HP17 - **HISTONE DEACETYLASE INHIBITORS AS AN ALTENATIVE FOR THE TREATMENT OF TOXOPLASMOSIS**

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Toxoplasmosis is a cosmopolitan zoonosis, caused by the obligate intracellular protozoa *Toxoplasma gondii*. *T. gondii* infection can cause uveitis, congenital diseases and encephalitis in immunocompromised individuals. The treatment for toxoplasmosis is restricted to few drugs, which are commonly related to several side effects. Besides, the available drugs are only effective against the acute stage of the disease and do not promote the parasitological cure to the chronic stage of the disease. Thus, the discovery of new effective treatments for toxoplasmosis is necessary. Recent studies show that the histone deacetylase inhibitors are potential chemotherapeutic agents for the treatment of parasitic infections. In this work, we evaluated the anti-*T. gondii* effects of three histone deacetylase inhibitors, Tubastatin A (TST), Suberoylanilide Hydroxamic Acid (SAHA), Trichostatin A (TSA) *in vitro*. For that, epitelial LLC-MK_2 monolayers were infected with tachyzoites of *T. gondii*, RH strain, and then treated with different concentrations of the compounds for 24 or 48 hours. Two of the three compounds presented high activity against *T. gondii* resulting in IC_{50} values at nanomolar range. IC50 values after 24h or 48h of treatment with TST were 20 ± 0,06 nM and 734 ± 160 nM, respectively, while with SAHA were 40 ± 0,03 nM and 67 ± 36 nM, respectively. Cytotoxicity studies in LLC-MK_2, HFF (Human Forskin Fibroblast) and i.p mice macrophages using MTS assay showed that TST and SAHA have high selectivity for *T. gondii*, although TST showed lower toxicity to host cells with TD_{50} values > 10μM. Transmission electron microscopy analysis of the cellular effects of TST and SAHA showed that the treatment after 24 hours affected the process of individualization of *T. gondii* daughter cells and induced the disorganization of parasite organelles. These results indicate that TST and SAHA are promising compounds for the treatment of toxoplasmosis. **Supported by:**CNPq, Capes and Faperj **Keywords:** Histone deacetylase; hydroxamic acid; chemotherapy

HP18 - **USE OF HAMSTER (*MESOCRICETUS AURATUS*) AS A MODEL TO UNDERSTAND SPLEEN DISORGANIZATION IN INFECTION BY *LEISHMANIA INFANTUM*.**

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Spleen is involved in the course of visceral leishmaniasis. Splenomegaly and histological changes as lymphoid follicle hyperplasia followed by atrophy, depletion of leukocytes populations of the white pulp compartments, and red pulp hypercellurarity are all observed. To study white pulp disruption in visceral leishmaniasis, an experimental model was defined to study sequential alterations in the spleen. Fifty-six Golden Syrian hamsters were used in this experiment: 28 were infected with 2x107 stationary phase Leishmania infantum and the 28 controls where injected with saline solution. Seven hamsters of each group were euthanized at 30, 60, 120 and 150 days post infection. Emaciation was more frequent in the infected than in the uninfected animals after 120 days post infection (p=0,04) and after 150 days post infection the number of clinical signs of disease was higher in the infected (7/7) than in the uninfected animals group (0/7) (p=0,002). Splenomegaly was more frequent in infected than in uninfected animals at 120 (p<0,0001) and 150 days post infection (p=0,006). Five out of seven animals of the infected group and 1/7 animals of control group had moderate to intense disorganization in the spleen white pulp 150 (p=0,03) days post infection. Morphometry study revealed a decreased of the white pulp/red pulp ratio in the animals of the infected group in comparison with the uninfected animals on 120 days of follow-up (p=0,008). The experimental model of visceral leishmaniasis in hamsters reproduces the spleen changes observed in dogs and humans. We are now preforming, the transcriptomic analysis of the spleen of these animals aiming potential signaling pathways and differentially expressed molecules, which may be related to spleen disruption. **Supported by:**Capes, Fapesb, CNPQ **Keywords:** Visceral leishmaniasis; animal model; spleen disruption

HP19 - **EVALUATION OF PARASITE LOAD AFTER USE OF PGE2 RECEPTOR AGONISTS IN SPLEEN CELLS OF DOGS NATURALLY INFECTED WITH VISCERAL LEISHMANIASIS**

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Introduction: Canine visceral leishmaniasis (CVL) is caused by the intracellular parasite Leishmania infantum. Prostaglandin-E2 (PGE-2) has potent regulatory properties of the immune system, and can bind to EP1, EP2 and EP4 receptors that generate cellular activation or EP3 that generates inhibition of cellular responses. The regulatory role of PGE2 has not yet been studied CVL, so this study aimed to evaluate the PGE2 receptors in the spleen of healthy dogs and dogs naturally infected with visceral leishmaniasis (VL), and the parasite load on the spleen of dogs naturally infected with VL. Methods and Results: 20 dogs with seropositive VL were selected by indirect ELISA. The control group consisted of 13 healthy dogs, all seronegative for VL by indirect ELISA. Evaluation of the expression of EP1, EP2, EP3 and EP4 receptors on spleen leukocytes was performed by flow cytometry using polyclonal antibodies, EP1, EP2, EP3 and EP4 (Santa Cruz Biothecnology™), and (PE) conjugated to secondary antibody (Santa Cruz Biothecnology™), at the dilution (1:100) the respective control isotypes. Quantification of the parasite in the cell culture was performed by flow cytometry, where the spleen cells of LV dogs were incubated with PGE2 receptors agonists, COX-1 and COX-2 inhibitors and PGE2. The statistical test used to evaluate the receptors between the groups was Mann-Whitney, for the parasitic load the Friedman test was used. The EP2 receptor decreased in total leukocytes of the spleen of the infected group when compared to the healthy group. The parasite load decreased in the presence of the EP3 agonist, the COX-1 inhibitor (AH-6809) and in the presence of PGE2 in spleen leukocyte from dogs naturally infected with VL. Conclusion: Leishmania infection is modulating EP2 receptor expression and the signaling by PGE-2 can regulate parasite load in leukocytes of the spleen of dogs naturally infected with VL. **Supported by:**FAPESP **Keywords:** Leishmaniasis; dog ; prostaglandin e2

HP20 - **FABP4 AND PPAR-GAMMA INVOLVEMENT IN *L. (L.) AMAZONENSIS*-INFECTED MACROPHAGES**

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Amastigote forms of *Leishmania* reside in parasitophorous vacuoles (PV) of mononuclear phagocytic cells. Currently, little is known about the molecular mechanisms controlling *Leishmania* metabolism in these organelles, concerning both biosynthesis of macromolecules and the metabolic control of the host cell by the parasite itself [McConville et al., 2015; doi: 10.12688/f1000research.6724.1]. Our previous transcriptomic analysis showed that FABP4 (fatty acid binding protein 4) transcript levels are increased in macrophages infected with *L. (L.) amazonensis* after 48h. FABPs are small intracellular proteins that access the cell nucleus under certain physiological conditions, functioning as transporters of fatty acids that modulate transcription factors, such as the peroxisome proliferator-activated receptor [Boss et al., 2015; doi:10.1016/j.atherosclerosis.2015.03.042]. Based on the FABP4 biological properties, in addition to the fact that amastigotes are dependent on fatty acid uptake for amino acid biosynthesis, it is reasonable to postulate that macrophagic FABP4 may play an important role in lipid homeostasis during *Leishmania* infection. In this study, we aim to investigate role of FABP4 and PPAR-gamma involvement in the progression of *L. (L.) amazonensis* in vitro infections. Our results indicate that PPARgKO macrophages are unable to sustain amastigote proliferation for up to 96h when compared to WT macrophages. Also, infected cells were incubated with a selective FABP4 inhibitor at 20 µM and no differences were observed for treated vs. untreated PPARgKO macrophages. Additionally, PV area was quantified and WT cells exhibited larger PVs with increased number of parasites when compared to PPARgKO macrophages. Further experiments are being conducted in order to determine FABP4 abundance in the presence and absence of macrophagic PPARg. Our findings will allow the understanding of FABP4/PPARg interplay in the

development of macrophage infection by *Leishmania*. **Supported by:**FAPESP **Keywords:** Leishmania; macrophage ; ppargamma

HP21 - **EVALUATION OF TWO MESOIONIC SALTS DERIVED FROM 1, 3, 4 TIADIAZOLIUM ON *LEISHMANIA AMAZONENSIS IN VITRO***

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Leishmaniases are a group of diseases caused by protozoan parasites of the genus *Leishmania*. The clinical manifestation varies according to *Leishmania* species and host immune response. The current treatment have high toxicity, cost and cases of resistance have been reported. For these reasons the search for new substances for the treatment of these diseases is intense, in order to find new compounds more efficient and safe. Mesoionic compounds are widely studied molecules with anti-inflammatory, antioxidant, antibacterial, antifungal, anticancer and leishmanicidal action. The aim of this work was to analyze the cytotoxic effects of the mesoionic compounds 4-phenyl-5-(X-phenyl)-1,3,4-thiadiazolium-2-phenylamine (X=4Cl (MI-4Cl); 3,4 diCl (MI-3,4diCl) on promastigotes and amastigotes forms of *Leishmania amazonensis in vitro*. First, promastigotes forms were incubated with different concentrations of MI-4Cl and MI-3,4diCl for 72h and parasite growth were analyzed by counting. The results showed that MI-3,4diCl at 50 and 25 µM inhibited 100% and 70%, respectively, of parasite growth at 48h. MI-4Cl at 50 µM inhibited 86% at 72h of parasite growth compared to control. IC50 values of MI-4Cl and MI-3,4diCl at 72h are 37.8 and 18.4 µM, respectively. In addition, promastigote mitochondrial viability was evaluated by XTT assay. The data demonstrated that MI-4Cl and MI-3,4diCl inhibit around 50% of parasite mitochondrial viability after 48 h. The cytotoxic effect of the mesoionic salts to the host cell, tested by XTT assay, demonstrated that up to 100 µM of MI-4Cl and MI-3,4diCl, were not toxic to macrophages. Amastigote infected macrophages were treated with 50 and 25 µM the salts for 48 h. Preliminary results showed that both compounds at 50uM inhibited around 50% of amastigotes survival inside macrophages compared to control. These results suggest that these compounds could be promising candidates against *Leishmania amazonensis*. **Supported by:**CNPq **Keywords:** Leishmaniasis; mesoionic salts; treatment

HP22 - **ABSTENCE OF REACTIVE OXYGEN SPECIES PROMOTES RESISTANCE TO *TOXOPLASMA GONDII* INFECTION IN MICE**

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Background: *T. gondii* is an obligate intracellular parasite that infects several types of nucleated cells, especially those related to innate immune response, such as macrophages and dendritic cells. By oral infection, long-lived and non-immunogenic cysts containing bradyzoites can spread throughout the body and can infect multiple organs and tissues, such as the brain and muscles. The parasite survives intracellularly exposed to antimicrobial mechanisms, such as nitric oxide (NO), secretion of pro-inflammatory cytokines and production of reactive oxygen species (ROS). Objective: Several studies try to elucidate the importance of ROS in the control of this infection, but its role in eliminating parasites and host survival is still not understood. So our goal is evaluate the role of ROS during infection. Methods and Results: To understand the role of ROS in *T. gondii* infection, we used mice deficient in the production of ROS via NADPH oxidase (Phox KO) and evaluated its effects *in vivo* and *in vitro* infections. *In vivo* experiments, Phox KO and WT mice were infected with *T. gondii* cysts and we analyzed the weight loss; parasitic load on brain, liver and intestine; and cytokine production during 7 and 15 days of infection. *In vitro* experiments, Phox KO and WT macrophages were infected with tachyzoites and the infection index was evaluated. We observed that from 9th day of infection, WT mice had marked weight loss compared to Phox KO mice, in addition to increased production of NO in the blood and production of IFN-γ. However, we did not observe differences in parasite load between groups. *In vitro*, we did not observed difference in infection index between WT and Phox KO macrophages infected with *T. gondii*. Conclusion: Our results indicate that despite both groups presents the same parasite load, Phox KO mice seems to be more resistant to *T. gondii* infection. However, more experiments are needed to establish the role of ROS during *T. gondii* infection. **Supported by:**FAPEMIG, CAPES e CNPq **Keywords:** T. gondii; ros; phox ko

HP23 - **MICROSCOPY-BASED OBSERVATIONS OF TRYPANOSOMA CRUZI EGRESS**

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Trypanosoma cruzi is the causative agent of Chagas' disease. T. cruzi trypomastigotes infect host cells and differentiate into intracellular amastigotes. After replication, they differentiate into bloodstream trypomastigotes that disrupt host cell accessing the extracellular medium. Several studies addressed cell invasion by T. cruzi but less is known about their egress. Some researches demonstrate that, similar to other intracellular pathogens, disruptions occur in the three cytoskeleton-forming filaments during parasite development. Others suggest that calcium influx may occur due to increased cell permeability, promoting release of parasite proteases. Some reports demonstrate that egress mediated by host cell apoptosis may favor maintenance of infection, although the role of cell death is controversial. Using confocal microscopy of living cells and later prepared for scanning electron microscopy (correlative), we investigated some aspects of T. cruzi egress. Our results showed that parasite egress is a sudden event; cells about to disrupt maintain membrane integrity until parasites are release, and promptly after parasite egress little remain of host cell structures. In addition, there are apparent actin cytoskeleton modifications from normal F-actin meshwork in non-infected cells to "circular-shaped" F-actin rings that seem to initiate in cells infected with amastigotes becoming more evident in cells containing trypomastigotes, indicating that egress events may initiate when parasites are still on replicative stages. We also observed that in some egress events the release of amastigote forms showing that premature lysis may be related to the origin of infective extracellular amastigotes. Finally, in experiments using a protocol to remove membrane components but preserving host cell actin cytoskeleton we observed that TCTs infected cells present disorganized actin cytoskeleton and in some cases actin seems to be disrupted next to intracellular parasites. **Supported by:**Fundação de Amparo a Pesquisa do Estado de São Paulo-FAPESP **Keywords:** Trypanosoma cruzi; egress; live cell imaging

HP24 - **FUNCTIONAL GENOMICS FOR THE VALIDATION OF PUTATIVE MOLECULAR TARGETS OF PLATINUM AND PALLADIUM-BASED COMPOUNDS SYNTHETIZED AS ANTICHAGASIC AGENTS**

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For more than 50 years, Chagas' disease treatment has been based on two broad spectrum drugs: Nifurtimox and Benznidazol. Nevertheless, the effectiveness of these drugs in chronic patients is limited and several side effects are produced due to its toxicity. In this context, a new organometallic platinum compound, Pt-dppf-mpo, has been synthesized and characterized as anti-*T. cruzi* agent. Morphological changes in *T. cruzi* epimastigotes are observed after treatment and a mitochondrial dysfunction with a collapse of the mitochondrial membrane potential is induced. These events suggest that Pt-dppf-mpo incubation leads to cell necrosis with no evidence of early apoptotic-like markers. Moreover, we analyzed global changes in transcriptome and proteome of parasites incubated with the complex aiming to identify affected pathways and further characterize its mode of action. Currently, several proteins are being studied as molecular targets to get a deeper knowledge of the mechanism of action of a promising antitrypanosomal compound. **Supported by:**PEDECIBA; CSIC; ANII **Keywords:** Antichagasic agents; platinum and palladium-based compounds; molecular targets

HP25 - **IDENTIFICATION OF POSSIBLE TARGETS OF 1.8 CINEOL, ALPHA-PINENE AND P-CYMENE IN LEISHMANIA AMAZONENSIS IN VITRO.**

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Leishmaniasis are neglected diseases that affect more than 12 million people around the world caused by parasites from the genus Leishmania. Leishmania amazonensis is the causative agent of cutaneous and diffuse cutaneous leishmaniasis in the Americas. The current treatments used are highly toxic, expensive and the appearance of resistant parasites has stimulated the search for new substances with antileishmanial activity, including the prospecting of natural products. Previously, we demonstrated that the three major components isolated of the essential oil (EO) from Burseracea: 1.8 cineol, alpha-pinene and p-cymene were toxic for amastigotes inside macrophages in a dose-dependent manner and it is not modulated by NO production in macrophages. In this work, in order to confirm the anti-amastigote activity of the compounds, infected-macrophages were treated with 50 µg/mL of each one of the compounds and parasite load in vitro was evaluated by ability of amastigotes-promastigotes transformation. The results showed that parasite load reduced around 60% in relation to control for all compounds. The phagocytosis ability of macrophages in vitro was not affected by them. Lipid analysis of treated promastigotes with 50µg/mL of the compounds showed that 1.8 cineol decreased 50% of fatty acid and 1.3 diacylglycerol (DAG) content, and 36 % of reduction was observed in sterol 1.2 DAG compared to control. A tendency of reduction was observed in triacylglycerol (TAG) on treated parasites. Alpha-pinene treated promastigotes presented an increased in TAG compared to control, while p-cymene treatment did not affect parasite lipidic composition. These results suggest a negative modulation by 1,8 cineol in key enzymes involved in acylated lipids and sterol biosynthesis pathways. As our next step, we intend to evaluate different enzymes involved in these routes through Real-Time PCR to identify a possible target of this compound. **Supported by:**CAPES, FAPERJ **Keywords:** leishmaniasis; essentials oils; burseracea

HP26 - **EVALUATION OF HUMAN PERIPHERAL BLOOD FIBROCYTE INFECTION BY *LEISHMANIA (L.) AMAZONESIS***

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Fibrocytes, originate in the marrow, express the pan-leukocyte CD45 protein and produce matrix proteins. Studies have defined the fibrocytes participation in pathological processes suggesting a central role in the repair of the lesion in infectious diseases. In analyses of in vitro infection by *Leishmania (L.) amazonensis* promastigotes, the fibrocytes from BALB/c mice endocytosed and killed parasites after 72h of interaction. However, there are no studies on the role of human fibrocytes in leishmaniasis. Then we began the analysis of human fibrocyte endocytic capacity using *Leishmania (L.) amazonensis* promastigotes. Primary cultures were established and, from the 15th days onwards, they were infected and the interaction kinetics were performed. Anti-CD45 and anti-HSP47 were used in the immunostaining and analysed under epifluorescence microscopy, which 100% of the cells had a double marking. After evaluated the fibrocytes interaction with the promastigotes, we verified that the fibrocytes endocytosed the parasite, and after 15 days of interaction they resolved the infection. To analyse fibrocyte morphology with promastigotes, these were fixed, postfixed and analysed by scanning electron microscopy (SEM) and transmission (TEM). In SEM, we have seen that the fibrocytes emit cytoplasmic projections and the parasites adhere by the body and flagellum to the cell surface, and by TEM, we observed the conversion of promastigotes into amastigotes inside parasitophorous vacuoles. These results allow us to suggest that human fibrocytes may serve as host cells and may play an important role in the inflammatory response caused by the presence of *Leishmania (L.) amazonensis*. **Supported by:**CEP-IOC/Fiocruz, SH-HUCFF/UFRJ and Capes. **Keywords:** Fibrocyte; host-parasite interaction ; leishmania

HP27 - **FAST AND EFFICIENT CRISPR/CAS9 TAGGING AND SINGLE KNOCKOUT OF MCM PROTEINS INVOLVED ON DNA REPLICATION OF TRYPANOSOMA CRUZI**

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Trypanosoma cruzi, during its life cycle, vary among non-replicative/infective forms and replicative forms. This transition is poorly understood, however recently it has been implicated on parasite drug resistance by non-replicating amastigotes, pointing the importance on understanding replication mechanism in this parasite. From proteins involved on DNA replication, there is a complex formed by 6 proteins (MCM2-7) that have a ring shape structure that involve the double stranded DNA and unwind it during the replication process. We have already showed that MCM7 have different expression levels during *T. cruzi* life cycle, not being expressed on tripomastigote forms that can potentially be involved in replicative/non-replicative transition. Recently genome editing by the CRISPR/Cas9 has been widely used as an efficient tool and is especially valuable on *T. cruzi.* So we wanted to evaluate the MCM2-7 expression on *T. cruzi* by genetic reverse through CRISP/Cas9. Here epimastigotes constitutively expressing Cas9 enzyme were used to both tag and knockout genes for MCMs on T. cruzi (CL Brener) genome. From the 6 MCM proteins, 5 were efficiently tagged at the C terminal region (MCM2, 3, 4, 6 and 7) and also their single genes were knocked out. All tagged MCMs showed nuclear localization on epimastigote forms and clonal population of MCM 2, 3, 4 and 6 could be observed on nuclear space at all cell cycle stages of epimastigotes of clonal population, further experiments on life stages forms will be performed to evaluate these proteins. These results show that CRISPR/Cas9 genome editing is being efficient on *T. cruzi* (CL Brener genome) and polyclonal cell lines are generated just after 2 weeks under drug selection. Moreover, the tagged proteins with fluorescent protein NeonGreen seem to be functional once showed nuclear localization and normal Edu incorporation. So CRISPR/Cas9 is a very powerfull tool available on *T. cruzi*, with promising applications. **Supported by:**FAPESP **Keywords:** Crispr/cas9; gene tagging; gene knockout

HP28 - ***TRYPANOSOMA CRUZI* CYCLOPHILIN 19 IS SECRETED BY MAMMALIAN STAGES OF THE PARASITE**

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Cyclophilins are ubiquitous and evolutionarily conserved enzymes, known for its peptide-prolyl-cis-trans-isomerase activity. Several types of cyclophilins are expressed from prokaryotes to eukaryotes. Mammalian cyclophilin A (CypA) is an intracellular enzyme and its expression is augmented under stress situations, which also causes its secretion into extracellular environment. The extracellular CypA (eCypA) has recently been shown to play an important role during inflammatory processes such as cell migration and proliferation, T-cells activation and chemotaxis. Inhibition of eCypA expression and activity, decrease inflammation by preventing the recruitment of T-cells and reducing the production of metalloproteinase-9 (MMP-9) and IL-6. *Trypanosoma cruzi*, the protozoan parasite that causes Chagas disease, encodes cyclophilin 19 (TcCyp19), which is highly similar to CypA. Therefore, it could have a role in the inflammatory process occurring in the acute phase of the disease. Here we found that TcCyp19 is expressed and distributed in the parasite cytosol of all lifecycle stages. Tissue culture trypomastigotes exposed to medium lacking glucose differentiated into amastigotes and secreted preferentially several cytosolic proteins, including TcCyp19 in the soluble form. Differentiation and secretion were prevented by proteasome inhibitors, suggesting that the release of cytosolic proteins is a consequence of cellular transformation. By using TcCyp19 tagged with the HA epitope, we observed that intracellular amastigotes also release TcCyp19 in the host cell cytosol 48 hours post infection. When cells were filled with parasites, before parasite exit TcCyp19 was found in the host cell leading edges and in culture supernatant. Taking together, these results, suggest that TcCyp19 released by mammalian stages of T. cruzi could have a similar role as CypA and affect the pathogenesis of Chagas disease. **Supported by:**CNPq; CAPES; FAPESP **Keywords:** Cyclophilin; chagas disease; inflammation

HP29 - **ORIGINS AND EVOLUTION OF THE SAP (SERINE-, ALANINE- AND PROLINE-RICH PROTEIN) MULTIGENE FAMILY THAT IS INVOLVED IN *T. CRUZI*-HOST CELL INTERACTIONS**

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SAP proteins (Serine-, alanine- and proline-rich protein) of T. cruzi share a conserved central motif (SAP-CD) that is involved in the interaction of parasite with the mammalian cells and activation of signal transduction pathways. In this work we show that SAP is distributed across all T. cruzi DTUs (Discrete Typing Units), including Tc bat and T. cruzi marinkellei. SAP is absent from other trypanosomes such as T. grayi, T. carassii T. congolense, T. evansi, T. theileri, T. vivax, T. brucei, T. rangeli, T. conorhini, T. dionisii). It is noteworthy that SAP is absent in T. dionisii, a trypanosome isolated from bats, and also in T. rangeli that shares hosts and geographical distribution with T. cruzi. MDS (Multi-Dimensional Scaling) analysis validated the previous classification of SAP into 4 groups and showed that SAP sequences from different DTUs are very closely related to each other and they are distantly related to SAP sequences from Tc marinkellei. These results suggest that Tc marinkellei sequences could have evolved independently from other SAP sequences.
SAP proteins share similarity with a few members of T. cruzi mucins (TcMUC, TcMUCII) and MASP (Mucin Associated Surface Protein). Searching for SAP conserved domains (CDD) we identified the mucin domain (pfam01456) in several SAP sequences which is consistent with a phylogenetic relationship between SAP, MASP and mucins. SAP, MASP and TcMUC genes were mapped in the same genomic regions suggesting that they may have evolved together. We hypothesized that SAP and MASP could have evolved from mucins during the expansion of this multigenic family in T. cruzi. Alternatively, SAP could has originated from the MASP ancestor gene, followed by duplication and diversification of the current SAP repertoire. Multigene family expansions are mainly species-specific and involved genes that play a role in the invasion and multiplication of T. cruzi in mammalian cells. **Supported by:**FAPESP, CNPq **Keywords:** Sap; multigene family; host cell interactions

HP30 - **SOCS2 IS ESSENTIAL IN NON-HEMATOPOIETIC CELLS MODULATING POTASSIUM CURRENTS AND VENTRICULAR REPOLARIZATION TIME AND DETRIMENTAL IN HEMATOPOIETIC CELLS FOR PARASITE CONTROL DURING TRYPANOSOMA CRUZI INFECTION**

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

HP31 - **DIRECTION OF FLAGELLUM BEAT PROPAGATION IS CONTROLLED BY PROXIMAL/DISTAL OUTER DYNEIN ARM ASYMMETRY**

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Trypanosoma brucei and *Leishmania mexicana* have complex life cycles in which they encounter a variety of different environments; the ability to move and navigate in these different environments is crucial for their success as parasites. They movement is powered by the flagellum, which has an apparently symmetrical 9+2 axoneme structure. Recently, asymmetries along the length of motile flagella have been identified in a number of organisms, typically in the inner and outer dynein arms. Flagellum beat waveforms are adapted for different functions and they may start either near the flagellar tip or near its base (and may be symmetrical or asymmetrical). We hypothesised that proximal/distal asymmetry in the molecular composition of the axoneme may control the site of waveform initiation and direction of waveform propagation. *T. brucei* and *L. mexicana* often switch between tip-to-base and base-to-tip waveforms, making them ideal for analysis of this phenomenon. We show here that the proximal and distal portions of the flagellum contain distinct outer dynein arm docking complex heterodimers. This proximal/distal asymmetry is produced and maintained through growth by a concentration gradient of the proximal docking complex, generated by intraflagellar transport. Furthermore, this asymmetry is involved in regulating whether a tip-to-base or base-to-tip beat occurs, which is linked to a calcium-dependent switch. Our data show that the mechanism for generating proximal/distal flagellar asymmetry can control waveform initiation and propagation direction, which will be crucial for navigating complex, *in vivo* environments **Supported by:**Wellcome Trust **Keywords:** Flagellum; flagellar beat; intraflagellar transport

HP32 - **PRODUCTION AND EXPRESSION OF INFLAMMATORY AND ANGIOGENIC FACTORS IN THE ACUTE PHASE OF EXPERIMENTAL INFECTION BY *TRYPANOSOMA CRUZI***

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The protozoan *Trypanosoma cruzi* causes cardiomyopathy in mammalian hosts. The inflammation is an important key to the development of this cardiomyopathy and to promote, directly or indirectly, local angiogenesis. This angiogenesis would promote oxigen supply to the damaged organ and also would intensify the leukocyte migration into the tissues worsening and/ior repairing the inflammation. Here, we evaluated the production and expression of inflammatory and angiogenic mediators in male C57BL/6 mice infected with 100 trypomastigote forms of the *T. cruzi* (VL-10 strain), for 24 days in acute phase. The parasitemia was performed during every day and, after the euthanasia, at 24th day of infection, heart and plasma samples were collected from mice. The heart was used for histological/immunohistochemistry analysis (expression of pro and anti-angiogenic factors: Ang-1, Ang-2, Thromb-1, Thromb-2, VEGF and production of inflammatory mediators TNF, CCL2, CCL5, IL-10, IL-17 and CCL3. The presence of the *T. cruzi* was able to reduce the number of blood vessels when compared to the uninfected group and to elevate the plasma IL-17, TNF, IL-10 and CCL5 but not the CCL3. Besides, the presence of the parasite was related to the expression of Ang-2 and the CCR2 and CCR5 receptors in the cardiac tissue. However, no significant differences were observed in the expression of VEGF as well as in Ang-1 and Thromb-1 and -2. Our preliminary data suggest that the infection with VL-10 strain of *T. cruzi* was related to the expression/production of inflammatory and angiogenic mediators and reduction of new vessels formation during the experimental acute phase. **Supported by:**CAPES,TWAS, CNPq, FAPEMIG, UFOP **Keywords:** angiogenesis; inflammation; trypanosoma cruzi

HP33 - **EFFECTS OF DIFFERENT DOSES OF NITAZOXANIDE IN THE ACUTE *TRYPANOSOMA CRUZI* INFECTION**

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Over more than 100 year of its discovery, the human Trypanosoma cruzi infection still needs an effective therapeutically strategy. The benznidazole the only available drug used for elimination of this parasite, presents its effectiveness dependent on the time to start the therapy, the genetic background of parasites and others. For this reason, new pharmacological strategies have been proposed aiming the elimininations of parasites and the modulating of the inflammation, the key element for the cardiac pathogenesis associated to this protozoan. In this study, different doses of Nitazoxanide - NTZ (100, 200, 400, 600, 800, 1000 and 1200 mg/kg) were administrated as oral gavage, during 10 days,C57BL/6 mice (n=10/group) infected with 4.000 trypomastigote forms of T. cruzi (Y strain). The parasitemia was performed daily and, after the euthanasia (11 th day of infection),blood and heart samples were excised to immune (TNF and CCL2) and biochemical (TGO and TGP) analysis. We observed an increase of in the parasitemia peak in all infected animals treated with NTZ,when compared to the untreated mice. There was no alteration in the heart mass after the NTZ treatment,and the survival curve demonstrated a high mortality observed in animals treated with the highest dose (1200 mg / kg), in which 40% of the animals died. In addition, we observed that TNF and CCL2 reduced in the treated groups relative to the untreated infected group. Regarding TGO and TGP, our preliminary data show no changes to the TGP,however the TGO presented high levels using 800, 1000 and 1200 mg/kg of NTZ. In conclusion, we can assume that treatment with NTZ, at lower doses, may regulate partially the acute inflammatory process, induced by Y strain of the T. cruzi infection. in C57BL/6 animals. **Supported by:**CAPES, UFOP, CNPq. **Keywords:** Trypanosoma cruz; nitazoxanide; inflammation

HP34 - **EVALUATION OF SPLEEN IMMUNE RESPONSE IN SYMPTOMATIC DOGS INFECTED WITH L. INFANTUM SUBMITTED TO IMMUNOTHERAPY WITH LBMPL VACCINE**

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L. infantum is responsible for the most fatal form of visceral leishmaniasis. Dogs are very susceptible to infection presenting pathological and immunological alterations very similar to human d and are considered the most important model for evaluation of new treatment strategies suc erapy. Recently our group evaluated the immunotherapeutic effect of the vaccine com s and monophosphoryl lipid A (LBMPL) antigens in symptomatic dogs natural rein, it was proposed to analyze the spleen immune response trough th NF-α, IL-12, IL-10, TGF-β1) and iNOS expression, the para he correlation between these cytokines and the parasitis to three series of immunotherapy with LBMPL vaccine submitted to immunotherapy with MPL alone or anima dogs-ID). Our major results showed that animals subje gher levels of IFN-γ, TNF-α, IL-12 in the spleen when co , we observed higher expression of iNOS only in the LBM ted showed higher levels of IL-10 and TGF-β1 when co BMPL vaccine also promoted a reduction in parasite bu of infected dogs, being even possible to observe some when compared with ID and MPL groups. The correlation I the vaccine therapy demonstrated negative correlations with parasitism and positive correlation with IL-10 and the parasite burden. Ta upport the development of a strong antigen-specific immune response in spl to the vaccine therapy with LBMPL. **Supported by:**FAPEMIG-PPP, CNPq, UFO APES **Keywords:** Visceral leishmaniasis; lbmpl vaccine; spleen immune response

HP35 - **THE FOCAL ADHESION KINASE IS INVOLVED IN INTERNALIZATION OF HOST CELLS BY *TRYPANOSOMA CRUZI***

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Focal Adhesion Kinase (FAK), a protein that in humans is encoded by the *PTK2* gene, is involved in cellular adhesion. Its activation elicits intracellular signal transduction pathways that promote contacts with the extracellular matrix. We investigated whether FAK plays a role in the process of host cell invasion by metacyclic trypomastigote (MT) forms of *T. cruzi*. To that end, two approaches were pursued. In one set of experiments, human HeLa cells were pretreated or not for 30 min with FAK inhibitor PF573228 at different concentrations (10, 20 and 40 µM) and then incubated for 1 h with MT of *T. cruzi* strain G (TcI) or CL (TcVI). Invasion by CL strain MT was significantly inhibited by PF573228 at all concentrations. At 40 µM, the inhibition was ~80%. Significant inhibition of G strain MT invasion was achieved only at 40 µM (~40%). Another approach consisted in submitting HeLa cells to lentiviral delivery of shRNAi to specifically deplete FAK. By western blot analysis, two cell lines were confirmed to have reduced levels of FAK. They were tested in MT invasion assays using CL strain and G strains. The number of CL strain MT that entered FAK-depleted cells was significantly higher, as compared to control wild type cells. No such increase in the number of internalized parasites toward FAK-depleted cells was observed when G strain was used. As MT invasion is associated with the disruption of actin microfilaments and the spreading of lysosomes from the perinuclear region to the cell periphery, experiments are under way to examine the architecture of actin cytoskeleton and the distribution of lysosomes in cells treated with FAK inhibitor or in FAK depleted cells. Still to be clarified is the question related to the difference between G and CL strains as concerns the involvement of FAK in target cell invasion and the intriguing results showing the decrease in CL strain MT invasion of HeLa cells treated with FAK inhibitor and the increase in FAK-deficient cells. **Supported by:**Sao Paulo Research Foundation (FAPESP) and Conselho Nacional de Desenvolvimento Científico e Tecnoló **Keywords:** Focal adhesion kinase; host cell invasion; metacyclic trypomastigote of trypanosoma cruzi

HP36 - ***IN SITU* CHARACTERIZATION OF THE REGULATORY IMMUNE RESPONSE IN HUMAN CUTANEOUS LESION CAUSED BY *LEISHMANIA (VIANNIA) PANAMENSIS*.**

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Leishmania (Viannia) parasites are the most prevalent etiologic agents of human cutaneous leishmaniasis (CL) in the Americas. In Panama, *Leishmania (V.) panamensis* is the most prevalent agent and responsible for the majority of human cutaneous leishmaniosis. In infectious diseases, Treg cells regulate the intensity and duration of cellular immune responses, limiting the tissue damage. It has been demonstrated that these cells contribute to pathology and parasite persistence in leishmaniosis. Despite the increasing knowledge of immunopathological mechanisms that contribute to disease progression, the role of Treg cells during *L. (V.) panamensis* infection remains unclear. In this sense, this study has the objective to characterize the regulatory immune response in skin lesions of patients with CL caused by *L. (V.) panamensis* in order to better understand the pathogenesis of the infection caused by this species of parasite in Panama. Biopsies (n = 46) from panamanian patients with localized CL were collected and processed by usual histological techniques. *L. (V.) panamensis* infection was proven by *in vitro* isolation and characterization of the parasites by HSP70-RFLP. In situ regulatory immune response was evaluated by immunohistochemistry using anti-IL10, anti-CD4, anti-FoxP3 and anti-TGF-β antibodies. Quantitative morphometric analysis showed density of CD4+=914.5±51.76, FoxP3+=648.5±38.03, IL-10+=537.5±30.94 and TGF-β+=132.2±9.50 cells/mm2. Positive correlations were found among CD4+, FoxP3+, IL-10+ cells ($p<0.001$). There was a tendency to increase in the number of CD4+, FoxP3+, IL-10+ and TGF-β+ cells in non-ulcerate and granulomatous lesions, as well as decrease on IL-10+ cells and increase on FoxP3+ cells in lesions >30 days. These data suggest that Treg cells contribute to the immunopathology of the skin lesions caused by *L. (V.) panamensis* regulating Th1 cellular immune response, mainly through the production of IL-10. **Supported by:**FAPESP 2014/50315-0, 2017/03141-5 and LIM50 HC-FMUSP **Keywords:** Leishmania panamensis; t regulatory cells; leishmaniasis

HP37 - **EFFECT OF PHOSPHATIDYLSERINE BLOCKING ANTIBODIES ON INFECTION BY *LEISHMANIA AMAZONENSIS***

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Leishmaniasis is a neglected tropical disease caused by protozoan parasites of the genus Leishmania. Amastigotes infect and proliferate within macrophages of vertebrate hosts. As infection strategy, parasites have virulence factors that are recognized by receptors of macrophages that induce the endocytosis of the pathogen and regulate the leishmanicidal activity of the host cell. Among the virulence factors, stands out the phosphatidylserine (PS), which is a phospholipid that induces endocytosis of the pathogen and the production of TGF-B and IL-10. These cytokines have anti-inflammatory and immunosuppressive actions on infected macrophages and adjacent cells. Thus, PS is an important therapeutic target in this infection. We aimed to evaluate the effects of PS blocking antibodies and their purified Fab regions during Leishmania amazonensis infection. PS blocking antibodies when compared to untreated parasites reduced infection. Interestingly, anti-PS-treated parasites were able to proliferate, displaying infectivity indexes similar to untreated parasites 48 h post infection. This was not observed when annexin V-treated amastigotes were used as control of PS blockade. This is corroborated by the fact that anti-PS antibodies were not able to block the amastigote-dependent modulation of the macrophage inflammatory response 48h post infection. There was no difference between complete antibodies and purified Fab portions. Preliminary results suggest that mice treated with antibodies i. p. displayed histological modifications when compared to control mice. Anti-PS antibodies and their purified Fab regions were able to partially block the infection and drastically decrease the formation of parasitophore vacuoles in vivo. More experiments are being carried out to conclude the role of anti PS antibodies in the inhibition of infection and the modulation of the inflammatory response. **Supported by:**CAPES **Keywords:** Leishmania; antibodies; phosphatidylserine

HP38 - **ROLE OF FAK IN *TRYPANOSOMA CRUZI* INDUCED CARDIAC HYPERTROPHY**

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Chagas disease, an important neglected tropical disease, is the leading cause of heart failure in Latin America. Hypertrophy and cardiac fibrosis are among the main symptoms of chronic chagasic cardiomyopathy (CCC). Hypertrophy can be triggered by a variety of external stimuli through multiple signaling pathways. Focal adhesion kinase (FAK), a non-receptor protein tyrosine kinase, has emerged as a signaling pathway regulating idiopathic cardiac hypertrophy. Endothelin 1 (ET-1), a vasoconstrictor produced by endothelial cells, cardiomyocytes among others, plays an important role in CCC and has been pointed as a modulator of FAK activation. Thus, in this study we evaluated the participation of FAK in the modulation of hypertrophy induced by *T. cruzi*.
C57B16 mice were infected with *T. cruzi* (Brazil strain) and evaluated for electrocardiographic (ECG) disorders and activation of signaling pathways involved in cardiac hypertrophy, including ERK1/2 and FAK, as well as the expression of hypertrophy markers and extracellular matrix components (fibronectin and collagen), in the different stages of infection development (60 to 270 dpi).
Parasitemia was followed up to 40 days post infection (dpi) showing low parasite burden ($7x10^4$/mL) at the parasitemia peak (26 dpi). Arrhythmia, bradycardia and atrioventricular block were alterations evidenced in ECG analysis, which were prominent at 150 - 240 dpi. ECG changes are accompanied by increased expression of extracellular matrix components such as fibronectin. Activation of extracellular signal-regulated kinases 1/2 (ERK1/2), a regulator of cardiac hypertrophy, was evidenced in early and late stages of *T. cruzi* infection. Our preliminary results revealed activation of FAK signaling pathway, demonstrated by increased level of FAK Tyr397 phosphorylation (180 dpi). Together, these results suggest that FAK may be involved in the regulation of heart hypertrophy. **Supported by:**Fiocruz, FAPERJ, CAPES e CNPq **Keywords:** Trypanosoma cruzi; focal adhesion kinase; hypertrophy

HP39 - **ACTIN CYTOSKELETON AND RHO GTPASES REGULATE THE INTERNALIZATION OF *T. CRUZI* EXTRACELLULAR AMASTIGOTES IN RAW 264.7 MURINE MACROPHAGES**

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Extracellular amastigotes (EAs) of *T. cruzi* can colonize professional and non-professional phagocytic cells. In both cells types, cell invasion process by EAs is dependent on actin cytoskeleton of host cells. It has been described that Rho GTPases (Rac1, Cdc42 and RhoA) are key mediators of the actin cytoskeleton, promoting actin polymerization by the Arp2/3 complex, participating in phagocytosis induced in non-professional phagocytic HeLa cells. Thus, the goal of this study was to evaluate whether the role of actin and Rho GTPases is conserved during EAs invasion in professional phagocytic cells (RAW 264.7). Using confocal microscopy it was observed that EAs induce recruitment of actin in RAW cells (activated or not with LPS + IFN-γ) similar to actin recruitment induced in non-phagocytic HeLa cells. Using lentiviral transduction of shRNAis we depleted endogenous expression of Rho GTPases in RAW cells for invasion assays. These assays showed that depletion for Rac1 and Cdc42 inhibited EAs invasion in activated and non-activated RAW cells, but this was not observed for RhoA. Thus, these results show that Rho GTPases promote EAs internalization in professional phagocytic cell (RAW 264.7) similarly to the mechanisms engaged in non-phagocytic cells (HeLa cells). **Supported by:**FAPESP, CAPES, CNPq **Keywords:** Trypanosoma cruzi; extracellular amastigotes; phagocytosis

HP40 - **CDC42, RHOA AND RAC1 GTPASES CONTROL THE ACTIN CYTOSKELETON DURING HELA CELL INVASION BY *TRYPANOSOMA CRUZI* METACYCLIC AND TISSUE CULTURE TRYPOMASTIGOTES.**

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Cellular invasion by trypomastigotes of *Trypanosoma cruzi* is complex and involves parasite and host-cell intricate interactions. While metacyclic trypomastigote forms rely on lysosome exocytosis the bloodstream (tissue-culture) trypomastigotes forms can also subvert host PI3k signaling. Rho GTPases modulate actin cytoskeleton, being RhoA, Rac1 and Cdc42 responsible for the formation of stress fibers, lamellipodia and filopodia, respectively. Participation of host cell actin cytoskeleton and invasion by trypomastigote forms has not been fully elucidated in non-phagocytic cells. In order to investigate this question, the aim of this study was to evaluate the role of RhoA, Rac1 and Cdc42 on the actin cytoskeleton during the host cell invasion by metacyclic and tissue culture trypomastigote forms of *T. cruzi*. Stably depleted HeLa cell lines for each studied GTPase were established by shRNAi lentiviral transduction. Our results demonstrated that depletion of RhoA, Rac1 and Cdc42 inhibited the invasion of tissue culture trypomastigotes. On the other hand, only RhoA depletion and Cdc42 but not Rac1 inhibited metacyclic trypomastigote invasion. Despite the current controversy over the role of actin cytoskeleton in trypomastigote invasion, our results demonstrated that these Rho GTPases are linked to the host cell invasion process by trypomastigote forms of *Trypanosoma cruzi*, however, they do not seem to act in the same way. In near future other approaches will be carried out to clarify the role of actin cytoskeleton and the GTPases RhoA, Rac1 and Cdc42 during trypomastigote internalization process. **Supported by:**FAPESP; CAPES; CNPq **Keywords:** Trypanosoma cruzi; trypomastigotes; rho gtpases

HP41 - **A ROUTE TO TOXOPLASMA GONDII NATURAL EGRESS**

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The study of *Toxoplasma gondii* egress has been addressed with the aid of inducers, such as calcium ionophores. Although this and other calcium transients have been successful in triggering *T. gondii* egress, the structural panorama of “natural” and artificial events should match. Herein we approached the natural egress of this parasite using super-resolution and electron microscopy, revealing lytic and non-lytic events of individual egress. The present work corroborates the use of calcium ionophore as a reliable tool to trigger parasite egress and indicates that different signalling routes can converge to similar structural aspects in natural and induced egress. **Supported by:**CAPES **Keywords:** Toxoplasma; egress; microscopy

HP42 - **INTERLEUKIN-9 PRETREATMENT DECREASED INVASION AND MULTIPLICATION OF *TRYPANOSOMA CRUZI* IN MURINE C2C12 MYOBLASTS**

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Chagasic cardiomyopathy is related to a chronic inflammatory process that occurs in cardiac striated tissue as a response to parasite persistence. Patients' myocardium may present several foci of inflammatory infiltrate, cardiac fiber impairment, intense fibrosis and high production of key cytokines that lead the course and outcome of the disease. Some studies have observed that IL-9 is elevated in chagasic patients’s blood and in T. cruzi-infected Balb/c mice. In addition, this interleukin is responsible for stimulating CD4 T lymphocytes differentiation to Th17 and activating Treg cells, important cellular profiles that act to inhibit *T. cruzi* intracellular multiplication and modulate the immune response during chronic phase of Chagas' disease. The present study aimed to evaluate the role of IL-9 in the progression of *T. cruzi* infection *in vitro*. For this purpose, myoblasts were pretreated with IL-9 (25ng/mL) for 24 hours and the cells were then infected with *T. cruzi* Y strain trypomastigotes to evaluate invasion rates (3h), nitric oxide production and parasite multiplication (48h, 72h e 96h). Myoblasts pretreated with IL-9 showed a significant reduction in the percentage of infected cells (37,5% vs. 27%, respectively) and number of internalized parasites compared to the control group (191 and 112 parasites/300 cells, respectively). However, there was no significant differences in nitric oxide production during parasite invasion among the groups. IL-9 pretreatment significantly reduced parasite multiplication for 48h, 72h, and 96h compared to the control group. Concluding, we demonstrated that IL-9 interacted with myoblasts and presented a beneficial effect on the cellular response against the parasite, thus indicating the IL-9 as an important cytokine in the control of *T. cruzi* infection. Further research regarding IL-9 knock out cells and laboratory animals are currently in progress to confirm and support these data. **Supported by:**FAPESP/CNPq/Capes **Keywords:** Trypanosoma cruzi ; il-9 ; myoblasts

HP43 - **THE GP82 SURFACE MOLECULE OF TRYPANOSOMA CRUZI METACYCLIC FORMS INTERACTS WITH LAMP2 DURING THE PROCESS OF CELL INVASION**

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Mechanisms of host cell invasion by Trypanosoma cruzi, a crucial process for the establishment of infection, have been partially elucidated. However, many questions remain to be investigated. One of these refers to the role of lysosome associated membrane proteins (LAMP) in invasion by metacyclic trypomastigotes (MT), which relies on MT-specific surface molecule gp82 to induce host cell lysosome spreading and exocytosis required for the parasitophorous vacuole formation. Invasion assays performed with human HeLa cells in the presence of antibody to LAMP1 or LAMP2 showed a significant inhibition of MT invasion by anti-LAMP2 antibody but not by anti-LAMP1 antibody. To further confirm the requirement of LAMP2 in MT invasion, HeLa cells depleted in LAMP1 or LAMP2 were generated using lentiviral transduction methodology. Extensive depletion of LAMP1 was confirmed in two cell lines and of LAMP2 in one cell line by western blot analysis and confocal microscopy. MT entry into LAMP2-depleted cells was significantly reduced. Invasion of LAMP1-deficient and wild type cells showed similar susceptibility to MT invasion. Extracellular amastigote invasion increased in LAMP-deficient cells. We also addressed the question related to still unknown gp82 receptor. The possibility that LAMP2 might be the receptor for gp82 was examined by co-immunoprecipitation assays.Magnetic beads crosslinked with antibody directed to LAMP1 or LAMP2 were incubated with detergent soluble HeLa cell extract and then with MT lysate, followed by washings and elution. The eluted samples were analyzed by western blotting using anti-LAMP and anti-gp82 antibodies. Eluate from LAMP2 beads contained LAMP2 and gp82. Eluate from LAMP1 beads did not contain gp82. Another experiment consisted in incubating beads crosslinked to anti-gp82 monoclonal antibody, first with MT lysates and then with HeLa cell extracts. LAMP2 and gp82 were detected in the eluate. These data indicate that gp82 binds to LAMP2. **Supported by:**Fundação de Amparo à Pesquisa do Estado de São Paulo - FAPESP **Keywords:** Trypanosoma cruzi; lysosome membrane proteins; host cell invasion

HP44 - **UNVEILING THE INFECTION OF FIBROBLASTS BY LEISHMANIA PROMASTIGOTES: CALCIUM SIGNALING, LYSOSOMES RECRUITMENT AND EXOCYTOSIS CULMINATE WITH CYTOSKELETON-INDEPENDENT INVASION.**

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Intracellular parasites of the genus Leishmania are the etiological agents of leishmaniasis. The disease is transmitted by the bite of a sandfly vector which inoculates the infective promastigotes into the skin of mammalian hosts, including man. During chronic infection the parasites replicate in macrophages but other cell types and even non phagocytic cells have been found infected. The mechanisms by which Leishmania invades non-professional phagocytes, such as fibroblasts, were not studied to date. Here we show that infective promastigotes can actively induce its entry in fibroblasts independent of the actin cytoskeleton activity. Invasion involves subversion of host functions such as calcium signaling, plasma membrane repair and recruitment and exocytosis of lysosomes whose positioning and integrity directly interfered in invasion. Parasites completed life cycle and remained viable in fibroblasts which shows that these and possibly other non-phagocytic nucleated cells could be invaded and serve as parasite hideout, notably at the beginning of infection. **Supported by:**FAPEMIG **Keywords:** Leishmania ; invasion ; non-phagocitic cells

HP45 - **KINETOPLASTID PHYLOGENOMICS USING TOTAL ORTHOLOG MEDIAN MATRIX (TOMM) APPROACH**

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Kinetoplastid protozoans present a great biological variety, from free-living to parasitic organisms. Because of medically important genera (Leishmania and Trypanosoma) and the biological diversity, kinetoplastids are an interesting model to understanding the evolution of parasitism. Large-scale sequencing data for several kinetoplastids have powered the molecular evolutionary analysis through Phylogenomics. Here, our aim was built a phylogenetic tree containing 46 kinetoplastids with available genomes using a novel Phylogenomics approach, named TOMM (Total Ortholog Median Matrix), which is based on evolutionary changes in all orthologous protein-coding genes for a group. The pipeline consisted of (1) downloading all protein sequences from a genome when available (NCBI or TriTryDB) or (2) translating the coding regions from genomic sequences by obtaining open reading frames using EMBOSS tool and adjusting starting Metionine by BLASTX to Protozoa-RefSeq. Then, the kinetoplastid “proteomes” were pairwise submitted to the (3) Reciprocal Smallest Distance (RSD) program to obtain matrices of orthologs and their amino acid distances. Customized programs named RSDmaker, Batcher and RSD2Table were wrote to help the orthologs identification. The distance values of TOMM were input in hierarchical clustering R packages, HCLUST and PVCLUST, to obtain a phylogram that was annotated using APE R package and MEGA7 software. The resulting single phylogram was built compiling an average of 5655 ortholog proteins, with the minimum number of ortholog sequences found for the Perkinsela/Phytomonas serpens pair, (1473) and the maximum for the Crithidia fasciculata/Leptomonas pyrrhocoris pair (8434). The major clades clustered species from the main subfamilies Leishmaniinae, Phytomonadinae, Strigomonodinae and Trypanosomatinae with high statistical node support. To the best of our knowledge, this genome-wide phylogram is the most comprehensive obtained for kinetoplastids so far. **Supported by:**FAPESP, grant 2016/20258-0; CAPES **Keywords:** Kinetoplastid; phylogenomics; genome-wide ortholog proteins

HP46 - **THE TNF RECEPTOR 1 IN *LEISHMANIA AMAZONENSIS* INFECTION: PROMOTION AND REGULATION OF INFLAMMATION.**

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The Th1/Th2 paradigm that explains most models of infection with *Leishmania major* does not apply to the experimental models of infection with *L. amazonensis*. In this latter model, mixed immunological responses rather than polarization are observed, in contrast with the Th1/Th2 paradigm of resistance and susceptibility. TNF is a pleiotropic cytokine that mediates inflammation, lymphoid tissue development and homeostasis, extracellular matrix destruction, among other functions. TNF binds to two receptors, TNFR1 and TNFR2. Both receptors trigger inflammatory responses but only by binding to TNFR1 TNF mediates regulation or supression of inflammation, through induction of apoptosis via caspase 8/3. Hence, our work aimed at the identification of the role of TNFR1 the mouse model of infection with *L. amazonensis*. Our data reveal the importance of TNFR1 in the control of lesion development, but not on the control of parasite replication. This control was more efficient in the subcutaneous model of infection where, in addition to promoting lesion control, TNFR1 was importante in the maintainance of tissue homeostasis, probably due to IL-10 production. Furthermore, in the accute phase of the subcutaneous infection, TNFR1 mediated recruitment of mieloid cells and lymphocytes to the site of infection and, in the chronic phase, regulated cell recruitment. TNFR1-mediated apoptosis was inhibited during intradermic infection by L. amazonensis, and an alternative pathway was in play. However, our data indicate that apoptosis seems to be inhibited by the pro-inflammatory signaling through TNFR1, since apoptosis was lesser in wild-type mice. In conclusion, our data suggest the involvement of TNFR1 in resistance to *L. amazonensis*. **Supported by:**Capes **Keywords:** Leishmania amazonensis; tnfr1; apoptosis

HP47 - **RELATIONSHIP OF PERIPHERAL BLOOD MONONUCLEAR CELLS MIRNA EXPRESSION AND PARASITIC LOAD IN CANINE VISCERAL LEISHMANIASIS**

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Introduction: Visceral Leishmaniasis in humans is a chronic and often fatal disease if left untreated. Dogs are powerful transmitters of the parasite to humans by the phlebotomain vector. Immune response can be modulated by microRNAs (miRNAs). Methods and Results: We evaluated the miRNAs differentially expressed in peripheral blood mononuclear cells (PBMCs) of symptomatic dogs naturally infected with Leishmania infantum (n = 10) compared to healthy dogs (n = 5). In the microarray, miRNAs miR 21, miR 424, miR 194 and miR 451 had an increase in expression on the order of 3-fold change and miRNAs miR 192, miR 503 and miR 371 had an increase in expression on the order of 2-fold change. The miR 150 and miR 574 had decrease in expression on the order of 2-fold change. Real-time PCR validated microarray results for miRNAs miR 21, miR 150, miR 451, miR 192, miR 194 and miR 371. Parasite load of PBMCs was measured by real-time PCR and correlated to differentially expressed miRNAs. The parasite load showed a strong positive correlation with miR 194 expression, regular positive correlation with miR 371 expression and moderate negative correlation with miR 150 expression. Targets and pathways analysis were performed in Ingenuity Pathway Analysis (IPA) program and 63 canonical pathways were obtained, among them, p53 signaling pathway, antiproliferative role of TOB in T cell signaling, STAT3, signaling of death receptor and crosstalk between dendritic cells and natural killer cells, that could be regulating the immune response on Canine Visceral Leishmaniasis (CVL). The canonical pathway crosstalk between dendritic cells and natural killer cells targets important molecules involved in the immunopathogenesis of CVL, such as NFκB, TNF-α, CD80, IFN-γ and DNAM-1. Conclusion: These findings suggest that miRNAs interfere on the immune response of dogs infected with L. infantum and their correlation with parasite load may help in the identification of therapeutic targets in LVC.
Supported by:FAPESP, CNPq **Keywords:** Visceral leishmaniasis; mirna; immune response

HP48 - **MICRORNA EXPRESSION IN SPLEEN LEUKOCYTES FROM DOGS WITH VISCERAL LEISHMANIASIS**

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Canine visceral leishmaniasis (VL) the cell immune suppression being determinant of disease progression and the regulation of immune response appears to depend on miRNAs. The objective of this study was the characterization of the miRNAs in spleen leukocytes (SL) from dogs naturally infected by L. infantum. A group of 5 healthy dogs and 10 dogs with VL was studied. miRNA was extracted from SL using Mirvana Kit (Invitrogen™). The miRNA RNA integrity number upper 8 (RIN=8) was used to microarray using Affymetrix™ miRNA4.1 Strip according to manufacturer's recommendations. cDNA production was performed using the miScript RTII kit (Qiagen™), and qPCR was performed using the inventoried dog miRNAs (Qiagen,USA) and SYBR Green (kit miScript SYBR Green PCR, Qiagen™), according to the manufacturer's recommendation. After analysis of the pathways, then the LS from infected dogs were transfected with miR21 using miScript miRNA Mimics and Inhibitor (Qiagen,US) according to manufacturer's recommendations. After the transfection LE the transcription factor T-bet and GATA 3, parasite load were measured by flow cytometry and IL-12 was quantified by ELISA Kit (R&D Systems). The analyses of microarray was performed on Expression Console, Transcriptome Analysis Console (Affymetrix™); ANOVA, qPCR validation data was analyzed by Mann-Whitney test, the canonical pathway of miRNA differentially expressed in IPA® (Ingenuity Pathway Analysis) with the Bonferroni test, the transcription factor, parasite load and expression of IL-12 was analysed by Friedman test, with the level of significance of $p < 0.05$. We showed miR148a, miR21, miR7, miR615 were upregulated and miR150, miR125a and miR125b were downregulated. The IPA showed 127 pathways regulated by these miRNAs, including pathways which regulate immunity. After transfection, the miR21 inhibitors increased T-bet and IL12 and decreased parasitic load. The miR21 regulates cellular immunity and may be therapeutic targets in canine VL.
Supported by:FAPESP 2015/16101-6 **Keywords:** Visceral leishmaniasis; mirna; immuno response

HP49 - **METABOLOME FINGERPRINTING OF MURINE MACROPHAGES INFECTED WITH *LEISHMANIA AMAZONENSIS*; CORRELATION WITH GENE EXPRESSION OF L-ARGININE METABOLISM**

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The *Leishmania amazonensis* could modulates infected-macrophages gene expression, inhibiting Nitric Oxide Sintase (NOS 2) and consequently the production of nitric oxide (NO). This modulation directs L-arginine for production of polyamines by increasing the expression of host arginase I (ARG1), fating the survival of the parasite. Moreover, parasite arginase competes with NOS2 for L-arginine during macrophages infection and the absence of its activity proved to be an attenuating factor of infectivity of BALB/c macrophages. The aim is to investigate metabolic changes in BALB/c-murine macrophages infected with L. amazonensis wild type (*La*-WT) or arginase knockout (*La*-arg-) and compared to uninfected macrophages, focusing in the L-arginine metabolism. CE-MS was the technique used for a non-targeted metabolomics study. Using this platform, more than 89 compounds were identified as statistically significant in *La*-WT-infected macrophages compared to uninfected, contrasting with 80 metabolites *La*-arg--infected macrophages. In the comparison of *La*-WT versus *La*-arg--infected, only 14 metabolites were altered. *L. amazonensis* infection increased the levels of proline, glutamic acid, glutamine, L-arginine, ornithine and putrescine, activating the polyamine pathways. However, an increase of citrulline levels indicates the metabolization of L-arginine by NOS2 and/or arginine deiminase. The absence of parasite arginase activity reduces the amount of ornithine, proline and tripanothione in infected macrophages, but increases argininic acid and citrulline. The amount of Arg1 and L-arginine cationic transporters mRNAs were increased in *La*-WT- or *La*-arg- infected macrophages. Indeed Nos2 mRNA, NOS2 protein and NO levels were increased only in La-arg- infected macrophages. We concluded that *L. amazonensis* infection alters the metabolome fingerprint of macrophages, which parasite arginase due an important role to subvert the host immune responses. **Supported by:**FAPESP, CNPq **Keywords:** Metabolome; leishmania; macrophage

HP50 - **MAST CELLS COUPLE TISSUE PARASITISM TO INFLAMMATORY NEOVASCULARIZATION IN A HAMSTER MODEL OF *TRYPANOSOMA CRUZI* INFECTION**

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Microvascular leakage induced by proangiogenic factors fosters the formation of a provisional fibrin matrix that supports the migration of endothelial-tip cells at the onset of neovascularization. We reported that plasma leakage, a mast cell (MC)--driven inflammatory response propagated via cycles of bradykinin release and contact system activation, fuels heart parasitism, myocarditis and fibrosis. Here we investigated the impact of tissue parasitism in the microcirculation of the hamster cheek pouch (HCP) following challenge by wt Dm28c TCTs or Dm28c TCTs expressing GFP protein and luciferase. Intravital microscopy (IVM) showed angiogenesis in HCP at 7 d.p.i. Neovascularization was associated to PMN infiltration and increased density of MCs. Bioluminescence analysis revealed that Dm28c-luciferase reached other tissues, in a few cases, including the heart on 14, 21 and 30 dp.i. The link between parasitism and angiogenesis was supported by evidences that (i) injection of the same dose of Dm28c epimastigotes did not induce microvascular changes in the HCP (ii) Benznidazole (BZN) treatment initiated 24 h after TCT inoculation abrogated inflammatory neovascularization at 7 d.p.i. Since angiogenesis developed within a narrow time –window (3 days), we next examined the HCP at early time points (3 d.p.i.). There was no evidence of vessel sprouting in spite of the massive tissue parasitism observed at this time-point, although non-vascular fluorescence (dextran-FITC) was slightly increased, suggesting that endothelial barrier function is altered at 3 d.p.i. Next, we conducted proteomic analysis in infected HCP (3 d.p.i) versus infected/BZN-treated hamsters and found upregulated levels of chymase, a MC protease, previously appointed as a driver of angiotensin II-dependent angiogenesis in the hamster sponge model. T.cruzi- induced angiogenesis was blocked by an angiotensin II converting enzyme inhibitor (captopril), chymostatin and TY51469- a selective chymase inhibitor. **Supported by:**CNPQ, FAPERJ, CAPES, INBEB **Keywords:** Mast cells; angiogenesis; trypanosoma cruzi

HP51 - **NEW TREATMENTS TO AMELIORATE HEART FIBROSIS DURING CHAGAS DISEASE CARDIOMYOPATHY**

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Extracellular matrix (ECM) components can accumulate in the cardiac tissue during Chagas disease (CD), leading to fibrosis. Clinical evidence revealed a correlation of fibrosis level to cardiac performance impairment in patients. Furthermore, Benznidazole reduces the parasite burden during the chronic phase, but doesn't improve the clinical outcome in cardiac patients, what suggests that the development of therapies aiming fibrosis recovery are necessary in combination with trypanocidal agents to provide a clinical improvement when cardiomyopathy is advanced. Our goal is the identification of inhibitors of T. cruzi-induced collagen accumulation in the heart by screening of anti-fibrosis compounds using cardiac fibroblast (CF) cultures. Since inflammation is important for fibrosis establishment, our study targeted p-38 and c-Jun signaling pathways and modulation of cytokine activity by glycosaminoglycans (GAG`s). Our data showed that CF display ECM accumulation together with c-Jun and p38 signaling after T. cruzi infection. Sirius Red/Fast green dye was used as a readout assay to quantify both the collagen expression and total proteins content, allowing cytotoxicity measurement. T. cruzi infection and serum from infected mice lead to a significant raise in collagen detection in CF. We identified an anti-fibrotic effect for Pirfenidone (TGF-β and TNF-α inhibitor, EC50 114.3 μM), Losmapimod (p38 inhibitor, EC50 17.6 μM) and SP600125 (c-Jun inhibitor, EC50 3.9 μM). This effect is independent of CF proliferation since these compounds do not affect T. cruzi induced host cell multiplication measured by BRDU incorporation. Heparin derivatives were able to inhibit collagen accumulation by a dual effect on CF proliferation and reduction of parasite burden. These results propose a new approach for fibrosis therapy in CD providing a prospect of possible new treatments to reduce excessive synthesis of ECM proteins during T. cruzi infection. **Supported by:**CAPES **Keywords:** Fibrosis; signaling pathway; cardiomyopathy

HP52 - **MODULATION OF THE PRODUCTION OF LIPID DROPLETS BY FLAVONOIDS IN LEISHMANIA AMAZONENSIS INFECTION**

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Leishmaniasis is a neglected zoonosis endemic in Rio de Janeiro state. As an obligate i[illegible]llular parasite, infective forms of L. amazonensis preferentially invade macrophages. m[illegible]ction fundamental in the development of this disease. There is a correlatio[illegible]s of cytoplasmic lipid droplets (LDs) and intracellular parasite infecti[illegible]les may differ in their metabolic activity, especially in th[illegible]th signaling and cellular morphogenesis. In a[illegible]f inflammatory lipid mediators. In the [illegible]matory mediators dependent on [illegible]oactive natural flavonoids can m[illegible]ation of LDs during the infectio[illegible] and 48 hours post infection we [illegible] amastigotes inside the parasitophorous [illegible]tween the infection kinetics and the biogenesis of [illegible]ible role of bioactive natural flavonoids in the biogenesis of L[illegible] lipid-dependent inflammatory activity of macrophages are being c[illegible] **d by:**CNPQ **Keywords:** Leishmania; lipid bodies; flavonoids

HP53 - **THE ROLE OF INTERLEUKIN 15 DURING TRYPANOSOMA CRUZI INFECTION**

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Interleukin 15 (IL-15) is a pleiotropic cytokine implicated in the immune response against different infectious agents. Although its role during *Trypanosoma cruzi* infection has not yet been fully investigated, yeast two-hybrid assays provide evidences indicating that IL-15 are among the host cell proteins with which *T. cruzi* amastigote surface proteins interact during the parasite intracellular development. Using ELISA, western blotting and immunofluorescence (IF) assays, we analyzed the production of IL-15 by murine peritoneal macrophages, L6 myoblasts, LLCMK2 cells and H9C2 in response to the infection with *T. cruzi*. Increased levels of intracellular IL-15 were found in infected cells compared to uninfected fibroblast and macrophages. IF analyses showed an uniformed distribution of IL-15 in the cytoplasm and nuclei of uninfected macrophages whereas, in infected cells, IL-15 was predominantly found localized in the cytoplasm. In *T. cruzi* infected macrophages and L6 cells the localization of IL-15 in the cytoplasm suggests that this cytokine interact with the membrane of amastigotes. A role of IL-15 as a factor that contributes to the infection by *T. cruzi* was revealed by RNAi inhibition as well as infection of knockout mice. Reduced expression of IL-15 in bone marrow derived macrophages (BMDM) transduced with lentiviral vectors to deliver an short hairpin RNA (shRNA) with IL-15 sequences resulted in 40% inhibition of infection rates as well as a 42% reduction in the number of tissue culture trypomastigotes (TCTs) released in the culture supernatant when compared to the BMDMs transfected with a vector delivering a non-target shRNA. Similarly, macrophages from IL-15-/- mice infected by *T. cruzi*, showed a 50% reduction in the number of amastigotes per infected cells when compared to the wild type macrophages, further suggesting a role of the cytoplasmic isoform of IL-15 for the viability of the intracellular stage of the parasite. **Supported by:**CNPq **Keywords:** Il-15; trypanosoma cruzi; amastigotes

HP54 - **MODULATION OF MUCIN EXPRESSION IN INTESTINAL CELLS INFECTED WITH *GIARDIA LAMBLIA***

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Giardiasis, is a parasitic disease caused by the intestinal protoza Giardia lamblia, and affects thousands of individuals every year worldwide. Infection occurs after the ingestion of cysts that, upon reaching the stomach, desencyst releasing two trophozoites. The adhesion of trophozoites to the intestinal epithelium is a key step in the establishment of the disease, and occurs after trophozoites have crossed the mucus layer formed mainly by highly glycosylated proteins of the mucin family that covers all gastrointestinal tract (GIT). According to some studies, treatment of mammalian cells with mucinogenic compounds such as short chain fatty acid (sodium butyrate) as well as infection with some enteropathogenic organisms modulate the expression of mucins. Thus, the objective of this work was to evaluate how infection with Giardia influences the levels of transcription and translation of mucins in control cells, in cells treated with 1 □M butyrate of sodium for 36 h and infected with Giardia trophozoites. For this, Caco-2, LS174T and HT-29 cells (colon adenocarcinomas) and Hutu-80 cells (duodenum) were evaluated by indirect immunofluorescence microscopy, RT-PCR and qPCR after interaction with the parasite and treatment with sodium butyrate. The results show that the levels of both MUC2 and MUC5Ac mRNAs and the secretion of mucis were upregulated in the presence of Giardia lamblia trophozoites. **Supported by:**FAPESP, CNPq **Keywords:** Giardia lamblia; mucin expression; host-parasite interaction

HP55 - **HIGH PROTECTION IN THE FORMATION OF BRAIN CYSTS, USING SIMULTANEOUS IMMUNIZATION WITH DIFFERENT IRRADIATED *TOXOPLASMA GONDII* STRAINS.**

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Toxoplasmosis is a globally disease caused by the protozoan *Toxoplasma gondii*, which has the ability to invade and multiply inside of nucleated cells of the host. It is an opportunistic parasite mainly affects immunosuppressed and pregnant people, causing significant ocular involvement and congenital affection, generating severe sequelae. Ionizing radiation has been used as a tool in the production of immunogen, with promising results in several parasites. Therefore, we evaluated the humoral and protective immune response induced in mice immunized simultaneously with 03 different strains of *T. gondii* tachyzoites irradiated at 255 Gy of Cobalt 60 (Co-60). By ELISA we observed that immunization using the junction of three irradiated strains induced significant levels of specific antibodies (IgG, IgM and IgA), predominantly IgG. Regarding the subclasses of the IgG antibody, we noticed that mice immunized with the VEG and ME-49 255 Gy strains showed an increase of the IgG1 subtype, indicating a Th2 type response. The groups immunized with the strain RH 255 Gy and the association of the strains, RH+VEG+ME-49 255 Gy induced higher levels of the IgG2a subtype, corresponding to the Th1 type response profile. The induced protection in animals challenged with the ME-49 cystogenic strain was satisfactory in all immunized groups. By conventional optical microscopy and Real-Time PCR we observed that the groups immunized by the association of the three strains showed partial or total decrease in the number of cerebral cysts. Our results show that ionizing radiation may be an important promising tool in the development of an immunogen for toxoplasmosis with possible use in animals. **Supported by: Keywords:** Toxoplasma gondii; ionizing radiation; protection

HP56 - **PRELIMINARY ANALYSIS OF THE D-GALACTOSE EFFECT ON THE VIRULENCE OF *E. DISPAR***

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Introduction: *Entamoeba dispar* is a species considered commensal by most authors, although it has been isolated from patients with non-dysenteric colitis. The strains isolated were able to cause lesions in experimental animals similar to those caused by *E. histolytica.* The mechanisms of pathogenicity of trophozoites are mainly due to surface molecules, which bind to target cells, causing damage to the host. Among the ligands, there are lectins which bind to sugars such as D-galactose and N-acetylgalactosamine, present on the surface of some cells, such as erythrocytes. Objectives: To evaluate the influence of D-galactose on the virulence and pathogenicity of *E. dispar* strains. Materials and methods: For virulence, was performed erythrophagocytosis assay using the MCR and ACFN strains cultured. The assay was performed in duplicate, with a tube without the presence of D-galactose concentration and other with 55mmol.L-1 in 30 minutes, then trophozoites were interacted to erythrocytes. After this, 100 amoebae were counted per strain as well as the number of erythrocytes phagocytosed. To evaluate the pathogenicity, 100,000 trophozoites of the MCR strain associated or not with D-galactose were inoculated into the left lobe of hamster liver. Results: MCR and ACFN strains decreased their ability to phagocyte erythrocytes with D-galactose in the culture medium compared to the non-sugar tubes. Statistical difference was observed for the MCR strain (Mann Whitney test $p<0.0001$) and for ACFN (Wilcoxon signed rank test, $p=0.0007$). After necropsy of the hamsters, it was observed that two hamsters from the group inoculated with the MCR strain associated with D-galactose had no lesions. In the other three hamsters, the lesions were less extensive than the MCR group. Conclusion: D-galactose is effective in reducing the ability of erythrophagocytosis, as well as in interfering in the ability to produce hepatic lesions, reducing their extent. **Supported by:**FAPEMIG; CNPq; PRPq/UFMG **Keywords:** Entamoeba dispar; amebic liver abscess; d-galactose

HP57 - **UNRAVELLING RECEPTOR-TYPE ADENYLATE CYCLASE FUNCTIONS IN *TRYPANOSOMA CRUZI* USING A DOMINANT-NEGATIVE STRATEGY**

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Trypanosomatid parasites causing neglected diseases appear to differ considerably from the host in signalling mechanisms, with the exception of receptor-type adenylate cyclases (AC) that are topologically similar to receptor-type guanylate cyclase of higher eukaryotes but seem to control a new class of cAMP targets of unknown function, the cAMP response proteins (CARPs). Several trypanosomatid differentiation events were thought to be controlled by cAMP, such as the metacyclogenesis in Trypanosoma cruzi, a crucial cell differention event occurring in the digestive tract of the insect, which corresponds to the transformation from noninfective epimastigotes to infective metacyclic trypomastigote forms. In vitro monitoring of the T. cruzi metacyclogenesis induced by starvation showed that AC activity is stimulated in a two-step process with a constitutive upregulation of AC that are moving from the flagellar membrane to the flagellum. The objective of the present work is to study the functions of the putative receptor-type ACs of T. cruzi, whose genome encodes around 14 different AC isoforms, in order to provide new insights on the dialog between the parasite and its hosts. A constitutive mutant and a wild-type copy GFP-tagged were generated in a dominant-negative (DN) strategy in order to further determine their in vitro and in vivo phenotypes. DN phenotype will be assessed first by assaying cyclase activity and measuring in vitro cell proliferation and differentiation. We will next in vivo monitoring the insect colonization and the virulence in mice (host cell invasion and infectivity for mice) of the several AC cells lines by qPCR and BLI imaging using bioluminescent transgenic cell lines. **Supported by:** **Keywords:** Trypanosoma cruzi ; adenylate cyclase receptor; dominant-negative strategy

HP58 - **ERYTHROPHAGOCYTOSIS AS A VIRULENCE MARKER OF *ENTAMOEBA DISPAR***

DA SILVA, C.A.V.*[1]; DE OLIVEIRA, I.M.C.[1]; CRUZ, R.E.[1]; OLIVEIRA, F.M.S.[1]; GOMES, M.A.[1]; CALIARI, M.V.[1]
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Introduction: *Entamoeba histolytica* causes amoebiasis, an infectious disease affecting the human intestine that has an annual mortality of about 100,000 people worldwide. *E. dispar* is morphologically indistinguishable from *E. histolytica* and lives in the same habitat, but is considered noninvasive. There are few studies evaluating factors inherent to the biology of *E. dispar*, which appears to be reasonably more prevalent than *E. histolytica*. Materials and Methods: Virulence of the strains ACFN, ICS, ADO and VEJ of *E. dispar* were determined based on their ability to erythrophagocytosis and cause lesions in hamster livers. One ratio of one amoeba per 100 erythrocytes was chosen for the erythrophagocytosis assays and the trophozoites that phagocytosed and the erythrocytes in each trophozoite were quantified. Five hamsters per strain were inoculated with $1x10^6$ trophozoites and, on the third day post-infection, livers were collected and processed for histopathology and digital morphometry. Results: All strains of *E. dispar* were able to phagocyte erythrocytes with no significant differences between them. By contrast, there were significant differences between strains with respect to their ability to cause hepatic injury. The ACFN strain produced active amoebic liver abscesses with extensive areas of necrosis in 100% of infected animals. The ICS and VEJ strains caused minor lesions in inoculated animals. Amoebic liver abscesses under repair were only observed in animals inoculated with the ADO strain, suggesting that the host was able to rapidly control infection with this strain, favoring clearance. Conclusions: These results indicate that erythrophagocitosis is an ineffective method to evaluate the virulence of *E. dispar.* Studies involving other *E. dispar* strains and hosts may clarify the mechanisms related to the generation of lesions by *E. dispar* and lead to better amoebiasis control strategies. **Supported by:**CNPq, FAPEMIG e PRPq-UFMG **Keywords:** Entamoeba dispar; amoebic liver abscess; erythrophagocytosis

HP59 - **MORPHOLOGICAL STUDY OF KINETICS OF AMEBIC LIVER ABSCESS INDUCED BY STRAINS OF *ENTAMOEBA DISPAR***

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Introduction: *Entamoeba histolytica* produces amebiasis, an infectious disease that affects the human intestine, second largest cause of death among parasitic diseases. After the recognition of *E. dispar* in 1997 (WHO), several studies were started comparing this species of amoeba with *E. histolytica*. Considered commensal, some authors experimentally reproduced colitis and amebic liver abscess (ALA) using *E. dispar.* Some strains isolated by us have not been tested yet for their pathogenicity and the evolution of ALA is unknown after 8 days of *E. dispar* infection. Materials and methods: Fifteen hamsters for each strain of *E. dispar* (ACFN, MCR, ICS, ADO and VEJ) were inoculated into the left lobe of liver with 1x105 trophozoites in 0.1 mL/saline. Five of each group were euthanized at 8, 12 and 16 days post-infection (DAI) for necropsy, liver collection and processing for histopathology. Results: ACFN strain was shown to be pathogenic in the hamster, being able to produce ALA and 100% mortality in periods longer than 8 DAI. MCR, however, produced 23% mortality from the 8th to the 16th DAI, whereas the ICS, VEJ and ADO strains did not cause mortality. ALA was observed in all groups of hamsters, being more frequent in the inoculated with ACFN, MCR and ICS strains. The extent of necrosis was associated with intense parasitism and inflammatory infiltrate (granulocyte, histiocytic or mixed, focal and/or diffuse). At the 12th DAI, the last active abscesses were observed and the first ones under repair. Chronic granulomatous inflammation was more frequent in the 8th and 12th DAI, varying between groups. Most hamsters in the 16th DAI presented preservation of most of the liver parenchyma. Conclusions: Regeneration is the predominant healing mechanism in the experimental amebiasis produced by *E. dispar*. Healing occurs in small areas, despite large extensions of granulation tissue. Thus, with the exception of ACFN, all other strains were able to produce self-limiting liver damage. **Supported by:**CNPq, FAPEMIG e PRPq-UFMG
Keywords: Entamoeba dispar; amebic liver abscess; amebic lesions

HP60 - **THE ROLE OF IL-18 : THE MAINTENANCE OF CUTANEOUS LESIONS IN MODEL OF INFECTION WITH L. AMAZONENSIS**

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In infection by *Leishmania amazonensis* the classic model of polarization Th1 and Th2, found in the model of infection with *Leishmania major*, does not apply. What is described is a mixture of responses by changing the profiles of susceptibility and resistance in mice models. The IL-18 is a pro-inflammatory cytokine that has a capability to polarizes T lymphocytes to Th1, Th17 and Th2 phenotypes, depending on the environment in which it finds. Our work aims to identify the role of the IL-18 in the course of infection and development of skin lesions in mice infected by *L. amazonensis*. Our data showed that the wild-type animals (C57BL/6) have larger lesions than those of IL-18 KO since the beginning of the infection. In addition, the times analyzed, the two strains have the same parasite burden when infected with 10^2,10^4 and 10^5 promastigotes forms of *L. amazonensis*. On the cytokine profile, we found considerable levels of IL-12, TNF in both strains, but we found higher levels of IL-10 in the KO mice. Analyzing the cellular profile, we note that the wt lesions tends to have a larger population of important inflammatory cells. During the course of infection we identified a massive percentage of T cells CD4+ expressing the receptor for IL-18 after the fourth week of infection. This coincides with the appearance of the lesion exacerbated in wt mice and can be associated with the peak production of IL-18 seen by ELISA. We decided treat the infected mice, KO and wt, with anti-CD4(GK1.5) and we also look the profile of activation of the CD4+ T cells before, during and after the treatment with GK.1.5. To validade our hypothesis, we infected the IL-18 KO mice and treated these animals with CD4+ T cells from a wt GFP+ mice. Our data suggests that the IL-18 is partially involved in susceptibility to infection by *L. amazonensis*. This is likely due to the interaction of IL-18 on T lymphocytes helping the maintenance of the lesion. **Supported by:**CNPq, CAPES, FAPEMIG **Keywords:** Il-18; cd4+ t cell; leishmania amazonensis

HP61 - **PKA SIGNALING PATHWAY INVOLVED IN THE INTERACTION OF *TRYPANOSOMA CRUZI* WITH EXTRACELLULAR MATRIX ISOLATED FROM DIFFERENT CELL LINES**

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Trypanosoma cruzi, the protozoan agent of Chagas' disease, has a complex life cycle involving distinct developmental stages in the insect and mammalian hosts. Signaling triggered by the adhesion of *T. cruzi* trypomastigotes (Ty) to the extracellular matrix (ECM) from the mammalian host is an important step in the invasion process and leads to post-translational modifications of the proteins. Adhesion of Ty to ECM elements, such as laminin-111(α1β1γ1), fibronectin, collagen, heparan sulfate or thrombospondin are early events in the host cell infection. Despite the relevance of ECM in this process, the signaling pathways triggered by the Ty-ECM interaction are barely known. Dephosphorylation of α-tubulin, paraflagellar rod proteins (PAR) and ERK1/2 in Ty incubated with laminin or fibronectin were recently described by our group (Mattos et al., 2012). Also, unpublished data suggest the reprogramming of TY metabolism by ECM. This led us to analyze the influence of the ECM composition on the phosphorylation level of Ty proteins by isolating ECM from different cell lines (U87, HT29, LLCMK2) and Geltrex. The adhesion of Ty to the isolated ECMs was compatible with the infection data and therefore the ECM composition was determined by LC-MS/MS. The main components of ECM, such as glycoproteins, proteoglycans, collagens and other components varied among the ECMs. In general, Ty-ECM (MTy) interaction led to changes in the phosphorylation profile of Ty proteins, including the catalytic subunit of PKA, as well as to an increase in cAMP production between 5 to 15 min, depending on the original cell line. In summary, distinct ECM composition affects differently the protein phosphorylation levels in Ty, which is more intensely observed in trypomastigotes incubated with ECM derived from U87 cells. **Supported by:**FAPESP (2017/06717-5; 17/19854-0). **Keywords:** Trypanosoma cruzi; extracellular matrix; signaling pathway

HP62 - **THE MINIMAL ESSENTIAL HOST CELL COMPONENTS FOR LEISHMANIA INTRACELLULAR SURVIVAL: DUAL RNA-SEQ OF ENUCLEATED HOST CELLS INFECTED WITH LEISHMANIA AMAZONENSIS**

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Enucleated cells or cytoplasts (cells which nucleus is removed in vitro) represent an unexplored biologic model for intracellular infection studies due the abrupt interruption of nuclear processing and new RNA synthesis of host cell machinery in response to pathogen entry. Accordingly, murine cells enucleated by centrifugation with cytochalasin B host Leishmania amazonensis amastigotes for >48 hours, allowing parasite multiplication and biogenesis of large parasitophorous vacuoles. Cytoplasts were processed for a dual RNA sequencing to score both host cell and parasite transcripts at 48 hours post-infection, to identify: which host transcripts were preserved to support parasite intracellular multiplication; which host transcripts were more conserved or decay more rapidly due to L. amazonensis hosting and; what were the repertoire of transcripts expressed by the parasite in the absence of a parasite-stimulated differential gene expression of host cells. Cytoplasts displayed a functional enrichment of transcripts related to mitochondrial protein synthesis and mRNA alternative splicing machinery, potentially implicated in sustaining cytoplasts lifespan. Infected cytoplasts conserved cell transcripts related to metabolic, biosynthetic and signaling processes, while transcripts decay related to immune system processes. Cytoplasts hosting L. amazonensis displayed a >200-fold enrichment of a cluster of serine or cysteine protease inhibitors clade B (SerpinBs) transcripts, especially SerpinB9 and SerpinB8, which are related to increased cell survival and hindering important innate immune responses. Concerning parasite transcripts, the absence of host cell nucleus increases the expression of RNA binding protein transcripts potentially implicated in the preservation of cytoplasts transcripts. In conclusion, we presented preliminary evidence that there is a parasite-mediated control of host cell transcripts half-life, beneficial to parasite intracellular multiplication. **Supported by:**FAPESP, CNPq, CAPES **Keywords:** Leishmania amazonensis; rna-seq; cytoplasts

HP63 - **ROLE OF ENZYME ENOLASE AS A VIRULENCE FACTOR OF *LEISHMANIA AMAZONENSIS.***

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Leishmaniasis is a group of diseases caused by *Leishmania* parasites, which comprises cutaneous, mucocutaneous and visceral clinical forms. *Leishmania* cycle involves vertebrates (mammals) and *phlebotomine* insect vectors hosts. Promastigotes are transmitted at the time of blood feeding and infect preferentially macrophages, where they differentiate to amastigote forms, obligate intracellular parasites. Surface molecules present in the parasite are considered virulence factors because they are fundamental in the parasite-host interaction and control the different ways of infecting the macrophage. Some of these factors are associated with the parasite's ability to express surface molecules similar to those expressed by apoptotic cells, in a mechanism called apoptotic mimicry. This strategy allows macrophages to recognize *Leishmania* parasites, promoting phagocytosis and a damped inflammatory response. Phosphatidylserine (PS) is a well-studied molecule although there are other molecules expressed by apoptotic cells with similar functions, such as glycolytic enzymes expressed at the surface of apoptotic cells. We aimed to evaluate the presence of enolase, a glycolytic enzyme, in *L. amazonensis* infection. To compare parasites of different evolutionary forms and infective capacities we analyzed enolase surface expression in metacyclic and non-metacyclic promastigotes by flow cytometry. Promastigote forms in stationary phase were able to express surface enolase. It was possible to observe that in the population of promastigotes of smaller size (FSClow), and therefore more infective, 62% of the parasites express enolase. While in the less infective population (FSChigh,) 32% are able to express the protein. These results suggest that enolase surface expression is correlated to the infective capacity of *L. amazonensis* promastigotes. Enolase blockade at the surface of the parasites to evaluate the impact on the infection and macrophage inflammatory activity are being conducted. **Supported by:** **Keywords:** Leishmaniasis; apoptotic mimicry; promastigote

HP64 - **L. (L.) AMAZONENSIS MACROPHAGIC INFECTION IS MEDIATED BY THE FATTY ACID BINDING PROTEIN TYPE 4**

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Leishmania spp. is transmitted to vertebrate hosts through the bite of infected sandflies. In mammalian hosts, promastigotes infect several cells of the phagocytic system, mainly macrophages, and remain in parasitophorous vacuoles where they differentiate into amastigotes and modulate the environment to survive. It has been previously shown that the incorporation of fatty acids for amino acid biosynthesis is essential for the amastigote persistence (Saunders et al., 2014; https://doi.org/10.1371/journal.ppat.1003888). In macrophages, the fatty acid binding protein type 4 (FABP4) is abundant and plays a major role in controlling the availability of lipids and fatty acids in the cytosol (Furuhashi et al., 2014; https://dx.doi.org/10.4137%2FCMC.S17067). Because parasites metabolically depend on these molecules, this protein may exert an influence on the establishment of the infection. In order to investigate this hypothesis, we quantified fabp4 transcript levels in bone marrow derived macrophages after infections with *Leishmania (L.) amazonensis* by qRT-PCR. After 48h, transcript levels were ~7-fold increased in infected vs. non-infected macrophages. Also, after different time points (1, 48 and 96h), protein extracts and coverslips were obtained from which Western blot and indirect immunofluorescence experiments were performed. FABP4 levels augmented as the amastigote intracellular number increased as well. Immunofluorescence analysis indicated the accumulation of the protein around the parasitophorous vacuole over time. In parallel, infected cells incubated with 10 and 20 microM of a specific FABP4 inhibitor showed reduction in the parasite burden after 24 and 48h. Further assays are under way to confirm that FABP4 plays a major role in *L. (L.) amazonensis* infection. **Supported by:**FAPESP **Keywords:** Leishmania; parasitophorous vacuoles; fabp4

HP65 - **THE LEISHMANIA LYPOPHOSPHOGLYCAN TRIGGERS CASPASE-11 AND THE NON-CANONICAL ACTIVATION OF THE NLRP3 INFLAMMASOME**

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The activation of innate immune receptors by Leishmania parasites is critical for the outcome of leishmaniasis, a disease that affects over 12 million people worldwide. The innate immune response against Leishmania spp., including the activation of the family of nucleotide-binding and oligomerization domain-containing leucine-rich repeats (NLRs), plays an important role on the restriction of the parasite. Although many groups have demonstrated the importance of NLRP3 during Leishmania infection, the mechanisms by which the NLRP3 inflammasome is activated is unknown. Thus, the aim of this work is to evaluate the contribution of the caspase-11-mediated non-canonical inflammasome during Leishmania infection. We demonstrate that caspase-11 is activated in response to infection by Leishmania species and trigger the non-canonical activation of the NLRP3 inflammasome, a process that accounts for host resistance to infection in macrophages and in vivo. We also identified the parasite membrane glycoconjugate Lypophosphoglycan (LPG) as the molecule involved in caspase-11 activation. Intracellular delivery of Leishmania LPG in macrophages triggers caspase-11 activation. Accordingly, infections performed with Lpg1-/- parasites reduced the caspase-11-mediated non-canonical activation of the NLRP3. But different from bacterial LPS, LPG does not physically interact with caspase-11, suggesting the participation of additional molecules/receptors in LPG-mediated caspase-11 activation. LPG is extremely down regulated in the intracellular stage of the parasite, suggesting a mechanism to avoid caspase-11 activation and parasite killing. Collectively, our findings demonstrate an important role of caspase-11 in the control of Leishmania parasites and reveal the first parasite molecule involved in caspase-11 activation. **Supported by:**fapesp **Keywords:** Lipophosphoglycan; inflammasome; caspase-11

HP66 - **USING POPULATION GENOMICS APPROACHES TO UNDERSTAND POPULATION DYNAMICS IN BRAZILIAN L. INFANTUM AND IDENTIFY VIRULENCE FACTORS**

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At present, we are underutilizing advances in whole genome sequencing methodologies to understand the population structure of Leishmania in Brazil, and the benefits these data types can provide in facilitating better treatment of visceral leishmaniasis. Clinical trials for miltefosine treatment, which has a high efficacy in treating Leishmaniasis in India, has resulted in a high rate of treatment failure in Brazil. From 26 Piauí isolates we were able to strongly associate parasite genotype with patient outcome, demonstrating patient outcome is a heritable trait of the parasite. Using a genetic marker of miltefosine resistance, alongside other clinical manifestations, we were able to identify a distinct cline of this marker, and regional distinctions in the parasite genotype across Brazil. In order to understand the parasite genome component of patient treatment outcome, and more fully understand the genomic landscape of Brazil, we have embarked on the first large L. infantum population study using genomic sequencing. The high level of heritability, low genetic diversity and evidence of sexual recombination we have observed in these strains, and the additional genomes we have since collected, suggest that GWAS will facilitate the identification of genotypes under selective pressure and balancing selection with a larger sample size. Population genomic research with Plasmodium malaria parasites has been able to identify genes that are mediate host-parasite cellular interactions. These genes have become promising vaccines. A localised excess of genetic diversity caused by ‘balancing selection’ is one indicator of a gene involved in host-parasite interaction. Preliminary analysis has already identified robust signatures of balancing selection in multiple regions of the L. infantum genome. With analysis of other genetic signatures and increased population sampling important host-interacting genes, and domains within genes, can be identified. **Supported by:**Wellcome Trust **Keywords:** Genomics ; population genetics ; snps

HP67 - **THE SERINE-PYRUVATE PATHWAY IN TRYPANOSOMA CRUZI**

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Trypanosoma cruzi is the causative agent of Chagas disease. This parasite can use amino acids as energy sources and to support several biological processes such as differentiation, resistance to stress conditions and host-cell invasion. Metabolites containing -SH groups (such glutathione, trypanothion, cysteine, and some of its intermediates such as cystathionine) are relevant to buffer the redox state of the different sub-cellular compartments of this organism. Moreover, the redox pathways are a central aspect of the oxidative and energetic metabolism. Therefore, the study of pathways involving cysteine (Cys) is of major relevance. In the present work we intend to analyse the cysteine synthesis from serine, through reverse transulfurization pathway, which involves two enzymatic steps mediated by the enzymes Serine acetyl transferase (SAT) and cysteine synthase (CS). This pathway uses SH2 as the source of sulfur, and is directly connected to glycolysis. For both enzymes we already have identified the putative genes in T. cruzi genome databases (TriTryps). We obtained an active version of the recombinant TcSAT, which showed the predicted molecular mass (38 kDa). Additionally, we showed that O-Acetyl Serine (OAS), the product of SAT, was able to maintain the viability of epimastigotes in similar levels than histidine (control) when the cells were submitted to severe metabolic stress. Even more, we could evidence the ability of OAS to trigger mitochondrial respiration activity (free routine respiration), showing that this metabolite is able to trigger T. cruzi respiration. Summarizing, our results show that OAS, an intermediate between Ser and Cys, participates of the bioenergetics of T. cruzi epimastigotes. **Supported by:**FAPESP **Keywords:** Trypanosoma cruzi; cysteine synthesis; o-acetyl serine

HP68 - **SIGNALING PATHWAYS INVOLVED IN LEISHMANIA INFANTUM AMASTIGOTES INDUCED NETOSIS**

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Netosis cell death leads to the release of web-structures composed of chromatin, cytosolic and granular proteins (NETs). NETs can be extruded by two mechanisms: a reactive oxygen species (ROS)-dependent classical and a ROS-independent early/rapid netosis. NETs formation requires chromatin decondensation accomplished by the enzymes peptidyl arginine deiminase 4 (PAD4), elastase and myeloperoxidase (MPO). Our group has been studying the interaction of NETs and Leishmania promastigotes. However, amastigotes are the parasite form that keeps the active infection in the vertebrate and the presence of NETs interacting with amastigotes has been demonstrated in human lesions. In this study we try to unveil the mechanisms involved in NET formation induced by Leishmania infantum amastigotes. For this, human neutrophils, purified from blood of healthy donors, were incubated for 10 or 90min with different numbers of axenic amastigotes and NET-DNA released in the supernatant was measured with the Picogreen dye. Our data show that amastigotes induce netosis in a dose dependent way. Moreover, flow cytometry analysis show that amastigotes induce the production of ROS as assessed by the DHR 123 fluorogenic probe. We did not observe the activation of the early/rapid netosis. To investigate the signaling pathways involved in the formation of NETs, neutrophils were pretreated with several different chemical inhibitors for 30 min before the addition of the amastigotes. Blockage of the enzymes PAD4, NADPH oxidase, elastase and MPO completely abrogates NET release by amastigotes-activated neutrophils. Moreover, inhibition of the PI3K pathway (AS-604850 inhibitor), calcium mobilization (BAPTA - a calcium chelator) or glucose-6-phosphate dehydrogenase (6-AN inhibitor) impairs the release of NET. We can conclude that amastigotes induce netosis via a ROS-dependent mechanism with participation of elastase, MPO, PAD4, calcium mobilization, PI3Kγ and the pentose phosphate pathway. **Supported by:**CNPq, CAPES e FAPERJ **Keywords:** Visceral leishmaniasis; leishmania infantum; netosis

HP69 - **INTERACTION OF *TRYPANOSOMA CRUZI* WITH EXTRACELLULAR MATRIX TRIGGERS CHANGES IN THEIR PROTEINS PHOSPHORYLATION PROFILES**

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Adhesion of trypomastigotes (Ty) to ECM elements, such as laminin-111(α1β1γ1), fibronectin, collagen, heparan sulfate or thrombospondin are early events in the host cell infection. Despite the relevance of ECM in this process, the signaling pathways triggered by the Ty-ECM interaction are barely known. The characterization of Ty phosphoproteome upon interaction with ECM (MTy) showed important metabolic switches, being detected a decrease in the phosphorylation of hexokinase, phosphofructokinase, fructose-2, 6-bisphosphatase, phosphoglucomutase, phosphoglycerate kinase. Inhibition of hexokinase (HK), pyruvate kinase (PK) and lactate dehydrogenase (LDH) activities were observed at MTy cell extracts and this correlated with the phosphorylation levels of the respective enzymes. The enzyme activity was measured at total cell extract treated or not with alkaline phosphatase, showing the phosphorylation effect on the enzymes activities. The Ty cAMP intracellular concentrations were determined in different ECM incubation times and those correlate with a decrease of the total phosphodiesterase (PDE) activity in the first 45 min of MTy incubation when compared with the Ty levels. The phosphorylation of PKA was followed at the same time incubation times, by an specific anti-phospho-PKA antibody, suggesting differences at the phosphorylation levels of this kinase at the first 60 min of Ty-ECM interaction. In conclusion our results show that the contact of Ty with ECM, results in modulation of metabolic enzyme activites drived by changes of intracellular cAMP levels, phosphorylation profile and PDE activity. **Supported by:**CNPq
Keywords: Trypanosoma'; cell signalling ; phosphodiesterase

HP70 - **PROGRESSION OF SKIN INFECTION AND PRODUCTION OF CYTOKINES IN SKIN AND SERUM OF C57BL/6 AND BALB/C INFECTED WITH *LEISHMANIA (LEISHMANIA) AMAZONENSIS***

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Leishmaniasis is a disease caused by species of the genus *Leishmania*, an intracellular protozoan. According to different clinical manifestations, leishmaniasis can be: tegument form (subforms: cutaneous or localized cutaneous, diffuse cutaneous and mucocutaneous) or visceral form; thus, according to the genetic conditioning and development of the immune response of the host and also of the species *Leishmania spp*., one can have divergent profiles of immune response. Due to the genetic variability of both host and parasite species, the classic and well established pattern of Th1 and Th2 profiles doesn't represent the standardized and well segmented immune response to leishmaniasis, requiring others studies based on murine experimental infections. In this study, the progression of cutaneous infection and the production of cytokines in the skin and serum of C57BL/6 and BALB/c infected with *Leishmania (Leishmania) amazonensis*, both susceptible but exhibiting divergent clinical courses, were analyzed. We evaluated the lesions after 30 and 60 days of infection and observed early emergence accompanied by more severe lesions in BALB/c animals with a more frequent presence of intensely parasitized macrophage vacuoles, as well as a higher parasitic load, as well as less nitrergic activity since acute phase (30 days) of the infection. In contrast, C57BL/6 animals presented milder lesions, lower parasite load, higher nitergic activity and increased production of IL-4, IL-6 and TNF-α cytokines during the chronic phase of infection (60 days). We conclude that the infection is similar about BALB/c and C57BL/6, but lesions are divergents and these facts are related to the profile or intensity of immune response developed by each of these animals. **Supported by:**CAPES e IEC/SVS/MS. **Keywords:** Leishmaniasis; isogenic animals; immune response

HP71 - **THE ROLE OF TRYPANOSOMA CRUZI DEUBIQUITINASES IN PROTEIN SECRETION AND SURFACE PROTEIN TURNOVER**

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In eukaryotic cells, the ubiquitin-proteasome pathway (UPP) is the factor involved in regulating of protein levels and secretory pathways. UPP is composed of ubiquitin ligases and deubiquitinases. Ubiquitin ligases E1, E2, and E3 are required to link ubiquitin to each specific protein substrate protein that can then either targeted to proteasome degradation, or recycled by deubiquitination proteases (DUB) to couple diverse functions, including membrane trafficking. In T. brucei two DUBs (TbUSP7 and TbVdu1) were found to participate in the turnover of invariant surface glycoproteins. Knockdown of TbUSP7 produced an abnormal morphology and decreased cell growth. Similar DUBs are detected in Trypanosoma cruzi genome and there we asked whether these orthologous proteins (TcUSP7 and TcVdu) participate in the regulation of secretion of and turnover of surface proteins. We found that TcUSP7::GFP and TcVdu::GFP overexpressors showed different cytoplasmic fluorescence patterns with were similar to localization observed for T. brucei. Both parasites multiplied similarly to GFP overexpressors although TcVdu::GFP line presented decreased cell infectivity and delayed parasite egress from the host cell. Gene editing on TcDUBs for knockdown was made by transfecting the complex SaCas9/sgRNA in epimastigotes overexpressing TcUSP7::GFP and TcVDU::GFP. More than 50% of parasites showed no GFP expression and were selected by flow cytometry. The selected epimastigotes showed no large morphological differences and we are currently investigating whether both overexpressors or knockouts presents differences in the protein surfaces or in the secretory pathway. **Supported by:**FAPESP **Keywords:** Deubiquitinase; protein turnover; ubiquitin-proteasome pathway

HP72 - **PROFILE OF THE CELLULAR IMMUNE RESPONSE INDUCED IN C57BL/6J MICE IMMUNIZED WITH THE ASSOCIATION OF DIFFERENT STRAINS OF *TOXOPLASMA GONDII* TACHYZOITES IRRADIATED BY COBALT-60**

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Toxoplasmosis is a disease described worldwide that affects about 1/5 of the human population. In immunocompetent patients, significant ocular involvement and frequent congenital involvement can occur, with severe sequelae. The disease is acquired by oral ingestion of infectious forms such as cysts and oocysts and the mucous membranes are the first place to combat the agent. In toxoplasmosis, the use of irradiated parasites induces effective post-challenge protective immunity using *T. gondii* cystogenic strains. In this work we evaluated the cellular immune response of C57Bl/6j mice immunized simultaneously with three different strains of *T. gondii* tachyzoites irradiated at 255 Gy of Cobalt 60 (Co-60). We quantified the production of cytokines: IL-10, IL-6, IL-4, TNF-alpha, IFN-γ and IL-2 by CBA (Cytometric Bead Array) in spleen cells from previously immunized mice. The cytokine profile showed a classical profile of Th1 response with a typical increase of IFN-γ in relation to the other cytokines in all groups evaluated. In the groups immunized with the VEG 255 Gy strain and the RH+VEG 255 Gy and RH+ME-49+VEG 255 Gy groups we observed a significant difference in IL-2 production ($p < 0.001$) compared to the reference group RH 255 Gy. In relation to the cellular profile developed after the immunization, we detected a significant increase of the $CD3^+CD4^+$ phenotype in the immunized group with the association of the three irradiated strains. The proportion of the $CD19^+$ profile was higher in the groups immunized with the RH 255 Gy strain and the associations RH+VEG 255 Gy, RH+VEG 255 Gy and RH+ME-49+VEG 255 Gy. Our results are promising and show that immunization composed of more than one strain seems to induce a more efficient immune response. This would be of great importance for the development of a vaccine directed to use in felids, thus breaking the causal network of the disease and significantly reducing environmental contamination. **Supported by:**FAPESP **Keywords:** Toxoplasma gondii; vaccine; ionizing radiation

HP73 - **IN VITRO MODULATION OF TIGHT JUNCTION PROTEINS IN INTESTINAL CELLS INFECTED WITH *GIARDIA LAMBLIA***

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The gastrointestinal tract (GI) is formed by a continuous layer of epithelial cells that act as a semipermeable barrier. The barrier function results from a multiproteic complex that allows the selective passage of ions, solutes and water across the epithelium. This complex is composed of intercellular junctions, which are responsible for the paracellular permeability. Giardiasis is one of the most common intestinal disorder worldwide with high incidence and prevalence, particularly in tropical and subtropical areas. The infection is caused by *Giardia lamblia* is a microaerophilic parasite that colonizes the upper smal intestine of humans and other mammals worldwide. Its infection presents a large spectrum of symptoms varying from asymptomatic to acute or chronic symptomatic. Little is known about the pathophysiological mechanisms leading to giardiasis. However, it is well established that upon infection with *Giardia*, the intestinal cells shows morphological and functional damages such as villus shortening and apoptosis. In the present study, we show that *Giardia lamblia* infection, under low oxygen conditions, does not affect the paracellular flux of FITC-dextran (3 – 5 kDa) and decreases transepithelial electrical resistance values about 80% in HuTu-80 cells (duodenum cell lineage) after 4 hours of exposure. Western blotting analysis revealed that *Giardia* decreases the expression levels of claudin-4, occludin, ZO-1 and ZO-2 and increases the expression levels of claudin-1 and claudin-7. Immunofluorescent analysis shows the disruption of ZO-1 and ZO-2, corroborating with the WB analysis. In cocnlusion we provide evidence that *Giardia* infection causes epitelial dysfunction altering tight junction proteins (claudins, occludin and ZOs) in the duodenum at low O2 levels. **Supported by:**FAPESP and CAPES **Keywords:** Tight junctions; giardia lamblia; post-translational modifications

HP74 - ***LEISHMANIA AMAZONENSIS* LYSOPHOSPHOSPHOLIPIDS ARE HEMOLYTIC AND MAY ACCOUNT FOR PARASITE'S PORE-FORMING ACTIVITY.**

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In previous studies, we have described a pore-forming cytolysin present in L. amazonensis promastigotes extracts that acts optimally at 37°C and pH 5.5. Since these are the conditions found in the mammalian host we have postulated that it could be involved in phagolysosome and plasma membrane rupture, contributing to parasite survival and infection recrudescence. Here, using ionic exchange, molecular filtration and reverse phase chromatography, we obtained fractions from L. amazonensis promastigotes extracts containing hemolytic activity. Using mass spectrometry, we found that the hemolytic fractions were devoid of protein and contained one abundant peak of mass/charge ratio (M/Z) 518 and two others of M/Z 520, which were further identified as lysophospholipids of phosphatidylcholine (LPCs). Total lipids obtained from promastigotes extracts, also devoid of protein, retained nearly all hemolytic activity present in the extracts , thus suggesting that lipids, most probably the LPCs, are the major responsible for the hemolytic activity of L. amazonensis promastigotes extracts. Since LPCs are produced by the action of phospholipases A, we investigated the hemolytic activity of promastigotes grown in the presence of a phospholipase A2 inhibitor. Indeed, we found that parasites whose phospholipases A2 were inhibited totally lost their hemolytic activity. Together our results indicate that L. amazonensis hemolytic activity is due to phospholipase A2-derived LPCs that can directly lead to cell lysis. We are currently investigating whether LPCs lyse cells by pore formation. Since previous work from our group strongly indicated that L. amazonensis pore-forming cytolysin also depend on a protein, we are currently investigating whether this cytolysin would be a multi-molecular complex possibly composed by proteins and lipids. **Supported by:**CAPES; CNPq; FAPEMIG **Keywords:** Leishmania; cytolysin; lysophospholipids

HP75 - **PARTICIPATION OF THE CD36 SCAVENGER RECEPTOR IN THE PREFERENTIAL PROCESSING OF ANTIGENIC EXTRACTS OF *TOXOPLASMA GONDII* TACHYZOITES SUBMITTED TO COBALT-60 IRRADIATION**

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Toxoplasma gondii is an obligate intracellular parasite that affects warm-blooded animals, without a safer vaccine. Co-60 irradiated extracts of *T. gondii* tachyzoites induce better immunity without adjuvants in mice compared to native extracts. Scavenger receptors (SR) are a family of cell surface receptors, including CD36, responsible for internalizing oxidized products. Oxidized proteins is the phenomena induced by protein irradiation similar to neutrophil myeloperoxidase action. SR CD36 appears to participate in the presentation of antigens as its absence affecting immune response in C57Bl/6j knockout mice $CD36^{-/-}$ ($KoCD36^{-/-}$). In order to clarify the action of ionizing radiation of those extracts, we produced extracts of *T. gondii* tachyzoites labeled biosynthetically by ^{3}H proline ($STAG^{3}H$) in cell culture, a non-oxidative process. We assay these extracts both in vitro macrophage (MΦ) uptake and in mice immunization for detection of induced immune response both in wild type and $KoCD36^{-/-}$ C57Bl/6j mice. STAG3H binding assays in peritoneal MΦ of $WtCD36^{+/+}$ showed that irradiated antigens presented greater uptake by MΦ as compared to native antigen ($p<0.05$) while these effect are absent in $KoCD36^{-/-}$ MΦ ($p<0.05$). In $WtCD36^{+/+}$ mice with the same immunization, we detected higher production of specific IgG anti-*T. gondii*, in animals immunized with STAG 1500 Gy ($p<0.01$), with high avidity IgG ($p <0.05$), which demonstrates a greater adaptive immune response, resulting in protection after challenge with the *T. gondii* RH strain. In deficient mice of CD36 receptor, we did not detect significant production of specific IgG antibodies when immunized with native or irradiated STAG. Animals $CD36^{-/-}$ were extremely susceptible to *Toxoplasma* after challenge with viable parasite independent of immunization schedule. CD36 may participate in the uptake of irradiated by APCs resulting in the enhancement of immune response and protection induced by those irradiated antigen extracts. **Supported by:**FAPESP **Keywords:** Toxoplasma gondii; scavenger receptor; irradiated proteins

HP76 - **EFFECT OF TRYPANOSOMA CRUZI INFECTION IN HOST CELL DNA REPAIR AND SURVIVAL**

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It has been reported anticancer properties of Trypanosoma cruzi, but little is known about which mechanisms and molecules are involved with this therapeutic effect. Some hypothesis suggests that the oxidative stress response of T. cruzi, with its repair system, may protect host chromosomes reducing cancer development. To characterize that, differences in cell survival and DNA repair should be shown between infected and not-infected cells. The DNA damage was induced through cisplatin exposure, a drug known to induce DSBs. Methodology: The assay was performed using L6 cells. Three T. cruzi's strains (Berenice, Colombiana and CL Brener) were used for infection, separately, and one more group was infected with promastigote forms of Leishmania braziliensis. Three days after the infection, damage with cisplatin was performed with different concentrations and different exposure times. Cells was harvested immediately after damage or after a 24h recuperation period, then fixed with ethanol 70% and stained with Propidium Iodide. The cell cycle, considering DNA fluorescence, was analyzed using cytometry, and the sub-G1 phase was considered to determine DNA degradation and cell death. The cells were also submitted to a comet assay, to confirm DNA degradation. Results: Some differences in cell survival and DNA degradation were detected between the infected/ not-infected group, especially the infected with Berenice strain. Apparently, some T. cruzi strains induces cells death when damaged, while control cells not damaged don't show differences in survival if infected or not. Conclusion: T. cruzi infection seems to perform some influence in cell repair DNA and cell survival under damage. This influence depends of the strain, which is coherent with the literature, that indicates that different strains have different isoforms of some repair enzymes, such as MSH2. This can be a field to research possible mechanism that explain the influence of T. cruzi in cancer cells. **Supported by: Keywords:** Trypanosoma cruzi; repair system; cisplatin

HP77 - **TH17 LYMPHOCYTE IN NON-ULCERATED OR ATYPICAL CUTANEOUS LEISHMANIASIS BY *LEISHMANIA* (*L.*) *INFANTUM CHAGASI* IN CENTROAMERICA.**

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Background: In Honduras, visceral leishmaniosis (VL) and non-ulcerated or atypical cutaneous leishmaniasis (NUCL) have as etiological agent L. (L) infantum chagasi. The lesions of NUCL are defined as papule, asymptomatic nodule, non-ulcerative, erythematous or not and often surrounded by a hypopigmented halo. The immune response in leishmaniasis is quite complex, while in some cases a spontaneous cure may occur, in others an exacerbation of the response could be present. Recently, the Th1/Th2 paradigm has been expanded, followed by the discovery of a third subtype of effector Th cells that produce IL-17 (Th17) and exhibit distinct effector functions of Th1 and Th2 cells. Thus, the aim of this study was to characterize the participation of Th17 cells in the skin lesions of patients with NUCL from the municipality of Amapala and Orocuina, Honduras. Methods: Biopsies (n=20) of patients with NUCL, with positive parasitological diagnosis, were collected and processed by usual histological techniques. The presence of Th17 cells in situ was evaluated by immunohistochemistry using anti IL-17, anti-IL-6, anti-TGF-β and anti-IL-23 antibodies. For the development of the reaction, a NOVOLINK kit was used. The number of immunolabeled cells was counted by quantitative morphometric analysis using an image analysis system (Zeiss). Results: IL-17+, IL-6+, TGF-β+ and IL-23+ cells were observed in the dermal inflammatory infiltrate in all the studied cases, varying in intensity according to the histopathological features. The densities of these cellular types were 185.5 cells/mm2 for IL-17$^+$ cells, 298.1 for IL-6$^+$, 78.63 for TGF-β$^+$ and 285.2 for IL-23$^+$. We observed a positive correlation between IL-17$^+$ and TGF-β$^+$ cells (p=0.01947). Conclusion: Preliminary data showed the presence of IL-17$^+$, IL-6$^+$, TGF-β$^+$ and IL-23$^+$ cells, which could play an important role in the immunopathogenesis of NUCL. **Supported by:**FAPESP, CAPES, CNPq and LIM50 HC/FMUSP **Keywords:** Non-ulcerated or atypical cutaneous leishmaniasis; th17 cells; immunohistochemistry

HP78 - **EVIDENCE OF CD8 T CELLS RESPONSE IN NON-ULCERATED OR ATYPICAL CUTANEOUS LEISHMANIASIS BY *LEISHMANIA* (*L.*) *INFANTUM CHAGASI* IN CENTROAMERICA**

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Background: In Central America, especially in Honduras, non-ulcerated or atypical cutaneous leishmaniasis (NUCL) and visceral leishmaniasis (VL) are caused by the same etiologic agent Leishmania (L.) infantum chagasi and occur in the same geographic region. NUCL lesions are defined as: papule, plaque or nodule asymptomatic, non-ulcerative, erythematous or skin-colored and often the presence of a hypo-pigmented halo. Microscopically, the lesions are characterized by a mononuclear inflammatory infiltrate in the dermis composed mainly of lymphocytes, followed by macrophages and few plasma cells. The intensity of the inflammation is variable with discrete parasitism. The aim of this study was to characterize the role of T lymphocytes in NUCL. Methods: Twenty skin biopsies from patients with parasitological diagnosis was used. Immunohistochemistry, was evaluated using the primary antibodies to T lymphocytes (CD4, CD8), nitric oxide synthase (NOS2) and interferon-gamma (IFN-γ). For the development of the reaction, a NOVOLINK kit was used. The number of immunolabeled cells was counted by quantitative morphometric analysis using an image analysis system (Zeiss). Results: The immunohistochemical reaction showed an inflammatory response composed mainly of CD8$^+$ T lymphocytes (785.83 cells/mm2), followed by CD4$^+$ (296.58) and the presence of NOS2$^+$ (219.5) and IFN-γ$^+$ cells (671.4). We observed a positive correlation between CD8$^+$ T lymphocytes and IFN-γ+ cells (p = 0.00001). The evolution time of the lesion varied from 1 month to 20 years and showed no correlation with the inflammatory response in the tissue. Conclusion: The data suggest an efficient cellular immune response characterized by a high number of CD8$^+$ cells related to the activation of macrophages (IFN-γ$^+$ and iNOS$^+$), which is could be responsible for the low tissue parasitism helping to prevent the evolution of the lesion size in NUCL

Supported by:FAPESP, CAPES, CNPq and LIM50 HC/FMUSP **Keywords:** Cd8+ t lymphocytes ; non-ulcerated or atypical cutaneous leishmaniasis; immunohistochemistry

HP79 - **EFFECTS OF METAL-COORDINATED COMPOUNDS ON THE PHYSIOLOGY OF LEISHMANIA AMAZONENSIS AND LEISHMANIA CHAGASI**

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Leishmaniasis is a disease caused by flagellated protozoa belonging to Leishmania genus. The drugs used in its treatment present serious problems and the search for anti-leishmania compounds remains a major goal. In this context, our objective is to evaluate the effects of metal-coordinated Ag+-phendione and Cu2+-phendione compounds as potential drugs to be used in a chemotherapy against two of the most relevant Leishmania species in the country, L. amazonensis and L. chagasi. Our results showed that L. amazonensis and L. chagasi presented a dose-dependent reduction in growth in the presence of Ag+-phendione and Cu2+-phendione, being the IC50 value calculated for L. amazonensis as 7,8 nM and 7,5 nM, respectively. The same effect was observed on the growth of L. chagasi, being the IC50 calculated as 24 nM and 20 nM for Ag+-phendione and Cu2+-phendione, respectively. The optical microscopy analysis and scanning electron microscopy showed that metallocompounds caused morphological changes in promastigotes, such as shortening of the cell body and shortening or loss of the flagellum. In addition, the treatment with Ag+-phendione and Cu2+-phendione modulated the expression of gp63, an important virulence factor of Leishmania spp. The treatment with these compounds also affected the mitochondrial membrane potential of promastigotes, as observed by incubation with Rhodamine 123, besides inducing the production of reactive oxygen species in the parasites. The pre-treatment with Ag+-phendione and Cu2+-phendione inhibited the interaction of L. amazonensis with RAW macrophages; additionally, RAW cells previously infected with L. amazonenis and then post-treated with the metallocompounds presented a significant reduction in the viability of intracellular amastigotes. The results presented may contribute to the development of new drugs able to act in a selective and effective way against the diseases caused by Leishmania, being an alternative chemotherapy for leishmaniasis.**Supported by:**Faperj CNPq, CAPES
Keywords: Leishmania; metallocompounds ; chemotherapy

HP80 - **EVALUATION OF ANTI-TRYPANOSOMA CRUZI ANTIBODIES AND AUTOANTIBODIES IN ACUTE AND CHRONIC CHAGAS' DISEASE**

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Introduction: Chagas disease (CD) is the most important parasitic disease in the Americas, with 7 million people infected. Nearly 30% of infected individuals will develop cardiac, digestive or neurological dysfunction during the chronic phase. Factors that lead to the development of clinical manifestations have not been fully elucidated yet, existing hypotheses that suggest from the degradation of tissue directly affected by the presence of the parasite to the possibility of autoimmune disease that is not directly dependent on the persistent critical infection. Objective: To analyze the rate of production of IgM and IgG specific anti-T.cruzi, anti-cardiac and intestinal proteins in mice infected with different strains of T. cruzi, in the acute (DCA) and chronic Chagas. Methods: Sixty male and female BALB/c mice were divided into three groups, according to the infective strain (Colombian, DTU I, Y, DTU II, CL Brener, DTU VI), and analyzed at 30 and 90 days postinfection (dpi). Ten uninfected mice (5 males and 5 females) were selected for the negative group. Serum was separated for Enzyme-Linked Immunoabsorbent Assay (ELISA) indirect. Results: There was a high positivity for all isotypes against the T. cruzi antigen, in which the animals of the chronic phase were 100% reactive for IgG. Rates of anti-proteins antibodies were low in DCA, however increased in DCC, notably in relation to intestinal proteins. The Colombian strain was significantly higher in relation to the presence of anti-heart autoantibodies. Conclusions: The ELISA technique proved to be very effective for detecting of pathology. Our results confirm the participation of autoimmune response in CHD, which varies according to T. cruzi lineage. New experiments are essential to establish the actual association of such autoantibodies with the evolution of the clinical manifestations of CD. **Keywords:** Autoantibodies; autoimmunity; t. cruzi

HP81 - **DETECTION OF MMP/TIMP IN 3T3 FIBROBLASTS INFECTED WITH LEISHMANIA BRAZILIENSIS.**

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Cutaneous Leishmaniasis is a neglected disease that occurs in several countries, including Brazil. In mammalian tissues, phagocytes are like cells of predilection of *Leishmania spp.*, however, the parasite can also be phagocytosed by non-phagocytic cells, such as fibroblasts. In addition, the migration of Leishmania to other tissues may involve metalloproteinases (MMP), due to the degradation of various components of the extracellular matrix. MMPs are modulated by endogenous tissue inhibitors of MMP, the TIMPs. Therefore, the objective of this study is to investigate the participation of MMP/TIMP in fibroblasts infected with *Leishmania braziliensis*. 3T3 fibroblasts were infected at a ratio of 1:10 (Fibroblast:Leishmania) for 3h at 34 ° C and maintained in culture for 24h and 96h. After the formation of the monolayer, the fibroblasts were subjected to the in vitro scratching assay, then infected and maintaining in culture for 24h. Immunofluorescence marking was performed for MMP-2 and TIMP-2. The supernatant was subjected to the zimography technique to evaluate the extracellular activity of MMP. The fibroblasts infected or not, presented active MMP-2 in 24h and 96h after infection, however, with no significant statistical difference. In immunofluorescence, MMP-2 and TIMP-2 were detected in all groups, mainly near the nucleus or dispersed by the cytoplasm. It can be suggested that there was cell migration of the fibroblasts submitted to the lesion. The infected fibroblasts for 24h were stained to TIMP-2 and showed an increased intracellular labeling compared to MMP-2, presumably a possible attempt by the cells to control the excess of MMP-2 already released in the extracellular medium. Thus, it is suggested that the production of MMP/TIMP by fibroblasts plays a relevant role in the control of infection. **Supported by:**CNPq **Keywords:** Leishmania; mmp; timp

HP82 - **EVALUATION OF THE INVASION PROCESS OF DIFFERENT SPECIES OF *LEISHMANIA* IN NON-PROFESSIONAL PHAGOCYTIC CELLS**

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Leishmaniasis forms a group of anthropozoonosis transmitted to humans and other mammals by sand flies vectors and are considered neglected tropical diseases. The disease is endemic in 98 countries and it's estimated that 350 million people live in areas at risk of infection and approximately 2 million of new cases are reported per year. Although macrophages are considered the most important host cells during parasite infection, the fibroblasts may also be host cells for *Leishmania*, but the initial form of the interaction between *Leishmania* and fibroblast has not been well clarify. Thus, our study used scanning microscopy (for observation of the ultrastructure), fluorescence microscopy (observation of the actin filaments and Rab5a) and bright field microscopy (counting of adhered parasites) to study the interaction between the cells in different periods (30 minutes, 1 h, 3 h, 5 h and 18 h). In the ultrastructure results filopodia, lamelipodia and *ruffles* were observed on the surface of the infected fibroblasts. The *L. braziliensis* presented the highest number of parasites adhered to the surface of fibroblasts compared to *L. amazonensis*. The Rab5a was detected in fibroblasts for 5h and 18h of interaction with *L. amazonensis* and *L. braziliensis*, respectively. In both species the presence of Rab5a was detect, but in different times. Therefore, the fibroblasts are potential hosts for *Leishmania*, possibly endociting the parasite by non-classical pathways, such as macropyocytosis. **Supported by:**CNPq **Keywords:** Fibroblast; leishmania; ultrastructure

HP83 - **ROLE OF SAPA REPEATS PRESENT IN THE TRYPANOSOMA CRUZI TRANS-SIALIDASE DURING INFECTION**

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Trans-sialidases are enzymes from T. cruzi whose function is to transfer sialic acid from host glycoconjugates to mucin-type molecules at the surface of the parasite, an activity involved in invasion of the host cell and immune evasion mechanisms developed by the parasite. Trans-sialidases of group I (TcTS-I) exhibit a repeat domain at the C-terminus, named SAPA (Shed Acute Parasite Antigen) repeat. Genomic comparisons suggested that the avirulent cloned T. cruzi strain CL-14 have fewer SAPA repeats than the virulent CL Brener clone. Transcriptome analyses showed that the expression of TcTS-I genes are up-regulated during the final stages of the intracellular development whereas other members of the TS family are up-regulated in trypomastigotes. To test the role of SAPA repeats as a virulence factor we transfected CL-14 parasites with a TS gene containing 19 SAPA repeats and with the same gene without repeats as well as with the empty pROCK expression vector. In vitro infection assays showed that cells infected with parasites overexpressing the transgene TS genes with or without the repeats have similar numbers of intracellular amastigotes and trypomastigotes released into the supernatant. To test effect of the distinct TS forms, we generated recombinant versions of the protein with and without their repeat domains as well the protein containing only SAPA repeats. The different antigens were used to immunize animals before challenging with a virulent strain of the parasite. Production of IgG antibodies against all portions of the proteins were observed in the immunized animals. Confirming the expression data obtained from RNA-seq analyses, antibodies generated against the recombinant TS containing the SAPA repeats recognized high molecular weight proteins only in late stages amastigotes and trypomastigote but not in epimastigotes. The effect over the infection of the immunization with the different TS antigens is currently being investigated.

Supported by: CNPq **Keywords:** Trans sialidase; sapa repeats; immune response

HP84 - **IMMUNOMETABOLISM IN THE SYMBIOTIC RELATIONSHIP BETWEEN THE AEDES FLUVIATILIS MOSQUITO AND BACTERIUM WOLBACHIA PIPIENTIS**

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Wolbachia pipientis is a Gram-negative bacteria that has attracted considerable interest in promoting distinct effects on its hosts, such as the reduction of the replication capacity of pathogens in its hosts. It was recently seen that Wolbachia strain (wFLU) promotes a modulation of glycogen metabolism in its natural endosymbiont, the mosquito Aedes fluviatilis. When silencing the enzyme glycogen synthase kinase 3 (gsk3), the enzyme regulating glycogen synthesis, the amount of this bacterium and glycogen in Aedes fluviatilis increases. Thus, a better understanding of the interaction of Wolbachia with the Aedes fluviatilis mosquito is necessary because we believe that this bacterium can change the communication between the Gsk3 and the immune system of its host. The aim of this work is study the intersections between the Toll-mediated immune response and the metabolic control by the action of the enzyme GSK3. To investigate whether Toll pathway acts to control the amount of Wolbachia in mosquitoes, we reduced the levels of Relish1 in adult females and their eggs by RNAi. The knock-down of Relish1 leads to embryonic lethality and the amount of Wolbachia increases. Therefore, our results suggest that the Toll pathway is directly involved in the symbiotic relationship between A. fluviatilis and Wolbachia. Next steps will be to silence Relish1, quantify the glycogen in the injected mosquitoes and assess the levels of expression of the wnt pathway. **Supported by:**CNPQ, CAPES, FAPERJ, INCT-EM **Keywords:** Wolbachia; aedes fluviatilis; glycogen synthase kinase 3 (gsk3)

HP85 - **GENE EXPRESSION AND MORPHOLOGICAL ALTERATIONS OF *ONCOPELTUS FASCIATUS* (HEMIPTERA, LYGAEIDAE) SPECIES INFECTED BY *LEPTOMONAS WALLACEI* (TRYPANOSOMATIDAE)**

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The milkweed bug *Oncopeltus fasciatus* is commonly found infected by *Leptomonas wallacei* in nature. Previous studies from our group showed that uninfected insects have larger body, wings and appendages; they are less deformed, copulate more, lay more eggs and live longer than infected ones. The aim of this study is to test the correlation between *L. wallacei* infection with morphological changes in reproductive organs and the expression of the *intersex* gene (*ix*). The *ix* gene is of great interest, since it is associated with the development of the reproductive system of these insects. For morphometric analysis, females from both colonies (infected and uninfected) were dissected (n = 40) and photographed on graph paper. Ovopositor/forewing rates were measured by Analyzing Digital Images software. No significant differences on the external morphology of the two colonies were found. For gene expression analysis, adults had their ovaries (n=12) dissected, photographed and RNA extracted. A quantitative PCR was performed and the data showed that ovaries of infected females showed a decrease of 71.84% (± 12.64) in *ix* expression, as compared to uninfected females. For gene silencing using RNAi, 4th instar nymphs were separated (n = 20) and induced to ingest specific dsRNA for the *ix* gene. Preliminary data showed that ovaries of uninfected silenced females were atrophied. Previous studies have shown that *ix* silencing 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 with the reduction of sexual activity, oviposition and offspring, along with the reabsorption of the eggs. This study shows a correlation between the expression of *ix* gene with the atrophy of ovaries in *O. fasciatus*, which is clearly more evident in *L. wallacei*-infected *O. fasciatus*.
Supported by:CNPq, FAPERJ, INCT-EM, CAPES **Keywords:** Intersex; qpcr; rnai

HP86 - **PD-1 REGULATES MICROBICIDE ACTIVITY AND IL-17 IN CANINE VISCERAL LEISHMANIASIS**

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Leishmaniasis is an immunosuppressive disease caused by protozoa of the genus Leishmania for which dogs are the domestic reservoir. The programmed cell death 1 molecule (PD-1) is highly expressed in leukocyte cells of dogs with visceral leishmaniasis and it promotes T lymphocyte exhaustion and suppression of cytokine secretion. Because PD-1 has a suppressive function regarding cell immunity, we evaluated the effect of PD-1 blocking antibodies on NO, Nitrite, ROS, IL-17 production and parasite load in spleen leukocyte cultures from naturally infected dogs. First, we determined the PD-1 levels in spleen leukocytes from infected and uninfected dogs by means of flow cytometry (BD AccuriTM C5 Flow Cytometer, San Diego, CA, USA). For NO and ROS determination, 5x105 spleen leukocytes from naturally infected dogs, were cultured at 37°C with 5% CO2, in the presence of 5mg/ml blocking monoclonal antibody against PD-1 (anti-human CD279, eBioscience Inc, San Diego, CA, USA) for 18 hours to NO and ROS intracellular analysis by flow cytometry. IL 17 and nitrite was measured after 72 hour of in culture supernatant and parasitic load was determined in splenic leuckocytes. Nitrite dosage was determined using the Griess method. IL-17 in was determined using capture ELISA. The intracellular quantification of the parasite load was determined by optical microscopy,. In vitro, PD-1 blocking promoted increased levels of intracellular NO and Nitrite and reduced the levels of IL-17 in the culture supernatant, in addition to reducing the parasite load, but do not change ROS levels. We conclude that PD-1 participates in regulation of the immune response in spleen leukocytes and that the blocking antibody is effective in

restoring host microbicidal activity. **Supported by:**CAPES, FAPESP 201516072-7, CNPq 400063/2016-6 **Keywords:** Spleen leukocytes; protozoa; cd279

HP87 - **PLASMONIC ELISA FOR THE ULTRA-SENSITIVE DETECTION OF *ANTI-LEISHMANIA SP.* IGG ANTIBODIES - PRELIMINARY DATA**

MACIEL, M.O.S.*[1]; SOARES, M.F.[1]; COSTA, S.F.[1]; BRAGATO, J.P.[1]; VENTURIN, G.L.[1]; HERNANDEZ, F.M.O.[1]; FREITAS, J.H.[1]; MELO, L.M.[1]; REBECH, G.T.[1]; LIMA, V.M.F.[1]
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In Visceral Canine Leishmaniasis (VCL) most dogs develop a humoral immune response, with high titers of IgG *anti-Leishmania sp.* However, in endemic areas many infected dogs are clinically "healthy" and with low antibody titers, which are undetectable by conventional serological methods. Due to the zoonotic importance of the disease, the study of new methods of diagnosis is important, since it may constitute new tools for the control of VCL. Therefore, the objective of this study was the characterization of canine samples to validate a plasmonic ELISA strategy (pELISA) for the ultra-sensitive detection of IgG anti-Leishmania sp. For this purpose, 180 blood samples from adult dogs of both sexes were obtained from the different sectors of the city of Araçatuba/SP, in addition to 35 blood samples from dogs obtained from the laboratory routine of an area not endemic to the disease (São Vicente/SP). In the endemic area, 20.55% (37/180) of the samples had a confirmed diagnosis of VCL by serology, using the rapid test DPP® Visceral Canine Leishmaniasis and conventional ELISA. In the non-endemic area all samples were negative for both tests. Among the infected dogs, 7/37 showed mild-moderate disease (level I) and 30/37 showed moderate disease (level II), based on a physical examination, laboratory findings and on their levels of anti-Leishmania antibodies. The most frequent clinical sign was onychogryphosis, affecting 48.65% (18/38) of the animals, followed by lymphadenopathy with 45.95% (17/38) of the animals. The main biochemical alterations were hypergammaglobulinemia, hypoalbuminemia and hyperproteinemia. Hematological alterations were also observed, such as a decrease in CHCM associated with hematocrit decrease and thrombocytopenia. The results considered to date are similar to those described by several authors in endemic areas, demonstrating that this is a representative sample of the canine population positive for LVC, enabling the p-ELISA validation. **Supported by:**FAPESP 2017/11016-6; 2017/25821-8; 2015/16072-7 **Keywords:** Diagnosis; visceral canine leishmaniasis; plasmonic elisa

HP88 - **ANALYSIS OF THE T- BET (T1) AND GATA-3 (T2) PROFILE IN SPLENIC LEUKOCYTES OF DOGS WITH VISCERAL LEISHMANIASIS**

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The progression of Canine Visceral Leishmaniasis (CVL) is accompanied by a suppression of cellular immunity but the polarization of T1 or T2 in spleen tissues of symptomatic dogs with LVC is not characterized. The objective of the present study was to evaluate the T1 and T2 immune response profile in splenic leukocytes of dogs with LVC. Seven dogs with ages between 2 and 5 years of age, of both sexes, not defined race and with clinical signs for LVC from Araçatuba-S.P, Brazil were selected. The diagnosis was confirmed by indirect ELISA for antibody detection anti-Leishmania spp. A spleen fragment was collected and processed to obtain the splenic leukocytes. To evaluate the response profile, 104 cells were used. The cells were fixed with commercial buffer (Flow Cytometry Fixation Buffer 1X, R & D System), after centrifugation at 300g, the cells were resuspended with Flow Cytometry Permeabilization Buffer 1X (R & D System) and incubated at 4º C for 1h:30m with Human T- bet/TBX21 Fluorescein-conjugated Antibody (T1) and Human GATA-3 PE conjugated Antibody (T2) (R & D System). After incubation, the cells were washed and resuspended with pH 7.2 saline phosphate buffer and the fluorescence mean of 10.000 events was read on the Flow Cytometer (BD Biosciences). We observed a higher of T-bet in cells from splenic leukocytes compared to GATA-3 ($p < 0.05$, Wilcoxon test). We concluded predominance of a T1 profile response in the spleen of dogs with CVL and suggests that the cellular immune response is activated, but not enough to control the disease. **Supported by:**FAPESP 2015/16072-7, 2017/10906-8 **Keywords:** Dog; visceral leishmaniases; leukocytes

HP89 - **EVALUATION OF THE PHOSPHATIDYLSERINE (PS) EXPOSURE BY *PLASMODIUM CHABAUDI***

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Malaria is one of the most prolific parasitic diseases in the tropical countries. The infection of mice by *Plasmodium chabaudi* is one of the closest models to the human disease caused by *P. Fal[illegible]parum*. An evasive mechanism that inhibits the nitric oxide (microbicide agent) production i[illegible]hages was shown in the parasitic protozoan *Leishmania amazonensis*, *T[illegible]* and *Trypanosoma cruzi*. Such mechanism consists in the exposure [illegible]), a phospholipid present in the plasmatic membrane that usuall[illegible]l is the main connector involved in the recognition of the a[illegible]he PS exposure, apoptotic cells generate inflam[illegible]ed. The PS exposure for those protozoan [illegible]. To verify if this mechanism is present in [illegible]s in vitro-activated peritoneal macrophage[illegible]audi* population expose PS leading to the [illegible]48 h of macrophages interaction. Further[illegible]nd blebs formation in macrophages. Mice [illegible]fected with parasites with PS blocked by annexyn [illegible]posing parasites that resulted in high parasitemia and death [illegible]ations of the liver and spleen showed inflammatory process in a[illegible]posing parasites, in contrast with animals infected with parasites that had th[illegible]sults suggest that *P. chabaudi* performs the “apoptotic mimicry”, reinforcing the hyp[illegible]hat such mechanism is common to different parasitic protozoan, indicating that the PS exposure has a leading role in the infective process of parasitic protozoan. **Supported by:**FAPERJ
Keywords: Plasmodium chabaudi; phosphatidylserine; nitric oxide

HP90 - **TRYPANOTHIONE SYNTHETASE CONTRIBUTES TO PARASITE SURVIVAL TOWARDS OXIDATIVE STRESS AND TRYPANOCIDAL DRUGS BENZNIDAZOL AND NIFURTIMOX IN *TRYPANOSOMA CRUZI***

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The antioxidant network of *Trypanosoma cruzi* is known to be determinant in its survival upon the oxidative response displayed by innate immunity cells, and thereby allow the establishment of the infection. Besides, some members of this system are promising targets for the development of new treatment strategies for Chagas disease since they are trypanosomatid-specific enzymes, absent in the vertebrate host. One of these, named trypanothione synthetase (TryS), mediates biosynthesis of a low MW thiol formed by spermidine and two molecules of glutathione, called trypanothione. This metabolite is extremely important for the recycling of antioxidant oxidoreductases and the neutralization of ROS in the parasite cell. We have overexpressed TryS in *T. cruzi* SylvioX10 strain, recombinant parasites ($TryS^{hi}$) exhibited stable overexpression (>2-fold increase) of the TryS protein and a significant increase in TryS enzymatic activity as compared to controls. Herein we report that $TryS^{hi}$ epimastigotes as well as infective trypomastigote forms, exhibited higher resistance to H_2O_2 (50-1000 mM), compared to wild type parasites (36-71%, respectively). Even more, epimastigote and trypomastigote forms of $TryS^{hi}$ (*vs.* control) tolerated higher doses of benznidazole and nifurtimox, the drugs currently administered for acute Chagas disease treatment. Further, treatment with TryS specific inhibitors (5-30 mM) was beneficial in increasing parasite’s sensitivity to anti-parasitic drugs. Our results firstly suggest that TryS provides a sort of benefit to the pathogen to develop resistance against currently used anti-trypanosomal drugs and secondly, its inhibition could be useful for the design of drug combination therapy against Chagas disease. **Supported by:**
Keywords: Trypanosoma cruzi; trypanothione synthetase; anti-parasite drugs

HP91 - **LAMININ AND POLYMERIZED LAMININ TRIGGERS NEUTROPHIL EXTRACELLULAR TRAPS (NETS) AND MODULATES NETOSIS INDUCED BY LEISHMANIA AMAZONENSIS**

OLIVEIRA, G.S.[*1]; GOMES, C.S.O.[2]; LACERDA, L.L.[1]; RIEDERER, I.[2]; SAMPAIO, T.C.[1]; SARAIVA, E.M.[1]

1.UFRJ, RJ, Brazil; 2.FUNDAÇÃO OSWALDO CRUZ, RJ, Brazil.

Neutrophils are the first cells recruited and infected in the early stages of the Leishmania infection. Upon interaction with the parasites they also extrude structures known as neutrophil extracellular traps (NETs). All these initial steps occur in close contact with proteins from the extracellular matrix (ECM), such as the glycoprotein laminin. The release of NETs with ECM involvement has been reported for fibronectin associated with β-glucan, where the integrin VLA-5, a fibronectin receptor, regulates NETosis. Here, we aim to study the interaction between neutrophils, Leishmania amazonensis promastigotes and different isoforms of laminins either, isolated or in association, analyzing the induction of NETs. Thus, initially, we observed that neutrophils stimulated with laminin isoforms (111, 211, 332, 411, 421 and 511) in solution, released NETs. The same happens when neutrophils interacted with the polymerized laminin (Polylaminin) 111, 411 and 511. We also showed that about 70% of neutrophils express the α6 integrin subunit (CD49f), which together with subunits β1 or β4 integrins forms the laminin receptor. The pretreatment with anti-α6 integrin subunit antibody decreased NETs release by 32% and 35% when stimulated with laminins 411 and 511 respectively. In the context of NETs molecular mechanism, we demonstrated that neutrophils stimulated with laminin 511 release NET on a ROS dependent way, however the 411 isoform is independent of ROS generation. Furthermore, NET trigger by these laminins are dependent on neutrophil elastase and peptidylarginine deiminase 4 (PAD4), enzymes that participates in the chromatin decondensation process. Finally, we demonstrated that laminins 411 and 511 increase NETs release by Leishmania amazonensis promastigotes. **Supported by:** CNPq, FAPERJ. **Keywords:** Laminin; nets; neutrophil

HP92 - **MAST CELLS EXTRACELULAR TRAPS INDUCED BY LEISHMANIA**

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Leishmania are inoculated by the insect vector in a pool of blood, whereby the parasite interacts with different immune cells and with the extracellular matrix proteins. Mast cells are residents' granulocytes that release extracellular traps composed of chromatin and granular proteins (MCETs), by the etosis mechanism. Similarly to neutrophil extracellular traps, MCETs are capable to retain and kill microorganisms. Although different studies imply that mast cells are the first line of defense against Leishmania, little is known about the induction and the importance of the MCETs. Therefore our objective here is to evaluate if L. amazonensis promastigotes induce MCETs release and if these traps are toxic for the parasites. We also want to understand the importance of laminin, an extracellular matrix protein, in this process. In our study, we used a human mast cell lineage, HMC-1, which was maintained in DMEM culture medium supplemented with 20% fetal bovine serum and 40 µg/ml of gentamicin at 37oC, 5% CO2. Regarding the MCETs release assay, HMC-1 was incubated in RPMI without serum with promastigotes and the release was quantified in the culture supernatants by Quanti-it™PicoGreen®. Our results have shown that promastigotes induce MCETs release by HMC-1 by the rapid and classic etosis mechanisms. In order to simulate the environment that these parasites interact with the host cells, we tested the release of MCETs in the presence of laminin. Our results confirmed that MCETs are release in the presence by Leishmania amazonensis promastigotes and that laminin, either in their polymerized form or in their soluble form, potentiated this release. HMC-1 cells were also treated with nerve growth factor (NGF), an important factor for mast cell growth and activation, which was added every 2 days for 10 days. The cells were incubated with promastigotes and the NET release was measured in the same way as before. Our results showed that NGF-treated cells also released MCETs. **Supported by:**CAPES, CNPq, FAPERJ **Keywords:** Leishmania; mast cells; extracellular matrix

HP93 - **LOWER NITRIC OXIDE PRODUCTION AFTER CLASSICAL ACTIVATION AND GROWTH OF TOXOPLASMA GONDII IN MACROPHAGES CULTURED IN COLLAGEN**

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Culture of cell on polystyrene surface is common. Culturing cells on collagen (COL) is an interesting alternative due to the similarity with the living organism, its abundance in the extracellular matrix and three dimensional environment. Macrophages are phagocytic cells commonly cultured in vitro that are active players in the immune response, eliminating pathogens, and responsible for tissue homeostasis. When activated they are classified as M1 or M2. M1 macrophages produce high amounts of reactive nitrogen/oxygen species resulting in high microbicidal activity. Nitric oxide (NO) is important in the microbicidal process being produced in the infection site, controlling the growth of Toxoplasma gondii, the causative agent of toxoplasmosis, a worldwide zoonosis. Infection with T. gondii modulates macrophages, inhibiting production of NO. Here we compared if the culture of macrophages on COL changes NO production of these cells after M1 activation, and how it influences the modulation of NO production by T. gondii and the growth of the parasite. COL was obtained from the tail of rats. RAW 264.7 macrophage cell line was plated on biofilms of COL or over polystyrene. Macrophages were classically activated with lipopolysaccharide and interferon-γ for 24 h, subsequently infected with a 1:1 ratio of cells:tachyzoite of T. gondii and cultured for 24 h. Infected macrophages cultured on COL produced less NO than macrophages grown in polystyrene. Lower production of NO as well as a tendency of higher growth of tachyzoites was found in macrophages cultured on COL. COL can be used as a substrate for culture and interaction analysis of macrophages and T. gondii, and is a promising alternative for more realistic elucidation of the functioning of the immune system and the relationship of cells with pathogens. **Supported by:**CNPq, CAPEs, UENF
Keywords: Collagen; macrophage; toxoplasma gondii

HP94 - **BRADYKININ MEDIATES ADHESION OF *PLASMODIUM FALCIPARUM* INFECTED ERYTHROCYTES TO ENDOTHELIAL CELLS AND BLOOD-BRAIN BARRIER DISRUPTION.**

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INTRODUCTION: Cerebral malaria (CM), a complication in *P. falciparum* infection, is associated to the sequestration of infected eryhtrocytes (iRBCs) into the brain microvasculature and blood-brain barrier (BBB) disruption. Sequestration depends on the interaction between parasite-derived proteins on the surface of iRBCs and adhesion molecules in the endothelial cells. Our group have previously demonstrated that BK levels are increased in the supernatant of iRBCs. However, its role in iRBCs adhesion to endothelial cells and BBB disruption have never been addressed.
OBJECTIVE: This work aims to investigate the role of BK in the adhesion of iRBCs to endothelial cells and BBB disruption.
METHODS: Health A-type human RBCs infected with *P. falciparum* W2 strain and a human brain endothelial cell line (BMEC) were used in adhesion assays. The expression of ZO-1 and β-catenin were performed by immunofluorescence.
RESULTS: Pretreatment of BMECs with 10-7 M BK induced a 2-fold increase in iRBCs-BMEC adhesion (n=3). Similarly, the iRBCs culture supernatant produced the same effect (n=3), suggesting the presence of BK in the conditioned medium (CM). Accordingly, the stimulatory effect of both BK and conditioned medium was abolished by 10-7 M of the HOE-140 or 10-7 M DALBK, B2 and B1 antagonists, respectively (n=3). Next, we determined the influence of BK and culture supernatant in the endothelial barrier disruption accessed by albumin permeability using BSA-FITC as a tracer and expression of ZO-1 and β-catenin by immunofluorescence. Both BK and CM induced 4-fold increase in albumin permeability (n=3) which was followed by the reduction in ZO-1and β-catenin expression (n=3). These effects were completely prevented by HOE-140 (n=3), suggesting a role of B2 receptor.
CONCLUSION: BK is a major soluble factor produced during *P. falciparum* infection that increases iRBCs adhesion and induces BBB disruption in B2R dependent manner. **Supported by:**CNPq, CAPES, FAPERJ
Keywords: Malaria; bradykinin; adhesion

HP95 - **ENGINEERED BACTERIAL OUTER MEMBRANE VESICLES AS A TECHNOLOGICAL PLATFORM FOR THE DEVELOPMENT OF A CHAGAS DISEASE VACCINE**

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Outer membrane vesicles (OMVs) are nanoparticles released from bacteria rich in immunomodulatory proteins and lipopolysaccharides. Three of the most promising characteristics of OMVs are their high adjuvant capacity, their safety, and the possibility of generating genetically engineered vesicles carrying foreign polypeptides. Therefore, the utilization of OMVs as vaccines offers promising potential against a wide range of diseases. With this in mind we proposed to evaluate the potential role of engineered OMVs carrying different Trypanosoma cruzi antigens as an experimental immunogen against Chagas disease. For this, we selected two well known antigens which have been extensively evaluated in vaccination models against T. cruzi, Tc24 and Tc52. The rational of selecting these antigens is that as a first step we propose to elucidate the advantage of using OMVs as carriers of parasite antigens and evaluate their adjuvant properties. As the first time reported, we were able to obtain recombinant OMVs with the selected T. cruzi antigens expressed not only on the outside of the vesicles but packaged within the lumen as well. These rOMVs were preliminarily evaluated in a murine prime-boot-challenge scheme for Chagas disease. For this purpose mice were injected intradermally with three separately doses of rOMVs. During the vaccination stage, a slight increase in IFN-□ production was detected in immunized animals. Thirty days after the last dose mice were challenged with a lethal dose of virulent parasites. Although not significant, a mild decrease in parasite load in vaccinated animals versus control groups could be detected. Several factors still need to be tested in order to optimize the use of rOMVs as a possible vaccine. In summary, the results so far obtained indicate that genetically designed OMVs could be a possible path for the development of novel strategies for trypanosomatids immunization. Funding: Fundación Bunge y Born y Florencio Fiorini. **Supported by:**Fundación Bunge y Born y Florencio Fiorini **Keywords:** Omv; vaccine; chagas disease

HP96 - **EVALUATION OF THE EFFICACY OF A NEW COUMARIN MOLECULE IN CURING SWISS MICE INFECTED WITH TRYPANOSOMA CRUZI**

SILVA, L.M.[*1]; XAVIER, V.F.[1]; MARQUES, F.S.[1]; DUARTE, T.H.C.[1]; FERRAZ, A.T.[1]; DAS MERCÊS, A.C.[1]; CARVALHO, T.V.[1]; TAYLOR, J.G.[1]; CARNEIRO, C.M.[1]; VIEIRA, P.M.A.[1]

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Chagas disease is an infection caused by the protozoan Trypanosoma cruzi (T. cruzi). Although many classes of substances have already been tested in vitro and in vivo against T. cruzi, the specific chemotherapy of Chagas' disease remains limited to Benznidazol (BZ) and Nifurtimox. These drugs are associated with a number of undesirable features that make them not ideal for the treatment of the disease. This work aims to evaluate the efficacy of a new Coumarin molecule in curing Swiss mice infected with T. cruzi. To achieve this goal, 70 Swiss mice, male and female, 30 days old were divided into different groups for the accomplishment of two tests. The Acute Toxicity test was performed according to the guidelines established by ANVISA, with the objective to determine the DMT (Maximum Tolerated Dose) of this compound. The doses tested were 1000, 500, 250, 100 and 50mg/kg, being n=4 animals/dose. Qualitative histopathological analyzes were performed: kidneys, liver, spleen, colon, heart, lung, brain and duodenum. For evaluation of therapeutic efficacy, animals infected with Y Strain, during acute phase, were divided into five groups (n=10/group): infected and untreated; infected and treated with BZ for 5 days; and three other groups infected and treated for 5 days with Coumarin at concentrations 50, 100 and 250 mg/kg. The parasitemia of the animals was performed until negativation for 5 consecutive days. In the evaluation of acute toxicity no histopathological changes were found in the analyzed organs. In the therapeutic efficacy test, there were significant differences in the area under the curve for parasitemia between the Coumarin and the BZ treated groups. The BZ treated group showed a significant reduction of parasitemia, whereas in the groups treated with Cumarina there was no significant reduction of parasitemia. Therefore, the use of Coumarin did not prove to be an option for the treatment of the experimental infection by Strain Y of T. cruzi. **Supported by:**FAPEMIG, CAPES e UFOP **Keywords:** Coumarin; trypanosoma cruzi; evaluation of therapeutic efficacy

HP97 - **THE ACTIVITY OF COFFEE DITERPENES AGAINST *TRYPANOSOMA CRUZI***

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Chagas disease is one of the seventeen Neglected Tropical Diseases, which affect approximately 8 million people in the world, mostly in Latin America. The current etiological treatment is restricted to two nitroheterocyclic drugs, benznidazole and nifurtimox, that were developed more than five decades ago. Both are far from ideal due to high incidence of collateral effects and the effectiveness decreases with advancement of the infection. In this context, an intensive research program has focused upon the search for alternative natural, semi-synthetic and synthetic lead compounds. In this study we investigated the effect of two diterpenes, cafestol and kahweol (C&K) isolated from green Arabica coffee beans. Coffee is the beverage consumed by more than 80% of the world's adult population and therefore its effects on human health have been investigated for decades. More recently, attention has focused on its chemical constituents with bioactive properties, among which caffeine, chlorogenic acids, and the diterpenes cafestol and kahweol. These diterpenes displayed anticarcinogenic, antioxidant, anti-inflammatory, anti-osteoclastogenic and hypercholesterolemic activities, and were evaluated against the infective bloodstream trypomastigote (Y strain) and intracellular amastigotes (Tulahuen strain) of *Trypanosoma cruzi*. Kahweol was two times more activity to trypomastigotes (Y strain) than cafestol. The C&K mixture was de most active to trypomastigotes and showed the lowest toxicity for the mammalian cell. Using another parasite strain and DTU, our data show that kahweol showed no activity against amastigote forms (Tulahuen strain) at concentration tested, and, on the other hand, cafestol and the C&K mixture present considerable reduction in the number of intracellular parasites. We propose that these diterpenes will be interesting potential drugs, or starting materials for new drugs. This message supports our argument that coffee is indeed good for our health. **Supported by: Keywords:** Doença de chagas; diterpenos do café; quimioterapia

HP98 - **ANGIOTENSIN II/AT1 RECEPTOR PATHWAY MEDIATES MALARIA-INDUCED ACUTE KIDNEY INJURY**

PINHEIRO, A.S.*[1]; SILVA, L.S.[1]; SANTOS, E.C.[1]; TEIXEIRA, D.E.[1]; PERUCHETTI, D.B.[1]; SILVA-AGUIAR, R.P.[1]; CARUSO-NEVES, C.[1]; PINHEIRO, A.A.S.[1]

1.UFRJ, RJ, Brazil.

INTRODUCTION: Malaria-induced acute kidney injury (MAKI) is a life-threatening complication in severe malaria. Previously, our group demonstrated that Ang II/AT1 receptor pathway was implicated in modulation of immune cells and brain damage in experimental CM. Although AT1R activation is clearly observed during renal diseases, the role of Ang II/AT1R axis in MAKI is poorly known.
OBJECTIVE: The aim of this work was to study the role of the angiotensin II (Ang II)/AT1 receptor pathway in the development of MAKI.
METHODS: C57BL/6 mice infected by *Plasmodium berghei* ANKA (PbA-infected mice) were treated or not with 20 mg/kg/day losartan, an antagonist of AT1 receptor, or captopril, an angiotensin-converting enzyme inhibitor (n=9/group).
RESULTS: Infection induced a 2-fold increase in plasma creatinine and blood urea nitrogen with a significant decrease in creatinine clearance (3-fold), indicating glomerular injury. PbA-infected mice had proteinuria and a 2-fold increase in urinary γ-glumatyltransferase activity. These functional changes were associated with a 3-fold increase in collagen deposition and interstitial space, showing tubule-interstitial injury. All these changes were prevented by treatment with losartan or captopril. Despite a discrete reduction in parasitemia, the treatments abolished the increase in renal cortex TNF-α, IL-6, IL-17, and IFN-γ induced by PbA infection. These results suggest a role of Ang II/AT1 signaling in host immune response during MAKI. Next, we tested the influence of PbA-activated immune cells in this process. When splenic T cells collected from PbA-infected mice were transferred to naïve receptor mice, we observed proteinuria after day 2 post transference (n=4), as observed in donors infected mice.
CONCLUSION: Our data allowed us to postulate that Ang II/AT1 axis is involved in MAKI development during PbA infection. These results open new avenues in possible coadjuvant therapies in malaria. **Supported by:**CNPq, CAPES, FAPERJ **Keywords:** Malaria; angiotensin ii; renal disease

HP99 - **POTENTIAL ANTILEISHMANIAL EFFECT OF NANOCOMPOSITE AGNPS-PVP-GLUCANTIME® DURING INFECTION OF PARASITE *LEISHMANIA AMAZONENSIS IN VITRO*.**

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Due to the resistance generated by parasites of the genus *Leishmania* to current drugs, used in the treatment of leishmaniasis the need arrives to develop new drugs capable of acting in the parasite, without causing damage to the host and being economically viable. In this study, the leishmanicidal effect of the AgNPs-PVP-Glucantime® nanocomposite was evaluated, *In Vitro*, on macrophages of BALB / c mice and, later, in the *Leishmania amazonensis* parasite. Different characterization techniques were used for the nanocomposite: transmission electronic microscopy (MET), X-ray diffraction (XRD) and X-ray dispersive energy spectroscopy (EDS). After characterization, we observed an inhibitory effect in the cellular viability of promastigote forms with a value of 65.67%, in relation to the control group. The infection rate was decreased after treatment with the nanocomposite generating an index of 23%, compared with 17% generated by Amphotericin B, as a positive control. Moreover, the production of proinflammatory cytokines IL-6, INF-γ and TNF-α were observed in infected and treated macrophages. The results allowed to conclude that the AgNPs-PVP-Glucantime® nanocomposite has leishmanicidal potential on the parasite *Leishmania amazonensis*, being an option of use for topical treatment against cutaneous leishmaniasis. **Supported by:**CAPES
Keywords: Leishmaniasis; antileishmanial effect; nanocomposite

HP100 - **ATP6V0D2 CONTROLS LEISHMANIA PARASITOPHOROUS VACUOLE BIOGENESIS VIA CHOLESTEROL HOMEOSTASIS**

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V-ATPases compose the membrane of pathogen-containing vacuoles, although their function in intracellular infection is elusive. Besides organelle acidification, V-ATPases are alternatively implicated in membrane fusion and anti-inflammatory functions that are controlled by ATP6V0d2, d subunit variant of V-ATPase complex. Therefore, we evaluated the role of ATP6V0d2 in the biogenesis of pathogen-containing vacuoles using ATP6V0d2-knocked-down macrophages infected by protozoan parasite *Leishmania* (*L*.) *amazonensis*. These parasites survive within inflammatory macrophages, sheltered within large/fusogenic parasitophorous vacuoles (PVs) and ATP6V0d2 is upregulated by the parasite in IFN-gamma/LPS-mediated inflammatory macrophages. ATP6V0d2 knock-down decreases macrophage cholesterol levels and inhibits PV enlargement without interfering in parasite multiplication. However, parasite requires ATP6V0d2 to promote the influx of cholesterol but enlargement of PV was achieved using oxLDL in ATP6V0d2-knocked-down macrophages, replenishing macrophage cholesterol pools, likely through an independent receptor. We reveal here a novel parasite-mediated subversion of host V-ATPase functions towards cholesterol retention, both required for establishing an inflammation-resistant intracellular parasite niche. **Supported by:**FAPESP; CAPES; CNPq **Keywords:** Atp6v0d2; leishmania; cholesterol

HP101 - **DIFFERENCES IN THE EXPRESSION OF A SUBSET OF CODING AND NON-CODING RNAS FROM THE HOST CELL DURING INFECTION WITH TRYPANOSOMA CRUZI STRAINS PRESENTING DIFFERENCES IN VIRULENCE**

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Global gene expression analyses are invaluable tools to allow the molecular characterization of host-pathogen interactions during infection by intracellular parasites such as Trypanosoma cruzi. We compared changes in the expression of parasite and host genes during infection of human foreskin fibroblasts (HFF) with two cloned T. cruzi strains that present contrasting outcomes of the infection: the virulent CL-Brener clone, which causes parasitemia in different animal models of infection, and the CL-14 clone, which is non-virulent, even when inoculated in immune deficient mice. Total RNA was extracted from HFF cells at 60 and 96 hours post-infection (hpi) with each strain, as well from uninfected samples. Differential expression analysis showed similar transcriptome profiles of protein coding genes (PCG) and non-coding RNAs (ncRNAs) at 60 hpi in cells infected with both strains compared with uninfected samples. However, at 96 hpi, cells infected with CL Brener showed significant differential expression of 3342 PCG and 379 ncRNAs when compared to uninfected cells whereas cells infected with CL-14 showed 2469 differentially expressed PCG and 368 ncRNA. To better identify the differences in the host cell pathways we used the Ingenuity Pathway (IPA) analysis. Similar enriched pathways related to immune response were identified during infection with both strains, such as Death Receptor Signaling, EIF2 Signaling, Interferon Signaling, mTOR Signaling and Antigen Presentation Pathway. However, at 96 hpi, a subset of PCG and ncRNAs were differentially expressed exclusively during CL Brener infection (1409 and 102) and CL-14 infection (603 and 93), as a subset of cytokines and chemokines (CXCL5, CXCL6, IL1A, IL1B, IL11, IL24, IL33) and ncRNAs (MIR3142HG, LINC00161, H19, SBF2-AS1, FAM198B-AS1) exclusive expressed in cells infected with CL Brener at 96 hpi indicating that these genes may constitute factors that could be correlated with the distinct infection outcomes. **Supported by:**CAPES e CNPQ **Keywords:** Trypanosoma cruzi; rnaseq; host response

HP102 - **ANALYSIS OF NATURALLY ACQUIRED HUMORAL IMMUNE RESPONSES AGAINST *PLASMODIUM MALARIAE*, *P. VIVAX* AND *P. FALCIPARUM* MSP1 RECOMBINANT PROTEINS IN NON-HUMAN PRIMATES FROM THE BRAZILIAN AMAZON AND ATLANTIC FOREST BY A MULTIPLEX ASSAY**

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The merozoite surface protein 1 (MSP1) is involved in the erythrocyte invasion process being one of the most studied target in malaria vaccine experiments. Here we evaluated the presence of antibodies against this protein in sera of non-human primates (NHP) from two malaria endemic regions of Brazil, the Amazon (AM, 128 samples) and Atlantic Forest (AF, 132 samples), using the Bioplex assay (BioRad) with MSP1 recombinant peptides coupled to beads. Glutathione S-transferase (GST) fusion peptides of *P. malariae* MSP1 ($PmMSP1_{F1}$, $PmMSP1_{F2}$, $PmMSP1_{F3}$, $PmMSP1_{F4}$, $PmMSP1_{19}$), *P. vivax* ($PvMSP1_{19}$) and *P. falciparum* ($PfMSP1_{19}$) were used in the assay. Percent of sera reacting to the recombinant peptides was similar in both cohorts (43% AM; 40% AF), with levels of reactivity being very similar between the two sets. The most reactant peptide with sera from AM was $PmMSP1_{F1}$, while $PvMSP1_{19}$ was with sera from AF. Moreover, $PmMSP1_{F1}$ and $PmMSP1_{19}$ were the most reactant with sera from AF, with high and similar reaction levels of reactivity, indicating that these peptides on MSP1 are the most immunogenic in natural infections by *P. malariae*. The *P. falciparum* $PfMSP1_{19}$ was positive in 5% of sera from both regions. However, the $PfMSP1_{19}$ positive sera classified as AF were all from Old World or Amazonian animals kept at the São Paulo Zoo. This study validates the use of recombinant peptides in multiplex immune-assay to measure naturally raised IgG antibodies against the parasite MSP1 protein and demonstrate that this technique is an important tool for making serum-epidemiological surveys. Lastly, the high prevalence of antibodies against *P. vivax* and *P. malariae* (and *P. falciparum* in the AM) in free-living or captive monkeys supports the hypothesis that these animals are potential reservoirs of malaria parasites in these regions.
Supported by:FAPESP **Keywords:** Plasmodium malariae; msp1; multiplex

HP103 - **IMMUNOLOGICAL PROFILE IN BALB/C MICE WITH LEISHMANIA INFANTUM "SUICIDAL" STRAINS.**

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Visceral leishmaniasis, caused by Leishmania infantum, has high morbidity and mortality. Despite the variety of drugs that are available for the treatment of leishmaniasis, most of them cause serious side effects and the parasites are showing someresistance. The development of attenuated parasite vaccines for the prevention of leishmaniasis may be a promising alternative. Our group developed the plasmid pFL-AMA, which restricts the expression of amastin, a specific protein in amastigote phase. In this plasmid, a sequence encoding mCherry (non-toxic) protein, and toxic proteins such as the active form of trypsin (TrypTox) and egg avidin (AviTox) were inserted, both proteins are intended to cause damage to the proteome of the parasite during the differentiation process. In this work we evaluated the immunological profile induced by vaccination with these "suicidal" parasites (pFL-AMA-TrypTox; pFL-AMA-AviTox). The humoral immune response was observed by ELISA. Both immunized and infected groups had high levels of significant IgG and IgG1 ($p < 0.001$), anti-Leishmania IgG2a ($p < 0.05$) after the 3rd week of immunization as compared to the control group, and over time the level of antibodies decrease, except the production of IgG1 antibodies. Spleen cell populations were determined by flow cytometry, and we observed a significant increase in the T helper ($CD3^+CD4^+$), cytotoxic T ($CD3^+$ $CD8^+$) and B ($CD3^-CD19^+$) lymphocytes population after 20 weeks of immunization with "suicidal" parasites. Cellular activation was observed by increased CD69 expression in TCD4, TCD8 and B lymphocytes in immunized animals after 10 weeks. Infected mice showed increased CD69 expression in TCD8 and B lymphocytes after 10 and 20 weeks, respectively. "Suicidal" parasites have the ideal characteristics of a vaccine model and it may be an alternative for the development of a future vaccine for leishmaniasis. **Supported by:**FAPESP **Keywords:** Leishmania infantum; vaccine; humoral immune response

HP104 - **EVALUATION OF THE COLON INJURIES IN INFECTION CAUSED BY METACYCLIC OR BLOOD TRYPOMASTIGOTES FORMS OF *TRYPANOSOMA CRUZI* IN MICE**

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Chagas disease is a protozoonose endemic in 21 countries of Latin America. The disease has as etiologic agent *Trypanosoma cruzi* (*T. cruzi*) and there two infective forms - blood trypomastigotes (BT) and metacyclic trypomastigotes (MT). It is already known that despite presenting differents surface molecules, both infective forms are capable to promote infection. In this way, this work aimed to evaluate pathological parameters related to experimental infection in *Swiss* mice infected with BT or MT forms of T. cruzi VL-10 strain. To reach this goal, 126 *Swiss* mice were divided in 3 experimental groups: non-infected, infected with BT forms and infected with MT forms. Was evaluated parasitemia, tissue parasitism, inflammatory process and the area immunomarked for neurons in muscle layer of colon. Parasitemia data showed that animals infected with BT forms showed a significant area under the curve when compared with MT group, however mice infected with MT forms presented a higher peak of parasitemia. Inflammatory process in the colon showed that mice infected with both forms presented inflammatory infiltrate in the acute phase of infection, but in the chronic phase only mice infected with BT forms presented absence of inflammatory process. However, the analysis of tissue parasitism showed that despite presenting a greater level of parasitism in acute phase, mice infected with MT forms presented a decrease in the number of parasites in chronic phase. Counting marked area for neuron showed that there was a decrease in the area of neurons in both infected groups, however in mice infected with MT forms the drop of immunolabelling area already begins in the acute phase of the disease. These results suggest that infection with MT forms leads to a higher parasitism in the acute phase of infection, which can be related to the earlier process of denervation of the myoenteric plexus in colon and consequently could be associated to the gastrointestinal disturbances of disease. **Supported by:**CAPES, UFOP **Keywords:** Chagas disease; infective forms; colon

HP105 - *TOXOPLASMA GONDII* INFECTION IMPAIRS MYOGENESIS *IN VITRO*

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Toxoplasma gondii is an intracellular parasite capable of crossing the transplacental barrier and infecting fetal tissues, causing congenital toxoplasmosis. The parasite has a strong tropism for the skeletal muscle cells, in which it forms tissue cysts. In the present work we investigated the molecular mechanisms that could interfere in the myogenic program during *T. gondii* infection. The mouse myoblast C2C12 cell line was infected with ME49 strain of *T. gondii* 24 h after plating. 24 h after infection proliferation medium was replaced for differentiation medium. At 24 or 120 h after induction, cells were processed to immunofluorescence or qRT-PCR. In addition, at the same points the conditioned medium was collected for cytokines/chemokines profiling using Cytokine Bead Array (CBA) or ELISA for TGF-β1. MMP activity was determined by zymography. The infection drastically reduced the differentiation and fusion indexes, as well as the number of mature myotubes and the diameter of such myotubes. The expression of myosin heavy chain, a marker of terminal myogenesis was reduced in infected cells. Expression of myogenic regulatory factors (MyoD, Myf5, Mrf4, myogenin) was also altered by infection. After 120 h of cultivation in differentiation medium, infected cells showed a significant increase of Ki67-positive cells, indicating increased proliferation. IL-6, MCP-1 and MMP-3 were highly increased in *T. gondii* infected cultures whereas TGF-β was decreased. In order to investigate upstream steps of the myogenic program, we looked for nuclear translocation of β-catenin. 10 hours after induction with differentiation medium, infected cultures showed decreased β-catenin intensity and decreased rates of nuclear staining. *T. gondii* induces a severe disarray of the skeletal muscle cell culture, altering its secretory profile, leading to an unresposiveness to Wnt/β-catenin pathway activation, which in turn induced the culture to remain in a proliferative, undifferentiated state. **Supported by:**CNPq, Fiocruz **Keywords:** Congenital toxoplasmosis; myogenesis; myogenic regulatory factors

HP106 - ROLE OF KININS GENERATED DURING MALARIA INFECTION IN MONOCYTE ADHESION TO ENDOTHELIAL CELLS

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INTRODUCTION: *falciparum*-malaria is considered the most severe form of the disease in humans. Monocytes display activated phenotypes during acute malaria. Our group have described that bradykinin (BK) levels are increased in *P. falciparum* culture supernatant. Kinins have role in adhesion of leukocytes into HUVEC, however how BK modulates monocytes adhesion into endothelial cells during malaria is still to be determined.
OBJECTIVES: This work aims to study the effect of kinins generated during malaria infection in monocyte adhesion to endothelial cells.
METHODS: Health A-type human RBCs infected with *P. falciparum* W2 strain were used to obtain the Pf-conditioned medium (PfCM). THP-1 monocytes and a human brain endothelial cell line (BMEC) were used in adhesion assays.
RESULTS: Initially, we determined the time course of 10^{-6} M BK effect in monocyte adhesion to BMEC cells. The pretreatment of THP-1 cells with BK, increases adhesion after 1 h incubation (n=2), reaching a maximum of 2-fold increase. Similar results were obtained when cells were incubated with 20% PfCM (n=2). To further characterize the effect of this peptide, we incubated THP-1 cells with increasing concentrations of BK (10^{-9} up to 10^{-6} M) or PfCM (1 up to 20%). We observed that both BK and PfCM increased monocyte adhesion with maximal effect (2 times) observed at 10-7 M and 20%, respectively (n=2). Since BK is easily degraded, we performed the same experimental approach in the presence of 10^{-7} M captopril, a well-known angiotensin converting enzyme inhibitor. It was observed that in the presence of captopril the effect of BK was enhanced even in physiological concentrations (10^{-9} M), reaching a 10-times fold increase in monocyte adhesion at 10^{-6} M.
CONCLUSION: These results allowed us to postulate a possible role of BK, produced during malaria infection, on monocyte adhesion to endothelial cells. Our work opens new avenues in the knowledge of malaria pathogenesis **Supported by:**CNPq, CAPES, FAPERJ **Keywords:** Malaria; bradykinin; monocytes

HP107 - **INHIBITION OF PROTEIN KINASE C INTERFERES IN PROLIFERATION AND CELL CYCLE OF *LEISHMANIA AMAZONENSIS***

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Protein Kinase C (PKC) comprises a group of multifunctional proteins that catalyze phosphorylation in serine and threonine residues. The PKC was characterized in promastigote of *Leishmania amazonensis*. During its life cycle, the parasite oscillates between procyclical promastigote and metacyclic promastigote, inside the invertebrate host. In vertebrate host, the promastigote differentiates into amastigotes inside the infected macrophage. These morphological and metabolic changes are regulated by protein kinases. The aim of this study was demonstrate the role of PKC in proliferation and in cell cycle of *L. amazonensis*. The proliferation was analyzed with CFSE. The promastigotes were incubated with RO32-0432, inhibitor of PKC, (0.1nM-1μM) for 120h. Each 24 hours, the proliferation was analyzed by flow cytometry (FACS Calibur). RO32-0432 was capable to inhibit the proliferation of promastigotes of *L. amazonensis* in a dose dependent manner. To evaluate the participation of PKC in cell cycle, promastigotes were cultivated with RO32-0432. Increase concentration of R032-0432 interferes, in a dose-dependent manner, on cell cycle of *L. amazonensis*. Was observed an increased in the percentage of cells retained in the G0/G1 region and G1 region when incubated with RO32-0432. Taken together, these results demonstrated that inhibition of PKC interferes in the processes of cellular proliferation and cell cycle on promastigotes of *Leishmania amazonensis.* **Supported by:**FAPERJ, CNPq, PAPES, CAPES, IOC/Fiocruz **Keywords:** Protein kinase c; leishmania amazonensis; leishmaniasis

HP108 - **TRYPANOSOMA CRUZI EARLY INFECTION ALTERS THE LIPID METABOLISM IN LIVER VIA CAMKK2-LKB1-AMPK PATHWAY**

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The protozoan *Trypanosoma cruzi* is the etiological agent of Chagas' disease, transmitted by triatomine of genus Triatoma. About 7 million people are infected worldwide, making it a public health issue. Once in the bloodstream, T. cruzi infects organs such as liver, adipose tissue and heart. The liver is essential for many functions such as making proteins and blood clotting factors, manufacturing triglycerides and cholesterol, glycogen synthesis and bile production. Independent studies have identified a lipid flow from the vertebrate host to trypanosomatids. This mechanism is often observed in parasites that present incomplete de novo lipid biosynthetic pathways. Parasites avidly take up lipids from the vertebrate bloodstream, satisfying their requirements for growth and differentiation. The protein kinase activated by AMP (AMPK) is the key enzyme in cellular energy metabolism, involved in multiple signaling pathways such as lipid and protein metabolism modulating factors and enzymes by phosphorylation. Thus, the aim of this work was to characterize the lipid metabolism during acute T. cruzi infection in swiss mice liver and to verify the involvement of the AMPK enzyme in this process. For this end, two mice groups (control and infected, n=8 ~ 50g) were subjected to infection with 106 parasites by intraperitoneal injection. After 7 days, liver and plasma were collected. Liver samples were processed; lipids were extracted and subjected to thin-layer chromatography. Significant increase in triacylglycerol was observed in infected group, a result confirmed by enzymatic colorimetric assay. Plasma profile showed hypertriglyceridemia, hypoglycemia, normocolesterolemia and normoproteinemia in infected animals. AMPK phosphorilation levels showed a significant decrease in infected animals, as well as to CAMKK2 and LKB1, the major AMPK activating kinases. These results show that the acute infection modulates the lipid metabolism by regulation of CAMKK2-LKB1-AMPK pathway. **Supported by:**CNPQ, FAPERJ e INCT **Keywords:** Ampk; trypanosoma cruzi; metabolismo de lipídios

HP109 - **ANALYSIS OF THE INFECTIVITY OF CRITHIDIA FASCICULATA IN VITRO AND IN VIVO**

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The family Trypanosomatidae has parasites of plants, insects and vertebrates. Despite the fact that the family diversity is extraordinarily extensive, only two genera are human parasites: Leishmania and Trypanosoma. However, since the 1980s, the isolation of insect trypanosomatids in vertebrate hosts has been described, including mammals. While the theoretical postulation doesn't predict these parasites as vertebrate pathogens, the reports of occasional infection in humans are increasing, evidencing that normally non-infectious species can explore new ecological niches in vertebrates. The Fiocruz Protozoa Culture Collection receives routinely isolates for deposit and taxonomic identification. Among these, there was a deposit from a presumably monoxenic species, isolated from a skin lesion of an immunocompetent patient in the city of Cusco (Peru), which was characterized as Crithidia fasciculata. Considering the low number of reports in the literature that demonstrate the isolation of monoxenic trypanosomatids in vertebrate hosts, an in-depth study of this isolate will provide a significant increase in the knowledge about the possible role of these parasites in pathogenic processes. In this context, we evaluated the infectivity of this parasite in murine peritoneal macrophages in vitro and also in an animal model. The results obtained up to now indicate that this isolate is capable of surviving and causing cutaneous lesions in Balb/c mice. These experiments allowed the re-isolation of the parasite. In vitro assays showed that this parasite persists in murine macrophages and, surprisingly, a higher infection rate (86%) was observed in recently isolated parasites. Taken together, the data obtained indicate that this strain may possess intrinsic characteristics that helps to explain its infection ability that deserves further investigation. In addition, they warn of an important role of virulence factors in monoxenic trypanosomatids. **Supported by:**CNPq, CAPES, FAPERJ and Fiocruz **Keywords:** Crithidia fasciculata; infectivity; trypanosomatids

HP110 - **THE HUMAN AFRICAN TRYPANOSOMIASIS CONTROL PERSPECTIVES IN THE WORLD: ADVANCES AND CHALLENGES**

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Introduction: The human African trypanosomiasis (HAT), a neglected tropical disease, is caused by subspecies of the protozoan *Trypanosoma brucei* (*T. brucei gambiense* and *T. brucei rhodesiense*); despite the high mortality rates, remains without effective treatment specially when there is late diagnosis. Once the parasite spreads in the vertebrate host organism and the disease is established, may cause neurological impairment and psychiatric disorders, leading to death individual. Objectives: Examine, from an epidemiological perspective, the amount of HAT cases in the world in order to provide an historical snapshot and make predictions for future trends of the disease, as well as analyze the main factors accountable for the progressive decrease of cases in the last 20 years. Methods: This study examined the published literature on two databases, PubMed and UpToDate, using the keywords descriptors: "human african trypanosomiasis" and "sleeping sickness". It was also analyzed the World Health Organization (WHO) data on worldwide HAT cases over a 20-year time period. Results: The HAT cases analysis goes from 1997 to 2016. There was drastic decrease in the number of total cases of the disease, with 91% reduction by subspecies *T. brucei rhodesiense* and 94% by *T. brucei gambiense*. The main cause these reductions was the investment of WHO, along with the action of NGOs, in mobile teams for screening and epidemiological surveillance – mainly in hard-to-reach African areas, campaigns educative and vector control. The main current challenge is to instill in the international medical community the need for research into HAT in tourists with febrile syndrome post-trip in Africa in order to improve the prevention and the promotion of traveler's health. Conclusion: It's possible to observe advances in control as well as reduction in the incidence of sleeping sickness. **Supported by:**The authors are grateful to CNPq and PROAPP / FADIP for their financial support. **Keywords:** Trypanosomiasis; epidemiology; neglected tropical disease

HP111 - **INFLUENCE OF 1,8-CINEOL IN THE SEQUESTRATION OF ERYTHROCYTES INFECTED WITH *PLASMODIUM FALCIPARUM* IN ENDOTHELIAL CELLS**

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INTRODUCTION: *Plasmodium falciparum*, the human malaria parasite, has at least one strain resistant to every known antimalarial. 1,8-cineol (1,8Cin), a monoterpene, has anti-inflammatory, microbicidal and antiplasmodial effects; however, its effect in falciparum-malaria need to be characterized.
OBJECTIVES: We aimed to evaluate the effect of 1,8Cin in the adhesion of erythrocytes infected with *P. falciparum* to endothelial cells.
METHODS: Health A-type human RBCs infected with *P. falciparum* W2 strain and a human brain endothelial cell line (BMEC) were used in adhesion assays. Parasite schizonts (1% parasitemia) were used to evaluate ring formation rate by optical microscopy.
RESULTS: Increasing concentrations of 1,8Cin (10 – 1,000 µg/mL) reduced ring formation in a dose-dependent way, with maximum effect at 1,000 µg/mL and IC50 of 154 µg/mL (n=5), without any hemolytic effect. The inhibitory effect of 1,8Cin seemed to be dependent on parasitemia. We observed that 150 µg/mL 1,8Cin induced 55,7% and 36,7% inhibition of new ring formation at 0,5 and 3% parasitemia, respectively (n=3). To evaluate whether the inhibitory effect of 1,8Cin was reversible, cells treated with 150, 500 or 1,000 µg/mL 1,8Cin for 24 h, were washed and re-cultured without compound. The effect of 1,8Cin was maintained for at least 72h incubation (n=3). Interestingly, cultures treated with 1,000 µg/mL, which apparently lost viability, recovered growth 10 days after compound washing (n=3). At this time point, parasitemia was around 4,4%, whereas the control reached 11,0% parasitemia at day 4. Next, we determine the influence of 1,8Cin on infected erythrocyte adhesion to BMEC cells. We observed that 1,8Cin significantly reduced the infected erythrocytes by 60% (n=4).
CONCLUSION: Our results allow us to conclude that 1,8-cineol impairs P. falciparum erythocytic cycle by inhibiting the generation of new ring forms, and consequently, reduction in adhesion of infected erythrocytes to endothelial cells. **Supported by:**CNPq, CAPES, FAPERJ **Keywords:** Malaria; 1,8-cineol; adhesion

HP112 - **EVOLUTIONARY EXPANSION OF GP63 GENES FROM *LEISHMANIA BRAZILIENSIS***

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The GP63 proteins, the major Leishmania surface antigens, are proteases with functions related to adhesion and nutrition within the invertebrate vector and to protection against the humoral immunity in the vertebrate host. The number of GP63 genes vary substantially between Leishmania species, with L. braziliensis having the greatest number of genes (39 annotated so far) when compared to other human pathogenic species, such as L. infantum (8 genes) or L. major (6 genes). The reasons behind these differences remain unknown but clarifying those would greatly contribute to a better understanding of the parasite biology. This study sought to investigate the true number of GP63 genes in L. braziliensis, investigate events that led to the expansion in gene numbers and understand their impact on the biology of this pathogen. First, PCR assays with degenerate primers were performed, followed by cloning and sequencing of the gene fragments. The PCR results found a total of 32 sequences not yet described in the databases, mostly sequences associated with chromosome 10, with only one linked to chromosome 31. These and other sequences were used to build phylogenetic trees and investigated for recombination events. The trees showed a clear correlation between Leishmania sp. GP63 genes from chromosomes 28 and 31 with genes from trypanosomatids that infect plants or with a monoxenous life cycle. In contrast, chromosome 10 GP63 genes clustered mainly with genes from human pathogenic species and experienced independent expansions in numbers in Leishmania, especially in L. braziliensis. Intragenic recombination analysis showed that 60% of the chromosome 10 genes had recombinant motifs, located mainly within their N and C terminal regions. These results suggest a greater role for the sequence variation found among the chromosome 10 GP63 genes for the pathogenesis of L. braziliensis and closely related species within the mammalian host. **Supported by:**Capes, CNPq **Keywords:** Gp63; leishmania braziliensis; virulence protein

HP113 - **TRYPANOSOMA BRUCEI ATR: A PROTEIN KINASE ESSENTIAL FOR PARASITE GENOME MAINTENANCE AND HOST IMMUNE EVASION**

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To prevent clearance by the mammalian host immune system, blood stage Trypanosoma brucei parasites stochastically switch their variant surface glycoprotein (VSG) coat; approximately 1000 VSG gene variants are available from which the parasites can select. VSG expression is monoallelic meaning at any given time, each cell expresses a single VSG from a subtelomeric expression site (ES) though exactly how monallelic expression is maintained remains unknown. To date, chromatin status, nuclear membrane structure, telomere maintenance and factors specific to kinetoplastids have all been shown to act in this process. VSG switching by recombination requires DNA repair processes and such processes have not previously been linked to monoallelic expression. Signalling the presence of a DNA lesion is vital to initiate an appropriate repair response yet little work has been performed to investigate this in protozoan parasites. Here, we show that RNAi-mediated loss of ATR, an atypical PK central to DNA repair in other eukaryotes, compromises cell proliferation, de-regulates the cell cycle and leads to widespread nuclear and chromosomal damage. Beyond these core and conserved repair functions, ATR RNAi results in loss of monoallelic expression, as seen by increased VSG transcripts from the silent ESs, increased transcription from genes proximal to the ES promoter and the appearance of cells expressing two VSGs on their surface. Furthermore, the sub-cellular localization of a kinetoplastid-specific factor, VEX1, is altered. VEX1 is a factor required for ES monallelic expression thus revealing a clear link between DNA damage signaling and VSG expression control.**Supported by:**BBSRC **Keywords:** Vsg; trypanosoma brucei; host immune evasion

HP114 - **CARDIAC REGENERATION AFTER TGF-β INHIBITOR THERAPY IN A PRE-CLINICAL STUDY OF CHRONIC CHAGAS DISEASE**

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Studies published by our group demonstrated the involvement of TGF-β in Chagas cardiomyopathy development in T. cruzi-infected animals during the acute phase of Chagas disease. Activation of TGF-β signaling pathway was observed in the cardiac tissue of infected animals during the acute phase, favoring the increase of extracellular matrix proteins expression. TGF-β is the most import protein involved in fibrosis process. The aim of this study is investigate the effect of GW788388 treatment in TGF-β signaling pathway during the chronic experimental model of Chagas disease. C57BL/6 mice were infected with Trypanosoma cruzi (10 2 parasites from the Colombian strain) and treated orally with 3mg/kg GW788388 starting at 120 days post-infection (dpi), when 100% of the infected mice show cardiac damage, and following two distinct treatment schedules: i) one dose per week; or ii) three doses per week during 30 days. The treatment with GW788388 improved several cardiac parameters: reduction of the prolonged PR and QTc intervals, increased heart rate, and reversal of sinus arrhythmia, and of atrial and atrioventricular conduction disorders. At 180 dpi, after 30 days of treatment interruption, the GW3x treated group remained in a better cardiac functional condition. Further, GW788388 treatment reversed the altered formation of connexin-43 enriched intercellular plaques and reduced fibrosis of the cardiac tissue. Inhibition of the TGF-β signaling pathway reduced TGF-β/pSmad2/3, increased MMP-9 and Sca-1, reduced TIMP-1/TIMP-2/TIMP-4 and partially restored GATA-6 and Tbox-5 transcription, supporting cardiac regeneration. The therapeutic effects of GW788388 are promising and suggest a new possibility to treat cardiac fibrosis in the chronic phase of Chagas disease by TGF-β inhibitors. **Supported by:**INSERM / FIOCRUZ / CNPq / FAPERJ / DECIT **Keywords:** :chagas disease; fibrosis; tgf

HP115 - **TRANSCRIPTOME MAPPING OF HOST GENES WITH ALTERED EXPRESSION IN EXPERIMENTAL VISCERAL LEISHMANIASIS**

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Visceral leishmaniasis is often associated with anemia, splenomegaly and downregulation of immunity in infected individuals. Although the immune status of the patient plays an important role in disease progression, parasite-derived factors may contribute to pathology. We used 2 viscerotropic species, *L. infantum* (NCL) and *L. donovani* (LV9) to infect BALB/c mice, in order to investigate whether these strains have distinct transcriptomic signatures, and whether this can be attributed to either the host, the parasite, or the heterogeneity of both. Pathways contributing to pathology in the spleen and liver were identified through the collection of RNA samples at day 35 post-infection, and host and parasite transcripts were analysed. Distinct and reproducible (n=5 per group) transcriptomic profiles were observed, in which non-infected and infected host profiles clustered separately in both the spleen and liver. There was a large increase in both the number of differentially expressed genes (DEG) in infected animals compared to uninfected. In the spleen, we found 473 DEGs log2FC in infected mice compared to uninfected, while in the liver there were >600 DEGs. In contrast, very few genes were DE between the mice infected with *L. donovani* and *L. infantum*, or the parasites themselves, revealing that host heterogeneity is much greater than parasite heterogeneity during infection. In the spleen, DEGs were grouped in 10 KEGG pathways that could be further clustered in 3 major groups: metabolism (2 subgroups), transcriptional regulation (2 subgroups), and host defence (6 subgroups). Furthermore, ~2% of DEGs were of host peptidases or natural peptidase inhibitors, mainly belonging to the S1A family of serine peptidases. 12 DEGs in the spleen were chosen for further validation by qPCR, in addition, an increase in myeloperoxidase expression was validated in infected mice through non-invasive imaging, whilst immunohistochemistry was used to analyse serine peptidases and inhibitors.**Supported by:** **Keywords:** Transcriptomics; differential expression; visceral leishmaniasis

HP116 - **L-ARGININE SUPPLEMENTATION AND THROMBOXANE SYNTHASE INHIBITION REVERSE CEREBRAL ISCHEMIA IN MURINE CEREBRAL MALARIA BY *PLASMODIUM BERGHEI* ANKA**

MOREIRA, A.S.*[1]; ALMEIDA, V.E.F.[1]; BIANCO JÚNIOR, C.[1]; DANIEL RIBEIRO, C.T.[1]; TIBIRIÇÁ, E.V.[1]; CARVALHO, L.J.M.[1]
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Cerebral malaria (CM) is one of the most severe and lethal complications of *Plasmodium falciparum* infection. The mainstay treatment for CM is intravenous artesunate, nevertheless 15-20% of [illegible]tients receiving this drug still die. Vascular dysfunction, with vasoconstriction, leads to decre[illegible]lood flow, ischemia, tissue hypoxia and death in CM. Nitric oxide (NO) and arachi[illegible]are important regulators of physiological cerebral blood flow through the[illegible]ve properties. The objective of this study was to determine the e[illegible]d pharmacological inhibition of thromboxane A_2 (TXA_2) synt[illegible]l cerebral malaria (ECM). C57BL/6 mice infected[illegible]wing clinical signs of ECM were treated subcut[illegible]g) and/or the thromboxane synthase inhibitor ozag[illegible]d by *Laser Speckle Contrast Imaging* (LSCI). Th[illegible] artesunate on survival of mice with late-stage E[illegible]M showed a significant decrease (25%) in cere[illegible]se (22.5%) in cerebral blood flow in mice with EC[illegible]ion of ozagrel, which prevents the generation of t[illegible]ncreased (16.5%) cerebral blood flow in animals with E[illegible]sed levels of brain TXA_2 in ozagrel-treated compared to saline-tre[illegible] ozagrel and L-arginine showed no synergistic effect on cerebral blood flow[illegible] with late-stage ECM with artesunate plus L-arginine increased survival com[illegible]ate plus saline. Conclusion: L-arginine and ozagrel show potential as adjuvant therapy for [illegible]alaria complication.**Supported by:**FIOCRUZ, CAPES, CNPq, FAPERJ **Keywords:** Experimental cerebral malaria; vascular dysfunction ; adjuvant therapy

HP117 - **ACT USING REPOSITIONED DRUG IN MALARIA CHEMOTHERAPY**

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Malaria is a severe, potentially fatal parasitic disease with high incidences, according to WHO in 2016, causing at least 216 million recorded cases, which resulted in 445,000 deaths, including 306,000 children under 5 in 91 countries. This infection affecting the poorer areas of the globe, posing great social impact due to the inefficiency of its control by factors such as the absence of effective vaccines and resistance of the pathogens to the drugs of choice. Therefore, the search for new drugs is a pressing demand. The study aimed at producing a new combination of artemisinin (ACT artemisinin combination therapy) with a repositioned drug (patent pending) in the treatment of malaria. We tested the combination in vivo in Plasmodium berghei-infected mice by treated by gavage or intraperitoneal injection for 4 days. Parasites inside red blood cells obtained from the peripheral blood for up to 45 days were analyzed by transmission electron microscopy to determine the possible modes action of ACT and eventual mechanisms of death involved. The parasites obtained from animals submitted to the treatment showed enlarged endoplasmic reticulum cisternae and large accumulation of membrane fragments surrounding the cytostoma. Hemozoin crystals were observed in heterogeneous configurations and eventually free in the cytoplasm, which sometimes presented areas of reduced electron density. The parasites were apparently destroyed by necrosis triggered by permeabilization of the digestive vacuole (autolysis), presumably promoted by oxidative stress. These approaches may help not only the development of an effective therapeutic regimen for malaria, but also hamper the emergence of parasite resistance and reduce the side effects of therapy. **Supported by:**CNPq, FAPESB and Fiocruz **Keywords:** Malaria; act; electron microscop

HP118 - **PROTECTIVE ROLE OF SKIN MICROBIOTA IN *LEISHMANIA AMAZONENSIS* INFECTION**

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Cutaneous leishmaniasis is an infectious disorder caused by several members of the genus *Leishmania*. Recent studies have demonstrated that various inflammatory disorders, including cutaneous leishmaniasis, are associated with a dysbiotic microbiota, reflecting the importance of resident microbes for these diseases. On the other hand, certain skin commensal bacteria have been shown to produce antimicrobial peptides (AMPs) that have protective effects. Here we aimed to investigate the possible protective effect of specific skin microbes in cutaneous leishmaniasis. Germfree mice (GF) were monoassociated with either *Staphylococcus epidermidis*, S*taphylococcus aureus* or *Corynebacterium accolens* and, seven days later, infected with 10^4 *L. amazonensis* in both ears. *C. accolens* or *S. epidermidis* mice presented lower numbers of $CD11b^+$ cells when compared with GF or *S. aureus* mice 24 hours and two weeks after infection. *S. aureus* showed reduced numbers of Treg cells. Interestingly, a new influx of neutrophils was observed in GF and mice monoassociated with *S. aureus* two weeks after infection. These results show that distinct skin bacteria could influence immune responses differently during infections. To evaluate a protective effect, SPF mice were infected with *L. amazonensis* and 3 weeks later were topically treated with either a mixed culture of bacteria (BAC) isolated from the skin of a healthy mouse or with the culture supernatant (SUP). Mice treated with SUP showed smaller lesions when compared to control or BAC group up to 10 weeks of infection. Moreover, SUP mice presented reduced numbers of $CD11b^+$ cells and lower production of TNF and IFN-γ at 6 weeks after infection. Our results indicate that bacterial metabolites from the skin microbiota could have a positive effect on the outcome of Leishmania infection. It is possible that AMPs produced by commensal microbes could help restore the microbiota homeostasis and contribute to the control of the infection.**Supported by:**CAPES, CNPq **Keywords:** Leishmania; microbiota; skin

HP119 - **OVEREXPRESSION OF THE DIMERIC MUTANT OF THE NUCLEOSIDE IPHOSPHATE KINASE B (*LM*NDKB) IN *LEISHMANIA MAJOR***

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The nucleoside diphosphate kinase catalyzes the transference of a γ-ph a NTP to a NDP, involving a phosphohistidine intermediate state, by t was described that NDK is secreted by intracellular pathogens du ania, preventing ATP-induced macrophages cytolysis, there the benefit of the parasite. The *Lm*NDKb has a h studies involving mutations on the *Kpn* and C-term . Although with dimeric structure, the mutant protein s mpared with the wild type protein. Here, we obtained verexpressing the dimeric mutant of *Lm*NDKb and 100S/Δ5 coding sequence was amplified by PCR pX63NEO vector using *Bam*HI site. Promastigote the construction and selected using plates containi evaluated by growth curve of the parasites in the pr observed when compared with the wild-type parasite. overexpression was confirmed by SDS-PAGE of the e virulence of parasites overexpressing the mutant protein was a esion size of mice and a significative decrease of virulence was observed. Th of the dimeric mutant decreased the virulence of the promastigotes indicating an imp of the hexameric state in the protein function during parasite infection process. **Sup ted by:**Fapesp, CNPq **Keywords:** Ndk; leishmania; overexpression

HP120 - **EVALUATION OF PROTECTOR RESPONSE IN MICE TREATED WITH ECTI INHIBITOR AND INFECTED BY TRYPANOSOMA CRUZI.**

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Chagas disease is caused by the intracellular pathogen Trypanosoma cruzi and it is estimated hat 8 million people are infected by this parasite. The chronic phase presents numerous co that can lead to increased morbidity. CD8+ T cells control T. cruzi infection th of proinflammatory cytokines, and the release of cytotoxic granules. s e. However, the cellular immune response alone is not able t nt heart damage. Currently the treatment for Chagas di nifurtimox drugs, but both have adverse effect is a need for the development of n tion. The Kunitz EcTI type inhibitor (Er om the seeds of Enterolobium conto metallo-proteases. This inhibitor has HeLa cells infected with T. cruzi, but s with 150 forms of Y strain of T. cruzi and p and 1 mg/kg of EcTI the treated group. Although t y, treated group presented lower parasitemia compared w day. This data show that EcTI can be a promising drug against T. cru rther experiments need to be done to establish dosages and the effects of EcT on with other protease inhibitor against T. cruzi. **Supported by:**CNPq, FAPESP and CAP **Keywords:** Ecti; trypanosoma cruzi; protease inhibitors

HP121 - **DEVELOPMENT OF A MUCOCUTANEOUS LEISHMANIASIS MURINE MODEL: TNFRP55-/- MICE INFECTED WITH LEISHMANIA BRAZILIENSIS**

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Leishmaniasis are a group of diseases caused by the parasite of the genus Leishmania. Clinical manifestations and symptoms can be varied. 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 L. major is not associated with mucocutaneous leishmaniasis. The aim of this study is the characterization of chronic infection by L. braziliensis in TNFRp55-/- mice. We infected WT mice (C57BL/6) and TNFRp55-/- KO mice in the ear with L. braziliensis and the lesions were followed for 10 weeks. After 10 weeks of infection we found a lower parasite load, but a larger lesion in the TNFp55-/- mice when we compared to WT mice, this suggests that it is not the parasite that is inducing inflammation. We also found L. braziliensis in the spleen, which suggests there was migration of the parasite. To make the in vitro experiments, we derived macrophages from bone marrow cells of the C57BL/6 and TNFp55 -/- animals and infected with L. braziliensis in the presence of different stimuli. The results suggest that the macrophages from TNFp55 -/- animals have a greater capacity to eliminate the parasite or simply present a failure in their phagocytosis process. This work aims, therefore, to develop a murine model for mucocutaneous leishmaniasis, which reproduces the clinical manifestations that affect humans.**Supported by:**CAPES **Keywords:** Tnfrp55-/- ; leishmania braziliensis ; mucocutaneous leishmaniasis

HP122 - **INHIBITION OF NITRIC OXIDE PRODUCTION IN ACTIVATED MACROPHAGES CAUSED BY *TOXOPLASMA GONDII* INFECTION OCCURS BY DISTINCT MECHANISMS IN DIFFERENT CELL LINES**

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Toxoplasmosis is a worldwide zoonosis caused by *Toxoplasma gondii* infection. *T. gondii* is an obligate intracellular parasite able to infect any nucleated cell, including macrophages. Macrophage populations are extremely heterogeneous, responding differently to stimuli and to parasite infection. *T. gondii* infection inhibits nitric oxide (NO) in activated macrophages. However, parasite effectors responsible for this evasion mechanism still remain elusive. The aim of this study was to evaluated the NO production inhibition caused by *T. gondii* infection of J774-A1 and RAW 264.7 macrophages and to identify the role of several known parasite virulence factors in this phenotype. Infection of activated macrophages from both macrophage lines reduced NO production, however the mechanism of this decrease was different. Consistent with previous reports, infected J774-A1 macrophages had reduced iNOS expression and lower number of iNOS positive cells. In contrast, *T. gondii* infection of RAW 264.7 macrophages did not alter iNOS expression or the number of iNOS positive cells, and yet it led to lower levels of NO production. Supplementation of culture medium with extra L-arginine did not reversed the NO production inhibition. Deletion of a number of previously defined virulence factors including ROP kinases that disrupt innate immune factors, TgIST which blocks STAT1 activation, as well as the secretory trafficking proteins ASP5 and MYR1, did not alter the phenotype of decreased NO production. Taken together our findings indicate that *T. gondii* infection inhibits NO production of activated macrophages by different mechanisms that involve iNOS degradation vs. iNOS impairment and suggest that a novel parasite effector is involved in modulating this important host defense pathway.**Supported by:**CAPES, CNPq, NIH **Keywords:** Toxoplasma gondii; virulence factors; nitric oxide

HP123 - **EXPRESSION OF A *TRYPANOSOMA CRUZI* ASCORBATE PEROXIDASE (APX) BY *TRYPANOSOMA RANGELI* ALTERS THE PARASITE GROWTH IN VITRO**

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Trypanosoma rangeli and *Trypanosoma cruzi* are parasites that infect several mammalian species in South and Central America. While *T. cruzi* is the causative agent of Chagas Disease, *T. rangeli* is considered as non-pathogenic to mammalian hosts and it is primarily transmitted via the bite of triatomine vectors. Facing environmental drastic changes during their life cycles, these Kinetoplastids have efficient but distinct antioxidant defence systems. Such as *T. brucei, T. rangeli* lacks a functional ascorbate peroxidase (APx) and is more sensitive to H_2O_2 than *T. cruzi*. Thus, we hypothesized whether the absence of a functional APx in *T. rangeli* is related to a putatively extracellular lifestyle of this parasite within its mammalian hosts. Therefore, using functional rescue assays to assess phenotypic changes of the expression of *T. cruzi* APx (*Tc*APX) by *T. rangeli,* we have comparatively evaluated in vitro growth rates, infectivity to Balb/C mice and parasitemia of the transfectants (*Tr*APx$^+$) with wild-type (WT) parasites. WT parasites have a slight but significant higher growth rate than *Tr*APx$^+$ parasites during the log phase ($p<0.01/0.05$). However, when the number of live parasites gradually start to decrease from the stationary growth phase (day 8) onwards, *Tr*APx$^+$ parasites sustained larger numbers of live parasites than WT, presenting normal movement and morphology in culture up to day 14. No significant differences were observed on the infectivity and parasitemia of WT and *Tr*APx$^+$ in Balb/C mice, being in accordance to previous studies. These results seem to indicate that *Tr*APx$^+$ have an improved resistance to stress under culture conditions.**Supported by:**CAPES, CNPq and UFSC **Keywords:** Antioxidant system; oxidative stress; t. rangeli

HP124 - ***TRYPANOSOMA CRUZI* MASP FAMILY: EXPRESSION VARIABILITY AND ITS ROLE IN THE PARASITE INFECTIVITY**

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Trypanosoma cruzi, the etiological agent of Chagas' disease, is a heterogeneous species with high genetic and phenotypic diversity that circulates among the vector and different species of mammalian hosts. The genome sequencing of the CL Brener strain of *T. cruzi* revealed that there is a massive expansion of genes encoding surface proteins families such Trans-sialidase, mucins and MASP. MASP is the second largest multigene family of CL Brener *T. cruzi* with about 1400 members. The large polymorphism of the MASP family associated with its location on the surface of infective forms of *T. cruzi* suggest that MASP participates in mechanisms of host-parasite interactions. In this work, we investigated the expression profile of some MASP members selected based on RNA-seq analysis and antigenicity prediction. Polyclonal antibodies against these proteins were generated and flow cytometry and immunofluorescence assays were performed using CL Brener trypomastigotes. The results demonstrated that these MASPs are not expressed in all parasites in the population. The anti-M18 polyclonal antibody had a higher labeling percentage (80% of the the population), anti-M2 labelled 62,5% of the parasites, whereas the anti-M13 labelled a lower percentage (14%). Monoclonal antibodies were also generated against M2 which labelled 68,1% of the parasites. Binding assays were performed on L6 cells to investigate the possible involvement of MASP in the invasion of the host cell. The results suggest that different MASPs are capable of binding on the surface of L6 cell but not with the same affinity: M2, M12, M13 and M20 appear to bind with higher affinity, while M1, M6, M7 and M18 have a lower binding affinity. Pre-treatment of L6 cells with the M13 recombinant protein reduced the parasite infection by 58% compared to non-treated cells. These results reveal a heterogeneous expression of MASP in the parasite population and suggest the involvement of different MASP variants in host cell invasion.**Supported by:**FAPEMIG CAPES CNPq **Keywords:** Variability; masp; t. cruzi

HP125 - **CROTOXIN DERIVED FROM *CROTALUS DURISSUS TERRIFICUS* VENOM IS EFFECTIVE AGAINST *LEISHMANIA (VIANNIA) BRAZILIENSIS.***

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Leishmaniasis present a broad spectrum of clinical manifestations. In the Amazon region, *Leishmania (Viannia) braziliensis* is one of the main species that causes Cutaneous Leishmaniasis (CL) and Mucocutaneous Leishmaniasis (ML), a severe form of disease. The chemotherapy is one of the most effective treatments for leishmaniasis, although the antileishmanial drugs available are in general toxic, expensive and require long term treatment. Thus, the development of drugs from natural products to treat leishmaniasis has become a priority. Animal venoms are known to exhibit a variety of pharmacological activities including action against pathogen. Therefore, in this study we evaluated the activity of crotoxin (CTX), toxin in *Crotalus durissus terrificus* snake venom, against *L. (V.) braziliensis* and macrophages. MTT toxicity assay were performed on macrophages cell line (J774.A1) and on *L. (V.) braziliensis* treated at different concentrations of CTX (1,2; 2,4; 4,8; 9,6 µg/mL). CTX did not present cytotoxic action on macrophages. However, we observed a reduction in the viability of promastigote treated with 1,2 (4.9%); 2,4 (11.7%) and 4,8 µg/mL (29.5%) of CTX compared to the untreated control, after 96 hours of treatment (IC50: 8.3 µg/mL). The endocytic index showed a reduction in the number of amastigotes (35.56% and 59.35% with 2,4 and 4,8 µg/mL, respectively). The mechanism for the leishmanicidal action of CTX was investigated using the Annexin V-FITC by fluorimetric assay. The results demonstrated that parasite cell death is not associated with apoptosis. However, ultrastructural analysis showed that *L. (V.) braziliensis* promastigotes presented morphological feature of autophagy. Flow cytometry analysis of infected macrophages, treated with 2,4 µg/mL CTX for 72 h, demonstrated decreased secretion of TNF-α levels. Therefore, these results showed that CTX effectively promotes antileishmanicidal activity and has no cytotoxic effects on host cell.**Supported by:**FAPESPA, CNPQ, INCT - INBEB **Keywords:** Crotoxin; leishmaniasis ; leishmania braziliensis

HP126 - **THE ROLE OF NEUTROPHILS AND REACTIVE OXYGEN SPECIES IN INTRADERMAL INFECTION BY TRYPANOSOMA CRUZI**

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Recent works have demonstrated that ROS could be a signal to parasite proliferation inside macrophages. However, the role of these molecules in other types of cells, like neutrophils is not undestood. In vivo, when C57BL/6 WT and Phox KO mice (knockout to NADPH oxidase) were infected by IP route we did not observe differences in parasitemia level. Herein we focused on understanding the interaction between neutrophils and T. cruzi, exploring ROS involvement and the influence of infection route in this infection using WT and Phox KO mice. When in vivo infection was performed by ID route, Phox KO mice had lower levels of parasitemia than WT, corroborating our in vitro results that show that a signal induced by ROS is important for the replication of this parasite in macrophages. To elucidate what happens with neutrophils, bone marrow neutrophils were infected with trypomastigotes and the parasite burden and the occurrence of apoptosis was analyzed by flow cytometry. Both neutrophils, from WT and Phox KO, uptook parasites similarly. However, in contrast with we observed in macrophages, the parasite burden in Phox KO neutrophils was significantly higher than in WT neutrophils. Besides, Phox KO had significantly less apoptotic neutrophils than WT. In vivo, the cellular infiltrate after ear ID infection was evaluated by flow cytometry. The number of inflammatory monocytes was significantly higher in WT mice than Phox KO. In contrast, inoculation of T. cruzi in Phox KO mice induced a neutrophil accumulation that was not observed in WT mice. Our results shows that the absence of ROS affects cellular recruitment after intradermal T. cruzi infection and interfere with the parasite burden and apoptotic events in neutrophils. Besides, our results indicate that the inoculation route can influence the development of the disease, impacting parasite load and infection phenotypes. So, it is important to be taken into account during experimental designing. **Supported by:**CAPES, CNPq **Keywords:** Ros; trypanosoma cruzi; neutrophils

HP127 - **ANTI-PHOSPHATIDYLSERINE ANTIBODIES BLOCKS *TOXOPLASMA GONDII* ENTRANCE IN LLC-MK2 CELLS**

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Toxoplasmosis is caused by the protozoan *Toxoplasma gondii*. A subpopulation of tachyzoites of *T. gondii* exposes the phospholipid phosphatidylserine (PS) on the outer leaflet of the plasma membrane, mimicking apoptotic cells. The subpopulation that exposes PS (PS^+) actively penetrates macrophages and is found in tight-fitting vacuoles. However, the subpopulation that does not expose (PS^-) is phagocytosed and is found in loose-fitting vacuoles. Thus, it seems that exposure of PS is necessary for active penetration of *T. gondii*. To further test this hypothesis, anti-phosphatidylserine (anti-PS) antibodies were used to block PS exposed on *T. gondii* before interaction with a non-professional phagocityc cell line. For this, LLC-MK2 cell line was infected with untreated or treated tachyzoites with anti-PS antibodies (Fab portion, entire antibody or isotype control) and annexin-V. Tachyzoites treated with Fab portion, entire anti-PS antibody or annexin V presented significant reduced entrance in the host cells in comparison to parasites treated with isotype control or non-treated. Thus, the block of PS with anti-PS antibodies suggests the importance of this phospholipid in the active penetration process of *T. gondii*. We will next explore the effect of these anti-PS antibodies on NO production of *T. gondii* infected macrophages and parasite growth.**Supported by:**FAPERJ, CAPES, CNPq, UENF, UFRJ **Keywords:** Toxoplasma gondii; phosphatidylserine; antibodies

HP128 - **L-SERINE AND L-THREONINE ARE ABLE TO FUEL THE RESPIRATORY CHAIN IN TRYPANOSOMA CRUZI EPIMASTIGOTES.**

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Along its journey between mammalian and invertebrate hosts, Trypanosoma cruzi is constantly subjected to stressors in dynamic environments, such as: nutritional stress (NS), temperature changes, osmolarity changes and redox stress. More specifically, T. cruzi has to endure long periods of NS. It has been demonstrated, in other organisms, that Ser/Thr have a critical role as carbon sources in glucose deprivation conditions. This protection requires: (i) Ser/Thr obtention via de novo biosyntesis, uptake from extracelular enviroment or protein degradation; and (ii) a deamination step, usually catalyzed by Ser/Thr ammonia lyase (4.3.1.19), and which converts Ser or Thr into pyruvate or 2-oxobutanoate respectively, both able to feed the TCA cycle. Interestingly, no putative genes for de novo biosynthesis of Ser/Thr have been found in T. cruzi genome databases until now. Thus, assuming that neither Ser nor Thr are synthesized de novo, we characterized the uptake of Ser (KM: 5.89 ± 0.68 mM and Vmax: 4.24± 0.35 nmol L-Ser per 2 x 107 cells per min), the role of Ser/Thr on epimastigotes (Epis) bioenergetics and their capacity of supporting the parasite survival under servere NS. When incubated with Ser/Thr as a carbon source up to 96 h, Epis remained more viable than parasites without exogenous carbon sources. Ser and Thr were also able to stimulate the oxygen consumption of intact Epis, maintaining routine respiration (R) (Ser: 35.5 ± 4.2 and Thr: 36.2 ± 2.5 Slope pmol/(s*ml) O2). In addition, after titration with FCCP, we demonstrated Ser- and Thr-mediated stimuli on electron transport system (ETS), supporting the maximum capacity of this process (Ser: 38.5 ± 5.6 and Thr: 49.6 ± 6.6 pmol/(s*ml) O2). These results point to a unique route for the full catabolism of Ser/Thr in T. cruzi and to the participation of these amino acids in NS resistance.**Supported by:**CNPq, FAPESP **Keywords:** Serine and threonine metabolism; trypanosoma cruzi bioenergetics; ser/thr ammonia lyase

HP129 - **HEART LESIONS IN MICE INFECTED ORALLY WITH TRYPOMASTIGOTES METACYCLIC FROM BERENICE-78 *TRYPANOSOMA CRUZI* STRAIN**

RODRIGUES, T.G.*[1]; CARVALHO, T.V.[1]; CARVALHO, L.M.[1]; FERRAZ, A.T.[1]; DUARTE, T.H.C.[1]; MARQUES, F.S.[1]; XAVIER, V.F.[1]; SOUSA, L.R.D.[1]; GOMES, I.C.[1]; FARIA, J.Q.[1]; DAS MERCÊS, A.C.[1]; CARNEIRO, C.M.[1]; VIEIRA, P.M.A.[1]
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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 blood banks with high mortality. In this sense, a study on the cardiac injury caused by 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 were infected intraperitoneally (IR) or orally (OR) with metacyclic forms from Berenice-78 strain of T. cruzi and were euthanized at 14, 21, 28, 35, 42 and 180 days after infection (DAI). Heart was collected for evaluation of inflammatory process, expression of fibronectin (FN), parasite load by Real-Time PCR and serum concentration of CK-MB.The group IR presented a significant increase in the inflammatory process in the 42º DAI in relation to the 180º DAI and the group of uninfected animals (C), whereas in the OR group there was an increase of the inflammatory process in the 21º DAI in relation to the 14, 42 and 180º DAI and also in relation to the animals of group C at day 180. FN expression was assessed at 42 and 180º DAI. The OR group exhibited an increase in FN expression at the 42º DAI in relation to the 180º DAI. In addition, in relation to the 42º DAI, a greater expression of FN in the OR is observed when compared to the same time in groups C and IR. Both routes of infection showed an increased parasite load, wherein only at the 21º DAI the OR group presented an increased parasite load, however in the IR group this increase was found in 21 and 28º DAI. Besides that, OR group showed an increase in the CK-MB over time of infection being observed a greater concentration in 14 and 35 ºDAI. On the other hand, IR showed no changes in CK concentration during infection. Thus, these results show that the oral infection with metacyclic forms from Berenice-78 T. cruzi strain shows a different parasitological profile resulting in increased cardiac damage. **Supported by:**CNPQ, CAPES, FAPEMIG, UFOP. **Keywords:** Oral chagas disease ; fibronectin ; ck-mb

HP130 - **COMPOUND DERIVED FROM SCHIFF BASE CONTROL EXPERIMENTAL INFECTION BY TOXOPLASMA GONDII: INVOLVEMENT OF CCR2 RECEPTOR**

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Toxoplasma gondii(Tg)is the etiologic agent of Toxoplasmosis.It mainly affects the central ner ystem being its treatment ineffective, requiring therapeutic innovations. Have being demor ity of Schiff bases(BS) against bacteria and protozoa. Macrophages(MOs)are crucial i tion, andCCR2/CCR5 chemokine receptors are involved in the activation o an antiparasitic activity of BS and the requirement of CCR2 and/or C g-infected. MOs.Peritoneal MOs of C57B1/6(WT), CCR2 or C e plated, infected with Tg(RH strain;1:1;ratio Tg:cell ake, replication and pre-stimulus assays of the par 48h after infection/stimulus.Cytotoxicity assay(LDH or in vivo assay, C57BL/6(WT) mice were infected g/kg] started at 8h after infection followed daily until 7 red each 5 days up to 45 days post infection. The Tc cted by parasites pre-incubated with P8, decreased ed with non-stimulated MOs or untreated-parasite P ake by MO. LDH assay demonstrated that P8 was no oreover, the deficiency of CCR2-/-orCCR5-/- resulted in lo compared with WT cells. In addition, in the absence of CCR2, bu partially its anti-toxoplasmic effects. In vivo, we showed that mice trea lower weight loss when comparer with untreated infected mice.Collectively, our BS-P8 has anti-toxoplasmic activity that is partially dependent of CCR2. Financial suppo and FAPEMIG. **Suppored by:**Fapemig **Keywords:** Doença de chagas; macrophages; shiff ba

HP131 - **ARTIFICIAL IMMUNE SYSTEM: PRELIMINARY STUDIES OF IMMUNE RESPONSE TO *TRYPANOSOMA CRUZI* INFECTION**

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Introduction:The Chagas disease (ChD) is an important neglected parasitic infection, caused by *T.cruzi* protozoan transmitted in feces of insect of subfamily *Triatominae*. According WHO, about 8 millions of individual were infected in Latin America and 25 million are at risk of acquiring the disease in worldwide. The ChD is responsible by most common form of non-ischemic cardiomyopathy worldwide with 14000 annual deaths induced by heart failure. Despite none treatment to be effective in chronic phase, studies have pointed that in early stages of parasite infection the immune system and macrophages is capable of controlling infection. Objectives: The present investigation was carried out to present the creation of a modelling system and computational simulation of the immune system initially developed by Possi (2012) and adapted by Farago (2014) to perform *in silico* experiments in the acute phase of ChD. Methods:For protozoan modeling, T. cruzi agent was included and subsequently macrophage agent was added in order to simulate the main pathway of pathogen recognition by immune system in acute phase of disease. Results: The response pattern of the multiagent systems, pointed for a response more pronounced according virulence and number of strain inoculated over the clinical outcome of the infection in the acute phase. The simulation datas corroborate with that described by in vivo studies in literature emphasizing the relevant of inoculated strain characteristics over the mapping immune response profiles of host. Conclusion: Despite researches, involving immune response in condition of T. cruzi infection, several points on immunology and pathogenesis of the ChD are unclear. This way computational research could contribute for enabling the development of drugs and vaccines against the disease. Acknowledgments: The authors are grateful to CNPq and PROAPP / FADIP for their financial support.**Supported by:**CNPq; PROAPP / FADIP **Keywords:** Artificial imune system; chagas disease; macrophages

HP132 - **EVALUATION OF MORTALITY AND IMMUNE RESPONSE IN DIFFERENT LIFE STAGES OF *RHODNIUS PROLIXUS* INFECTED WITH *METARHIZIUM ANISOTPLIAE***

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The chemical control of triatomines, vectors of the protozoan *Trypanosoma cruzi*, an etiologic agent of Chagas' disease, has been threatened by the appearance of insect populations resistant to pyrethroids. Entomopathogenic fungi have been investigated as an alternative tool for the control of various insects, including the vector *Rhodnius prolixus*. However, studies showing the efficiency of *M. anisopliae* to control different life stages of *Rhodnius prolixus* still remain scarce. In addition, much less is known about immune response upon *M. anisopliae* infection at different life stages. The objective of the present work is to evaluate the mortality rate and investigate the immune response upon *M. anisopliae* infection throughout *Rhodnius* prolixus life cycle. For the mortality test, nymphs of 1st, 2nd, 3rd, 4th and 5th stages (3 hours after a blood meal) were sprayed with 0.05% Tween 80 using Potter's tower in the absence or presence of fungus (10^7 conidia). Fungal dispersal among insects was assessed by counting dead nymphs after putting two or ten infected insects in contact with other uninfected ones. Our results showed that nymphs of the 1st and 2nd stages were more susceptible than other stages. Mortality rate increased 4 days after infection, reaching 50% on the 6th day. Nymphs of 1st and 2nd stages presented 88% and 79% of mortality, respectively, on 21th day after infection. Nymphs of 3rd, 4th and 5th stages were more resistant to fungus, with mortality of 47%, 43% and 47%, respectively, on the 21th day. In terms of fungal dispersal among 1st stage nymphs, we showed 38% of mortality in those exposed to two infected nymphs, whereas nymphs exposed to ten presented 63%. For the analysis of immune response we are evaluating the expression of Defensin and Lisozymes (LysA, LysB) by means of RT-qPCR. Our results may help to understand the effect of *M. anisopliae* on *R. prolixus* in order to improve the strategy of vector control infection. **Supported by:**CAPES/ INCT - EM **Keywords:** Rhodnius prolixus; metarhizium anisopliae; biological control

HP133 - **CLINICAL-IMMUNOLOGICAL SPECTRUM CHARACTERIZATION OF THE HUMAN INFECTION BY *LEISHMANIA (L.) INFANTUM CHAGAS*I IN HONDURAS, CENTRAL AMERICA: PRELIMINARY RESULTS**

OCHOA, W.H.S.[*1]; ZUÑIGA, C.[2]; ARAUJO FLORES, G.V.[3]; SANDOVAL PACHECO, C.M.[3]; SILVEIRA, F.T.[4]; DA MATTA, V.L.R.[3]; TOMOKANE, T.Y.[3]; CORBETT, C.E.P.[3]; LAURENTI, M.D.[3]
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Introduction: In Honduras, *Leishmania (L.) infantum chagasi* is the etiological agent of visceral leishmaniasis and non-ulcerated cutaneous leishmaniosis (NUCL). The purpose of this study is to determine the prevalence and the clinical immunological profile of human infection by *Leishmania (L.) infantum chagasi* in the Municipality of Amapala, Honduras, Central America.
Methodology: 576 individuals from fifteen locations from the Municipality of Amapala, Honduras were evaluated. A transversal-type study was designed to determine the infection prevalence as well as the clinical immunological profiles (Asymptomatic Infection=AI, Symptomatic Infection=LV, Sub-clinical Oligosymptomatic Infection=SOI, Sub-clinical Resistant Infection=SRI and Initial Indeterminate Infection=III) using ELISA test to determine the antibody level and Delayed Type Hypersensitivity (DTH) to evaluate the cellular immune response against *Leishmania* parasite, both using specific antigen.
Results: 576 individuals were evaluated, out of which 58.33% were females and 41.67% males. *Leishmania (L.) infantum chagasi* was identified as the specie responsible for NUCL cases in the studied endemic area. According to the clinical exam and laboratory test diagnosis, ELISA and DTH, the prevalence of infection was 54% (314/576%). The clinical-immunological profile of infection was determined using data from 314 individuals and it as characterized as: AI 33.44%, LV 0%, SOI 2.23%, SRI 14.97%, III 6.24% and NUCI 33.12%.
Conclusions: The combination of ELISA and DTH tests allowed us to determine the clinical-immunological spectrum of human infection by *Leishmania (L.) infantum chagasi* on the Municipality of Amapala, including a new infection profile, the skin symptomatic infection called non-ulcerated or atypical cutaneous leishmaniosis characterized by a strong DTH response and slight antibody production point to a predominance of cellular immune response. **Supported by:**FAPESP, CAPES, CNPq and LIM-50 HC-FMUSP **Keywords:** Non-ulcerated cutaneous leishmaniosis; honduras; leishmania infantum chagasi

HP134 - **ASSESSING THE CHEMOTHERAPEUTIC POTENTIAL OF A HISTONE DEMETHYLASE INHIBITOR AGAINST CUTANEOUS LEISHMANIASIS CAUSED BY *LEISHMANIA BRAZILIENSIS***

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INTRODUCTION: Cutaneous leishmaniasis (CL) caused by L. braziliensis is the most common clinical form of the disease in Brazil. CL is associated with an intense chronic inflammatory response. The few therapeutic options, the high toxicity and the drug resistance reinforce the need for alternative treatments. Epigenetic mechanisms involving histone modification are deregulated in chronic inflammatory diseases. Thus, epigenetic regulators are considered promising targets for treating these diseases. The present work aims to evaluate in vitro and in vivo the chemotherapeutic potential of GSK-J4, a histone demethylase inhibitor with anti-inflammatory properties, against L. braziliensis in the murine model of cutaneous leishmaniasis. METHODS: The IC50 of GSK-J4 and its reversible inhibition for promastigote forms was determined by flow cytometry and for amastigote forms by direct counting using murine macrophages. A preliminary in vivo assay was performed using the BALB/C ear infection model with intralesional GSK-J4 administrations. RESULTS: GSK-J4 did not demonstrate cellular toxicity to uninfected host cells up to 100 µM after 24 hrs of treatment. In contrast, the compound showed leishmanicidal effect with IC50 of 888 nM for promastigotes and 4.5 µM for amastigotes after 24 hrs of treatment. In addition, treatment with 7.5 µM for 24 hrs showed an irreversible effect on the amastigote form. The intralesional treatment of the compound with 4.5 µM was not able to reduce lesion size and parasitic load on ears and lymph nodes. However, it shows an effect on the development of ulcers, since 75% treated mice did not develop ulcerated lesions. CONCLUSIONS: So far, data presented here reveals leishmanicidal properties in both evolutionary forms of the parasite without toxicity to the host cell. The next step is to evaluate higher concentrations of GSK-J4 in vivo and characterize its anti-inflammatory profile, what could result in higher cure rate and a faster healing time. **Keywords:** Histone demethylase inhibitor; inflammation; l. braziliensis

HP135 - **NEW THERAPEUTIC STRATEGIES FOR TREAMENT OF MICE ACUTELY INFECTED WITH VL-10 STRAIN T.CRUZI STRAIN**

MEDEIROS, L.F.*[1]; FONSECA, K.S.[1]; PERIN, L.H.[1]; PAIVA, N.C.N.[1]; MADEIRA, J.V.[1]; DUARTE, T.H.C.[1]; BARBEDO, P.C.[1]; VIEIRA, P.M.A.[1]; CARNEIRO, C.M.[1]
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Benznidazole (BNZ) presents toxicity and efficacy dependent on the strain and disease stage, although it is the reference drug in the Trypanosoma cruzi infection treatment. Thus, in addition to the searching for new drugs, a research strand aims to work with BNZ modifying formulation or therapeutic scheme. Therefore, this study evaluates the efficacy of new therapeutic strategies using BNZ in the Chagas disease acute phase. For this, 40 Swiss mice infected with VL-10 strain were divided into five groups: infected and untreated, infected and treated with BNZ 100 mg/kg/day for 20 days; infected and treated with BNZ 100 mg/kg/day for 40 days; infected and treated with BNZ 40 mg/kg/day for 20 days, infected and treated with BNZ 40 mg/kg/day for 40 days. The parasitemia curve was determined, the fresh blood exam (FBE) and the qPCR (blood, heart and colon) were performed after immunosuppression as well as histopathological analysis in the heart and colon. Treatments with BNZ 100 mg/kg/day for 20 or 40 days were able to reduce parasitemia compared to untreated animals and the group with the lowest peak of parasitemia was treated with BNZ 100 mg/kg/day for 20 days. For FBE and qPCR, mice treated with BNZ 100 mg/kg/day for 40 days showed 12% of cure and the other groups 0%. Regarding histopathology, a significantly higher inflammatory process was observed in the groups infected and treated for 40 days with 100 mg/kg/day (conventional treatment) and the reduced dose at 40 mg/kg/day. In the colon, only animals treated with 100 mg/kg/day during 40 days showed this increase. In conclusion, to date, the group treated with 100 mg/kg/day for an extended period (40 days) allowed a cure increase. However, an increase in the inflammatory process was also observed, suggesting tissue damage in both the heart and the colon. Further research is necessary to make a change possible in the therapeutic regimen, since despite the increase in efficacy, tissue damage was observed.**Supported by:**7º Programa Marco de la Comunidad Europea, CAPES, CNPq, FAPEMIG, UFOP. **Keywords:** Benznidazole; trypanosoma cruzi; chagas disease

HP136 - **EVALUATION OF HEPARIN EXTRACTED FROM *STYELA PLICATA* AS INHIBITOR OF INFECTION BY *LEISHMANIA AMAZONENSIS***

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Introduction: Receptors on the surface of Leishmania spp., Parasites that cause Leishmaniasis, bind to glycosaminoglycans (GAG) with a heparin-like structure, present in tissues and on the su of hosts. These receptors are known as heparin binding proteins (HBP), and influ on between parasite and host during infection. Objective: To investigate th d from the ascetic Styela plicata as an inhibitor of L. amazon f heparin extraction from S. plicata (HSP) was con imaging. Metacyclic promastigote for heparin (HEP) or PBS for me, they were wash ious parasites were was evaluated by light was analyzed by agarose gel e ssment was confirmed by NMR. There was a d reated promastigotes after 2 hours of infection at 5 µg / m at higher concentrations. After 48 hours of infection, a lower infectivity inc as observed. There was no significant difference in HSP treatment in amastigote infe Conclusion: the presence of HSP seems to decrease the infection by promastigotes of L. amazonensis, which may have an impact on the natural transmission of the disease. The binding of S. plicata-derived GAGs on the surface of promastigotes still needs to be defined.â€‹**Supported by:**FAPERJ **Keywords:** Leishmania; heparin; glycosaminoglycans

HP137 - **IN VIVO ANTILEISHMANIAL EFFICACY OF A TOPICAL TREATMENT OF CROTOXIN DERIVED FROM *CROTALUS DURISSUS TERRIFICUS* VENOM AGAINST *LEISHMANIA (LEISHMANIA) AMAZONENSIS***

MARTINS, A.J.E.[*1]; DA SILVA, B.J.M.[1]; HAGE, A.A.P.[1]; RODRIGUES, A.P.D.[2]; SAMPAIO, S.C.[3]; SILVA, E.O.[1]

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Leishmaniasis are neglected emerging diseases in 98 countries caused by several species of protozoa of the genus *Leishmania* and transmitted by phlebotomine sandflies. The treatment of tegumentar leishmaniasis is limited due to the several side effects and the development of protozoan parasites resistance against the drugs used. Thus, search for new medicaments from natural origin and high efficacy against the parasite without toxicity are needed. In this context, animal venoms and its purified compounds are know to exhibit a variety of pharmacological activities. In the present study it was evaluated the effects of of crotoxin (CTX), the predominant toxin in *Crotalus durissus terrificus* snake venom, on experimental cutaneous leishmaniasis caused by *Leishmania (Leishmania) amazonensis*. Animals (eight-week-old female BALB-c mice) were infected with 106 of *L. (L.) amazonensis* promastigotes during the stationary growth phase. The treatment was iniciated after five weeks of infection and the CTX formulation was applied topically to all lesions once daily for 30 days. Control groups were also maintained in parallel. After the treatment, animal were euthanized and tissues from lesions were processed for histopathological analysis and transmission electron microscopy analysis. CTX was able to decrease parasitic load and significantly reduce lesion size. Ultrastructural analysis and staining with Sirius Red showed the presence of collagen fibers compared to control, suggesting a healing process. In addition, CTX was able to alter the Th2 immune profile to Th1, as suggested by elevated production of the cytokines interleukin-6 and interferon-γ. In conclusion, our results reveal that treatment with CTX is associated with effective immune response against to the parasite, suggesting that CTX may be a possible topical therapy against tegumentar leishmaniasis.**Supported by:**FAPESPA, CNPQ, INCT - INBEB **Keywords:** Leishmaniasis; crotoxin; leishmania amazonensis

HP138 - **LEISHMANICIDAL ACTIVITY OF PLANTS POPULARLY USED TO TREAT HUMAN SKIN LESIONS**

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Leishmaniasis is a disease caused by protozoan parasites belonging to the Leishmania genus. There are two main clinical forms, the cutaneous and the visceral leishmaniasis. Drugs used in the therapy are considered toxic and outdated. The first-line drug, pentavalent antimonals have different effectivity in vivo, and serious side effects can be detected, including local pain and cardiotoxicity; furthermore, parasites resistant to this drug have been detected. Amphotericin b, a second-line drug, is effective against Leishmania, but the side effects are limiting factors. Thus, the objective of this study is to analyze the leishmanicidal activity of plants from Atlantic forest (SP- State) popularly used to treat skin problems. Fifteen elderly individuals were interviewed about plants used to skin problems. Fifty-two plants were indicated, but only twenty endemic specimens were tested, collected and identified at the Botany Institute of São Paulo. Ethanolic and hexanic extracts were produced and were assayed against promastigote and amastigote forms of Leishmania amazonensis . Cytotoxicity was assayed against J774 macrophages. Against promastigote forms, hexanic extract of the leaves from *S. terebinthifolius* was the most active, showing an effective concentration 50% of 13,9 µg/mL, while ethanolic and hexanic extracts from the leaves of *Alternathera brasiliana* were the least active against *L.amazonensis* promastigote forms. Cytotoxicity studies showed that the most toxic extract was hexanic produced with leaves of *S. terebinthifolius* and least active was ethanolic extract produced with *S. terebinthifolius* leaves. Concerning amastigote activity, it was observed that ethanolic extract produced with the leaves of *S. terebinthifolius* and *E. uniflora* were active, decreasing the infection index of macrophages by 79,8% (46µg/mL) and 71,3% (10µg/mL), respectively. This study suggests that S. terebinthifolius and *E. uniflora* extracts present interesting anti-Leishmania molecules. **Supported by:**FAPESP **Keywords:** Leishmanicida; conhecimento popular; l. amazonensis

HP139 - **LOCATION OF LIPID BODIES IN MACROPHAGES INFECTED BY LEISHMANIA AMAZONENSIS**

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Leishmaniasis is a zoonosis caused by a protozoan of the genus Leishmania, and it's an endemic disease, with reports in the state of Rio de Janeiro. A L. amazonensis is an intracellu ite, which infects macrophage preferentially. Studies relate a correlation bet oid corpuscles and infected macrophages. The importance of the l in controlling the syntax of lipid mediators in infecti r, since they are made up of lipids and p ancillary in the reproducti project is to s ensis. P azonensis produce of infection, the corpuscles are to be located close to or inside the parasitic cells may manifest overnight or the inside of the parasitoid v hat occurs a positive modulation of the biogenesis of these organelles d is still necessary to determine if there is a change between the corpuscles, the pa vacuole and the parasites. More studies are needed to improve these actions.**Supported by:**CNPQ **Keywords:** Macrophage; lipid corpuscles; leishmania amazonensis

HP140 - **ADHESIVENESS PROPERTIES OF THREE POTENTIAL VACCINE CANDIDATES IN VIVAX MALARIA**

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Background: Adhesiviness is an important phenomenon to study, especially when it's related to the pathogeny of a disease. In malaria, it can happen between parasitized and healthy erythrocytes, forming what is known as a rosette. This occurrence is well described when it comes to the malaria caused by *Plasmodium falciparum* and its correlation with severity of infection. However, in vivax malaria, little is known about rosettes or other adhesive phenomena. Given its part in parasite-host interaction, the adhesiveness of three potential vaccine candidates against vivax malaria were analyzed.
Methods: Using reverse vaccinology, the *P. vivax* genome was scanned for adhesive, antigenic and immunogenic proteins. Three selected genes were synthetically synthesized and cloned into the pEGFP-N1 plasmid, which has the green fluorescent protein (GFP) as a reporter but it's also modified to anchor the recombinant proteins to the outer cell surface. CHO-745 cells were transfected and adhesiveness assays were performed with different blood types' erythrocytes from healthy individuals.
Results and Conclusions: Although our analysis showed that the adhesive property of the selected vaccine candidates was predicted as higher than known important adhesive proteins in vivax malaria, there was no adhesion verified, at this point, between the candidates and healthy erythrocytes. The possibility that the adhesiveness can be between parasitized erythrocytes and reticulocytes or white blood cell receptors still needs to be investigated. This phenomenon can be complex and further studies with these proteins must be performed before they are ruled out as potential vaccine candidates or therapy targets.**Supported by:**CAPES **Keywords:** Malaria; adhesiveness; rosette

HP141 - **EARLY METASTATIC LESIONS IN MICE LACKING EOSINOPHILS INFECTED WITH LEISHMANIA AMAZONENSIS.**

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Both in mice and humans, eosinophils compose the cellular infiltrate in tissue infected by Leishmania spp., in association with the inflammatory milieu at the site of infection during early and late disease. dbl-GATA1 mice have a double deletion on the high-affinity GATA-binding site in the GATA1 promoter, which leads to a defect in the maturation of eosinophils. Previous experiments using Leishmania major showed that these mice develop early metastatic lesions compared to wild type. This work aims to evaluate how the lack of eosinophils promotes early dissemination of the parasites. BALB/c and dbl-GATA1 mice were infected in the footpad with stationary phase promastigotes of Leishmania amazonensis carrying integrated reporter genes (PH8-Luc+-RFP+). Lesions were measured weekly, and their development was expressed by the difference among infected and non-infected footpad. Parasite load was accessed by qPCR in blood samples. Metastatic lesions and parasite burden were measured by bioluminescence. There were no differences in the lesion development in the footpad between dbl-GATA1 mice and its background control. Also, no differences in parasite load were found between mice lineages at the same time of infection. As seen in the model using L. major, metastatic lesions appeared earlier in eosinophil's deficient mice. Lesions were replete with parasites as observed by the intensity of the luminescent signal in the metastatic lesions. Mean photon intensity was higher in eosinophil-deficient mice in the footpad and draining lymph nodes but were observed only after eight weeks post infection. Parasite burden in blood was also higher in dbl-GATA1 mice than BALB/c at nine weeks post-infection, suggesting intravascular dissemination. Flow cytometry analyses suggest amastigotes disseminate through blood within monocytes and neutrophils.More data is needed to clarify the role of this cell during leishmaniasis.**Supported by:**capes **Keywords:** Eosinophil; leishmania; dissemination

HP142 - **TRAFFICKING AND ASSORTMENT OF GPI-ANCHORED PROTEINS IN *TRYPANOSOMA CRUZI*.**

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At variance with mammals, trypanosomatids have a high percentage of proteins anchored by GPI in their plasma membrane. *T. cruzi* GPI-anchored virulence factors such as *trans*-sialidase (TS) and mucins are transported to the membrane by a non-conventional transport involving the contractile vacuole (CV) prior to reach the flagellar pocket and then the plasma membrane. Both proteins are only detectable in CV during the differentiation to trypomastigote. Once on the membrane, they are ordered in mutually exclusive domains, finding mucins in "lipid rafts-like" areas, whereas TS are not. Also, it is known that each protein has different GPI-lipidic composition. There is only scarce information concerning the signals that determine this arrangement on the plasma membrane or about the role of CV on this novel transport pathway. Here, the involvement of the lipid nature of the GPI anchor in this pathway and in the final membrane assortment were assessed. Recombinant *MucII* genes were constructed where their native GPI signaling or the TS-GPI signal were inserted and cloned in the inducible expression vector *pTcIndex*. We found that protein intracellular trafficking was not modified. Using this regulated system with the recombinant *MucII* genes, we observed that at low levels of expression, both proteins were localized on the membrane with no accumulation in the CV. However, at higher expression levels constant protein accumulation only is observed in the CV from 24h to 96h post-induction and not in the other organelles of the secretory pathway. This suggests that CV could function as a "bottle-neck". Then, the membrane arrangement of these proteins was analyzed. We have observed that the TS GPI-signal clearly change the surface protein domain pattern both in size and distribution as compared to the native GPI-signal, thus highlighting the relevance of the GPI anchor in the final destination of surface proteins.**Supported by:**NIH and ANPCyT **Keywords:** Trypanosoma cruzi; gpi anchored proteins; mucins/trans-sialidase

HP143 - **ANTI-INFLAMMATORY ROLE OF GALECTIN 8 IN TRYPANOSOMA CRUZI-INDUCED MYOCARDITIS**

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Galectins constitute a family of lectins that contain characteristic aminoacid sequences and hold affinity for β-galactosides. Galectin-8 (Gal-8) is included in the tandem repeat group. It has been involved in cellular adhesion, migration, apoptosis, etc., is widely distributed in different tissues and can works through an autocrine/paracrine way. In addition, different authors have proposed Gal-8 to be involved either in pro-inflammatory or anti-inflammatory events. It is known that T. cruzi cardiomyopathy is based on the induction of a strong inflammation by the parasite persistence in tissue. In order to analyse the role of Gal-8 in an inflammatory infectious context, we used a murine chronic T. cruzi infection model: C57BL/6J (B6, WT) and B6Gal-8 knock out (KO) mice, infected with T. cruzi (Ac strain DTU TcI). Mice were sacrificed at 4 months post-infection. KO cardiac tissue showed increased inflammation score in comparison with WT (p=0.0119). No differences were observed in fibrosis degree and tissue parasites level. In order to identify the immune population in inflamed cardiac tissue, a flow-cytometry analysis was performed. Similar values were found in the percentage of cardiac T lymphocytes (CD3+), and their different subpopulations, between KO and WT mice. We observed a rise in the frequency of macrophages (p=0.0021) in KO heart tissue compared to WT, in agreement with increased cardiac CCL-2 levels.
The increment in neutrophil numbers observed in KO versus WT (p=0.0097) did not correlate with cardiac and systemic CXCL1 and CXCL2 levels. These results suggest that neutrophil rise may be instead related to Gal-8 preaparesis induction mechanism.
Taken together, our results suggest that Gal-8 participate as an anti-inflammatory molecule in T. cruzi chronic infection. **Supported by: Keywords:** Galectin; t.cruzi; neutriphil

HP144 - **COMPLEMENT SUBVERSION BY *RHODNIUS PROLIXUS* AND ITS EFFECT ON *TRYPANOSOMA CRUZI* ESTABLISHMENT IN THE VECTOR OF CHAGAS DISEASE**

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One of the most important endemic diseases in Latin America is Chagas. The etiologic agent of ase is *T. cruzi* which transmission is related to the blood-feeding vectors, the triatomines, suc Since its description by Chagas in 1909, evidences from the literature indicated that s nt to parasite establishment in the bug. Having a clear understanding of how esis would allow us to uncover novel therapies against this disease. W dy using DIGE approach on *Rhodnius* gut tissues upon a bl *T. cruzi* infection. Surprisingly, very few proteins we to the presence of the parasite. Interestingly, we h post blood-feeding, the presence of the mamm plement cascade that is at the junction between the processing. We have thus tested the activity of th ibition assay. A significant inhibition of the classical M) and saliva while inhibition of the lectine pathwa , the alternative pathway wasn't affected by the A proteins C4BP, FH and vitronectin are captured by the g ment attack. The presence of these inhibitory molecules in the pathogens, such as *T. cruzi* which insect stage is sensitive to the cc tivity is a crucial component which governs the reservoir competence of the ho plement inhibitors may protect parasites, it is reasonable to suppose that inactivating ther their development.**Supported by: Keywords:** Host-parasite interaction; complement evas matophagous insects, proteomics