomes, resulting in the release of active cytokines IL-1 β e IL-18. In this work we investigated the effects of HA on the immune response against *T. cruzi*. Macrophages from peritoneal exudate (PE) were infected with *T. cruzi* and incubated in the presence or absence of HA. The infection was followed for 72 hours. Our results indicated a decrease of *T. cruzi* infection in HA-treated cells. To analyze the mechanism by which HA exerts its inhibitory action, quantification of superoxide (SO) and nitric oxide (NO) was performed. Our results show an increase in SO and NO production in treated cells, suggesting that HA could lead to an improvement of the microbicidal capacity of macrophages and the decrease of amastigotes survival. We also investigated the effects of HA on the cytokines of Th1/Th2 profile in *T. cruzi*-infected and non-infected cells. Our results showed a significant increase in TNF- α and IL6 in both non-treated and treated infected-cells compared with non-infected and non-treated cells. However no increment in the level of these cytokines was observed when infected-cells treated with HA were compared with their non-treated counterpart. A significant decrease in IL-2 and IL-4 was observed when peritoneal exudate cells were exposed to HA. Conversely, levels of the regulatory cytokine IL-10 increased significantly under this same condition. The 1 β e IL-18 increased their levels in response to *T. cruzi* infection, but not to the HA treatment. These results suggested that HA is able to immunomodulate the infection by *T. cruzi* in a mechanism independent on inflammasome. **Supported by:** Centro de Pesquisas Aggeu Magalhães, Fundação Oswaldo Cruz – PE **Keywords:** Inflammasome; *trypanosoma cruzi*; aluminum hydroxide

HP139 - EVALUATION OF *IN VITRO* METACYCLOGENESIS OF A STRAIN OF *LEISHMANIA AMAZONENSIS* ATTENUATED BY SUCCESSIVE PASSAGES IN AXENIC MEDIUM
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Axenic cultivation of parasites of the genus *Leishmania* for long periods was used for decades to obtain attenuated parasites. By comparing a wild strain of *L. amazonensis* (R0) and one obtained after 60 subsequent *in vitro* passages (R60), a study in our lab revealed differences in 67 metabolites, belonging to 8 metabolic pathways, highlighting the fatty acid metabolism as one of the main routes changed. The two strains also differed in their infectivity, which was reduced in the R60 line, in *in vitro* and *in vivo* assays. Considering that changes in the metacyclogenesis process may affect virulence, this study aimed to quantify the percentage of metacyclic promastigotes present in the 4th and 7th days of cultivation of the strains R0 and R60. Ficoll gradient separation and analysis by flow cytometry were performed with cultures grown to the 4^o (containing procyclic promastigotes) and 7^o days (containing metacyclic promastigotes). Statistics were performed by the Two-way ANOVA, followed by Bonferroni tests. There were no significant differences in growth rates between the two strains. According to the two methodologies employed, there were no significant differences in the percentage of metacyclic forms between the two strains. Using Ficoll gradient at the 4th day, R0 presented an average of 0.47%, ± 0.3 of metacyclic forms, while R60 showed an average of 0.44% ± 0.01 . By flow cytometry R0 presented an average of 0.55% (± 0.06), while R60 presented 0.79% (± 0.4) of metacyclic ($p > 0.05$). On the 7th day of cultivation the previous pattern was maintained for R0 (1.36% ± 0.2) and R60 (1.38% ± 0.14), by ficoll gradient and by flow cytometry, R0 presented 1.74% (± 0.8) and the R60 1.65 (± 0.3). Thus, differences in infectivity may not be linked to the ability to proliferate or to the production of metacyclic promastigotes, and may well be associated to other metabolic changes. **Supported by:** CNPq, CAPES, FAPEMIG

Keywords: Leishmania amazonensis; metacyclogenesis; attenuated

HP140 - ANALYSIS OF CARDIAC PARASITISM AND CREATINE KINASE IN MICE INFECTED ORALLY WITH METACYCLIC TRYPOMASTIGOTES FROM OF *TRYPANOSOMA CRUZI* BERENICE-78 STRAIN

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Oral transmission of Chagas disease has become the main route of contamination after the elimination of the central domestic vector and control of transmission by blood donation. A common feature of all these outbreaks by this transmission is the severity of the disease, where most patients have severe myocarditis, with a high mortality rate in the first two weeks after infection. In this sense, a study on the profile of parasitemia and cardiac injury in experimental oral infection allow an understanding of how the transmission route interferes with the interaction between *T. cruzi* /host promoting a more serious cardiac injury. For this, Swiss mice orally (OR) or intraperitoneally (IR) infected with metacyclic trypomastigotes from Berenice-78 strain were euthanized at 14, 21, 28, 35, 42 and 180 days after infection (DAI) and the heart was collected for evaluation of parasite load by Real-Time PCR and serum to the activity of CK-MB. The parasitemia curve was analyzed over time. Our results showed that OR had a maximum peak of parasitemia on 18th DAI with 1.151.000 trypomastigotes/0.1 mL of blood, while intraperitoneally infected animals showed 956.825 trypomastigotes/ 0.1 mL of blood in the same day. Both routes of infection showed an increased cardiac parasitism in the acute phase, at the 21th DAI the OR group presented a higher parasite load (23.471 parasites/ mg of tissue), occurring a reduction in the parasitic load on the 28th DAI. However, in the IR group increased parasite load was found in 28th DAI with 8.607 parasites/ mg of tissue, occurring a reduction in the parasitic load only on the 35th DAI. Besides that, OR group showed an increase in the CK-MB activity over time of infection being observed a peak of the levels of this enzyme at 35th DAI. On the other hand, IR showed no changes in CK activity during infection. Thus, these results show that the oral infection with metacyclic trypomastigotes from *Trypanosoma cruzi* Berenice-78 strain shows a different parasitological profile resulting in increased cardiac damage.

Supported by: FAPEMIG, CAPES E UFOP **Keywords:** Oral chagas disease; trypomastigotes metacyclic; parasitic load

HP141 - **ENDOPLASMIC RETICULUM STRESS CONTRIBUTE TO INDUCTION OF TH1 CELLS AND RESISTANCE AGAINST *L. MAJOR* INFECTION IN A NOD/RIP2-DEPENDENT MECHANISM**

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Endoplasmic reticulum (ER) stress is a major contributor to inflammatory responses. Recently, it was showed that ER stress participates on activation of innate sensors Nod1 and Nod2. Nod1 and Nod2 participate in the detection/control of several pathogens. However, the role of Nod1 and Nod2 during *Leishmania* infection is unknown. Here, we investigated the role of ER Stress in the Nod/Rip2 pathway activation in response to *L. major* infection. BMDMs and BMDCs derived from C57BL/6, *Nod1*^{-/-}, *Nod2*^{-/-} and *Rip2*^{-/-} mice were infected with *L. major*, treated with inhibitors or inducers of ER stress and analyzed the cytokine production and Th1 cells induction. The lesion development and parasite burden were measured in C57BL/6-, *Nod1*^{-/-}-, *Nod2*^{-/-}- and *Rip2*^{-/-}-infected mice. Dendritic cells activation and cytokines production *in vivo* were evaluated by flow cytometry. Finally, we analyzed the susceptibility and cytokines production in chimeras generated by irradiating recipient mice. *L. major*-infected C57BL/6 BMDMs and BMDCs treated with ER stress inhibitors had a decreased IL-12p40 and IL-6 production and an impaired Th1 cells induction. In contrast, *L. major*-infected *Nod1*^{-/-} and *Rip2*^{-/-} BMDMs and BMDCs treated with ER stress inducers increased IL-12p40 and IL-6 production and Th1 cells induction. Nod1 and Nod2 activation was crucial for *in vivo* parasite replication control and resolved cutaneous lesions. Rip2-dependent response was required for dendritic cells activation and induction of effective Th1 response *in vivo*. Additionally, Rip2-dependent signaling in radio-sensitive compartments was required for the control of the infection and induction of Th1 response. These studies indicate that ER stress induces a Nod/Rip2-dependent response that account for host resistance against *L. major* infection by mechanisms dependent of Th1 cytokine. Thus, this study providing a novel function for Nod-like receptors family in the parasite-host interactions. **Supported by:**FAPESP, CRID/FAPESP, INCTV/CNPq, CAPES. **Keywords:**Leishmania; er stress; nod like receptors

HP142 - **POTENTIAL INHIBITION OF MIGRATORY INHIBITORY FACTOR HOMOLOGUES FOR CONTROLLING OF PARASITIC DISEASES.**

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Migratory Inhibitory Factor is a preformed cytokine found in several immune cells. This cytokine possess an important role in controlling inflammation. As example of the importance of this control, parasites produces homologues of this cytokine. Thefhore, this work intended to evaluate differences *in silico* between the MIF homologues expressed in *Leishmania major*, *Toxoplasma gondii* and *Plasmodium falciparum*, in comparison with the human MIF, and averiguate the possibilite to inhibit only the MIF parasite. Firstly, we aligned the predicted amino acid sequences via Clustaw Omega and analyzed the 3D structure of the molecules using PDBeFold. The isoelectric point and molecular weight was calculated using Compute pI/Mw. Possible ligands and its binding sites was compared by Coach database. Additionally, it was performed an analysis of binding cavities of proteins that could probably interact with its ligands using MetaPocket 2.0. As result, invariant amino acids and conserved amino acids with similar biophysical properties at a given position were found. The theoretical isoelectric points and molecular weights obtained for MIF proteins were respectively 7.73 and 12519.28 Da of human 6.62 and 14675.70 Da of *Leishmania major*, 8.91 and 12254.15 of *Toxoplasma gondii* and 6.15 12844.51 Da of *Plasmodium falciparum*. The component YZ9 was related by homology with MIF of parasites and OX3 was identified as a ligand for human MIF. Both ligands interact with MIF proteins in conserved amino acids. Furthermore, the MetaPocket analysis demonstrated that one of the bindings cavities possess amino acids sequences that could be a target for a potential inhibitor. Also, the Human MIF was found to possess low similarity with parasites MIF. Thus, this work open a possibility for test the inhibition of the parasite MIF for controlling parasitic infection. **Supported by:**cnpq fapemig capes **Keywords:**Mif; mif parasite; mif homology

HP143 - SUBCELLULAR LOCALIZATION OF SUBTILISININ *LEISHMANIA SPP.*
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Subtilisin is part of S8A family of the serine proteases that are essential proteolytic enzymes to the organisms-physiological processes in different cells types. The protozoa parasites of the genus *Leishmania* causes leishmaniasis that is a diseases with a wide range of clinical symptoms, this disease affects millions of people world-wide and the presence of a subtilisin-family representative in species of genus *Leishmania* was observed by genome from *Leishmania* species. In this study, our objective was determine the localization and subcellular localization of subtilisin. First, we cloned the catalytic domain of subtilisin from *Leishmania amazonensis* and expressed recombinant protein. We used recombinant protein to produce a polyclonal anti-serum anti-subtilisin in rabbit. Thus, the subtilisin was identified by immuno-blot presenting a band with 150 kDa, as expected by molecular mass from genome. Then, we performed immunolocalization of *Leishmania amazonensis* and *L. major* by Apotome. These experiments showed that the subtilisin is present throughout the parasite, however, has a high concentration on the flagellar pocket region in both species. To determine subtilisin subcellular localization was performed Immunocytochemistry using electron microscopy with *L. amazonensis* promastigotes forms. This result demonstrated that subtilisin found in flagellar pocket and cytoplasmic vesicles of promastigote forms, indicating a possible role in the parasite's metabolism. **Supported by:**FAPERJ, CAPES.

Keywords:Subtilisin; leishmania spp; serino protease

HP144 - LEISHMANIASIS TRIGGERS CHANGES IN MONOCYTE SUBSETS IN DOGS
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Peripheral blood monocytes are the largest group of circulating progenitor cells. Its functions have been revealed through the study of their subpopulations, especially in humans and mice in pathological processes. In BRA, the parasite *L. infantum* is the cause of the canine visceral leishmaniasis. This study aimed to characterize subpopulations of peripheral blood monocytes from uninfected or *L. infantum* naturally infected dogs and compare their phenotypical and functional aspects. Blood samples were collected from uninfected and infected dogs, labeled by antibodies against different surface markers and analyzed by flow cytometry. It was observed two distinct populations according to CD14 expression: a population CD14High, constituting about 90% of the monocytes with greater phagocytic capacity, and another CD14Low, constituting about 10% of monocytes. The analysis of the other molecules showed different levels of expression between the two populations. In dogs with visceral leishmaniasis no difference in frequency was observed between populations, however there is an increase of CD14+ cells in infected dogs. We also noticed marked reduction of the expression of molecules related to the activation of monocytes, especially in the group of symptomatic dogs, indicating a possible inhibition on the capacity of these cells to control the disease.

Supported by:FAPEMIG, CAPES

Keywords:Leishmania; monocytes; leishmaniasis

HP145 - **STUDY OF AN EXPERIMENTAL MODEL OF VISCERAL LEISHMANIASIS IN MICE BY INFECTION WITH LEISHMANIA MAJOR OR LEISHMANIA AMAZONENSIS.**

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Visceral leishmaniasis is endemic to man and can kill if not treated. There are not good models of visceral leishmaniasis in mice. In general, the existing models develop minor injuries and cure in few weeks. Thus, the aim of this study is to evaluate a new model of visceral leishmaniasis in mice, using the infection by *L. major* or *L. amazonensis*. The evaluation of visceralization of the disease was realized by measuring the mass of organs such as liver and spleen, as well as body mass of mice experimentally infected. BALB/c and C57BL/6 mice were infected intraperitoneally with 2×10^6 stationary phase promastigotes of *Leishmania major* species (MHOM/IL/81/Friedlin) or *L. amazonensis* (IFLA/BR/67/PH8). Throughout the experimental period, the animals were weighed weekly until euthanized and the organs of interest were removed. Our results demonstrate, that BALB/c infected mice have a significant weight loss after 25 weeks of infection. Still, infected BALB/c mice showed increase of the spleen after 7 weeks of infection for both species of parasites, but after 28 weeks of infection, only the group infected with *L. major* showed this lesion. The results suggest that the process of visceralization promotes loss of body mass of the mice only in the chronic stage. Also, it was observed the development of necrotic lesions in the skin. More detailed analysis (histological, hematological and parasitic load) and new experiments are underway to determine the effectiveness of the experimental model of progressive visceral leishmaniasis in mice infected with *L. major* or *L. amazonensis*. **Supported by:** FAPEMIG

Keywords: Leishmania; mouse; visceral leishmaniasis

HP146 - **MONOCYTES AND PLATELETS AS BIOMARKERS IN CANINE VISCERAL LEISHMANIASIS**

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Canine visceral leishmaniasis is a chronic disease caused by *L. infantum* which affects dogs and promotes serious injury leading to death. Dogs are the main reservoir of this parasite, having a central role in the transmission to humans. In BRA canine visceral leishmaniasis have been treated for more than fifteen years. Recently, a new treatment protocol has been proposed, through immunotherapy with Leistech® vaccine associated with allopurinol, showing good results in the control of the disease in dogs. However, there's no reliable biomarker of prognosis or to measure the clinical recovery in infected animals. This study aimed to characterize monocytes and platelets as biomarkers in dogs naturally infected and treated in order to use this data as prognosis and for therapeutic management. The study included dogs naturally infected by *L. infantum*, symptomatic and treated with immunotherapy and allopurinol. Medical records containing information on the clinical status, hematological and biochemical tests were analyzed. Data were selected from the results obtained before the first administration of immunotherapy and tests performed after their last dose. Results showed WBC decrease in total leukocytes and monocytes, and an increase in platelets in 86% of dogs after treatment. The biochemical analysis showed decrease of globulin and albumin/globulin ratio after treatment. These results show that some hematologic data can be used to predict the prognosis of the disease.

Supported by: FAPEMIG, CAPES

Keywords: Leishmania; monocytes; platelets

HP147 - F2 DOMAIN OF LEISHMANIA NUCLEOSIDE HYDROLASE INDUCES RESISTANCE-ASSOCIATED T-CELL RESPONSES IN HUMAN VISCERAL LEISHMANIASIS.

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Protection against visceral leishmaniasis (VL) involves a Th1 immune response. Also, antigens targeting the CD8 and T-regulatory responses are relevant in VL pathogenesis and worthy of being included in a human vaccine. We assessed in active, cured and asymptomatic VL subjects the clinical signs, cytokine production and FACs in response to the *Leishmania donovani*-nucleoside hydrolase NH36 antigen and its F1, F2 and F3 domains. As markers of resistance and protection against VL, the F2 induced the highest levels of IFN- γ , IL-1 β and TNF- α and together with F1 promoted the strongest secretion of IL-17, IL-6 and IL-10 in DTH+ and cured subjects. F2 promoted the highest frequencies of CD4 cells producing IL-2, IL-2/TNF- α , IL-2/IFN- γ and IL-2/TNF- α /IFN- γ in cured and asymptomatic VL subjects. The IFN- γ and IL-17 increases were associated with decreased spleen and liver sizes and increased hematocrit, hemoglobin, monocytes and Hg counts. Conversely, as markers of susceptibility and advancement of the disease, the F1 and F3 increased the frequencies of CD8-IL-2, CD8-TNF- α /IL-2 and CD8-IL-2/TNF- α /IFN- γ T cells of VL patients before treatment, which correlate with increased spleen and liver sizes and decreased hemoglobin and hematocrit values. We showed that cure and natural resistance to VL correlate to the CD4+Th1, Th-17 and Treg-cell responses to the F2 and F1. Clinical outcomes of untreated patients correlate to the CD8+ T-cell responses towards the F3 and F1. Supporting our results, increased levels of IFN- γ were secreted in response to the synthetic in silico predicted CD4 T cell epitopes of F2 and F1, and CD8 T cell epitopes of F1. These epitopes are promiscuous, binding to many HL-DR and HLA and B allotypes and conserved in the genus *Leishmania*. We identified the NH domains and epitopes which are inducers of the CD4+ and CD8+ T cell immune response against NH36 and that could be combined to potentiate a human universal T-epitope vaccine against leishmaniasis. **Keywords:**Visceral leishmaniasis; epitopes; human vaccine

HP148 - IMMUNOTHERAPY AGAINST VISCERAL LEISHMANIASIS CAUSED BY L.(L.) CHAGASI WITH THE RECOMBINANT CHIMERA CONTAINING THE N- AND C- DOMAINS OF LEISHMANIA (L.) DONOVANI NUCLEOSIDE HYDROLASE

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The Nucleoside Hydrolase (NH36) is the main marker of the FML complex (Fucose and Mannose-ligand) of *Leishmania (L.) donovani*. The sequences of the chimera F1F3 including the F1 (N-terminal) and F3 (C-terminal) domains of the NH36 were cloned in tandem in the pET28b expression system. The recombinant chimera was obtained with optimized codons for *Escherichia coli*. In this investigation, we evaluated the therapeutic efficacy of the F1, F3 and F1F3 vaccines in formulation with saponin, against visceral leishmaniasis caused by L. (L.) chagasi. After immunotherapy all the vaccines increased the IgG, IgG1, IgG2a, IgG3 antibodies titers (p < 0.05). The F3 and the chimera F1F3 induced the strongest IDR at 24 and 48h after lysate injection (p < 0.05). The chimera also induced the strongest secretion of IFN- γ (97% higher than the control saline) and together with the F3 vaccine, the highest levels of TNF- α (828.3 and 702.45 pg/ml, respectively). All vaccines increased the IL-10 levels compared to the saline controls (p < 0.05). and the chimera showed the highest IL-10 secretion (p < 0.05). Supporting these results, the F3 and the chimera vaccines promoted the lowest hepatosplenomegaly and the chimera induced the smallest parasite load in livers (98.5% of cure). The F3 vaccine induced the highest proportions of CD4+IFN γ +, CD4+IL-2+IFN γ and CD4+IL2+TNF- α +IFN- γ + T cells. The chimera, on the other hand, induced the highest proportions of CD8+IFN γ + and CD8+TNF- α +IFN γ + T cells. Finally, our results demonstrate that both F3 and chimera vaccines induce a strong curative and TH1 immune response against visceral leishmaniasis. **Keywords:**Visceral leishmaniasis; immunotherapy; vaccine

HP149 - **EVALUATION OF THE GENETIC VARIABILITY OF GP63 VIRULENCE PROTEIN PARALOGS IN *LEISHMANIA BRASILIENSIS***

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Throughout their evolution *Leishmania* species have developed methods to evade the innate immune response from the mammalian host in order to invade and survive inside the macrophages. One of the main escape mechanisms is mediated by the virulence protein gp63, a major surface antigen in *Leishmania* but whose genes vary significantly in number among different species. In fact, species from the subgenus *Viannia* seem to have five to ten fold more gp63 genes than those species belonging to the subgenus *Leishmania*, but the draft nature of the available genomes impairs a better evaluation of their number and diversity. This study aimed to properly evaluate the genetic variability in gp63 sequences from a single *L. Brasiliensis* strain. Initially, with the goal of amplifying a large number of different gp63 genes, PCR reactions were designed using degenerated oligonucleotides which anneal to conserved motifs found in most, if not all, gp63 sequences. These reactions were performed with a high fidelity DNA polymerase and were followed by gene cloning and sequencing. A total of 27 genes were found, whose sequences displayed variable regions consistent with the known variability seen for gp63. Most of these, however, did not match the sequences already deposited in public databases, suggesting novel genes and a greater gene count than previously annotated. Phylogenetic trees were then constructed comparing the newly identified sequences with available *Leishmania* sequences. Most of the amplified sequences clustered with genes previously mapped to chromosome 10, confirming an expansion of the gp63 genes within this chromosome. Recombinant proteins encoded by equivalent fragments from four of these genes were also expressed in *Escherichia coli*, yielding polypeptides displaying variation in apparent molecular weight on gel electrophoresis. The sequence variation and the expansion of these paralogs may indicate adaptive mechanisms of the parasite to their environment.

Supported by:FACEPE **Keywords:**Leishmania Brasiliensis; gp63; genetic variability

HP150 - **THE ROLE OF NEUTROPHILS AND REACTIVE OXYGEN SPECIES IN EXPERIMENTAL CHAGAS DISEASE**

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Neutrophils are essential components of the early inflammatory response, acting as the first line of defense against invading pathogens. The involvement of neutrophils in *T. cruzi* infection has been poorly explored, and little information can be found about this interaction. Recent studies demonstrated that ROS could be a signal to parasite proliferation in macrophages. However, the role of these molecules in other types of cells remains to be elucidated. Herein we focused on understanding the interaction between neutrophils and *T. cruzi*, exploring ROS involvement. We conducted *in vitro* and *in vivo* assays using C57BL/6 wild-type mice (WT) and mice deficient in NADPH phagocyte oxidase (Phox KO) infected with Y strain of *T. cruzi*. Bone marrow neutrophils were infected with trypomastigotes and parasite burden and the occurrence of apoptosis were analyzed by flow cytometry. Both neutrophils, from WT and Phox KO, uptook parasites similarly. However, in contrast with what previously we observed in macrophages, after 24h, the parasite burden in Phox KO neutrophils was significantly higher than in WT neutrophils. At this time-point, Phox KO had significantly less apoptotic neutrophils than WT. *In vivo*, the cellular infiltrate after ear intradermal infection was evaluated by flow cytometry. The number of non-inflammatory cells decreases after infection for both groups. The number of inflammatory monocytes was significantly higher in WT mice than Phox KO after 24 and 48 hours of infection. In contrast, inoculation of *T. cruzi* in Phox KO mice induced neutrophil accumulation, peaking at 24 h. This increase was not observed in WT mice. Our results show that the absence of ROS affects cellular recruitment after intradermal *T. cruzi* infection and interfere with the parasite burden and apoptotic events in neutrophils. To understand the role of ROS in neutrophil infection and recruitment can be important to expand the knowledge related to the pathogenesis of Chagas disease. **Supported by:**FAPEMIG, CAPES and CNPq

Keywords:Trypanosoma cruzi; ros; neutrophils

HP151 - EFFECTS OF LEISHMANIA (L.) AMAZONENSIS LALRR17 PROTEIN IN MACROPHAGE INFECTION AND IDENTIFICATION OF ITS POSSIBLE LIGANDS

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The protozoan *Leishmania* is responsible for 2 million cases of leishmaniasis in the world and more than 50 thousand deaths per year. The parasite is transmitted by sand flies and once in the vertebrate host lives mainly inside macrophages. Several molecules named virulence factors permit the survival of *Leishmania* in macrophages, cells specialized in pathogen destruction. The LRR17 protein was previously identified in different *Leishmania* species and is characterized by leucine-rich repeats (LRR), commonly involved in protein-protein interactions. The protein was observed in the cytoplasm of infected macrophages, suggesting secretion and possible interaction with host cell proteins. Besides, overexpression of LRR17 in *L. (L.) amazonensis* leads to an increase in macrophage infection in vitro. In this work we show that not only overexpression but also the soluble recombinant protein increases the proportion of infected macrophages. To shed light on the mechanisms by which LRR17 affects infection, we searched for its possible protein ligands in the host cell. After producing the recombinant protein, we employed peptide phage display and identified sequences that were enriched after three selection cycles, which probably correspond to domains of proteins with high affinity for LRR17. We also used affinity chromatography to directly select for ligands using macrophage extracts. A single specific band bound to immobilized LRR17, and will be identified by mass spectrometry. The identification and comparison of the potential ligands identified by both methods will contribute to unravel the mechanisms of LRR17 associated virulence in this *Leishmania* species. **Supported by:**FAPESP

Keywords:Phage display ; leucine-rich repeat ; leishmania

HP152 - THE C-TYPE LECTIN FROM *BOTHROPS LEUCURUS* VENOM (BLL) HAS IMMUNOMODULATORY EFFECT ON MACROPHAGE INFECTION BY *LEISHMANIA BRASILIENSIS*

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Leishmania Brasiliensis is the main causative agent of American Tegumentary Leishmaniasis (ATL) in BRA. Because these parasites infect macrophages, the therapeutic response depends on both the host and microbial determinants. In this regard the host immune response to *L. brasiliensis* is essential for determining the outcome of infection, and the use of immunomodulatory agents can be a promising therapeutic approach. The aim of this study was to investigate the effects of a C-type lectin BLL on the immune response to *L. Brasiliensis* infection. Peritoneal macrophages (10^6 cells/mL) were seeded in multi-well culture plates and infected with promastigotes forms of *L. Brasiliensis*. The culture was incubated for 24h at different concentrations of BLL. After the treatment, untreated and treated-cells were stained with Giemsa and the Infection Index and the percentage of macrophage infection were determined. For quantification of ROS the supernatants were incubated with Griess reagent and the nitrite concentration was determined by spectrophotometry. The Th1/Th2/Th17 cytokine profile was evaluated by using BD CBA Mouse Cytokine Kit. Additionally, the cells were processed for transmission electron microscopy. BLL significantly decreased the survival of amastigote and inhibited the percentage of infected macrophage in 75%. The lectin treatment also induced a four-fold increase in the level of nitric oxide production by infected macrophage. Similarly, the release of proinflammatory cytokines (IL-1 β , IL-6, TNF- α and IL-17) but not Th2 cytokines, were also significantly increased. Our ultrastructural analysis showed that BLL induced morphological changes on amastigotes as loss of cytoplasmic content and cell lysis. Overall, our results suggest that the immunomodulatory actions of BLL are a possible mechanism responsible for the inhibition of *L. Brasiliensis* infection in the macrophages, opening new perspectives in the field of new therapeutic targets for leishmaniasis.

Supported by:CAPES, CNPQ, FACEPE AND FIOCRUZ

Keywords:Leishmaniasis; chemotherapy; c-type lectin

HP153 - DEVELOPMENT OF PROPHYLACTIC AND THERAPEUTIC MEASURES AGAINST MALARIA IN MICE

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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.

Keywords:Clec7a; plasmodium berghei; immune response

HP154 - TREATMENT WITH RECOMBINANT NCROP4 (RNCROP4) IS ABLE TO REDUCE THE INFLAMMATION DURING ACUTE TOXOPLASMOSIS AND DALAYING MORTALITY IN MALARIA CEREBRAL EXPERIMENTAL

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Toxoplasmosis and malaria are parasitic diseases caused by *Toxoplasma gondii* and *Plasmodium* spp., respectively. While toxoplasmosis affects approximately 50% of the world population, malaria is main cause of mortality by infectious disease in the world. Both parasites are protozoans from Apicomplexa phylum, sharing several characteristics, such as presence of secretory organelles (micronemes, rhoptries and dense granules). Another parasite of this phylum, closely related to *T. gondii* is *Neospora caninum*. In this work we aim to evaluate immunomodulatory potential of NcROP4, a rhoptry protein of *N. caninum*, during pathological processes generated by toxoplasmosis and malaria. For this purpose, the recombinant NcROP4 (rNcROP4) was produced in *Escherichia coli*. Then, C57Bl/6 and BALB/c mice were treated with rNcROP4, STAg (soluble *T. gondii* antigens) or PBS (control). Three days after treatment, mice were infected with 50 cysts of *T. gondii* (ME-49 strain). Peritoneal fluid and gut were collected seven days after infection to evaluate the IL-10 and IFN γ production. Histological analysis of intestinal segments were performed for evaluate the damages caused by infection. Moreover, morbidity and survival rate were evaluated in C57Bl/6 infected with *Plasmodium berghei* ANKA and treated with rNcROP4 or PBS (control) on day three and four after infection. Our results showed IL-10 up-regulation in rNcROP4 treated mice and infected with *T. gondii* both in the peritoneal fluid and in the gut (jejunum and ileum). IFN γ production was higher in STAg and PBS treated mice. rNcROP4 and STAg treated mice exhibited lower ileum damage mainly in BALB/c mice. Animals infected with *P. berghei* ANKA had lower morbidity and delayed in mortality when compared with control group. These results showed the anti-inflammatory properties of rNcROP4 with IL-10 up-regulation in acute *T. gondii*-induced ileitis and the capacity of delaying mortality in malaria experimental murine. **Supported by:**CAPES, CNPq and FAPEMIG **Keywords:**Ncrop4; toxoplasmosis; malaria

HP155 - MOLECULAR AND ULTRAESTRUTURAL CHARACTERIZATION OF ENDOPLASMIC RETICULUM STRESS ON *TRYPANOSOMA CRUZI*
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The endoplasmic reticulum (ER) is vital to mammalian cell survival. Adverse conditions in ER lead to organelle stress and trigger the unfolded protein response (UPR), which increases the expression of chaperones such as BiP and calreticulin (CTR), reduces protein synthesis leading to the degradation of unfolded proteins. Under persistent stress UPR lead to cell death. Despite its importance, studies on ER in *Trypanosoma cruzi* are scarce. This work aimed to assess the effects of tunicamycin (TM) and dithiothreitol (DTT) a well-known ER stressors on *T. cruzi*. Epimastigotes were incubated in the absence or presence of different concentrations of TM or DTT and cell growth was determined by cell counting. To assess whether TM or DTT was able to change the expression of Bip and CRT, western blotting and RT-PCR were performed. We used annexin V/PI to assess if long-term drug treatment induced cell death, and Rho123 and MitoSox to evaluate the effect of the drugs on parasite mitochondrion. DTT inhibited the cell growth, in dose dependent manner. DTT did not alter the BiP levels but significantly reduced the mRNA of BiP and calreticulin (CRT), suggesting that UPR in *T. cruzi* differs to those found in higher eukaryotes. DTT-induced persistent stress caused drastic morphological and physiological changes compatible with cell death. Conversely, TM showed strong trypanostatic effect, with no cell growth recovery after drug removal. The TM was also unable to change the BiP protein levels, but increased BiP and CRT mRNA levels. TM induced mitochondrial depolarization without a marked increase in the ROS and only 10% of treated-cells showed apoptotic phenotype. Ultrastructural changes compatible with autophagy were observed. Our results suggest that the DTT treatment compromises the whole cell function rather than acts as a specific inductor of ER stress. Conversely, The TM acts more specifically on the ER, triggering autophagy preventing the cell death. **Supported by:**CNPQ, FACEPE, CAPES, FIOCRUZ
Keywords:Trypanosoma cruzi; endoplasmic reticulum; cell death

HP156 - INFLAMMATORY PROFILE OF ORAL INFECTION WITH NEOSPORA CANINUM IN C57BL/6 MICE

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Neospora caninum is an obligate intracellular parasite with worldwide distribution, that causes abortions in livestock and over a billion dollars in economic losses annually. However, the mechanisms involved in this host-parasite relation remains unclear. Experimental models that assess host responses against the parasite are usually based on parenteral inoculations, but it is fundamental to understand the mechanisms involved in infection route. Thus, we aimed to evaluate the inflammatory profile and antibody production in mice orally infected with *N. caninum* (Nc-1 strain). Thus, C57BL/6 mice were orally infected by gavage with 3x10⁷ live tachyzoites, and were euthanized at 7, 14, 21 and 28 days post-infection (d.p.i.) to collect brains, lungs, livers and gut sections for histological analysis, parasite DNA quantification and specific IgG (IgG1 and IgG2) antibody detection. Histological analysis revealed a severe diffuse inflammation in livers (7 d.p.i.), lungs (14 d.p.i.) and central nervous system (21 d.p.i.). High parasitism was observed in the end portions of the gut (distal jejunum and ileum), while was not presenting any pathological changes. Liver and lung showed higher parasitism 7 and 14 d.p.i. same results of histology analysis. In the central nervous system was observed high parasitism 14 and 21 d.p.i. resembling the inflammatory process. We also observed crescent levels of specific IgG in the serum samples obtained during the infection, which were mainly composed of IgG2 antibodies, while IgG1 was lowest, differently of that usually observed in parenteral protocols. In that sense, we can infer that the experimental model proposed presents a promising tool for the study of the immunopathogenesis of neosporosis.

Supported by:CAPES, CNPq, FAPEMIG
Keywords:Neospora caninum; oral infection; neosporosis

HP157 - AGE ENHANCES ADVERSE REACTION TO ANTIMONY BUT DO NOT IMPAIR THERAPEUTIC RESPONSE IN CUTANEOUS LEISHMANIASIS

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Cutaneous leishmaniasis (CL) caused by *Leishmania Brasiliensis* is the main clinical form of American tegumentary leishmaniasis (ATL), observed mainly in male young adults. There is a lack of information about the disease in patients with age over 60 years. Here we compare the clinical presentation, frequency and severity of adverse reactions to antimony, and response to antimony therapy in elderly and young patients. Of the 66 participants 32 were elderly and 34 young, all were recruited in Corte de Pedra, Bahia. The skin test with soluble leishmania antigen was performed and a biopsy was obtained to identify parasite DNA by polymerase chain reaction technique. Patients were classified in two groups being the young patients with age range from 18 to 40 years, and old patients with age range from 60 to 75 years for women and 65 to 80 years for men. The inclusion criteria were presence of a classical CL ulcer, illness duration less than 60 days and a diagnosis of *L. Brasiliensis* determined by PCR. Exclusion criteria were patients with mucosal lesion, patients infected with HIV, diabetes and patients with kidney, heart or liver failure. All patients were treated with glucantime (Sanofi Aventis) in a dose of 20mg/Kg/weight for 20 days. Blood was obtained for laboratory tests on day 0 and 30 of therapy and electrocardiogram was also performed on day 0 and 30 of therapy. During therapy both hematocrit and hemoglobin levels decrease in the elderly and there was an enhancement in the creatinine levels in this group of patients. While ECG changes were observed in only 10% of the young patients it occurred in 90% of the elderly population. Elderly patients had less lymphadenopathy enlargement was no difference between the groups in the clinical presentation except for absence of lymphadenopathy that was more frequent in old patients. There was no difference regarding the percentage of failure to antimony but adverse reactions were much more frequent in the elderly group. **Supported by:**CNPq / NIH

Keywords:Cutaneous leishmaniasis; adverse reactions; age

HP158 - *LEISHMANIA BRASILIENSIS* AMASTIGOTES INDUCE THE EXPRESSION OF TNF- α AND IL-10 BY HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS IN A TLR4-DEPENDENT MANNER

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Expression of human Toll-like receptors (TLRs) such as TLR2, TLR4 and TLR9 has been demonstrated in lesions of Leishmaniasis patients. However, the role of TLRs in infections caused by *Leishmania Brasiliensis* is still unclear. The aim of this study was to evaluate whether TLR4 mediates cytokine production induced by amastigotes of *L. Brasiliensis* in human peripheral blood mononuclear cells (PBMCs) and whether TLR4 expression is altered in monocytes after interaction with parasites. Amastigote forms were obtained from mice infected with *L. Brasiliensis* (MHOM/BR/2003/IMG) and isolated by Percoll gradient. PBMCs were isolated from healthy donors and were cultured in absence or presence of rIFN- γ , TLR4 neutralizing antibodies, natural antagonist of TLR4 (*Bartonella* LPS), TLR4 agonist (*E. coli* Lipopolysaccharide - LPS) or amastigote forms. The concentrations of tumor necrosis factor (TNF- α) and interleukin 10 (IL-10) were assayed by ELISA and TLR4 expression by flow cytometry. Amastigote forms of *L. Brasiliensis* induced TNF- α and IL-10 production only in IFN- γ -primed PBMCs. TNF- α and IL-10 production was inhibited by TLR4 neutralization, both with anti-TLR4 and the antagonist *Bartonella* LPS. Interestingly, addition of *E. coli* LPS further increased TNF- α induced by *L. Brasiliensis* amastigotes, but not the IL-10 production. Amastigotes of *L. Brasiliensis* strongly reduced membrane TLR4 expression on monocytes/macrophages, apparently by internalization after the infection. Data show that *L. Brasiliensis* amastigotes can trigger TLR4 to induce TNF- α and IL-10 production, besides decreasing TLR4 expression in monocytes. Results suggest that *L. Brasiliensis* amastigotes use TLR4 internalization for infection or cytokine induction. Understanding the pathogenesis of *L. Brasiliensis* can contribute to new strategies for American Tegumentary Leishmaniasis (ATL) therapies, especially for ATL caused by *L. Brasiliensis*, the most relevant species in BRA and countries of South America. **Supported by:**CNPQ, CAPES E FAPEG. **Keywords:**Leishmania Brasiliensis; tlr4; cytokines

HP159 - TRANSGENIC MICE FOR HUMAN IL-32 γ ARE MORE RESISTANT TO INFECTION BY LEISHMANIA BRASILIENSIS THAN WILD TYPE MICE

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Interleukin 32 (IL-32) is a proinflammatory cytokine that is present in Leishmaniasis lesions. There are nine isoforms of IL-32, but only IL-32 γ is induced by *L. Brasiliensis*. Due to the absence of a homologous gene for IL-32 in rodents, transgenic mice for IL-32 γ human gene was used to investigate the role of this cytokine in *L. Brasiliensis* infection *in vivo*. C57BL/6 wild-type (WT) and IL-32 γ transgenic mice (IL-32 γ Tg) were infected with *L. Brasiliensis* (MHOM/BR/2003/IMG) promastigotes in stationary phase (10^5) and the ear thickness was monitored weekly for 9 weeks. The lesion size was lower in IL-32 γ Tg mice from the sixth until the ninth week post-infection than in WT mice. The tissue parasitism was determined by limiting dilution method and results demonstrated that IL-32 γ was able to reduce parasitism from the sixth week post infection. To examine the role of IL-32 γ on the profile of immune response during infection with *L. Brasiliensis*, draining lymph node cells from infected mice were stimulated with *L. Brasiliensis* antigen and the cytokines were assessed by ELISA. Data showed a higher IFN- γ production by lymph node cells of IL-32 γ Tg mice than WT mice (1, 3 and 6 weeks after infection). TNF- α production was increased in IL-32 γ Tg group on the 6th week of infection. In addition, it was also observed an increase in IL-10 production by IL-32 γ Tg mice (1, 6 and 9 weeks post infection). Bone marrow-derived macrophages (BMDMs) from WT or IL-32 γ Tg mice were infected with *L. Brasiliensis* promastigotes in the presence of IFN- γ . Production of NO was measured by Griess method (48 h). BMDMs of IL-32 γ Tg mice were more efficient in controlling parasite burden. This could be mediated by the highest level of NO produced by IL-32 γ Tg macrophages. Together, these data suggest that IL-32 γ is important for the control of *L. Brasiliensis* infection by the improvement of immune response mediated by IFN- γ /TNF- α /NO axis. **Supported by:**CNPQ, CAPES E FAPEG.

Keywords:Leishmania Brasiliensis; interleukin 32 (il-32); immune response

HP160 - PS EXPOSURE BY PLASMODIUM CHABAUDI IS ESSENTIAL FOR A SUCCESSFUL INFECTION

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Malaria is one of the most prominent parasitic diseases in tropical countries. Studying human malaria implies some adversities, thus, the use of experimental models has contributed deeply to the comprehension of the parasite biology and of the disease itself. Plasmodium chabaudi infection of mice is one of the most similar models to the human disease caused by *P. falciparum*. Nitric oxide is an inflammatory mediator with microbicidal effect produced by activated macrophages. An evasive mechanism that inhibits nitric oxide production by macrophages was demonstrated in others protozoan parasites. Such mechanism consists in the exposure of phosphatidylserine (PS), a phospholipid present in the plasma membrane that is usually concentrated at the inner leaflet of the lipid bilayer of live cells. However, PS is translocated to the outer leaflet of the plasma membrane being the major binder involved in the recognition of apoptotic cells and responsible for the anti-inflammatory response cause by apoptotic cells uptake. PS exposure by these protozoa consists in an evasive mechanism entitled "apoptotic mimicry". To verify if this mechanism occurs in *P. chabaudi*, this parasite was purified from mice blood cells and PS exposure analyzed. About 90% of the *P. chabaudi* erythrocytic population exposed PS. These parasites were able to completely inhibit nitric oxide production after interaction with activated macrophages. Infection caused cytoplasmic retraction and formation of blebs of infected macrophages. Purified parasites exposing PS were able to induce high parasitaemia and death of infected mice. However, mice infected with parasites that PS was blocked with annexine-V presented no parasitaemia and did not die. Our results suggest that *P. chabaudi* performs "apoptotic mimicry", reinforcing the hypothesis that such mechanism is commonly present in many protozoa parasite, suggesting that PS exposure hold a crucial role in the parasite infective process and disease generation. **Supported by:**FAPERJ

Keywords:Plasmodium chabaudi; phosphatidylserine; nitric oxide

HP161 - **GENERATION OF MOLECULAR TOOLS FOR GENOMIC EDITING OF *NEOSPORA CANINUM***

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The development of molecular genetics has greatly enhanced the study of the biology and pathology associated with parasites of phylum Apicomplexa. We have established a system specifically designed for *Neospora caninum*, used as a heterologous system for the expression of foreign genes. Plasmid constructs containing proteins from *Toxoplasma gondii* driven by *N. caninum* promoters were incorporated into *N. caninum* genome, as confirmed correctly targeted by immunofluorescence localization and Western blot. Therefore, these results showed a simple and stable proteins expression system. In addition, heterologous gene expression should be useful for studying the function of specific gene products and may allow the identification of genes responsible for the phenotypic differences observed between these two closely related apicomplexan parasites. Here, we demonstrate that *N. caninum* expressing *T. gondii* type I Rop16 kinase is biologically active and induces an immunological phenotype compatible with the originating parasite. *N. caninum* Rop16+ tachyzoites induced host STAT3 phosphorylation and reduction of IL-12 synthesis. These studies indicate that *Neospora* can be used as a model for the heterologous expression of genes, with the intent of observing targeting and function of *T. gondii* proteins. Those observations may prove to be useful for identifying and characterizing genes involved in the pathogenesis associated with toxoplasmosis and developing a safe vaccine to control toxoplasmosis and/or neosporosis.

Supported by:FAPEMIG, CNPq, CAPES

Keywords:*Neospora caninum*; molecular genetics; *Toxoplasma gondii*

HP162 - **GENE EXPRESSION ALTERATIONS OF *ONCOPELTUS FASCIATUS* (HEMIPTERA, LYGAEIDAE) SPECIES INFECTED BY *LEPTOMONAS WALLACEI* (TRYPANOSOMATIDAE)**

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The milkweed bug, *Oncopeltus fasciatus* (Hemiptera, Lygaeidae), is usually found naturally infected by trypanosomatid *Leptomonas wallacei*. Promastigotes forms infect the third and fourth ventricles of the host midgut. These forms are free in the midgut lumen or attached to the perimicrovillar membrane, that covers the epithelial microvilli wall; in the hindgut, the parasites are also observed in cyst-like forms. Uninfected insects are larger in size, have larger wings and appendages, are less deformed, copulate more and have a larger offspring, and live longer. In infected females, egg reabsorption on ovaries is usual. In that sense, the intersex gene seems to be of great interest, being associated with the development of this insect reproductive system. The aim of this study is to investigate if the presence of *L. wallacei* in the digestive tract alters intersex expression in the host *O. fasciatus*. For the two colonies (infected and not infected) fifth instar nymphs of both sexes (n = 12) were separated until they reach adult stage and sexual maturity (~14 days). After this period, ovaries and testicles were dissected, photographed for morphological studies and had their RNA extracted. A quantitative real-time PCR was performed using two pairs of primers, the constitutive gene *Eif3* and the gene of interest intersex (*ix*). Preliminary data showed that the ovaries of infected females show a decrease of 42.43% (± 16.42) in *ix* gene expression compared to uninfected females, while infected males showed an increase of 41.76% (± 30.87) in gene expression comparison. Preliminary studies have shown that the silencing of *ix* in *Oncopeltus* females generates an external morphology similar to the male. Therefore, the underexpression of *ix* gene in infected females may have a direct correlation in the reduction of sexual activity, oviposition and offspring, as well as in the reabsorption of eggs.

Supported by:FAPERJ, CNPq, Instituto Nacional de Ciências e Tecnologia em Entomologia Molecular (INCT-EM), CAPES **Keywords:**Reproductive system; quantitative pcr; milkweed bug

HP163 - **COMPARISON OF THE GP63 PROFILE BETWEEN LEISHMANIA (VIANNIA) BRASILIENSIS AND L. (LEISHMANIA) AMAZONENSIS**
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The protozoan parasites of *Leishmania* genus infects millions of humans. The parasite has a digenetic life cycle and is transmitted to humans by infected hematophagous female sand flies. In BRA, cutaneous lesions are related with *Leishmania* (*Viannia*) *Brasiliensis* and *L. (Leishmania) amazonensis*. These species presents adaptive mechanisms guided by their proteases, as zinc metalloprotease GP63 abundant on the promastigote surface. The GP63 is a pathogenicity factor that cleave host proteins, enabling the *Leishmania* sp to subvert processes such as transcription and translation. The goal of this work is verify the profile GP63 from exosomes and membrane of promastigotes, as well as, propose the cellular location of GP63 by in silico approaches and the quantitation of the respective genes, in *L. (V.) Brasiliensis* and *L. (L.) amazonensis* species. The proteinase activity assays were performed using fractions of promastigote supernatant enriched or not by treatment of parasites with phospholipase C. Then, these samples were processed by mass spectrometry to identify GP63. For in silico assays, the sequences were quantified from the survey in Genedb and then were submitted to predict its location. The proteolytic profiles of GP63 *L. (V.) Brasiliensis* and *L. (L.) amazonensis* present similar in both parasites. We have evidence that GP63 linked by anchors in *L. (V.) Brasiliensis* have higher diversity compared to *L. (L.) amazonensis*. This difference is also demonstrated the quantification of GP63 gene between both species assayed. Furthermore, topped was able to identify the location of the GP63 on the membrane surface and as excreted factor. Collectively the results indicate that promastigote of *L. (L.) amazonensis* and *L. V. Brasiliensis* exhibit a differentiated profile of GP63 in exosomes and on the promastigotes surface. Probably, this difference may represent an important factor of adaption. **Supported by:** Capes

Keywords: *Leishmania* (*L.*) *amazonensis*; *leishmania* (*v.*) *Brasiliensis*; metalloprotease gp63

HP164 - **IMMUNOMODULATORY EFFECT OF *AMBLYOMMA SCULPTUM* SALIVA DURING *TOXOPLASMA GONDII* INFECTION**

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The pathogenicity of *Toxoplasma gondii* varies according to the specie of host infected and any warm-blooded animal can serve as an intermediate host for the parasite. *Amblyomma sculptum* has a complex molecular saliva composition in order to maintain high levels of infestation, being capable of suppress Th1 type cytokines, while increasing anti-inflammatory cytokines. Saliva was pooled from engorged female ticks and its immunomodulatory effects were tested in mice during infection with *T. gondii*. Increased susceptibility of mice exposed to saliva was assessed by survival analysis, histopathology of target organs, parasite burden, cytokines quantification and specific quantification of total IgG. Mice exposed to *A. sculptum* saliva had worse clinical condition as a result of *T. gondii* infection due to a low percent of survival and areas of necrosis in liver. Parasitism in lungs was higher in comparison with mice not exposed to saliva. Additionally, saliva exposure induce higher production of IL-6 while reduce TNF production in peritoneal fluid. Surprisingly, there was no difference in production of IL-10 and IFN- γ in peritoneal lavage and liver and lungs homogenate, otherwise during antigenic recall, spleen cells derived from saliva exposed mice increased levels of IL-10 and reduce IFN- γ production, after stimulation with *T. gondii* tachyzoites. No significant differences were obtained in specific antibody production of *T. gondii*. The exposure to *A. sculptum* saliva increases susceptibility of mice infected with *T. gondii*, by an organ-specific immune response, thus enhancing replication capacity of protozoan during an earlier acute of infection. **Supported by:** CNPq, CAPES e FAPEMIG

Keywords: *Amblyomma sculptum*; *toxoplasma gondii*; immune effect

HP165 - A NOVEL EVASION STRATEGY OF HOST CELL DEFENSE BY LEISHMANIA:
INDUCTION OF THE PERK/eIF2ALPHA/ATF-4 AXIS.
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The Integrated Endoplasmic Reticulum Stress Response (IERSR) ensures cellular survival under ER stress. The PKR-like ER kinase (PERK) / eIF2a phosphorylation/ activating transcription factor 4 (ATF4) axis suppresses translation initiation to reduce the demand on protein folding while inducing the expression of genes required for restoring ER-homeostasis. ATF-4 also induces expression of genes that play critical roles in resistance to oxidative stress. In this work we sought to determine the role PERK/eIF2alpha/ATF-4 axis of IERSR in macrophage infection by *L. amazonensis*. Our studies demonstrate that *L. amazonensis* activates PERK/eIF2alpha/ATF4 arm of the IERSR. Lentiviral silencing of either PERK or ATF4 expression in macrophages impaired intracellular *L. amazonensis* growth. In CHIP assays, *L. amazonensis* infection induces the binding of ATF-4 in HO-1 promoter and knocking down PERK or ATF4 expression reduces the translocation of the NRF2 transcription factor and the expression of antioxidant genes, such as heme oxygenase (HO1), and increases Nitric Oxide (NO) concentration in infected cells. These data indicate that PERK/eIF2/ATF-4 axis protects parasites from oxidative stress in infected cells. Consistently, addition of anti-oxidants to PERK or ATF-4 knockdown cells restores *L. amazonensis* infection to wild type level. Importantly *L. Brasiliensis* infected lesions from human patients show a dramatic increase in ATF-4 mRNA levels. These data were corroborated in IH analysis of skin biopsies from human patients. Impressive, same regions showed an increase in dsRNA stained, suggesting viral load. We conclude that that *L. amazonensis* induces IERSR in macrophages which may play a role in the pathogenesis. **Supported by:**CNPq
Keywords:Er stress; leishmania; oxidative stress