HP-001 - **MicroRNAs containing AAGUGCU seed sequence are important for Leishmania amazonensis infection in human THP-1 macrophages**

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Leishmania amazonensis is a parasitic protozoan causing mainly cutaneous leishmaniasis in humans. Multiple mechanisms have been described regarding parasite-mediated rewiring of the immune response of host cell, especially macrophages, where *Leishmania*'s amastigotes multiply and establish infection. MicroRNAs (miRNAs) are small post-transcriptional regulators of gene expression leading to mRNA degradation or translational arrest by complementary binding of its seed sequence (nucleotides 2-8) with the 3' untranslated region of target mRNAs.
Although miRNAs have already been explored in multiple models of *Leishmania* infection, our approach is to compare their role in *L. amazonensis* infected human macrophages with the previously described one in murine macrophages. The expression of 84 immune response-related miRNAs was determined in human THP-1 derived macrophages infected with *L. amazonensis*. The upregulated miRNAs miR-372, miR-373, and miR-520d were further investigated since those miRNAs share the seed sequence AAGUGCU. This sequence is also present in the homologous murine miRNA mmu-miR-294, which was previously shown to target the nitric oxide synthase 2 mRNA. However, we could not find any significant modulation of mRNAs related to L-arginine metabolism in our model. Besides, the recognition site described in murine *Nos2* is mutated in humans.
Since the miRNAs of interest can potentially target similar pathways, we performed the functional inhibition assay with the individual miRNAs or in combination, to see if they could compensate the role of one, in case of the other being inhibited. We observed that inhibiting miR-372 or miR-520d, but not miR-373 alone reduced *Leishmania* infectivity while simultaneously inhibiting miR-372, miR-373 and miR-520d further decreased infectivity.
Here we report that a microRNA family, namely miR-372-373-520 is upregulated in *L. amazonensis* infected THP-1 macrophage correlating with infection susceptibility.
Supported by:FAPESP 2017/21906-9 **Keywords:** leishmaniasis.miRNA.regulation of gene expression

HP-002 - **MACROPHAGE SURFACE PROTEIN DISULFIDE ISOMERASE (PDI) ENHANCES INFECTION BY *Leishmania (L.) amazonensis***

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Leishmaniases are neglected parasitic diseases caused by parasites of the genus *Leishmania*, which are transmitted by female phlebotomine sandflies. Infections by different *Leishmania* species may lead to tegumentary or visceral complications in the vertebrate host. Chaperones present on macrophages surface play a fundamental role in the regulation of cellular homeostasis and may affect survival and infectivity of *Leishmania*. Protein disulfide isomerase (PDI) is one of the 20 most abundant chaperones of the endoplasmic reticulum (ER). The presence of PDI on the macrophage surface was associated with increased infection by *Leishmania chagasi*, a species associated with visceral leishmaniasis. The present study aimed to confirm the presence of PDI on the macrophage surface and evaluate its role on the infection by *Leishmania (L.) amazonensis*, a species responsible for cutaneous leishmaniasis. Cell membrane biotinylation assay was carried out to confirm the presence of PDI on macrophage surface. Bone marrow derived macrophages (BMDM) from BALB/c mice were then blocked with anti-PDI polyclonal antibody and infected with promastigotes of LV79 strain of *Leishmania (L.) amazonensis*. BMDM from transgenic mice overexpressing PDI (confirmed by *Western blot*) and wild type macrophages were also *in vitro* infected with *L. (L.) amazonensis*. *In vivo* imaging using M2269 La-LUC infection in transgenic mice overexpressing PDI and wild type mice is ongoing for comparison of lesion swelling and parasite load. The results of the biotinylation assay confirmed the presence of PDI on macrophage surface. Infection of macrophages blocked with anti-PDI was lower compared with infection in the presence of isotype antibody. Accordingly, infection of transgenic macrophages overexpressing PDI was higher than of the wild type counterparts. We expect that *in vivo* infections will lead to higher lesion swelling and parasite loads in PDI transgenic mice in comparison with the wild type counterparts. **Supported by:**(CAPES) 88887.360800/2019-00
Keywords: Leishmania (L.) amazonensis.PDI.Macrophage infection

HP-003 - **The *Trypanosoma cruzi* Enoyl-CoA hydratase, an enzyme involved in the catabolism of the branched chain amino acids, might be an interesting drug target**

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Trypanosoma cruzi, the causative agent of Chagas disease, depends on amino acids for many important biological functions. **Goals:** to increase our knowledge on the metabolism of branched chain amino acids (BCAA – Leu, Ile or Val) by *T. cruzi*. The enoyl-CoA hydratase (ECH) (also known as crotonase) catalyzes the fourth step of the BCAA metabolism. **Methodology:** In this work, the sequence encoding *T. cruzi* ECH (TcECH) was expressed and purified to determine its biochemical parameters. To assess the role of TcECH in the parasite biology, we obtained knockout mutants for both copies of *tcech* by using CRISPR/Cas9. We evaluated some phenotypic characteristics of these parasites. **Results:** The gene ablation resulted in the lack of crotonase activity as confirmed by measuring the enzyme activities in cell-free extracts. The knocked out parasites (D*tcech*) were submitted to nutritional stress in presence or not (control) of BCAA for 96 hours. Mutants and wild type parasites were resistant to starvation when incubated in the presence of Leu, but Ile and Val were deleterious for the mutants. Additionally, we found that D*tcech* parasites can complete the infection cycle in mammal cells at the same levels than control but have a decreased bursting of trypomastigotes from the infected cell when comparing with controls. **Conclusions**: Altogether, these data indicate that, the absence of crotonase activity induce the accumulation of a toxic intermediate in the presence of Ile and Val. Also the absence of TcECH affect the intracellular cycle of the parasite. Further experiments will allow us to better understand the role of the BCAA oxidation pathway for the biology of *T. cruzi*. **Supported by:** FAPESP 2017/14334-9 **Keywords:** Trypanosoma cruzi.Branched chain amino acids metabolism.Enoyl-CoA hydratase

HP-004 - **Identification and characterization of glycosomal iron transporters in *Leishmania***

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The protozoan parasites of the genus *Leishmania* are responsible for the diseases known as leishmaniases that affect millions of people worldwide. *Leishmania* life cycle includes invertebrate and vertebrate hosts. In the vertebrate host, the parasites are able to survive and replicate inside macrophages, despite the defense arsenal of these cells. One of the critical conditions found by *Leishmania* in the macrophage is the lack of various nutrients, such as iron, an essential cofactor of several enzymes. The identification and study of parasite genes involved in iron metabolism and transport revealed that the availability of iron plays a central role in virulence. Besides, it was also shown that iron deprivation modulates the expression of a series of genes whose function is still unknown. Among these, conserved genes encoding proteins addressed to the glycosomes, which are trypanosomatids unique organelles. Therefore, our goal is to identify and characterize genes involved in the transport of iron into the *Leishmania* glycosome. We initially performed the *in silico* analysis of the *Leishmania* transcripts significantly modulated by iron deprivation looking for those containing predicted glycosomal addressing signals (PTS1 or PTS2) and transmembrane domains. With this, we found 11 putative genes and selected 6 of them to confirm the subcellular localization of the encoded proteins. We cloned their ORFs into the *Leishmania* expression plasmids pXG-GFP+ and pXG-GFP2+, upstream or downstream of the GFP coding sequence. Thereby, we will confirm the subcellular localization of these proteins in *L. amazonensis* and characterize the overexpressing parasites regarding gene expression, replication and virulence. The identification of novel exclusive parasite' proteins may indicate potential targets for the development of new drugs for leishmaniases treatment. **Supported by:**FAPESP - 2019 nº 21185-5 - **Keywords:** Glycosomal.Iron.Transporters

HP-005 - **Characterization of Leishmania (L.) amazonensis Oligopeptidase B and its role in macrophage infection**

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Leishmania spp. are parasitic protozoa that cause leishmaniasis, a disease endemic in more than 98 countries, with more than 1 million new cases each year in the world. Symptomatic human infection may have different clinical forms, which are grouped into cutaneous and visceral. *Leishmania* promastigotes are transmitted to vertebrate hosts through the bite of female sandflies and differentiate into amastigotes within the host´s phagocytic cells. To survive the varied and hostile environments, the parasite has several virulence factors whose abundances vary according to the *Leishmania* species and the parasite stage. Oligopeptidase B (OPB) is a serine peptidase of the prolyl peptidase family (clan SC, family S9), characterized by the presence of a serine in the active site. This protein has been considered a virulence factor in different trypanosomatids. In fact, infections of mice with OPB-deficient *L. (L.) major* and *L. (L.) donovani* showed late lesion development, although little is known about the role of this protein, which is secreted in exosomes, in the parasite infection process. To fill the existing gaps, this project aimed to produce and characterize *L. (L.) amazonensis* recombinant OPB and to evaluate its effects on the infection of macrophages by this parasite species. We were able to produce and purify a recombinant soluble OPB in a bacterial system and used the recombinant protein to produce an anti-OPB serum in BALB/ c mice. We evaluated the activity and the enzymatic profile of the recombinant OPB, and our results indicate that the enzyme is active and has a profile similar to OPBs from other *Leishmania* species. Infection assays with BALB/ c bone marrow derived macrophages showed that OPB does not affect infection by *L. (L.) amazonensis* LV79 strain. Further assays are still required to determine the importance of this protein in *L. (L.) amazonensis* infection. **Supported by:**FAPESP- 2019/02391-3, CAPES - **Keywords:** L. (L.) amazonensis.Oligopeptidase B.Macrophage infection

HP-006 - **A map of PFR proteins and dissection of their functions in motility and assembly of the *Trypanosoma brucei* flagellum**

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Alongside the microtubule axoneme in many flagellated cells there are additional protein structures such as the paraflagellar rod (PFR) of *T. brucei*. The PFR has an intricate structure composed of three distinct domains (inner, middle and outer) that runs next to the axoneme within the flagellum and is important for flagellar beat regulation and cell motility. However, the specific contribution of each of the PFR domains to motility is unknown. The TrypTag project highlighted the complexity of the PFR with 146 proteins found in this structure. I determined by bioinformatics analysis, conservation patterns of these proteins across Euglenozoa and identified two major conserved sets of PFR proteins. The first set was conserved across Euglenozoa; whereas, the second set was conserved but not present in organisms with a reduced PFR, such as *Angomonas deanei*. A semi-automated analysis of TrypTag images, measuring the distance between the kinetoplast and the start of the PFR signal revealed a discontinuous start to the PFR and predicted the domain to which a PFR protein localised. These predictions were confirmed for a subset of 15 proteins by analysing the distance to known flagellum proteins. I analysed the function of these 15 proteins in cell motility and depletion of five of them disrupted motility, reduced cell growth and led to a ‘blob’ forming at the flagellum tip. The motility phenotypes were only observed in proteins present in the inner and very outer PFR domains and not present in organisms with a reduced PFR. However, for four of these proteins despite changes in motility, there was no obvious changes to the PFR2 localisation, suggesting these proteins are not required for PFR assembly. These results suggest that the motility function of the PFR can be separated from its assembly and that there are domains and sub-structures of the PFR with a specific role in motility. This is an important step in assigning specific functions to the individual PFR domains. **Supported by:**The Royal Society - NIF\R1\191618 - **Keywords:** Trypanosoma brucei.Paraflagellar Rod.functions

HP-007 - **P21 gene ablation of Trypanosoma cruzi induces changes in the virulence phenotypes**

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P21 is a protein expressed exclusively by *T. cruzi*. Studies using the recombinant form of P21 have shown that this protein plays essential roles at different steps of the infection of vertebrate host. Among the properties of P21 is the ability to induce signaling cascades in the host cell that results in the remodeling of the actin cytoskeleton, facilitating parasite internalization. Additionally, P21 interferes in the pathogenesis of Chagas disease, inhibiting angiogenesis, inducing inflammation, as well as controls amastigotes intracellular replication. Here we investigated whether the P21 deletion using CRISPR/Cas9 changes the parasite phenotypes involved in the infection of mammalian host. To obtain P21 knockout parasites, we have used the strategy of building a vector (pTREX/Cas9/sgRNA P21) carrying the Cas9 and the sgRNA-specific for the P21 gene. Transfected epimastigotes were selected with the antibiotics G418 and blasticidin, cloned by limiting dilution and four P21 KO clones were isolated. The absence of P21 mRNA and protein expression in the KO clones was verified by RT-PCR and immunofluorescence. Epimastigote growth kinetics showed that the P21 KO parasites have a slower growth rate than the wild-type parasites. In addition, incubation of HeLa cells with metacyclic forms for 4h demonstrated that the absence of P21 reduced the rate of invasion. However, the replication assay using HeLa cells infected with metacyclic forms showed that intracellular P21 KO amastigotes display increased replication rates when compared to control parasites. Together, our data show that P21 gene ablation alters the parasite growth, inhibits the invasion of metacyclic forms and increases the replication of intracellular amastigote. These results confirm that P21 may play a role in the development of non-proliferating intracellular amastigotes and may be responsible for the evasion of the host's immune system, constituting a crucial condition for the chronicity of the infection. **Supported by:**FAPESP, CNPq and CAPES - **Keywords:** P21 PROTEIN.CRISPR/Cas9.T. cruzi

HP-008 - **The importance of Sialic acids in Leishmania amazonensis and Leishmania infantum chagasi infection**

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Leishmania sp. are etiological agents of leishmaniasis, one of the most important parasitic diseases in the world. *Leishmania* promastigotes are covered by a dense glycocalyx composed by many glycoconjugates, which play an important role in *Leishmania* infectivity and survival. Sialic acids (Sias) are nine-carbon atoms sugars usually present as terminal residues of glycoproteins and glycolipids on the cell surface or secreted. The role of Sias in infections by protozoa such as *Trypanosoma cruzi* and *Leishmania donovani* was demonstrated in previous studies. The interaction between *Leishmania donovani* Sias and macrophage receptor Siglec-1 (Sialic acid-binding immunoglobulin-type lectins) contributes to the parasite's entry into the host cell. Due to the divergences among *Leishmania* species, the aim of this work was to evaluate the importance of Sias-Siglec-1 interaction in two endemic species in Brazil: *L. amazonensis* and *L. infantum chagasi*. For that, we treated parasites with sialidase, removing part of *Leishmania* Sias. *In vitro* infection assays using murine bone marrow-derived macrophages (BMDM) and cells of human lineage THP-1 showed that reduction of Sias decreased infection. These results were observed for both species and also for two different strains of *L. infantum chagasi*, MHOM/BR/1972/LD and MHOM/BR/2005/NLC, with the greatest impact of sialidase treatment for MHOM/BR/2005/NLC strain. We then analyzed Siglec-1 abundance in murine macrophages (BMDM) and human differentiated THP1 cells by flow cytometry. A low labeling was observed in BMDM, while more than 50% of THP1 cells expressed Siglec-1. Blocking of Siglec-1 had no impact in BMDM infection, perhaps due to the low abundance of this receptor in these cells. Blocking of Siglec-1 in THP1 cells is in course at this moment. Our results imply that sialic acid is important for *L. amazonensis and L. i. chagasi* infection and that the impact of sialidase treatment may vary between strains of the same species.
Supported by:CAPES, Processo: 88882.333062/2019-01 ; FAPESP **Keywords:** Leishmania.Sialic Acid.Macrophage infection

HP-009 - **The Cysteine Synthase enzyme plays an important role in the biological cycle of *Trypanosoma cruzi.***

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Trypanosoma cruzi 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 (glutathione, trypanothione, cysteine, and some intermediates) are relevant to buffer the redox state of the different sub-cellular compartments of this organism. The cysteine synthase (CS) catalyze the second step in the *de novo* biosynthesis of cysteine, however, its role in redox homeostasis has been unexplored in *T. cruzi*. In this work, we identified a *T. cruzi* sequence encoding a functional CS (TcCS). We obtained partial knockout lineages for *TcCS* by using CRISPR/Cas9. TcCS-knocked out epimastigotes showed a lower proliferation rates and a diminished resistance to a short-time (30 min) exposition to 120 uM H_2O_2 when compared with controls constitutively expressing Cas9. When these parasites were submitted to nutritional stress in the presence (or not as a control) of 5 mM L-Serine, 5 mM OAS or different concentrations (0.2, 0.4 or 1 mM) of L-Cys we observed that: i. L-Cys concentrations over of 200 mM were lethal to the mutants after 48 hours and; ii. L-Ser and OAS contributed to the survival of both, mutants and wild type parasites to severe starvation. We found that *DTcCS* parasites had diminished their ability to differentiate to metacyclic trypomastigotes. When these metacyclic trypomastigotes were assessed for their ability to infect mammalian host cells, we observed an over rate of infection after 48 hours and an increment of trypomastigote bursting, however, most of these trypomastigotes showed severe morphological alterations when compared to the control. Altogether, these data indicate that cysteine has an important role during epimastigotes proliferation, metacyclogenesis and the infection of mammalian cells. Further experiments will allow us to better understand the role of the cysteine biosynthesis *de novo* for the biology of *T. cruzi*. **Supported by:**FAPESP - **Keywords:** Trypanosoma cruzi.Cysteine biosynthesis.TcCS

HP-010 - ***Trypanosoma rangeli* mucins overexpression induces reduction of parasitemia and alters parasite immune response modulation in mice**

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The mucin-like glycoproteins are known to be sialic acid acceptors on the surface of *Trypanosoma cruzi*. This huge glycoprotein family is involved in parasite attachment to the host cell and blocking lytic antibodies recognition, among other functions. Despite acting as virulence factors in *T. cruzi*, the mucins-like expression was identified in *Trypanosoma rangeli*, a non-pathogenic and non-cell invasive parasite of mammals. Here, we report the characterization and effects of overexpression of *T. rangeli* mucins (*Tr*MUC) in the host-parasite interaction. The *Tr*MUC family is composed of 93 genes classified into two groups: *Tr*MUCg and *Tr*MUCp that encode putative glycoproteins showing shared features with *T. cruzi* mucins I and II. BALB/c mice infected with *T. rangeli* overexpressing *Tr*MUCg or *Tr*MUCp show parasitemia reduction ($p < 0.0001$ and $p= 0.0146$, respectively) compared to control groups. Interferon γ (IFN γ) levels in the spleen of mice infected with wild type strain were reduced ($p=0.0018$), however normal IFN γ levels were detected in mice infected by *Tr*MUCg parasite ($p = 0.396$). A similar effect was observed on interleukin-6 (IL-6) levels in LPS-activated macrophages (RAW264.7 cells) in the presence of these parasites. While *Tc*MUCII expression by *T. rangeli* increases infection in mice ($p < 0,0001$) without altering cytokines secretion profile. Our observations indicate that *T. rangeli* can negatively modulate the host pro-inflammatory response *in vivo* and *in vitro* and, *Tr*MUC overexpression diminish this phenomenon. Also, unlike the *T. cruzi* mucins, *Tr*MUCs are little or non-glycosylated despite the functional glycosylation machinery in *T. rangeli*. This *Tr*MUC characteristic probably affects their ability to act as sialic acid acceptors, which match the absence of trans-sialidase activity by *T. rangeli*. An accurate *Tr*MUC glycoprofile could provide detailed information about the sialic acid acceptor role of this new genomic family in *T. rangeli*.
Supported by:CNPq, CAPES, FINEP, UFSC - **Keywords:** glycoproteins.mice infection.non-pathogenic parasite

HP-011 - **Neurodegenerative development during *Plasmodium berghei* ANKA infection is reduced by Amido-coumarin co-treatment**

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The *Plasmodium berghei* ANKA(PbA) infection in mice closely recapitulates many aspects of severe malaria in human including cerebral malaria. Coumarins are a class of secondary metabolites that are widely present in plants and exhibit several pharmacological, biochemical, and therapeutic effects, for example, anti-inflammatory and neuroprotective agents. Our aim was to investigate the potencial neuroprotection of an amido-coumarin compound in the treatment of experimental severe malaria. C57Bl/6 mice were inoculated with 10^5red cells parasitized with PbA and 3 days after infection (dpi) were orally treated daily with amido-coumarin compound MJM220(MJM-10mg/kg) alone and/or in combination with chloroquine (CQ-30mg/kg) once per day. Parasitemia, body weight, survival, clinical score, memory, and immune cell profiles in the spleen and brain at 5dpi by flow cytometry, were analyzed. Our results showed that the treatment with MJM220 alone reduced the parasitemia at 6° and 7dpi, and improvement of the clinical score from 8° to 12dpi when compared with infected untreated mice. The animals treated with MJM+CQ showed a reduction in parasitemia in all analyzed kinetics (3° to 47dpi). Treatments with MJM and MJM+CQ increased the survival of infected mice (80% and 60%;28°dpi and 47°dpi, respectively). Treatment with MJM resulted in the protection of cognitive ability at 5dpi, and the combined treatment of MJM+CQ preserved cognition at 5dpi and 47dpi. Treatment with MJM+CQ resulted: (i) in the brain, reduction in the numbers of macrophages, dendritic cells, CD4 and CD8T cells producing IFNγ, CD4T cells producing IL17 and IL10, and (ii) in the spleen, reduction in the number of neutrophils, CD4T cells produzing IFNγ or IL17, and increased number of CD8T cells producing IFNγ. Colletively, our results suggest that the compound MJM220, based on starch-coumarin, has a potential neuroprotective activity being a promising compound for co-treatment during severe experimental malaria. **Supported by:**CNPq (474971/2013-9)
Keywords: Coumarin.neuroprotection.antimalarial

HP-012- **Study of the cross-regulation between transport of iron and heme mediated by LIT1 and LHR1 in *Leishmania***

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Leishmaniases, a spectrum of diseases caused by protozoan parasites of the genus *Leishmania*, affects millions of people around the world. During infection, nutrient availability within the parasitophorous vacuoles is known to have significant effects on parasite replication and virulence. This process requires the acquisition of essential nutrients such as iron and heme from the host since *Leishmania* does not have iron storage proteins and does not have heme biosynthesis capacity. Besides, iron and heme can be cytotoxic as they trigger the generation of free radicals in the presence of oxygen. *Leishmania*, therefore, must acquire heme and iron to survive in a hostile environment that restricts the availability of nutrients to the pathogen, a process called nutritional immunity. Identification of several proteins that participate in the transport of iron and heme was crucial for understanding these metabolic pathways in *Leishmania*, and the loss of one or both alleles of those transporters cause serious defects in the differentiation and/or multiplication of these parasites in the host. Hence, our goal is to investigate the cross-regulation between iron and heme transporters, LIT1 and LHR1. To begin, we used a single-step multi-fragment ligation strategy to assemble LIT1 targeting constructs. These constructs were transfected into *L. amazonensis* and LIT1 knockout clones were successfully selected. Currently, we are cloning LIT1 or LHR1 ORFs into the *Leishmania* expression vectors pXG-GFP2+ and pXG-GFP+. With these plasmids, we will generate mutant strains that overexpress LIT1 and LHR1 in the LHR1 single knockout and LIT1 knockout backgrounds, respectively. Those mutants will be evaluated regarding intracellular content of iron and heme, *in vitro* growth and virulence. Characterization of the regulation of the pathways related to iron and heme transport is critical for a better understanding of *Leishmania* physiology and the host-pathogen interaction. **Supported by:**FAPESP 2019/03861-3 **Keywords:** Leishmaniasis.Host-Parasite Interaction.Transition Metals

HP-013 - **Characterization of manganese transport mediated by the iron transporter LIR1 in *Leishmania***

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Leishmaniases are a group of diseases caused by protozoan parasites of the genus *Leishmania*. These parasites alternate between insect and mammalian hosts, going through dramatic changes with shifts in temperature, pH, and availability of nutrients. Among these nutrients, iron is an important cofactor of several enzymes, but can also be highly toxic when free and at high concentrations in the cytoplasm. Recently, Leishmania Iron Regulator 1 (LIR1) was identified and characterized in *Leishmania* as a plasma membrane transporter essential for iron efflux and regulation of the intracellular concentration of iron and other transition metals, such as manganese. LIR1 deficiency enhances the toxic effect of excess iron and manganese during promastigote replication. Similar to iron, manganese is also a cofactor of several *Leishmania* enzymes, in particular arginase, which is essential for parasite replication and infection establishment in mammalian host. Considering the significance of manganese for *Leishmania* and the evidences that LIR1 also modulates the intracellular levels of this metal, our main goal is to characterize the manganese transport mediated by LIR1 in *L. amazonensis*. For this, we quantified the total amount of parasite-associated manganese by inductively coupled plasma mass spectrometry (ICP-MS). Our preliminary findings revealed that LIR1 expression prevents manganese accumulation in *Leishmania.* Furthermore, western blot and arginase enzymatic activity assays indicate that *Leishmania*'s arginase is modulated by LIR1 expression. Therefore, our results corroborate a role of LIR1 in manganese homeostasis and its importance for parasite replication. Ultimately, we hope to evaluate the direct role of LIR1 in the transport of manganese across promastigotes plasma membrane. **Supported by:**FAPESP PROCESSO 2019/09715-9 **Keywords:** Leishmania.manganese.Arginase

HP-014 - **Alternatively activation of murine macrophage does not support infection by *Leishmania (V.) braziliensis***

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INTRODUCTION: Leishmania braziliensis is the main species responsible for American tegumentary leishmanias in Brazil. The control of Leishmania infection has been related to the classical activation of macrophages (cMo) that leads to parasite death. In the other way, macrophages polarized to alternative activation (AAMo) profile are described as susceptible to infection by *Leishmania spp*. The cytokines IL-4, IL-10 are important in polarizing different subtypes of AAMo. Thus, we aimed to evaluate *L. braziliensis* survival after infection of murine macrophage activated with INF-y and LPS, IL-4 or IL-10. METHODS: bone marrow-derived macrophages (BMDM) or Thioglycollate elicited peritoneal macrophages from C57BL/6 or BALB/c mice were stimulated with INF-y and LPS, IL-4 or IL-10 to evaluate nitric oxide production, arginase activity, and parasite load after infection with *L. braziliensis*. The number of infected macrophages and the parasite load were determined by light microscopy and by the recovery assay of promastigotes in culture. RESULTS: cMo cultured with INF-y and LPS produced high amount of NO (cMo 62.7 uM x CT 2.0 uM) but no increase in arginase activity, while AAMo cultured with IL-4 or IL-10 exhibited significant arginase activity (producing 538µg/mL and 515 µg/mL of urea respectively) and no NO production. In all situations there were significant decrease in the number of infected macrophages and amastigotes in infected macrophage cultured for 72 h. These results were confirmed by promastigote recovered in culture assay. CONCLUSION: These results suggest that control of *L. braziliensis* in murine macrophages occurs even with high arginase activity and independently of NO production. **Supported by:**FAPEG, CAPES, CNPq, INCT-IPH **Keywords:** Alternatively activation of macrophages.IL-4, IL-10.Leishmania (V.) braziliensis

HP-015 - **Glutamine is an important energy source for *Trypanosoma cruzi***

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Trypanosoma cruzi is the causative agent of American trypanosomiasis, also known as Chagas disease. Throughout its life cycle, *T. cruzi* alternates between mammalian hosts, humans among then, and the insect vector, a reduviid insect. Inside both hosts, the parasite needs to colonize distinct environments, such as the bloodstream and the cytoplasm of the cell in the mammalian host and the digestive tract in the insect vector. These environments present different nutrients availability and the parasite needs to adapt to the available nutrients. *T. cruzi* is able to use carbohydrates, amino acids and fatty acids as energy and carbon sources. In this work we demonstrate that *T. cruzi* is able to use glutamine (Gln) as an energy source. Gln can be completely oxidized to CO_2, fulfilling TCA cycle and sustaining the electron transport chain, contributing to ATP biosynthesis. In addition, exometabolomic analyses by ^{1}H-RMN showed that alanine and acetate are the main end products from Gln metabolism, demonstrating that Gln derived carbons are also feeding other metabolic pathways in epimastigotes. Taken together these data show that Gln is an important energy source for *T. cruzi*. **Supported by:**FAPESP, Processo número:2017/04808-3 **Keywords:** Trypanosoma cruzi.Bioenergetics.Glutamine

HP-016 - **Blood transcriptomics of human visceral leishmaniasis reveals insights into the long non-coding RNA and mRNA co-expression profile induced by Leishmania infantum infection.**

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Visceral leishmaniasis (VL) is an infectious, chronic and disseminated disease that can be potentially fatal if untreated. In Brazil, it is caused by *L. infantum* parasites transmitted by sand-fly vectors. Clinical outcomes of infection in leishmaniases are mainly mediated by host immune response and blood transcriptomics is powerful to assessing the molecular mechanisms of immunity. Thus, our aim was to perform analyses of bulk polyadenylated RNA-seq of VL patients (N=11) compared to non-diseased individuals: treated (T, cured VL patients; N=11), asymptomatic (A, resistant to disease; N=9) and healthy (H, control; N=9) in order to identify genes and molecular processes likely associated with immunopathophysiology that underlying the development of VL. Differential gene expression analysis, modular co-expression network analysis and gene features annotation used edgeR, cemitool and biomart R packages, respectively. Here, we focused on an integrated network analysis of mRNA with long non-coding RNAs (lncRNAs), which are key players of gene regulation during several pathological processes. We found 2,427 differentially expressed (DE) genes across all possible group comparisons, from which targeted approaches select a set of 1,512 genes to be explored by an integrated analysis of 147 DE lncRNAs (58 and 89 up- and down-regulated, respectively, in VL) and 1,263 DE mRNAs. Gene Set Enrichment Analysis identified co-expressed modules with significantly enriched pathways in the Over Representation Analysis, related mainly to “Hemostasis”, “Interferon Signaling”, “Cell Cycle Checkpoints”, “Neutrophil degranulation” and “Toll−like Receptor Cascades”. Co-expression networks based on highly correlated lncRNA-mRNA pairs (p-value < 5e-10) identified 6,060 positive and 1,351 negative pairs of correlations. Mining this lncRNA-mRNA correlated expression profile will provide insights into the lncRNA functions in immune-related processes triggered in visceral leishmaniasis patients. **Supported by:**FAPESP 2016/20258-0 **Keywords:** Leishmania infantum.blood transcriptomics.long non-coding RNA

HP-017 - **Gasdermin-D activation in response to Leishmania amazonensis induce a transient pore formation without cell death that promote NLRP3 activation and host resistance to infection**

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Leishmania is an obligate intracellular parasite the causes Leishmaniasis, a disease that affects millions of people worldwide. The parasites evade immune response by inhibiting cell death in many cell types. Here we investigated the role of Gasdermin-D (GSDMD, a pore-forming effector protein associated with pyroptosis) in *Leishmania amazonensis* infection and whether *Leishmania* inhibits pyroptosis-mediated cell death. We demonstrated that GSDMD is active in the early stages of infection, leading to low propidium iodide (PI) incorporation in live cells and low potassium efflux but without LDH release. Also, we observed that the *Gsdmd*$^{-/-}$ cells have less ASC puncta formation and secrets less IL-1β, suggesting that GSDMD pore formation contributes for the non-canonical activation of the NLRP3 inflammasome. Macrophages and mouse deficient in *Gsdmd*$^{-/-}$ mice were highly susceptible to *L. amazonensis* infection in vitro and in vivo, suggesting an important role of this molecule for non-canonical activation of NLRP3 and host resistance to infection. Altogether, our findings reveal that *Leishmania* infection trigger inflammasome-mediated GSDMD cleavage but not pyroptosis, and this process is very important for host immunity against these parasites.
Supported by:FAPESP **Keywords:** Leishmania.Inflammasome.GSDMD

HP-018 - **Genetic edition and drug free selection in Trypanosoma cruzi: new methodology for modifications by CRISPR/Cas9**

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Genetic engineering to tag genes using CRISPR/Cas9 technique is already well established in *T. cruzi* and usually inserts a drug resistance gene (DRG) so selection of the modified cells can be achieved. However a few problems arise from the need of drug selection such as: 1. the high price of drugs used for selection; 2. low number of antibiotics available which hinders the introduction of multiple modifications in *T. cruzi* genome simultaneously; 3. changes on the original gene 3' UTR that is important to mRNA stability and translational modulation. Considering these hindrances, we aim to validate a new methodology where target genes are tagged without insertion of a DRG and selection is performed by cloning process. For that purpose, epimastigotes constitutively expressing Cas9 and T7 RNA polymerase enzymes are transfected with two different PCR products: one for in vivo transcription of sgRNA by T7 RNA pol and another for homologous recombination. This latter is around 200 bp long which is composed of 3 copies of tag sequence (Myc, TY or HA) flanked by 30 bp of homology arms. We first attempted tagging the cell cycle kinase (CRK1) gene at the C terminal portion with Myc epitope, so epimastigotes were transfected with PCRs products and 24h later they were cloned. Seven clonal populations were obtained and their proteins were extracted and submitted to western blotting (anti-myc). 6 out of 7 clones showed a specific band around 35 kDa, which is the estimate mass of CRK1-Myc protein. Now we are trying to tag another two genes involved in cell cycle regulation: Cyclin 5 (HA tag at C terminal region) and Wee-1 (TY tag at N terminal region). Moreover, we also intend to modify two and three genes at once, so from one single eletroporation a monoclonal epimatigote population harboring three genes tagged can be obtained. So far we already can affirm that this methodology works and promises to be a valuable tool for *T. cruzi*. **Supported by:**FAPESP nº processo: 2019/17328-5 **Keywords:** T. cruzi.CRISPR/Cas9.Drug free selection

HP-019

Alterations and regulation of the mucosal barrier during mucin-*Giardia* interactions *in vitro*

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The extracellular secreted mucus prevent infection by the vast numbers of microorganisms that evolved a variety of mechanisms to cope with the mucosal barrier. In the case of *Giardia lamblia*, an extracellular protozoan parasite that colonizes the small intestine of humans and vertebrate animals, proliferative trophozoites have to penetrate or circumvent the mucus barrier to establish infection. In this study using in vitro co-cultures of *G. lamblia* trophozoites and intestinal epithelial cells (IECs), we have shown that mucin 2 (MUC2) and mucin 5AC (MUC5AC) are highly expressed in the human duodenum-derived adenocarcinoma cell line HuTu-80 in response to infection thus probably acts as a decoy to limit *Giardia* adherence to cell surface in the small intestine. By contrast, incubation of Caco-2 cells with trophozoites did not affected mucin production. Further, we evaluated whether trophozoites could promote degradation of mucins as a mechanism to control mucus abundance during infection. For this, trophozoites were incubated in a solution of isolated bovine submaxillary mucin or porcine gastric mucin and the expression pattern of MUC2 and MUC5AV were analyzed by Western blot. Our data show that trophozoites cleaves MUC5AC and totally degrades MUC2 independently of glycosylation as observed by Periodic-Acid Schiff (PAS) staining. Finally, we profiled phospho-signaling networks in IECs before and after exposure to trophozoites using the human Phospho-RTK array. IECs were incubated with trophozoites and the kinase activation patterns were compared to uninfected controls. CREB, Chk2, JNK, Akt1/2 and STAT2 phosphorylation increased in HuTu-80 cells exposed to *Giardia*, while phosphorylated p53 and STAT3 decreased. In Caco-2 cells infected with trophozoites the levels of phosphorylated p38a, EGF-R, MSK1/2 and STAT3 decreased however, no signaling cascades were activated in response to *Giardia*. Further studies are being conducted to confirm the observed signaling changes. **Supported by:**CNPq and FAPESP
Keywords: Mucus layer.intracellular signaling.giardiasis

HP-020 - **TNFR1, a conductor of inflammatory process against L. amazonensis**

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TNF is a cytokine involved in inflammation, tissue degeneration, tissue regeneration among other functions. TNF acts through two cognate receptors. TNFR1 seems to be more effective against intracellular parasites, including *Leishmania*. Studies about *L. major* elucidated the TH1-TH2 dichotomy. TH2 cells, through IL-4 and IL-13 secretion, active macrophages through the IL-4Rα leading to the metabolization of l-arginina by arginase I and production of polyamines, required to parasite replication. On the other hand, TH1 cells active macrophages through IFN-ɣ, which induces iNOS expression. iNOS, metabolizes L-arginine into citrulline and nitric oxide (NO), a leishmanicidal gas. Absence of TNF impairs signaling through its receptor and M1 activation, essential to control the parasite. However, what is the impact of TNFR1 in the infection by *L. amazonensis*? Our data reveal the importance of TNFR1 in iNOS and NO production expression by mononuclear phagocytes that leads to lesion control. Although expression of TNFR1 by wild type mice is not enough to eliminate the parasite, this receptor mediates control of parasite replication. Indeed, in *Leishmania* models of infection, sterile cure is not achieved. Recently NO was implicated in rewiring cells of the immune system by diminishing pro-inflammatory cytokine production that leads to the resolution phase of the inflammatory process. We observed a large inflammatory infiltrate, especially a larger number of lymphocytes at the site of infection in the absence of TNFR1. Furthermore, the few lymphocytes were regulatory T cells mainly, correlating with high levels of IL-10 at the chronic phase of infection. At the later time points macrophages present M2 phenotype, controlling immunopathology. We conclude that for successful clearance and wound healing during leishmaniasis, different phenotypes of macrophages need to appear coordinately in the appropriate time. TNF, through TNFR1, seems to act as this conductor.
Supported by:CAPES, CNPq, FAPEMIG **Keywords:** TNFR1.Macrophage polarization.Leishmania

HP-021 - **Role of STAT-1, STAT-3 and STAT-6 activation in modulating two *Trypanosoma cruzi* strains coinfection within human polarized macrophages M1 and M2**

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Introduction: Chagas' disease is classified as a neglected tropical disease by World Health Organization and affects approximately 8 million people worldwide. The disease is caused by the protozoan *Trypanosoma cruzi (T. cruzi)* and its genetic variability, host tissue tropism and host's immunological response are determinant factors for the disease progression and treatment. Transcription factors of JAK/STAT pathway presented trypanocidal activity, although parasite can modulate this pathway in order to thrive in intracellular environment. Moreover, coinfection with different *T. cruzi* strains is common at endemic areas. **Objective:** Evaluate the coinfection with two fluorescent-labelled *T. cruzi* strains (G-GFP and CL-DsRed), in comparison with single infection, in classic or alternatively activated human macrophages. **Methods:** Macrophages were derived from THP-1 cell line and polarized to M1 or M2 profiles to be further infected with trypomastigotes or extracellular amastigotes of *T. cruzi* (single or coinfection). We analyzed: % of infected cells, nitric oxide (NO), reactive oxygen species production and STAT-1, -3 and -6 phosphorylation levels. **Results:** Both strains were able to infect M0 macrophages, with different infectivity patterns. M1 demonstrated resistance and M2 susceptibility to infection of both strains and higher percentage of infected cells presented lower NO and vice-versa. STATs activation patterns were different among single and coinfection, demonstrating that both *T. cruzi* strains inside the macrophage elicited a new intracellular response. Moreover, we observed a paracrine effect in M2a non-infected cells next to infected cells with any strain, where M2a cells had some STATs dephosphorylated. **Conclusions:** Different activated STAT profiles were observed in coinfection in comparison with single infections; M2 profile may restrain the high inflammatory environment, being an important adjuster of tissue damage in response to *T. cruzi.* **Supported by:**FAPESP, Processo n. 2019/08933-2
Keywords: Trypanosoma cruzi.Coinfection.JAK/STAT Pathway

HP-022 - **Evaluation of different resins for ion exchange chromatography in the purification of T.evansi.**

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Trypanosoma evansi, a unicellular, hemoflagellate protozoan of the order Kinetoplastida, is morphologically indistinguishable from *T. brucei,* and characterized by a positively charged undulating membrane. Purification processes are important to isolate hemoparasites from other intrinsic components in the blood. In this context, the isolation of *T. evansi* is a process dependent on purification, which impacts diagnostic research. For this purpose, Diethylaminoethyl-cellulose (DEAE-Cellulose) is the resin commonly used to carry out ion exchange chromatography. The process of preparing the spine and the methodology to balance it present complications regarding the preparation period, in addition to its high cost. Seeking alternatives, our goal was to evaluate the purification efficiency and viability of *T. evansi* with porous silica chromatographic resins from Kopp Technologies (Purifica Y-N, Purifica Y-HONOH and Purifica Y-CNC3). Despite being intended for purification of several anionic compounds, it has never been tested for protozoa purification from blood samples. For this, we carried out the passage of samples through the three columns, where the efficiency of the processes in all resins was verified, through the observation of the protozoan viability by optical microscopy, Trypan blue and flow cytometry (propidium iodide). The results demonstrate the possibility of using these columns for the purification process of protozoa as a more affordable alternative, requiring further studies.
Supported by:FAPESC e CNPq **Keywords:** DEAE-cellulose.protozoan.viability

HP-023 - **Residence in a LAMP deficient intracellular environment interferes with biological and molecular characteristics of *Trypanosoma cruzi* trypomastigote forms**

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Trypanosoma cruzi invades non-professional phagocytic cells by subverting their membrane repair process, which is dependent on membrane injury and cell signaling, intracellular calcium increase and lysosome recruitment. Cells lacking Lysosome Associated Membrane Proteins 1 and 2 are less permissive to parasite invasion, however more prone to parasite intracellular multiplication. Several passages through a different intracellular environment can significantly change *T. cruzi*'s gene expression profile. Here, we evaluated whether one single passage through LAMP deficient (KO) or wild type (WT) fibroblasts could influence invasion ability of *T. cruzi* Y strain trypomastigotes in L6 myoblasts and WT fibroblasts. Parasites released from LAMP-2 KO cells (TcY-L2$^{-/-}$) showed higher invasion, calcium signaling and membrane injury rates when compared to those released from WT (TcY-WT) or LAMP-1/2 KO cells (TcY-L1/2$^{-/-}$) in L6 myoblasts. On the other hand, TcY-L1/2$^{-/-}$ showed higher invasion, calcium signaling and cell membrane injury rates compared to the others in WT fibroblasts. Albeit TcY-WT presented an intermediary invasion and calcium signaling rates in WT cells, they induced lower levels of injury, reinforcing that protein signaling may also have a significant contribution to parasite induced calcium signals. These results clearly show that parasites released from WT or LAMP KO cells are distinct from each other. Additionally, these parasites ability to invade the cell may be distinct depending with which cell type they interact to. Since these alterations most likely would reflect differences among parasite surface molecules, we also evaluated their membrane subproteome. Few protein complexes, membrane and secreted proteins were found regulated, such as some members of MASP, mucins, transialidases and gp63 proteins family, which may play an important role during parasite infection. **Supported by:**FAPEMIG/APQ-02974-17 **Keywords:** Trypanosoma cruzi infection.LAMP proteins.Proteomic profiling

HP-024 - **Assessing the infectivity of *Crithidia*-like parasites obtained from human visceral leishmaniasis cases through *in vitro* infection of macrophages**

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Visceral Leishmaniasis (VL) is caused by *Leishmania infantum* in Brazil and can be lethal if untreated. We have shown that some clinical isolates from VL cases in Sergipe, BR, do not belong to *Leishmania* and are phylogenetically related to *Crithidia*, a monoxenous genus considered non-pathogenic to humans. Here, we performed *in vitro* infection using mice bone marrow-derived macrophages (BMDM), J774 (murine) and THP-1 (human) cell lines. Cells were infected using *Crithidia*-like isolates, *L. infantum* and *C. fasciculata* strains during 24, 48 and 72 hours post infection (hpi). *Crithidia*-like isolates were able to infect all tested cell lines. The percentages of *Crithidia*-like infection using either J774 or THP-1 were, in average, 30% (24 hpi), 27.9% (48 hpi) and 22.15% (72 hpi) with an infection index of 57.5, 60.26 and 31.65, respectively. The highest porcentage of infection was obtained by *L. infantum* HUFS14 strain at 72 hpi (40% for J774 and 39.4% for THP-1) with an average infection index of 106,81. Likely, *C. fasciculata* was only phagocytosed by macrophages, rather than infected them, resulting in 5.6% and 4.3% of cells with internalized parasites at 24 hpi and 72 hpi and an infection index of 4.71 and 1.18, respectively. Interestingly, once BMDM were used, *Crithidia*-like showed a higher % of infection in all time courses compared to *L. infantum* PP75 strain. *Crithidia*-like infections reached up to 74% (24 hpi), while PP75 reached up to 28.25% (48 hpi). Infections using BMDM showed a better sensitivity of newly differentiated macrophages, corroborating that primary cells can be biologically more appropriated to use in infection assays. *In vitro* infections of *Crithidia*-like isolates will assist in the characterization of this new parasite. Moreover, infectivity assays will be performed using human peripheral blood mononuclear cells for evaluation of immune and microbicide responses, as well as gene expression induced by infection likely related to development of VL. **Supported by:**FAPESP grant 2016/20258-0, scholarship 2018/26799-9 and 2020/14011-8; Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) **Keywords:** In vitro Infection.Visceral Leishmaniasis.Crithidia-like

HP-025 - **Participation of enolase enzyme on the infection of promastigotes of *Leishmania amazonensis***

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Certain molecules on the surface of *Leishmania* are pathogenic factors, as they control the different infection pathways of host cells. Apoptotic mimicry is one of such pathways, consisting of the ability to express surface molecules similar to those of apoptotic cells. Thus, the macrophage recognizes *Leishmania* and phagocytes it, without generating a harmful inflammatory response. Although phosphatidylserine phospholipid is one of the main markers involved in apoptotic mimicry, there are other molecules expressed by apoptotic cells capable of modulating the inflammatory response. The proteins of the glycolytic pathway are directed to the cell surface and, regardless of the intracellular roles in metabolism, these proteins in an extracellular context are candidates for immunomodulators. The objective of the work was to evaluate the participation of the enolase on the surface of *Leishmania amazonensis* during the development of the parasite *in vitro*. For this, the role of enolase in the infection of macrophages was evaluated, as well as the capacity of the enzyme in modulating the inflammatory activity of the macrophage. In addition, enolase expression kinetics was performed in metacyclic and procyclic forms. Results showed that the percentage of promastigotes expressing enolase on the surface varied throughout development *in vitro*. The correlation coefficient of the mean of *FSC* and the mean of the enolase for the strains LV-78 and Josefa showed a correlation between the expression of enolase and the size of the parasite. In addition, there was a decrease in the infectivity rate of macrophages when they were infected with promastigotes treated with anti-enolase antibodies. Thus, the data suggest that promastigotes of *Leishmania amazonensis* express enolase on its surface and there is a variation of this expression throughout the growth and development of the parasite *in vitro*, in addition to demonstrating that the enzyme has a role in the infection of macrophages.
Supported by:CAPES **Keywords:** Leishmaniasis.metacyclic.apoptotic mimicry

HP-026 - **Visceral infection model with *Leishmania amazonensis***

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Leishmaniasis is a group of clinical manifestations caused by parasites of the Leishmania genus, being the principal clinical manifestations: the cutaneou (CL), mucocutaneou (MCL) and visceral (VL). The determining factors of clinical manifestations are the species of the parasite and the host's immune status. The species *Leishmania amazonensis (L.a)* is mainly related to cases of CL, MCL and disseminated, but there are descriptions of cases of VL caused by the species. In animal models L.a is mainly related to studies of CL. The route of administration of the inoculum in animal models is important to determine the trajectory of infection. Intravenous infections (I.V) are considered the best models for the study of the chronic phase of VL and can be used the study the acute phase. Few studies are carried with model visceralizarion of L.a. Thus, the objective of our work is to standardize an intravenous infection model with a species of *L.a* for studies of visceralization of the parasite. To do so, we separated two groups of BALB / C mice and infected via I.V with infected macrophages (G1) and 106 amastigotes (G2). We evaluated the parasite load in bone marrow, spleen, liver and lymph nodes after 7, 21, 35, 49 and 64 days post infection (d.p.i.), with the limiting dilution method (LDA) after 7 days. As a result, we found that mice in group G1 first presented infection in the bone marrow at 7 d.p.i. and the spleen and liver were the last to be parasitized. In group G2 we only found parasite in the bone marrow at 7 d.p.i. We did not find linearity in parasite loads and infections over the weeks, this variability is justifiable by the route of inoculation. We conclude that infections by infected macrophages lead to consistent visceral infection in less time. More experiments are being carried out to confirm the route of infection and dissemination of the parasite, but this study shows promise for the study of visceral manifestation with species of *L. amazonensis*. **Supported by:**CAPES
Keywords: Leishmania amazonensis.Intravenous infections .visceralization

HP-027 - **Trypanosoma cruzi inositol phosphorylceramide synthase as a potential drug target for Chagas disease**

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Sphingolipids (SLs) are lipids characterized by the presence of sphingosine and are essential components in eukaryotic cell membranes. Inositol phosphorylceramide (IPC) is an SL present in several protozoa, but absent in mammals. In *Trypanosoma cruzi*, the etiologic agent of Chagas disease, IPC is synthesized by IPC synthase (TcIPCS), a trans-membrane protein expressed in all forms of the parasite. Thus, TcIPCS constitutes a potential target for the development of new chemotherapeutics. In this study, we aim to validate TcIPCS as a potential therapeutic target for Chagas disease, through the generation and characterization of genetically modified parasites. For this, we generated null TcIPCS mutants by CRISPR-Cas9 technology and showed that deletion affected epimastigote proliferation and metacyclogenesis, decreased in vitro infection capacity, intracellular replication of amastigotes and release of trypomastigotes from host cells. Interestingly, the supernatant from cells infected with the null mutants showed the predominance of extracellular amastigotes (EA). In order to validate the results obtained with the null mutants, we generated an add back cell line by transfection with a construct containing the IPCS gene with an HA tag. Western blot analysis using anti HA antibody suggested protein re-expression by the add-back parasites and infection experiments are being carried out to assess whether there was phenotype restitution in these parasites. Now we plan to perform biochemical assays to measure IPC synthesis in the cell lines generated in this study and confirm the involvement of IPCS in this process. Experiments with polyclonal antibody generated against an antigenic C-terminal fraction of the protein are also being carried out to analyze the expression profile of TcIPCS in the parasites. So far, we can conclude that TcIPCS, despite being important for fitness, is not an essential enzyme of T. cruzi, since it was possible to obtain null mutants for TcIPCS.

Supported by:FINEP **Keywords:** IPC synthase.Sphingolipids.Trypanosoma cruzi

HP-028 - **NTL25 inhibit the cellular proliferation on promastigotes of Leishmania spp and interact with trypanothione reductase.**

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Leishmaniasis is a neglected tropical disease caused by the parasite of the genus Leishmania presenting two main clinical forms: tegumentary and visceral. The treatments for leishmaniasis are toxic and expensive. This study evaluated the in vitro effect of the NTL25 on promastigotes of L. infantum and L. amazonensis, as well as demonstrated the interaction of this molecule with the Tripanothione Reductase (TR), an enzyme involved in the redox balance and important for the survival of the parasite. To evaluate the effect of NTL25 against promastigotes of L. infantum and L. amazonensis, these cells were incubated in the absence or presence with an increased concentration of NTL25 in a 96-well plate for 72h, after this, resarzurin was added and the fluorescence was measured at excitation wavelength 560 nm and emission 590 nm. Both species demonstrated an inhibition profile in a concentration-dependent manner presenting an IC50 value of 0,41 mM and 0,011 mM for L. infantum and L. amazonensis respectively. The parameter of ADMET was obtained using the pkCSM platform. The molecule demonstrates the percentage of 61,8% human intestinal absorption indicating oral administration, and not demonstrated to be mutagenic, carcinogenic, and hepatotoxicity. We also performed the interaction of the NTL25 with TR, using the oxidative form (2JK6) and reductive form (4ADW) of TR, analyzing the higher prevalence and lower energy conformations. The lower energy conformation presented a ΔG value of -10.81Kcal/mol and -10.71 Kcal/mol and an inhibition constant (Ki) of 11.83 nM and 14.21 nM for 2JK6 and 4ADW, respectively. The higher prevalence conformation presented a ΔG value of -9.87 Kcal/mol and -9.84Kcal/mol, and a Ki of 58.23 nM and 60.89 nM for 2JK6 and 4ADW, respectively. In conclusion, the NTL25 is a compound that can be orally administered, presenting an antileishmania effect and as a possible mechanism of action, the inhibition of TR, compromising the redox balance.

Supported by:CAPES - **Keywords:** Leishmania.Flavonoid.Treatment

HP-029 - **Treatment with Suboptimal dose of Benznidazole Mitigates Immune Response Molecular Pathways in Mice with Chronic Chagasic Cardiomyopathy**

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Chronic chagasic cardiomyopathy (CCC) is the most frequent and severe form of Chagas disease, a neglected tropical illness caused by the protozoan *Trypanosoma cruzi*. Although efforts have been made to understand the signaling pathways and molecular mechanisms underlying CCC, we still lack information on the immunological signaling pathways regulated by the etiological treatment with benznidazole (Bz). In experimental CCC, Bz combined with the immunoregulatory agent pentoxifylline (PTX) has beneficial effects on CCC. C57BL/6 mice chronically infected with *T. cruzi* Colombian strain (TcI) showing electrocardiographic abnormalities were submitted to treatment with suboptimal dose of Bz or Bz+PTX from 120 to 150 days post-infection. Electrocardiographic alterations and heart parasite load were beneficially impacted by Bz and Bz+PTX. RT-qPCR TaqMan array was used to evaluate the expression of 92 genes related to the immune response in RNA extracted from heart tissues. In infected mice, 30 genes were upregulated and 31 were downregulated. Infection upregulated the cytokines IFN-γ, IL-12b, and IL-2 (126-, 44-, and 18-fold change, respectively) and the T-cell chemoattractants CCL3 and CCL5 (23- and 16-fold change, respectively). Bz therapy restored the expression of genes related to inflammatory response, cellular development, growth, and proliferation, and tissue development pathways, linked to the cardiac remodeling processes inherent to CCC, thus mitigating the Th1-driven response found in vehicle-treated infected mice. The combined Bz+PTX therapy affected pathways related to the modulation of cell death and survival and organismal survival, supporting that this strategy may mitigate the progression of CCC. Altogether, our results contribute to the better understanding of the molecular mechanisms of the immune response in the heart tissue in chronic Chagas disease, and reinforce that parasite persistence and dysregulated immune response underpin CCC severity. **Supported by:**CAPES, CNPq (311539/2020-3, BPP 306037/2019-0) and FAPERJ (JCNE, E-26/203.031/2018, CNE, E-26/ 210.190/2018) **Keywords:** Chagas disease.Immune response.Benznidazole

HP-030 - **Modulation of miR-145-5p and miR-146b-5p levels is linked to reduced parasite load in the H9C2 cardiomyoblast cells infected with *Trypanosoma cruzi***

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The microRNAs miR-145-5p and miR-146b-5p are involved in the regulation of genes related to immune response and inflammatory processes. In Chagas disease, miR-145-5p and miR-146b-5p have been shown to be downregulated and upregulated, respectively, in the heart tissue of acutely *Trypanosoma cruzi*-infected mice. Initially, we established an *in vitro* experimental model using the H9C2 rat cardiomyoblast cell line infected with the Colombian *T. cruzi* strain to investigate the effect of parasite infection on the regulation of miR-145-5p and miR-146b-5p levels by the host cell. Later, we explored the effects of treatment of *T. cruzi*-infected H9C2 cells with the trypanossomicidal drug Benznidazole (Bz) alone or combined with the immunoregulator Pentoxifylline (PTX) on parasite load and expression of these miRNAs. The infection of the H9C2 cells with trypomastigote forms allowed parasite cycle with intracellular forms multiplication and trypomastigote release. After 48 and 144 hours of infection, we detected upregulation of miR-145-5p and miR-146b-5b levels. Addition of 3 μM and 10 μM of Bz 48 hours after infection was able to reduce parasite load but did not interfere with miR-145-5p and miR-146b-5p levels. On the other hand, associated Bz+PTX treatment decreased the levels of both microRNAs, restoring the same levels detected in the non-infected H9C2 cells. The use of miR-145-5p and miR-146b-5p mimic/inhibitor systems before the H9C2 infection significantly decreased parasite load, 72 hours after infection. In parallel, when H9C2 cells were treated with miR-145-5p and miR-146b-5p mimic/inhibitor 48h after infection, all the used systems reduced the parasite load. Taken together, our results show the potential of miR-145-5p and miR-146b-5p to be further investigated as biomarkers of parasite control and tools to identify therapeutic targets to be used as adjuvant to etiological treatment in Chagas disease. **Supported by:**CAPES, CNPq (311539/2020-3, BPP 306037/2019-0) and FAPERJ (JCNE, E-26/203.031/2018, CNE, E-26/ 210.190/2018) - **Keywords:** Chagas disease.microRNA.Benznidazole

HP-031 - **Development of a qPCR method for parasite load quantification of *Leishmania spp***

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Leishmaniasis is a tropical and subtropical endemic disease caused by parasites of the *Leishmania* genus. Leishmaniasis present some clinical manifestations which depend on the infecting *Leishmania* species and the host immune response. The disiase can be classified into two types: tegumentary and visceral leishmaniasis. Since the isolated symptoms from leishmaniasis are not enough for the diagnosis, which is based on the visualization of amastigote forms of the parasite in biopsy tissue. Thus, the diagnosis confirmation needs other techniques with greater sensibility and capacity to identify infective species. The quantitative Polymerase Chain Reaction (qPCR) method presents a greater detection sensibility and it is capable of quantifying the *Leishmania* parasite load. In addiction, this method has shown some technical advantages such as speed, reduced risk of contamination and possibility to quantify the parasite. Therefore, we standardized a qPCR assay to quantify the parasite load of *Leishmania braziliensis* in infected bone marrow-derived macrophages, as well as in infected C57BL/6J and BALB/c mice tissues. This assay demonstrate to be able to obtain the parasite quantification from a standard curve of the number of parasites by the Ct (Threshold Cycle). Once the target gene is conserved among *Leishmania* species, the method developed could be to applied to identify and quantify other *Leishmania* parasites. Moreover, the species differentiation relies on the use of specific probes containing distinct fluorophores. So, the standardization of this method can be applied to diagnosis, treatment efficacy and epidemiological studies as well as vaccines assays.
Supported by:CAPES: 88887.517727/2020-00 **Keywords:** Parasite load.qPCR assay for Leishmania parasites.Molecular diagnosis

HP-032 - **Leishmania infection inhibits macrophages migration in a three-dimensional environment**

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Leishmaniasis results in a wide spectrum of clinical manifestations, ranging from skin lesions at the inoculation site to disseminated lesions to internal organs, such as spleen and liver. The ability of *Leishmania*-infected host cells to migrate may be important to lesion distribution on the host and the dissemination of disease. However, the mechanisms involved in parasite dissemination and the accompanying role played by host cells remains poorly understood. Previously published work has shown that *Leishmania* infection inhibits macrophage 2D migration by altering actin dynamics and impairing the expression of proteins that function in plasma membrane–extracellular matrix interactions. However, cell migration *in vivo* mostly occurs in 3-dimensional (3D) environments. The present study aimed to investigate the migration of macrophages infected by *Leishmania* and the mechanisms involved in this process, using a 3-dimensional environment. Following the infection of bone marrow-derived macrophages (BMMF,) by *L. amazonensis*, *L. braziliensis*, or *L. infantum*, cellular migration, the formation of adhesion complexes and actin polymerization were evaluated. We found that BMMF 3D amoeboid migration was inhibited following *Leishmania* infection. Reduced expression of proteins involved in adhesion complex formation and altered actin dynamics were also observed in *Leishmania*-infected BMMF. Taken together, our results show that *Leishmania* infection inhibits BMMF 3D amoeboid migration by altering actin dynamics and impairing the expression of proteins that function in plasma membrane extracellular matrix interactions.
Supported by:FAPESB, Nº 9092/2015 **Keywords:** Macrophages.3D Migration.Leishmania

HP-033 - **Single Nucleotide Polymorphisms of two *Trypanosoma cruzi* genes encoding truncated type-I nitroreductases (TcNTR-1 and TcOYE): *in vitro* resistance to Nitro-heterocyclic drugs and treatment failure**

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Currently, the two drugs approved for Chagas disease (CD) therapy, Benznidazole and Nifurtimox, have serious limitations, namely: prolonged treatment regimens, substantial side effects and failures. A relationship between parasite genetic diversity and drug susceptibility has been observed, but its epidemiological significance remains unclear. To gain information on this issue, we sequenced two *T. cruzi* genes involved in *in vitro* resistance to nitro-heterocyclic drugs. The nucleotide sequences of type I nitroreductase (TcNTR1) and old yellow enzyme (TcOYE) genes were identified in 76 strains and isolates belonging to different discrete typing units from vectors, reservoirs and patients (oral CD, chronic, congenital and CD reactivation) with variable degree of susceptibility. The genes showed ORFs of 941 bp (TcNTR1) and 1120 bp (TcOYE), encoding proteins of 313 and 373 amino acids (aa), respectively. The deduced aa sequences of TcNTR1 and TcOYE exhibited 94% and 72% of average homology among samples. Among TcNTR sequences, ten SNPs were detected, the most recurrent SNPs were G/A transitions (n=13 isolates) at aa positions 18, 239, and 243, which generated premature stop codons at the hypothetical carboxyl-terminal FMN binding site. The TcOYE gene sequence analysis showed six SNPs that also originated premature stop codons; the most frequent SNPs were deletions at positions 28 and 38 that generated a UGA codon at aa 27 (n=7 isolates) and deletions at ntds 180, 504, 519, 560, 712, 735, 750 that led to a UAA codon at aa 253 (n=13 isolates). These stop codons affected positions located at the hypothetical FMN and substrate binding sites. All these aa changes correlated with reference strains already characterized as resistant phenotypes plus 15 new isolates that can be classified as putative resistant to nitro-heterocyclic drugs at a genetic level, some of them isolated from oral CD patients infected with Tc I strains refractory to treatment. **Supported by:**ERANET LAC HD 328 - **Keywords:** Trypanosoma cruzi.drug resistance genes .single nucleotide polymorphism

HP-034 - **Natural Populations of *Trypanosoma cruzi* in Oral Chagas Disease Patients Refractory to Benznidazole Chemotherapy: Molecular characterization through 9 years of Follow-Up**

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We have characterized the genetic profiles of natural populations of *T. cruzi* present in patients with poor response to etiological treatment, affected by the outbreak of Oral Chagas Disease (OCD) in a rural school at the community of Chichiriviche de la Costa, Venezuela. Six hemocultures (HCs) and 102 venous blood samples were obtained from twenty-nine OCD patients at the time of diagnosis and along nine years of Post-treatment (Tx) follow-up. The IgG serology, *T. cruzi* discrete typing units (DTU), satellite DNA-qPCR parasitic loads, and minicircle signatures (Ms) were determined in all samples. The serological titers and parasitic loads changed after treatment, with a significant decrease of IgG titers (Spearman's r value= -0.961) and median parasite loads from 2.869 [IQR = 2.113 to 3.720] to 0.105 [IQR = -1.147 to 1.761] log10 par eq./mL at Pre-Tx and Post-Tx, respectively, suggesting infection evolution from acute to chronic phase, without seroconversion or parasitological suppress. All patients were infected with *T. cruzi* DTUI populations. The median of the Jaccard similarity coefficient in Pre-Tx samples was 0.775 [IQR = 0.708 to 0.882], decreasing in genetic variability towards the end of follow-up (Mann-Whitney U test p= 0.0031). Interestingly, no Post-Tx Ms was identical to its Pre-Tx counterpart population in the same patient, revealing a selection of parasite subpopulations between the primary infection and Post-Tx. Parasitic populations isolated from HCs showed a lower number of bands in the Ms with respect to the signatures obtained directly from the patients' blood samples, demonstrating a process of parasitic selection and reduction of the population variability that initially infected the patients. Decrease of parasitic loads after Tx as well as Pre and Post-Tx intra-TcI diversity might be a consequence of both, a natural evolution of the acute infection to the chronic phase and persistence of refractory populations due to Tx selection.
Supported by:NHEPACHA NETWORK **Keywords:** ORAL CHAGAS DISEASE.Trypanosoma cruzi I genetic polymorphism.treatment failure

HP-035 - **The asparagine synthetases from Trypanosoma cruzi: from coding genes to function.**

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In view of the absence of an efficient prophylactic treatment for Chagas disease, in order to identify new targets and therapeutic approaches, several studies have been developed aiming to deepen the knowledge of the biology of Trypanosoma cruzi, the etiological agent of this parasitic burden. In this sense, the amino acid metabolism has come up as a rational study proposal, given its paramount role in several aspects of the parasite biology. Besides being precursors of protein biosynthesis, amino acids provide carbons and energy and are involved in several critical aspects of the biology of T. cruzi, such as cell differentiation, host cell invasion, cell cycle regulation and stress resistance. A specific enzyme involved in aminoacid metabolism, the asparagine synthetase (AS), catalyzes the production of asparagine using aspartate as substrate and glutamine or ammonia as a nitrogen source. In this work we propose the hypothesis that this enzyme is involved in the adçministration and availability of important metabolites derived from aspartate, glutamine, asparagine and glutamate, and as well as in the acceptance and transference of amino groups and concomitant resistance to NH_4+ in T. cruzi. We start by identifying the genes coding for putative asparagine synthetases in T. cruzi and the subsequent cloning and heterologous expression of the recombinant proteins with the aim of performing a biochemical characterization of the enzymes. We also produced mutants with deleted sequences for the respective genes using the CRISPR-Cas 9 system, which will provide information about the functional role of these enzymes in different aspects of the biology of T. cruzi.
Supported by:FAPESP - Fundação de Amparo à Pesquisa do Estado de São Paulo. Processo N° 2016/06034-219 **Keywords:** Trypanosoma cruzi.asparagine synthetase.Metabolism

HP-036 - ***Leishmania*-Induced Dendritic Cell Migration and its Potential Contribution to Parasite Dissemination**

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Leishmania, an intracellular parasite species, causes lesions on the skin and in the mucosa and internal organs. The dissemination of infected host cells containing *Leishmania* is crucial to parasite survival and the establishment of infection. Migratory phenomena and the mechanisms underlying the dissemination of *Leishmania*-infected human dendritic cells (hDCs) remain poorly understood. The present study aimed to investigate differences among factors involved in hDC migration by comparing infection with visceral leishmaniasis (VL) induced by *Leishmania infantum* with diverse clinical forms of tegumentary leishmaniasis (TL) induced by *Leishmania braziliensis* or *Leishmania amazonensis*. Following the infection of hDCs by isolates obtained from patients with different clinical forms of *Leishmania*, the formation of adhesion complexes, actin polymerization, and CCR7 expression were evaluated. In addition, we evaluated the role of LPG, a key molecule involved in *Leishmania*-host cell interaction, in the migration of human dendritic cells infected by *Leishmania*. We observed increased hDC migration following infection with isolates of *L. infantum* (VL), as well as disseminated (DL) and diffuse (DCL) forms of cutaneous leishmaniasis (CL) caused by *L. braziliensis* and *L. amazonensis*, respectively. Increased expression of proteins involved in adhesion complex formation and actin polymerization, as well as higher CCR7 expression, were seen in hDCs infected with *L. infantum*, DL and DCL isolates. Also, we observed reduced migration in hDC infected by *L. infantum* lpg2 knockout when compared to those infected with the wildtype parasites. Reduced expression of proteins involved in adhesion complex formation was also found in hDC infected by *L. infantum* lpg2 knockout. Together, our results suggest that hDCs play an important role in the dissemination of *Leishmania* parasites in the vertebrate host and that LPG plays a role in the modulation induced by these parasites in these cells. **Supported by:**Fapesb 9092/2015 **Keywords:** Dendritic cell; Leishmania.Migration; Dissemination.LPG

HP-037 - **Protein ANXA1 during an experimental infection by *T. gondii: in vitro* and *vivo***

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INTRODUCTION: Toxoplasmosis, caused by *Toxoplasma gondii* (Tg), is a public concern owing mainly to its neurotropic nature. Tg is an obligatory intracellular parasite, and macrophages and glial cells are crucial controlling this infection/inflammation. **AIM:** Annexin A1 (ANXA1) is a pro-resolving and anti-inflammatory protein, and its role during Tg infection is unknown and was evaluated here. **MATERIALS E METHODS:** Peritoneal macrophages and glial cells from Balb/c (WT) and ANXA1 knockout(KO) mice were infected with Tg RH strain, *in vitro*. WT and ANXA1 KO mice were infected or not with Tg ME49 strain and weight loss and survival was monitored. Moreover, the peritoneal leukocytes phenotype was distinguished and quantified during infection. **RESULTS:** Macrophages and glial cell ANXA1 KO, *in vitro*, increased the tachyzoites number compared to WT cells. *In vivo,* ANXA1deficiency increased the *T. gondii* infection susceptibility, but not weight loss, associated with increased number of brain cysts when compared to WT counterparts. Moreover, a reduction number of lymphocytes, but not macrophages and neutrophils, was found in the peritoneal cavity of infected ANXA1 mice when compared to WT. **CONCLUSION:** Our data suggested an important role of ANXA1 during Tg infection regulating lymphocytes migration and/or activation, and macrophages and glia cells antitoxoplasma activity.

Supported by: Cnpq, Fapemig, Capes **Keywords:** Toxoplasma gondii.Annexin A1.Immune response

HP-038 - **ANXIETY AND DEPRESSIVE-LIKE BEHAVIOR IN LONG-TERM Toxoplasma gondii INFECTION OF C57BL/6 MICE ARE ASSOCIATED WITH DISRUPTION OF THE BLOOD BRAIN BARRIER AND INCREASE IN SITU AND SYSTEMIC CYTOKINE EXPRESSION**

BARRIOS, L.M.C.[1]; PINHEIRO, A.P.[1]; GIBALDI, D.[1]; DA SILVA, A.A.[2]; SILVA, P.M.R.E.[1]; ROFFE, E.[3]; SANTIAGO, H.D.C.[4]; GAZZINELLI, R.T.[4]; MINEO, J.[5]; SILVA, N.[5]; LANNES-VIEIRA, J.[1].
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Toxoplasmosis is caused by the protozoan *Toxoplasma gondii*, a mandatory intracellular parasite. This illness is of medical importance due to the high prevalence worldwide, the ability to cause abortion and malformations in fetuses, and neurological alterations in immunocompromised persons, as HIV-infected individuals. In chronically infected immunocompetent individuals, this parasite forms tissue cysts mainly in the brain. In addition, *T. gondii* infection has been related to mental illnesses as schizophrenia and depression. Here, we evaluated the kinetics of behavioral alterations in chronic infection, assessing anxiety and depressive-like behavior, and their relationship with the number of parasite cysts in brain, blood-brain-barrier (BBB) integrity, and cytokine status in the brain and serum. Adult female C57BL/6 mice were infected (gavage, 5 cysts, ME-49 type II *T. gondii* strain) and analyzed as independent groups at 30, 60 and 90 days after infection (dpi). Anxiety and depressive-like behavior were detected in the early (30 dpi) and long-term (60 and 90 dpi) chronic *T. gondii* infection, in a direct association with the presence of parasite cysts and linked to BBB disruption. The behavioral alterations paralleled the upregulation of expression of tumor necrosis factor (TNF) and CC-chemokines (CCL2/MCP-1, CCL3/MIP-1α, CCL4/MIP-1β and CCL5/RANTES) in the brain tissue. In addition, the behavioral changes paralleled with peripheral blood levels of interferon-gamma (IFNγ), TNF and CCL2/MCP-1, at 30 and 60 dpi. Our data suggest that the persistence of parasite cysts in the CNS induces increase in cytokine expression and sustained BBB disruption, thus allowing leakage of cytokines of systemic circulating plasma into the brain tissue. Therefore, these factors may contribute to anxiety and depressive-like behavior in chronic *T. gondii* infection.

Keywords: Behavioral tests.ME-49 strain.Neuroinflammation

HP-039 - **HYPERACTIVITY IN A MURINE MODEL OF LONG-LASTING CHRONIC Toxoplasma gondii IS REFRACTARY TO SULFADIAZINE AND PYRIMETHAMINE THERAPY**

BARRIOS, L.M.C.[1]; PINHEIRO, A.P.[2]; GIBALDI, D.[2]; DA SILVA, A.A.[3]; SILVA, P.M.R.E.[1]; MINEO, J.[4]; SILVA, N.[4]; LANNES-VIEIRA, J.[1].

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Toxoplasma gondii, an intracellular protozoan, is the etiologic agent of toxoplasmosis, an infectious disease of medical importance. The *T. gondii* protozoan is found worldwide and currently one third of the world population is seropositive. The parasite has tropism for the central nervous system (CNS), where it remains for long periods influencing the behavior of the host. In fact, infection by *T. gondii* has been related to mental illnesses such as schizophrenia, bipolar disorder, obsessive-compulsive disorder, and other behavioral abnormalities. The aim of the present study was to determine whether the presence of *T. gondii* cysts in the CNS leads to hyperactivity in chronically infected animals. For that, 4-6 weeks old C57BL/6 mice were infected with the cystic form of the ME-49 type II *T. gondii* strain and evaluated at 30-, 60- and 90-days post-infection (dpi). Later, we tested the role of parasite in the behavioral alterations. Thus, chronically infected mice were orally treated for 30 days (30 to 60 dpi) with a combined therapeutic strategy with sulfadiazine (S) and pyrimethamine (P). The open field (OF) and elevated plus maze (EPM) tests, widely used to assess hyperactive and impulsive components of attention deficit hyperactivity disorder (ADHD), respectively, were used. Subsequently, the number of cysts present in the brain was evaluated. Hyperactivity was detected in the early (30 dpi) and long-term (60 and 90 dpi) chronic *T. gondii* infection. For example, at 60 dpi infected mice showed increase in walking speed in the OF than non-infected (NI) mice, supporting the presence of hyperactivity. In addition, infected animals remained longer time exploring the open arms of the EPM, when compared to NI, indicative of impulsivity. The S+P treatment reduced the number of cysts in the CNS, when compared to vehicle. However, there was no effect on the hyperactivity observed in the OF, nor on impulsivity in the EPM. **Keywords:** Behavioral tests.ME-49 strain.Hyperactivity

HP-040 - **Expression and functionality of co-inhibitory receptors TIGIT, TIM-3 and LAG-3 in CD4+ T cells from patients with chronic Chagas disease.**

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Chagas disease, caused by *Trypanosoma cruzi*, affects ~7 million people worldwide, mainly in Latin America where it is endemic. Unless treated early after infection, the disease progresses to a chronic form in which some patients develop cardiac or digestive alterations, while others stay asymptomatic. $CD4^+$ T cell response plays critical and diverse roles during infection, and becomes impaired over time with defective cytokine release. This process known as T cell exhaustion is also defined by the upregulation of inhibitory receptors, mainly PD-1 and CTLA-4. In this work, we aimed to explore the expression of the second line of inhibitory receptors TIGIT, TIM-3 and LAG-3 in parasite stimulated $CD4^+$ T cells from patients with different stages of chronic Chagas disease (CCD), and whether their blockade restores cell functionality. Antigen-specific $CD4^+$ T cells were identified by activation induced markers (AIM) assay, using the surface molecules Ox40 and CD25. CCD patients, independently of their clinical stage, showed an increased frequency of $CD4^+TIGIT^+$ T cells. $TIM\text{-}3^+$ $CD4^+$ T cells were more abundant in patients with cardiac manifestations, while $LAG\text{-}3^+$ cells were increased in asymptomatic CCD patients, with higher frequency within the non-activated cells subpopulation upon *T. cruzi* lysate stimulation. Preliminary data showed that TIM-3 blockade tended to increase $IFN\text{-}\gamma^+$ $CD4^+$ T cells in CCD patients with cardiomyopathy. Furthermore, in the same group of subjects, the incubation with an anti-TIGIT blocking antibody led to a greater frequency of $IL\text{-}10^+$ $CD4^+$ T cells compared to the isotype control antibody. Our results highlight the role of the inhibitory receptors TIGIT, TIM-3 and LAG-3 in the modulation of anti-*T. cruzi* $CD4^+$ T cell responses, in relation with the progression of chronic Chagas disease, and paves the way for the development of novel therapeutic strategies. **Supported by:**PIP 2015 **Keywords:** chronic Chagas disease.T-cell exhaustion.inhibitory receptors

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HOST IMMUNE RESPONSE PARTICIPATES IN MALARIA ACUTE KIDNEY INJURY: ROLE OF CD8+ T CELLS

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Severe malaria is attributed to *Plasmodium falciparum* infection and entail different pathologies caused by direct effects of parasite infection and host immune response. Malaria acute kidney injury (MAKI) is characterized by glomerular and tubular damage. This process is attributed to oxidative stress and obstruction of renal microvasculature caused by aggregates of parasitized red blood cells. However, little is known about the role of immune system in MAKI. The objective of the work was to evaluate the participation of T cells in MAKI pathogenesis. For this, we performed adoptive transfer of splenocytes-derived T cells from C57Bl6 mice infected with *P. berghei* ANKA to healthy acceptor animals. Renal function as well as homing and immune response were assessed. Adoptive transfer induced proteinuria (2-fold) and increased UPCr (protein and creatinine ratio; 2.3-fold). Markers of glomerular injury, creatinine clearance, plasma creatinine and plasma urea, did not change. On the other hand, an increase in gamma GT activation in urine (1.6-fold), a marker of renal tubular damage, was observed. These results indicate that malaria-responsive T cells induce renal tubular damage without glomerular involvement. We observed an increase in homing of malaria-responsive T cells to the spleen, brain and kidneys. An increase in renal proinflammatory cytokines INF□, IL-17 and IL-6 were also observed. When we evaluated the T cells in the kidney, adoptive transfer increased the frequency of renal CD8+ T cells as well as the expression of perforin in the renal cortex, a marker of cytotoxic T cell activity. These results indicate that CD8+ T cells are activated during malaria infection and can migrate to the kidney besides brain and spleen. In the kidney, perforin production has a role in inducing renal tubular damage. This work adds to the literature that MAKI could be also a consequence of an exacerbated host immune response. **Supported by:**Faperj, CNPq e Capes
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