

HP-05 - Trans-sialidase as a virulent factor of *Trypanosoma cruzi* involved with parasite egress and the production of pro-inflammatory cytokines by the infected cell

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Trans-sialidases (TS) are encoded by the largest gene family in the *Trypanosoma cruzi* genome in which 12 sequences encode enzymatically active TS (aTS). aTS are responsible for transferring sialic acid from host glycoconjugates to mucins present on the parasite surface. Using CRISPR/Cas9 technology, we generated aTS knockout parasites that display undetectable levels of TS activity. Disruption of aTS genes does not affect parasite infectivity and escape from the parasitophorous vacuole but resulted in impaired differentiation of amastigotes (AMA) into tissue culture derived trypomastigotes (TCT). Moreover, parasites lacking aTS are unable to establish infection even in the highly susceptible IFN-g knockout mice. To further investigate the role of aTS, we performed *in vitro* infection assays with wild type (WT) and aTS knockout (aTSKO) parasites in two cell models: HeLa (non-phagocytic) and THP1 (phagocytic). Our results confirmed that the lack of aTS does not limit parasite internalization and AMA multiplication in both cell types, but drastically affect release of TCT. Since it was previously shown that several genes associated with the immune response, particularly, type I IFN response are up-regulated in mammalian cells upon *T. cruzi* infection, we performed RT-PCR to determine the expression levels of pro-inflammatory cytokines in cells infected with WT and aTSKO parasites. Compared to cells infected with WT parasites, infection with aTSKO resulted in significantly lower levels of IL-1- β , IL-1- α , IL-6 and CXCL8. To further understand the role of aTS in the egress of the parasite, we developed a strategy to heterologous express aTS in the cell before the infection with aTSKO parasites. **Supported by:** CNPq, CAPES, INCTV **Keywords:** Active trans-sialidases (aTS); host cell egress; pro-inflammatory cytokines.

HP-06 - IMMUNE RESPONSE MODULATION IN CHRONIC CHAGAS DISEASE AND MHV-3 BETACORONAVIRUS COINFECTION: ROLE OF SOCS-2

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The balance of immune response (IR) is essential for the survival of both *Trypanosoma cruzi* and host during the acute phase of Chagas Disease (CD). Sars-cov2 infection can cause unbalanced IR associated to pulmonary and systemic damage in its host. Suppressor Cytokine Signaling (SOCS) 2 is an important regulator of the innate and adaptive response to various infections, such as viral and parasitic, but its role during chronic CD and MHV-3 infection are unknown. Our aim was to evaluate the role of SOCS2 during the chronic CD, MHV3, and coinfection chronic CD/MHV3 in mice. C57BL/6 (WT) and SOCS-2 (KO) animals were infected with trypomastigotes forms (Y strain), and after reaching the chronic CD (100 days), was co-infected or not with MHV-3. Deficiency of SOCS2 increased the gut and pulmonary tissue damage at chronic CD and acute MHV3 infection, respectively. During chronic CD, the coinfection with MHV3 increased the gut and cardiac tissue damage in SOCS2 KO mice when compared with WT counterparts. Moreover, a greater parasitism in the colon and higher viral load in the lung during coinfection was observed in SOCS-2 KO mice. The profile of infiltrating cells analyzed by flow cytometry demonstrated that the absence of SOCS2 in chronic CD increased in lung and gut the TNF production by macrophages and IFN-g by CD8 T cells and reduces IL-10 by Tregs. Absence of SOCS2 in MHV-3 infection increased TNF and reduced IL-10 production by all innate cells analyzed, and increased CD4- and CD8 T cells-producing IFN-g in both organs; in lung was observed an increased production of IL-17 by CD8 T cells. Deficiency of SOCS2 during coinfection was marked by a reduction in IL-10 production by all innate cells and lymphocytes analyzed, and by increased CD4- and CD8 T cells-producing IFN-g in lung and gut. In summary, SOCS-2 is essential to modulate the immune response and progression of pathogenesis, especially in co-infection by beta coronavirus MHV-3 in the chronic phase of CD. **Supported by:** CAPES, CNPQ e FAPEMIG **Keywords:** SOCS2; CHAGAS DISEASE; MHV-3.

HP-07 - Study of the molecular mechanisms of sexual differentiation in *Toxoplasma gondii*

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Toxoplasmosis is a disease of worldwide distribution caused by the intracellular parasite *Toxoplasma gondii*, causing high morbidity and mortality in the human population and farm animals. *T. gondii* has a complex life cycle, with asexual and sexual stages. During sexual differentiation, male and female gametes combine to form diploid zygotes that become encysted and provide the parasite with a window of opportunity for genetic admixing, a crucial step in the generation of genetic diversity. These mechanisms are of particular importance in our continent whereby genetic hypervariability is associated with exacerbated virulence phenotypes. Despite its inherent epidemiological importance, the fundamental aspects of sexual differentiation of *T. gondii* are still unknown. This is mainly due to sexual stages occurring only in the intestinal epithelium of members of the *Felidae* family, and access to them is limited. However, new technological breakthroughs have made it possible to mimic the molecular fingerprints that trigger the process, allowing it now to be modeled *in vitro*. In this context, we have pursued the study of *T. gondii* gametogenesis capitalizing on the unprecedented opportunity to generate these stages in 2D cell culture in non-felid cells. We have optimized an enrichment protocol of pre-sexual and sexual stages of *T. gondii* in traditional 2D cell culture, where we have mapped the increase in sexual-stages specific gene expression patterns by qRT-PCR and determined the subcellular localization of specific protein markers of these stages by indirect immunofluorescence. This will serve as a starting point for bettering our understanding of sexual stages, whose molecular and cellular aspects are so far unknown. Ultimately, will serve as a kick-off point for obtaining data essential to the rational design of strategies to prevent horizontal transmission of *T. gondii* and its genetic recombination in infected cats. **Supported by:** Pasteur network/ Ministerio de Educación y Cultura, Uruguay. **Keywords:** *Toxoplasma gondii*; horizontal transmission; sexual differentiation.

HP-08 - Determination of *Toxoplasma gondii*'s genetic variability in infections during pregnancy in Uruguay

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The paradigm for transmission and manifestations of human toxoplasmosis arose based on clinical data and experimentation in mice using strains of *Toxoplasma gondii* circulating in Europe and North America known as "typical." However, we now understand that the severity of human toxoplasmosis depends on the genotype of the parasite, which is closely linked to its geographic origin. Worldwide genotyping studies describe a higher incidence of atypical strains in South America, but this concept is mainly based on data obtained in Brazil, Colombia, and Argentina. Pioneering work in Uruguay described the existence of "atypical" serotypes in patients and the isolation of a genetically atypical strain of Uruguayan origin in France. Based on this, we hypothesize that there is ample genetic variability of circulating *T. gondii* strains in humans in our country. To address this, we genotyped *T. gondii* strains acquired during pregnancy, as determined by the patient's seroconversion detectable during routine pregnancy checkups. We amplified parasite DNA from peripheral blood, placental tissue, and umbilical cord blood from patients. *T. gondii* DNA was detectable in 14 of 21 patients. Positive samples were genotypes using in silico PCR-RFLP of nine polymorphic genetic markers. The presence of "atypical" strains was evidenced in 7 samples. We are currently advancing in their genetic characterization and pursuing the isolation of circulating strains with the aim of correlating genotype with phenotype. **Keywords:** *Toxoplasma gondii*; Pregnancy; Vertical transmission.

HP-09 - Identification and characterization of iron metabolism-related proteins in *Leishmania amazonensis* glycosomes

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The protozoan parasites of the genus *Leishmania* are the causative agents of a group of diseases collectively known as leishmaniasis that affect millions of people worldwide. *Leishmania* life cycle includes invertebrate and vertebrate hosts. In the vertebrate host, the parasites 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. Besides, it was also shown that iron deprivation modulates the expression of a series of genes whose function is still unknown. Among these, we found conserved genes containing predicted glycosomal addressing signals (PTS1 e PTS2). Therefore, our goal is to identify and characterize genes involved in the transport of iron into *Leishmania* glycosomes, which are trypanosomatids unique organelles. *In silico* analysis of 576 *Leishmania* transcripts modulated by iron deprivation revealed 11 putative genes containing predicted glycosomal addressing signals and transmembrane domains. By immunofluorescence, we confirmed the glycosomal compartmentalization of the proteins encoded by 2 of these genes in promastigotes: a putative gene encoding a hypothetical multipass protein, and an *aquaglyceroporin* (*aqp1*). Then, we used the CRISPR/Cas9 strategy to generate full and partial knockouts, and complemented strains, as confirmed by PCR analyses. The overexpression and knockout of these genes impacted promastigotes *in vitro* growth. Moreover, phenotypic analyses revealed that *aqp1* is implicated on parasite partial resistance to trivalent antimony (SbIII). In-depth characterization of these mutant strains will contribute to elucidate the mechanisms involved in the metabolism and transport of iron into *Leishmania* glycosomes and may indicate new targets for the development of chemotherapeutic agents for the treatment of leishmaniasis. **Supported by:**FAPESP 2022/04551-0 **Keywords:**Gene editing;Drug resistance;Immunofluorescence.

HP-10 - Exploring the function of RNA modifications during *Leishmania* stage differentiation.

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The *Leishmania* life cycle involves promastigotes present in the insect vector and amastigotes present in the mammalian host. Adapting to these hosts requires not only morphological but also translation and gene expression changes. Gene expression regulation in *Leishmania* happens mainly at the post-transcriptionally level by mechanisms involving mRNA stability/degradation. Several post-transcriptional modifications of RNA molecules have been described and are widely recognized as an additional mechanism for regulating gene expression. Among these modifications, N6-methyladenosine (m6A) and N1-methyladenosine (m1A) are the most abundant and present in RNAs from different organisms. m6A is added by the methyltransferases METTL3/METTL14 and removed by FTO/ALKBH5 demethylases. Although METTL3 orthologue is absent in trypanosomatids, it was demonstrated the presence of m6A in *Trypanosoma brucei* and its impact in regulating VSG genes. To study the role of m6A in the different *Leishmania* parasite stages we selected five species belonging to different subgenera (*Leishmania mexicana*, *L. amazonensis*, *L. infantum*, *L. major* and *L. braziliensis*), in order to demonstrate the presence of m6A. Using total RNA samples of the procyclic forms of each species, dot-blot assays with the specific antibody against m6A were performed and we found that in *L. mexicana* the levels of m6A were higher compared to other species, followed by *L. amazonensis* and *L. infantum*. Based on this data we choose *L. mexicana* to quantify m6A in the parasite stages, experiments that are in progress. Thus, with these analyses, we will help to understand the processes involved in gene expression regulation in *Leishmania*. **Supported by:**FAPESP; 2023/02341-1 **Keywords:**Leishmania;m6A;epitranscriptome.

HP-11 - Comparative systems immunology of non-human primate infection with *Plasmodium coatenyi* and *Plasmodium cynomolgi*

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Non-human primates can be infected with *P. coatenyi* (Pco) and *P. cynomolgi* (Pcy), which are similar to human infections with *P. falciparum* and *P. vivax* infection, respectively. In this context, the Malaria-Host Pathogen Interaction Center (MaHPIC) consortium set up experimental infections and acquired longitudinal data from *Macaca mulatta* infected with those *Plasmodium* species for 100 days. The datasets include parasitological and clinical laboratory data, immunological, transcriptomics, metabolomics, proteomics, and lipidomics data. We sought to compare the non-human primate response to infection by different *Plasmodium* species by analyzing and integrating different types of data. First, we evaluated the dynamics of parasitemia and laboratory clinical data for each individual experiment along the 100 days of infection. Pco induced high parasitemia during acute infection and developed chronic parasitemia that persisted along the 100 days. In contrast, Pcy induced high parasitemia during acute infection, but parasites were cleared from blood after 25 days. This species is known to form hypnozoites, which can cause relapses latter after acute infection is cleared. There were relapses after 50 days and latter after 80 days of infection. Whole blood cell counts increased and remained high along the course of infection with Pco, whereas Pcy induced dynamic changes along the infection, but counts seemed to be comparable to baseline at the end of 100 days. Pco infection reduced red blood cell (RBC) counts that remained low along the infection, while Pcy induced acute RBC loss that recovered with time. The same pattern was observed for hemoglobin and hematocrit levels. Infections induce different patterns of granulocyte, monocyte and lymphocyte counts along 100 days of infection. Taken together, our preliminary results suggest that *M. mulatta* engages different cellular and molecular responses that depend on the *Plasmodium* species. **Supported by:** Instituto Serrapilheira, CNPq, FUNAPE **Keywords:** Malaria; Systems Biology; Non-Human Primate.

HP-12 - Comparative study of biological and molecular characteristics of susceptibility to miltefosine by *Leishmania (L.) infantum* infecting dogs in Florianópolis, Santa Catarina, Brazil

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Visceral leishmaniasis (VL) is an endemic zoonosis in Brazil, where domestic dogs are urban parasite reservoirs. Between 2010 and 2023, over 900 autochthonous canine VL and 5 human cases were reported in Florianópolis (SC). In this study, we have assessed biological and molecular characteristics of *L. infantum* isolated from naturally infected dogs, before (T0) and after (T1) treatment with miltefosine (MIL). The growth kinetics of all isolated strains was similar but distinct from the PP75 reference strain. Strains isolated before and after *in vivo* treatment showed distinct infectivity to THP-1 cells. No differences were observed *in vitro* for the MIL IC₅₀ for both amastigotes and promastigotes (T0 and T1). The transcription of genes associated with susceptibility to MIL revealed a positive modulation in promastigote treated *in vitro*. No genetic resistance profile to MIL based on the LiMT and LiRos3 genes was observed. Proteomic analysis of MIL treated and untreated *L. infantum*-infected THP-1 carried out by MS resulted on the identification of a total of 4,729 unique proteins, from which 4,235 were from THP-1 and 494 from *L. infantum*, representing 5.8% of the predicted proteins in the JPCM5 reference strain genome. The function was assigned to 99% of these proteins, most of which being cytoplasmic (44.3%), followed by mitochondrial subcellular localization (19.9%). The signal peptide was detected for 2% of these proteins, and 7.3% have at least one transmembrane domain. A total of 225 proteins were shared among the six strains despite the experimental conditions. Proteins with significant differential abundance did not show alteration associated with the mechanism of action or resistance to MIL. Six B-cell epitopes from five proteins were identified, with five shared among *Leishmania* spp. species, and a single *L. infantum*-specific epitope. Despite some strain-specific variations, neither phenotype nor genotype associated with resistance to miltefosine was observed. **Supported by:** CNPq e CAPES **Keywords:** Canine Visceral Leishmaniasis; Miltefosine; Intracellular Amastigote.

HP-13 - Preliminary structural analysis of the Venus Flytrap domains of the ESAG4 receptor-like adenylate cyclase ectodomain in *Trypanosoma brucei*

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Trypanosoma brucei, a protozoan parasite responsible for causing Sleeping Sickness, a neglected tropical disease prevalent in Africa, possesses a large family of transmembrane receptor-like adenylate cyclases (RACs)¹, which are the only known signalling receptors at the cell surface. All RACs possess a conserved architecture, consisting of a large receptor ectodomain composed of two Venus Flytrap domains (VFT1 and VFT2), a transmembrane helix, and a cytosolic catalytic domain with cyclase activity. We have previously shown that activation of trypanosomal RACs expressed in the mammalian host, triggered by mild acid stress, leads to the production of large amounts of cAMP. This elevated cAMP level inhibits TNF- α synthesis in host myeloid cells, allowing immune evasion during the early stage of infection². Nevertheless, the molecular mechanisms triggering the activation of RACs through the ectodomain and the identification of their putative ligands remain unknown. Although the surface architecture of the bloodstream parasite, imposed by the VSG coat, may play a critical role in regulating cyclase activity³, the specific ligands that bind to VFT domains are currently unknown. This project aims to address this gap by identifying the ligands for a bloodstream-specific RAC ectodomain (named ESAG4) and further characterizing the structures of the ectodomain-ligand complexes. To characterize the ligands, several VFT2 soluble protein constructs were produced in bacteria and purified using affinity and size exclusion chromatography, followed by quality control assessments using biophysical techniques. The structural characterization of ESAG4 extracellular sensor domain and its interaction with ligands will potentially provide valuable insights for the development of novel strategies against trypanosome infections. 1. Alexandre S., et al. *Mol Biochem Parasitol.* 43, 279-88 (1990) 2. Salmon D., et al. *Science.* 337, 463-6 (2012) 3. Rolin S., et al. *J Biol Chem.* 271,10844-52 (1996) **Supported by:**CNPQ processo: 870092/1997-9

Keywords:Trypanosoma brucei;Adenylate cyclase;Structural biology.

HP-14 - Modifying to adapt: the impact of lysine deacetylases in *Leishmania* parasite stage differentiation

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Protein acetylation has been implicated in the regulation of essential cellular processes in diverse organisms. Proteomic analysis from our group revealed differential protein acetylation among the three main *Leishmania mexicana* stages. Thus, to expand our knowledge on how changes in protein acetylation affect *Leishmania* differentiation, we generated knockout and fluorescent endogenous tagged parasite strains using the CRISPR-Cas9 system of all lysine deacetylases of the parasite (DACs 1, 3, 4 and 5). We obtained only single-knockouts for DAC1/3, and null mutants for DAC4/5, suggesting that DAC1-3 are essential for procyclic stages. Using the fluorescent parasites, we found that DAC1/5 are cytoplasmatic, while 3/4 have nuclear localization in all stages. Phenotype analyses using the mutant parasites showed that: **i)** DAC1/3/5 affect procyclic multiplication; **ii)** DAC3/5 affect procyclic to amastigote stage differentiation; **iii)** all DACs are important during amastigotes to procyclics differentiation; **iv)** DAC1/5 directly affect *in vitro* differentiation of procyclic to metacyclic infective forms **v)** DAC3/5 impacts metacyclogenesis in experimental *in vivo* infection of *Lutzomyia longipalpis*; **vi)** *in vitro* infection with BMDMs showed decreased in the survival and proliferation for all DAC mutants; **vii)** A similar scenario was observed in the Balb/c *in vivo* infection assays for DAC5 mutants, with no apparent lesion development compared to parental parasites. Taken together, these results indicate that regulation of protein acetylation levels might be important for *L. mexicana* differentiation and pathogenesis, opening the opportunity to explore DACs as potential drug targets. **Supported by:**FAPESP 2021/13477-6 **Keywords:**Leishmania mexicana;acetylation;CRISPR.

HP-15 - Disruption of the inositol phosphorylceramide synthase gene affects *Trypanosoma cruzi* differentiation and infection capacity

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Sphingolipids (SLs) are essential components of all eukaryotic cellular membranes. In fungi, plants and many protozoa, the primary SL is inositol-phosphorylceramide (IPC), which is absent in mammals. *Trypanosoma cruzi* is a protozoan parasite that causes Chagas disease (CD), a chronic illness for which no vaccines or effective treatments are available. IPC synthase (IPCS) has been considered an ideal target enzyme for drug development because IPCS null mutants are not viable and the enzyme activity has been described in all parasite forms of *T. cruzi*. Furthermore, IPCS is an integral membrane protein conserved amongst other kinetoplastids, including *Leishmania major*, for which specific inhibitors have been identified. Using a CRISPR-Cas9 protocol, we generated *T. cruzi* knockout (KO) mutants in which both alleles of the IPCS gene were disrupted. We demonstrated that the lack of IPCS activity does not affect epimastigote proliferation or its susceptibility to compounds that have been identified as inhibitors of the *L. major* IPCS. However, disruption of the *T. cruzi* IPCS gene negatively affected epimastigote differentiation into metacyclic trypomastigotes as well as proliferation of intracellular amastigotes and differentiation of amastigotes into tissue culture-derived trypomastigotes. In accordance with previous studies suggesting that IPC is a membrane component essential for parasite survival in the mammalian host, we showed that *T. cruzi* IPCS null mutants are unable to establish an infection *in vivo*, even in immune deficient mice. **Keywords:** Sphingolipids; IPC synthase; Inositol-phosphorylceramide.

HP-16 - Molecular Detection and Genotyping of *Blastocystis* spp in samples from patients suffering from inflammatory bowel disease in Uruguay.

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Blastocystis spp is frequently detected in routine diagnosis of inflammatory bowel diseases (IBDs). However, the association between the presence of the agent and its impact on the pathology of these diseases is ill-understood. Moreover, whether there is a correlation between specific genotypes of *Blastocystis* spp and the histopathological manifestations of IBDs, has not been clearly established. Here, we set out to determine the *Blastocystis* spp genotypes present in patients suffering from inflammatory bowel diseases in Uruguay. First, we optimized tools for DNA extraction from fecal samples, and for molecular detection of the agent by PCR amplification of its SSU rRNA. Secondly, to gain insight into the genotypes present in the country, we assessed which *Blastocystis* subtypes were found in these samples by Sanger sequencing and BLAST analyses. We have additionally optimized the culture and cryopreservation of these parasites, generating a biobank of autochthonous isolates. Preliminary results on our study populations suggest that *Blastocystis* is 40% more likely to be detected in patients suffering from IBD than in control patients or patients suffering from unrelated intestinal diseases. The genotype found most often corresponds to ST6 which was detectable in 3 out of 4 positive samples. Overall, our results suggest that patients suffering from IBD could be especially vulnerable to colonization by specific *Blastocystis* genetic subtypes. To gain mechanistic insight into the clinical significance of these findings, we are currently analyzing the correlation between these genetic subtypes, the clinical outcomes in IBD patients, their inflammatory profiles and their associated changes in intestinal microbiota. **Keywords:** Blastocystis spp; Inflammatory bowel diseases; Parasite.

HP-17 - Strategies for the identification of genes involved in the Vertical Transmission of *Toxoplasma gondii*

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The most devastating consequences of toxoplasmosis are connected to the ability of *Toxoplasma gondii* to access and infect vital anatomical sites such as the placenta. Infection during pregnancy can cause miscarriages, stillbirths, premature births and babies born with severe debilitating neuropathies. In Uruguay *T. gondii* is the causative of 75% of abortions from infectious etiology in sheep, causing millionaire loses every year. Despite its importance, the mechanisms underlying transplacental transmission are poorly understood. Genome wide CRISPR screens are powerful tools to identify genes required for processes under selective biological pressure. The strategy involves a parasite population that expresses Cas9 and a single gRNAs per cell, targeting selected genes that are further subjected to a “challenge”. The readout of the experiment involves comparative whole genome sequencing pre and post challenge. Through the identification of gRNA profiles of each population genes that are lost or not upon the specific challenge are postulated as essential or dispensable candidates. In this project, we propose to use this approach to identify apicomplexan factors required for surviving the biological pressures upon vertical transmission strategies. The identification of parasite genes that are essential for transplacental passage may contribute to the development of prevention, control, and prophylaxis strategies to mitigate congenital toxoplasmosis and other related apicomplexan diseases. Furthermore, the work pipeline and resourced developed within this project represent a potential starting point for additional *in vivo* or *in vitro* experiments addressing other biological questions regarding the pathogenic mechanisms of parasites in this phylum.

Keywords: Vertical transmission; Placental tropism; CRISPR-Cas9 genome wide libraries.

HP-18 - Unveiling the Infectiveness-associated Proteins of *Trypanosoma cruzi* G and CL Strains: Insights from Proteomics, Phosphoproteomics, and N-Linked Glycoproteomics

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Trypanosoma cruzi extracellular amastigotes (EAs) are able to infect cultured cells and animals, establishing a sustainable infective cycle. EAs are either prematurely released from infected cells or generated by the extracellular differentiation of released trypomastigotes. Among the EA stage, *T. cruzi* I strains (such as the G strain) are more infective than *T. cruzi* II and VI strains (such as the Y and CL strains). Here we aimed to perform a comprehensive proteomics – including phosphoproteomics and N-linked glycoproteomics - of both EA G and CL strains. EA samples were obtained by differentiation of TCT forms in acid medium (pH 5.8), with 90-95% purity. Samples were lysed and digested with trypsin, labeled with TMT10plex (ThermoFisher) and enriched for phosphopeptides and glycopeptides (TiO₂ and deglycosylation with PNGaseF/SialidaseA). Sample complexity was reduced by pre-fractionation using high pH reverse phase chromatography (HpH-RP; Dionex Ultimate 3000). The LC-MS/MS analyses were done using the Exploris 480 mass spectrometer (ThermoFisher). Results were analyzed in the Maxquant program (V. 1.8.6.0) using a database of 47847 sequences for the G and CL strains, obtained from TritypDB database. The data was statistically analyzed in Perseus (V. 1.8.6.0) and DanteR (V. 1.0.0.10) programs. A total of 5532 proteins were identified from the total proteome, 785 of these were differentially regulated in the G strain, and 561 in the CL strain (P-value < 0.01). For the phosphoproteome and glycoproteome, 4959 phosphopeptides (2298 proteins) and 935 N-glycopeptides (666 proteins) were identified, respectively. Differentially regulated proteins play roles in the invasion process and establishment of infection in the host, such as trans-sialidase and mucin families, as well as calpain, cruzipain, and mevalonate kinase. This study shows, for the first time, the proteins potentially associated with the strains' infectiveness.

Keywords: Extracellular Amastigotes T; cruzi; Phosphoproteomics; Glycoproteomics.

HP-19 - DEVELOPMENT OF RNA VACCINES FOR LEISHMANIASIS AND COMPARISON WITH VACCINES BASED ON RECOMBINANT ANTIGENS

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Despite intensive efforts and studies in animal models indicating variable levels of protection achieved by immunization with defined subunit vaccines, to date, no vaccine for human leishmaniasis is available. Because, as demonstrated during COVID-19 pandemic, RNA vaccines have proven to be a technological breakthrough, we decided to develop an RNA vaccine and compare its protection level with the immune response and protection obtained with a recombinant vaccine based on the same antigen. The protein associated with kinetoplast (PAK), identified after two-dimensional gel analysis and mass spectrometry of *L. amazonensis* proteins using serum from mice immunized with total extract of the parasite, was selected as a target antigen. Immunization of mice with recombinant PAK, named DTL8, was able to generate a Th1 response that partially reduced the parasite load of animals after challenge with *L. infantum*. In vitro transcribed DTL8 RNA containing the appropriated 5' and 3' UTRs and a poly-A tail encapsulated in a lipidic nanoparticle (LNP) formulation was used to immunize C57BL/6 and BALB/c mice. In contrast to a weak antibody response in BALB/c mice, immunization of C57BL/6 mice with DTL8 RNA resulted in the induction of higher levels of antibodies compared to immunization with recombinant DTL8. To investigate whether the composition of the LNP influences antibody production, we immunized mice with the DTL8 RNA encapsulated in our LNP formulation or in a LNP formulation present in the Moderna Covid-19 RNA vaccine, but no differences in the antibody levels were observed. After challenging immunized BALB/c mice with *L. infantum*, only animals immunized with recombinant DTL8 were partially protected against the infection, a result that is probably due to the low antibody levels observed in this animal model. To evaluate the protection against infection with *L. amazonensis*, we are currently immunizing C57BL/6 mice with DTL8 RNA and with the recombinant antigen. **Supported by:** Cnpq

Keywords: Leishmaniasis; RNA vaccine; LNP formulation.

HP-20 - Selective and synergistic leishmanicidal action: an evaluation of repositioned and new compounds isolated and combined

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The search for safe and orally administered therapies persists as a demand for cutaneous leishmaniasis. Drug repositioning and combination are promising strategies to reduce development time, cost, and risk, as well as increase efficacy and reduce toxicity. Thus, we investigated the *in vitro* activity of POD1, AM and NAM (under patent secrecy) isolated and combined. For this, promastigotes of *Leishmania amazonensis* PH8 and J774 macrophages untreated or treated with different concentrations of compounds were used, serially diluted in 96-well microplates. The Alamar Blue assay was used to assess cell viability after 24h and to determine the IC₅₀, CC₅₀ and selectivity index (SI). Combinations were performed using the fixed proportions method to determine FIC and $\bar{x}\Sigma$ FIC, analyzed according to Odds, and plotted in isobolograms. The IC₅₀ values of POD1, AM and NAM in promastigotes were 317.06, 61.44 and 5.42 μ M, respectively. While the CC₅₀ in J774 cells were 1187.00, 241.50 and 194.20 μ M, in that order. Therefore, the following SI were obtained: SI_{POD1} = 3.74, SI_{AM} = 4.0, SI_{NAM} = 35.93. In combination, the IC₅₀ of POD1 (19.95 μ M), AM (1.37 μ M) and NAM (0.90 μ M) reduced by 16, 45 and 6-fold as compared to their isolated actions. All POD1:AM combinations based on FIC₅₀ were synergistic (≤ 0.5), as were the 3:2 and 2:3 POD1:NAM ratios. High synergistic activities (≤ 0.1) were achieved in combinations 4:1 in FIC₂₅ POD1:AM and 3:2 in FIC₇₅ POD1:NAM. The other proportions were also synergistic or additive (> 0.5 or ≤ 4.0), with no antagonistic combination (> 4.0). Analysis of $\bar{x}\Sigma$ FIC₅₀ demonstrated that the combination POD1:AM was synergistic (0.29) and POD1:NAM additive (0.90). Together, our data corroborate the potential of combinations to reduce concentrations and increase the leishmanicidal effect. **Supported by:** Fiocruz, IOC, CNPq, CAPES, FAPERJ, PPSUS **Keywords:** Leishmaniasis; Repositioned drugs; Drug combination.

HP-21 - PI(3,4,5)P3-dependent allosteric regulation of repressor-activator protein 1 (RAP1) controls antigenic switching in African trypanosomes

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African trypanosomes evade host immune clearance by antigenic variation expressing a surface coat of variant surface glycoproteins (VSGs). They transcribe one out of hundreds of VSG genes at a time from telomeric expression sites (ESs) and periodically change the VSG expressed by transcriptional switching or recombination. The mechanisms underlying the control of VSG switching and its developmental silencing remain elusive. We report that telomeric ES activation and silencing entail an on/off genetic switch controlled by a nuclear phosphoinositide signaling system. This system includes a nuclear phosphatidylinositol 5-phosphatase (PIP5Pase), its substrate PI(3,4,5)P3, and the repressor-activator protein 1 (RAP1). Cross-linking and mass spectrometry analysis revealed that these proteins are part of an interaction network including DNA/RNA-binding proteins, protein kinases, phosphatases, and nuclear lamina proteins, implying an ES signaling complex compartmentalization at the nuclear periphery. ChIP-seq assays show that RAP1 binds silent ESs at sequences flanking VSG genes, namely 70 bp and telomeric repeats. Gel shift and binding kinetics confirmed that RAP1 binds directly to 70 bp or telomeric repeats, which occurs via its Myb and Myb-like domains. In contrast, we found a villin headpiece domain in the N-terminus of RAP1 and show this region binds PI(3,4,5)P3. PI(3,4,5)P3 acts as an allosteric regulator of RAP1, and its binding removes RAP1 from 70 bp or telomeric repeats. Transient expression of catalytic inactive (D360A/N362A) PIP5Pase, which cannot dephosphorylate PI(3,4,5)P3, results in the accumulation of RAP1-bound PI(3,4,5)P3 in bloodstream forms. Importantly, ChIP-seq and RNA-seq in these cells confirmed the displacement of RAP1 from silent ESs *in vivo*, resulting in silent ES transcription and activation of VSG gene switching. The data provides a mechanism controlling reversible telomere silencing essential for the periodic switching in VSG expression. **Supported by:** Canadian Institutes of Health Research grant CIHR PJT-175222 (IC); The Natural Sciences and Engineering Research Council of Canada grant RGPIN-2019-05271 (IC); Fonds de Recherche du Québec - Nature et Technologie grant 2021-NC-288072 (IC); Canada Foundation for Innovation grant JELF 258389 (IC)

Keywords:Antigenic variation;transcription;nucleus.

HP-22 - In vitro analysis of Anilinoquinazolines: new perspectives for the treatment of toxoplasmosis.

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Toxoplasmosis is an important neglected disease related to congenital malformations, retinochoroiditis, and encephalitis in immunocompromised patients. Conventional treatment with sulfadiazine and pyrimethamine has limitations, such as toxic side effects, potential cases of resistance, and inactivity against the chronic phase of the infection. Therefore, new, and more selective compounds for the treatment of acute and chronic toxoplasmosis are needed. In this study, we tested the effect of six Anilinoquinazolines derivatives, a new group of compounds derived from quinazoline, which have recently been explored as antineoplastic and antiparasitic drugs.

Different concentrations (1, 2, and 5 μ M) of anilinoquinazolines (1A-6A) were tested in an antiproliferative assay with the highly virulent RH strain (Type I) in cultures of NHDF cells infected with tachyzoites for 24h. The cytotoxic effect of these compounds against host cells was evaluated by the MTS/PMS assay using concentrations of up to 10 μ M for 7 days. The 50% of effective parasite inhibition concentration (IC50) and the 50% cytotoxic concentration for the host cells (CC50) were calculated to obtain the Selective Index (SI), calculated as the CC50/IC50 ratio.

All six compounds tested were active against *T. gondii* tachyzoites with IC50 at the nano and micromolar range (0.73-2.9 μ M) after 24h of treatment. For four compounds (1A-4A), SI ranged from 9 to 68. Three of the six compounds (1A, 2A, and 4A) had an IC50 less than 1 μ M and SI greater than 20, values recommended by the World Health Organization. In addition, the analysis of the physicochemical properties of these compounds showed that they do not violate Lipinsky and Veber's rules, being favorable for future oral use. Thus our results confirmed that anilinoquinazolines control the growth of tachyzoites of *T. gondii*, pointing to a new class of compounds with the potential for developing a future treatment for toxoplasmosis. **Keywords:**Toxoplasma gondii;toxoplasmosis;treatment.

HP-23 - Screening of the antileishmanial activity of 103 extracts of endophytic fungi collected in the Amazon rainforest

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The treatment for leishmaniasis has few therapeutic options and recommended drugs are highly toxic, costly, and the treatment has long-lasting. Thus, the discovery of new therapeutic options has global importance. This work aimed to evaluate the *in vitro* anti-*Leishmania* potential of 103 extracts of endophytic fungi isolated from *Arrabidaea chica* (Bignoniaceae), popularly known as Crajiru. The fungi were isolated from the leaves and branches of different trees in the Amazon rainforest and are deposited in the Microbiological Collections Center (CCM/UEA). The screenings were carried out with *Leishmania* (*L.*) *amazonensis* (MHOM/BR/1973/M2269). The promastigotes forms were incubated for 24h with each extract, and the cell viability was assessed with PrestoBlue® dye. Extracts that showed the lowest IC₅₀ values in the promastigote test were evaluated for their ability to eliminate intracellular amastigote form in infected murine macrophages. Parasite cell cycle alteration and cell death were performed by flow cytometry using Annexin V markers and Propidium Iodide. The IC₅₀ values of the 103 tested extracts ranged from 67.1±1.66 µg/mL±SD to values greater than 1250 µg/mL, while the reference drug Pentamidine showed an IC₅₀ value of 0.38±0.09 µg/mL±SD. Two extracts showed lower IC₅₀ values (67.1±1.66 and 71.4±3.06 µg/mL±SD) and maintained their stability in the three independent experiments. Cytotoxicity tests were performed with murine macrophage RAW 264.7, showing CC₅₀ values thirteen times higher than the IC₅₀ values for the extracts and up to six times for the standard drug, showing greater toxicity to the parasite and not the host cell. These extracts showed a slight antioxidant activity and no antimicrobial effects. Identification of chemical compounds present in the extracts, their mechanism of action, and *in vivo* analyses will contribute to better evaluating the possibility of using these extracts/derived molecules in therapy for cutaneous leishmaniasis. **Supported by:**FAPESP/CNPQ/FAPEAM

Keywords:Leishmania;extracts;endophytic fungi.

HP-24 - *In vitro* susceptibility of macrophages obtained from *T. crassiceps* parasitized mice to *Leishmania major* and *L. braziliensis*

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For the development of leishmaniasis, macrophage must be infected by parasites. While the killing of *Leishmania* is associated with classically activated macrophages (CAMs), the survival and dissemination of parasites are associated with the presence of alternatively activated macrophages (AAMΦ). AAMΦ can be obtained by different methods, including recuperation of cells from helminthic infected mice. In this work, macrophages were obtained from mice injected with Thioglycolate (TG) or mice previously infected with *Taenia crassiceps* cysticerci (TC). The cells were characterized and used to evaluate the susceptibility to *Leishmania* (*L.*) *major* and *Leishmania* (*V.*) *braziliensis* infection. TC macrophages produced less NO after LPS stimulus and presented higher arginase activity when compared to TG macrophages. Additionally, TC macrophages were more susceptible to infection by *L. (L.) major* and *L. (V.) braziliensis* when compared to TG macrophages. The higher susceptibility of TC macrophages to *Leishmania* infection did not allow *L. (L.) braziliensis* to proliferate inside macrophages when infected macrophages were cultured for 10 days. In contrary, *L. (L.) major* proliferated inside TG and TC infected macrophage in the same period. Our data demonstrate that AAMΦ derived from helminthic infection are more susceptible to *Leishmania* infection, but control proliferation of *L. (L.) braziliensis*. **Supported by:**FAPEG (202110267000628); CAPES (001); INCTMCTI/CNPQ/Universal (14/2014)

Keywords:Leishmania;Cysticerci;Alternatively activated macrophages.

HP-25 - CRK3 role in modulation pathways of cell cycle and metacyclogenesis in *Trypanosoma cruzi*

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Trypanosoma cruzi, a clinically relevant protozoan parasite responsible for Chagas disease, undergoes several phenotypic and biochemical changes throughout its life cycle, indicating an intricate regulation of its cellular mechanisms. Understanding the interruption of the cell cycle in the infective (quiescent) forms and its reactivation in replicative stages is of great interest not only in the field of cell cycle research but also in areas such as pharmacology, as dormant amastigote parasites exhibit resistance to current treatments. Therefore, this study aims to characterize how CRK3 (Cdc2-related kinase 3), a kinase proven to take part in the cell cycle, modulates the cell cycle and life cycle of *T. cruzi* using techniques such as CRISPR/Cas9 DNA editing for knock-in and knock-out, cell cycle synchronization with hydroxyurea, immunofluorescence, and *in vitro* metacyclogenesis. Two *T. cruzi* strains were generated, with one strain expressing the Myc tag at the N-terminal of CRK3, while the other strain has a Myc tag at the C-terminal of CRK3. We validated these strains through sequencing, western blot, and growth curve analysis. With these strains, we observed no difference in the expression of CRK3 throughout the cell cycle of epimastigotes. However, in metacyclic trypomastigotes, the expression of CRK3 significantly decreased or was completely absent during metacyclogenesis. CRK3 was predominantly localized in the cytoplasm but exhibited discrete nuclear localization in some parasites. There was no difference in expression and localization between the two strains. These findings suggest that the regulation of CRK3 occurs predominantly through post-translational mechanisms, which involve nuclear-cytoplasmic shuttling and the involvement of other regulatory proteins, such as cyclins, CRK inhibitors, and possibly other unidentified proteins.

Supported by: FAPESP - 2022/05264-5 **Keywords:** CRISPR/Cas9; *Trypanosoma cruzi*; Ciclo Celular.

HP-26 - COMPARISON BETWEEN LIGHT MICROSCOPY COUNTING AND PROMASTIGOTE RECOVERY ASSAY TO ASSESS PARASITE BURDEN IN IN VITRO INFECTIONS WITH LEISHMANIA.

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INTRODUCTION: Light microscopy counting (LMC) and promastigote recovery assay (PRA) are classical methods of assessing parasite burden (PB) in *in vitro* infections with *Leishmania* parasites **OBJECTIVE:** Analyse the limitations and strengths of each PB assessment method using *in vitro* infection of macrophages with *L. major* (Lm) or *L. braziliensis* (Lb). **METHODS:** Macrophages from thioglycolate-elicited BALB/c mice were infected with *Lb* or *Lm* to evaluate PB 3h, 72h, 144h and 216h post infection (PI). LMC assay was conducted after staining cells to access percentage of infected cells (%IC) parasites per infected macrophage (P/C) infection index (II) and infection score. PRA was evaluated after incubation of infected macrophages in grace's insect medium at 25°C for 3 days (*Lm*) or 5 days (*Lb*) for each time PI. **RESULTS:** The LMC method was efficient in distinguishing the susceptibility of BALB/c macrophages to *Lm* infection evidencing PB growth from 3h to 72h, 144 and 216h PI respectively (%IC= 54,38±10,41%; 65,67±11,24%; 65,52±14,43%; 69,32±6,98%) (P/C= 2,05 ±0,58, 3,79±0,83; 4,62±0,91; 7,31±0,85) (II= 108,42 ±25,57; 251,50±78,39; 302,28±79,33; 510,09±99,13). It was also efficient in demonstrating macrophage resistance towards infection with *Lb* species with the decrease of PB from 3h to 144h and 216 PI respectively (P/C = 3,59±0,33; 2,33±0,18; 2,26±0,18) (II= 223,01±58; 107,80±21,71; 101,37±20,06). This method also allowed the evaluation of different cell populations that hosted varying quantities of amastigotes through different score for both parasites. The PRA efficiently demonstrated the contrasting phenomena of PB in infections with both parasite species but with parasite growth only being observed after 216h PI on *Lm* infections and parasite decrease after 144h on *Lb* infections. **CONCLUSION:** Both tests can distinguish the phenomena of susceptibility or resistance of macrophages to different leishmania species, but they offer more valuable information when used together. **Supported by:** INCT/MCTI/CNPQ/Universal 14/2014 **Keywords:** *Leishmania braziliensis*; *Leishmania major*; Peritoneal macrophages.

HP-27 - Vps32 plays an important role in endocytic traffic and cell cycle progression in *Trypanosoma brucei*

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Trypanosoma brucei is the causative agent of African trypanosomiasis in humans and cattle. This parasite presents two different developmental stages: the tse-tse fly procyclic form (PCF) and the mammalian bloodstream form (BSF). Stage differentiation is critical for successful life cycle progression, while endocytosis, exocytosis and autophagy are crucial to guaranteeing their survival. Endosomal Sorting Complex Required for Transport (ESCRT) consist of a series of complexes integrated by many proteins responsible for vesicles formation during intracellular transport. In trypanosomatids the most engaging is ESCRT-III, which is highly preserved in eukaryotes. A member of this complex, Vacuolar Protein Sorting 32 (Vps32) plays important roles in cytokinesis and endocytic traffic, as demonstrated in human and yeast models. Previously in our laboratory, we identified the Vps32 orthologue in *T. brucei* (TbVps32) and observed that TbVps32 downregulation decreases endocytosis and affects intracellular transport. In our current work our aim is to further investigate the role of this protein in the PCF stage. To achieve this we have obtained two different cell lines: one that over-expresses TbVps32 under a Tet-inducible regulatory system (HA-TbVps32) and another in which the protein expression can be silenced by an inducible interference RNA system (TbVps32-iRNA). With these cell lines, we have studied TbVps32 localization and dynamics. Furthermore, we are focusing our studies on determining the precise step in which TbVps32 is participating during endocytic traffic. In conclusion, we have demonstrated that both TbVps32 over-expression and silencing impairs cell proliferation, and its downregulation results in severe abnormal nucleus-kinetoplast configurations. Overall we propose that TbVps32 participates in endocytic traffic to the lysosome and is essential for *T. brucei* survival. **Supported by:**Proyectos de Investigación Plurianuales (PIP) 2021-2023. CONICET. Nro. 03073. "Señalización y regulación del ciclo celular en epimastigotes de *Trypanosoma cruzi*" **Keywords:**ESCRT;TbVps32;procyclic form.

HP-28 - Advancing Drug Discovery for Leishmaniasis: Targeting the Proteasome 20S with AI-driven Compound Selection

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Proteasomes are protein complexes that act as the main proteolytic system in eukaryotic cells, degrading unneeded or damaged proteins into small peptides that are recycled as amino acids. If inhibited, proteasomes lead to the accumulation of ubiquitinated proteins and vesicles, triggering an autophagic cell death pathway. Through a collaborative effort together with Atomwise, we have pre-selected potential anti-leishmanial compounds utilizing their proprietary technology, AtomNet®. For target-based hit discovery, a molecular library of million compounds was virtually screened against Leishmania proteasome 20S. A total of 84 potential compounds were selected as active against *L. infantum*. Further phenotypic screening against *L. amazonensis* and *L. braziliensis* intracellular amastigotes filtered the 20 most active compounds. Most of the selected compounds were nontoxic to THP-1 derived human macrophages. To confirm proteasome inhibition, a cell-based proteasome 20S activity assay was conducted using two compounds 1 [C1] and 4 [C4]. The assay measured the chymotrypsin-like (CT-L), trypsin-like (T-L), and caspase-like (C-L) proteases activity associated with the proteasome. Marizomib was used as proteasome inhibition positive control. Marizomib presented proteasome inhibition [IC50 = 0.72 to 1.43 nM] associated with CT-L protease activity for all Leishmania species. T-L and C-L protease activities were inhibited in a less expressive manner [IC50 = 2 to 458 nM]. C1 inhibited CT-L and C-L activities with IC50 of 1.47 and 751 nM, respectively; while T-L activity was inhibited by C4 at 1 µM, only on *L. amazonensis*. This suggests the presence of different targets in *L. braziliensis* and *L. infantum*. Since structural analysis revealed similarities among proteasome 20S from different trypanosomatids, these lead compounds could also be tested against human and animal trypanosomiasis. Through collaborative efforts, it is possible to accelerate the drug discovery process. **Supported by:**Fapemig, CAPES, CNPq, Fiocruz **Keywords:**Leishmania; Proteasome 20S inhibition;Drug discov.

HP-29 - Transcriptomic analysis of the Interferon pathways in macrophages during *Neospora caninum* and *Toxoplasma gondii* infections

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Neospora caninum and *Toxoplasma gondii* are obligatory intracellular parasites that affect a wide range of animals. Interferon-gamma (IFN- γ) is known to be fundamental in the immune response against these parasites. However, there is little or no data about the roles of type I and III Interferon pathways in controlling these infections. Therefore, our study aimed to compare, through transcriptome analysis, the differential gene expression of Interferon type I, II, and III pathways during the infection by *T. gondii* and *N. caninum*. For that purpose, we conducted RNASeq analysis of marrow-derived macrophages obtained from C57BL/6 mice, infected with both parasites, for 6 hours, and compared the gene expression of the targeted pathways using TPM (transcriptions per million) values. Using that approach, we found that the expression of most of the genes in the interferon pathways was increased during the infection with *N. caninum*, compared to the non-perturbed or downregulated genes. We observed a distinct profile in response to *T. gondii* infection, with a higher percentage of genes that did not undergo changes in expression. This phenotype was especially noted in type I and III Interferon pathways. Heatmap analysis of single genes divided by pathways further corroborated these observations, showing significant alterations in critical genes. These results highlight that, despite the phylogenetic similarities between these parasites, there are striking differences in host-parasite interactions, which may account for their distinct pathogenesis in mice and other hosts. **Supported by:** CNPq (313761/2020-5), FAPEMIG (RED-0313-16) **Keywords:** Innate immunity; RNASeq; Signaling pathways.

HP-30 - EFFICACY OF INTRALESIONAL MEGLUMINEANTIMONIATE IN THE TREATMENT OF CANINE TEGUMENTARY LEISHMANIASIS: A RANDOMIZED CONTROLLED TRIAL

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Background: Dogs living in areas of *Leishmania (Viannia) braziliensis* transmission may present canine tegumentary leishmaniasis (CTL) characterized by cutaneous or muzzle ulcers as well as asymptomatic *L. braziliensis* infection. It is not clear if dogs participate in the transmission chain of *L. braziliensis* to humans. However, dogs may remain with chronic ulcers for a long time, and as there are no public policies about CTL, these animals die or are sacrificed. **Objective:** We compare the efficacy of intralesional meglumine antimoniate with intralesional 0.9% NaCl solution in CTL treatment. **Methods:** This randomized control study included 32 dogs with cutaneous or muzzle lesions who had *L. braziliensis* DNA detected by PCR in tissue biopsied. Group one received 5ml of intralesional meglumine antimoniate, and group two received 5ml 0.9% NaCl solution, both applied in the four cardinal points on days 0, 15, and 30. Cure was defined as complete healing of the ulcers in the absence of raised borders on day 90. **Results:** There was no difference in animals' demographic and clinical features in the two groups ($p > .05$). While at the endpoint, the cure rate was 87.5% in the group test, in those who received 0.9 NaCl the cure rate was only 12.5% ($p < 0.01$). As important as the high cure rate, the healing time was faster in dogs treated with meglumine antimoniate than in those treated with saline ($p < .001$). **Conclusion:** Intralesional meglumine antimoniate is effective in the treatment of dogs with *L. braziliensis* infection and accelerates the healing time of CTL. **Supported by:** Instituto Nacional de Ciência e Tecnologia em Doenças Tropicais (INCT-DT), CNPq #465229/2014-0 **Keywords:** Leishmania braziliensis; Canine tegumentary leishmaniasis; Treatment of canine tegumentary leishmaniasis.

HP-31 - THE ROLE OF DOGS IN THE TRANSMISSION OF *Leishmania braziliensis* IN AN ENDEMIC AREA OF AMERICAN TEGUMENTARY LEISHMANIASIS

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Background: Cutaneous leishmaniasis (CL), caused by *Leishmania braziliensis*, is the most important presentation of American Tegumentary Leishmaniasis (ATL). Little is known about the importance of dogs in the transmission of *L. braziliensis* to humans. **Objectives:** In the present study, we determine the frequency of *L. braziliensis* infection in dogs with cutaneous and mucosal ulcers in Corte de Pedra, Bahia, Brazil, an endemic area of CL, describe the clinical manifestations and histopathologic features, and determine if the parasites isolated from dogs are genetically similar to those found in humans. Also, we determine the prevalence and incidence of both cutaneous tegumentary leishmaniasis (CTL) and subclinical (SC) *L. braziliensis* infection in dogs and evaluated if the presence of dogs with the CL or SC infection is associated with the occurrence of ATL in humans. **Methods:** SC infection and CTL were determined by PCR on biopsied healthy skin or on the ulcer or by detecting antibodies against soluble leishmania antigen (SLA). To determine if *L. braziliensis* infection in dogs was associated with human ATL we compared the occurrence of ATL in homes with or without dogs with CTL or SC infection. **Results:** The PCR was positive in 41 (67%) of animals, and the lesions in the snout, followed by the testicles and ears were the sites where parasite DNA was most detected. There were genotype similarities between *L. braziliensis* isolates from dogs and humans. The prevalence of SC infection was 35% and of CTL 31%. The incidence of SC infection in dogs was 4.6% and the incidence of CTL was 9.3%. The frequency of ATL in humans in homes with infected dogs was 50% (38/76), while it was 13% (7/56) in homes without evidence of *L. braziliensis* infection in dogs ($P < 0.01$). **Conclusions:** CTL and SC infection are highly prevalent, and dogs may participate in the transmission chain of *L. braziliensis*. **Supported by:** Instituto Nacional de Ciência e Tecnologia em Doenças Tropicais (INCT-DT), CNPq #465229/2014-0
Keywords: Canine tegumentary leishmaniasis; *Leishmania braziliensis*; Canine subclinical infection.

HP-32 - Evaluation of the amount of ROS in *T. cruzi* intracellular multiplication

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Upon infection with *T. cruzi*, host cells respond producing reactive oxygen species (ROS). Previous studies have shown that cardiomyocyte (CM) infection with *T. cruzi* JG strain (*T. cruzi* II) induces higher amounts of ROS, as well as higher amounts of intracellular parasites, when compared to those infected with *T. cruzi* Col1.7G2 strain (*T. cruzi* I). Additionally, inhibition of ROS was able to decrease the number of JG strain intracellular parasites, but not Col1.7G2. These results together suggested that, at least to some *T. cruzi* strains, ROS production might be signaling to the parasite and contributing to increasing its intracellular multiplication rate. We have also shown that Col1.7G2 produces more antioxidant enzymes, suggesting that it may be less affected by an oxidative environment. Whether ROS induction of intracellular multiplication is related to the parasite strain or just the amount of ROS is still not known. To test this, we evaluated *T. cruzi* Y strain (*T. cruzi* II) intracellular multiplication after H9c2 cardiomyocyte pre-treatment with different concentrations of H₂O₂. Using CM-H2DCFDA, a fluorescent indicator of ROS production, we showed that cells treated with 15 or 30 μM H₂O₂ presented 5- and 6-times higher amounts of ROS, respectively, when compared with the control, non-treated. We then infected H₂O₂ pre-treated cells and evaluated parasite intracellular multiplication 48h and 72h post parasite exposure. At 48 hours, the number of intracellular parasites was higher in cells treated with 30 μM H₂O₂, when compared to cells treated with 15 μM, being both higher than in non-treated cells. At 72h, the number of intracellular parasites was higher in cells treated with 15 μM H₂O₂, when compared to 30 μM H₂O₂ treated and non-treated cells. For the latter, the number of parasites seems to stagnate. This may indicate that the amount of ROS play a major role in parasite intracellular multiplication.

Keywords: *T. cruzi*; reactive oxygen species; intracellular multiplication.

HP-33 - Evaluation of the susceptibility of Brazilian isolates of *Toxoplasma gondii* to drugs used in the treatment of toxoplasmosis

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Toxoplasma gondii, the etiological agent of toxoplasmosis, is an obligate intracellular protozoan. Brazil has a great diversity of recombinant or unusual strains, which gives the species a high genetic variability in the country, which differ considerably from those that circulate in Europe and North America. Little is known about the susceptibility profile of Brazilian strains in the face of conventional chemotherapy (Reynolds et al., 2001; Doliwa et al., 2013). Thus, the objective of this study was to evaluate the effectiveness of the drugs pyrimethamine and sulfadiazine. Conventionally used for treatment in humans, to control the infection of three atypical strains of *T. gondii* (TgCTBr12, TgCTBr14 and TgCTBr18), isolated from congenitally infected children in in vitro assays. In vitro susceptibility assays showed that pyrimethamine at concentrations of 0.25 μM , 0.5 μM , 1 μM and 2 μM helped to reduce parasite proliferation in the three studied strains. The IC₅₀ value (mean inhibitory concentration) of pyrimethamine observed for the TgCTBr14 strain was 0.1469 μM , for the TgCTBr18 strain it was 0.06209 μM and for the TgCTBr12 strain it was 0.02913 μM . However, the strains studied are not susceptible to the different sulfadiazine doses tested (125 μM , 250 μM and 500 μM). Of the conventionally used drugs, pyrimethamine can be used to treat infection by the TgCTBr12, TgCTBr14 and TgCTBr18 strains of *T. gondii*. Furthermore, it would be interesting to evaluate the effectiveness of the association between these two drugs at different concentrations. New studies, which evaluate a treatment time greater than 48 hours, also need to be carried out, to confirm the lack of efficacy of sulfadiazine. **Supported by:** Conselho Nacional de Pesquisa (CNPq), Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) e Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). **Keywords:** *Toxoplasma gondii*; Atypical strains; Pattern of susceptibility.

HP-34 - NEUTROPHIL ELASTASE AND CATHEPSIN G CONTRIBUTE TO MACROPHAGE INFECTION BY *Leishmania amazonensis*

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Leishmania amazonensis depends on the production of type I interferons to establish an infection in macrophages. Our group has shown that the activity of a host serine peptidase, neutrophil elastase (NE), leads to the activation of a pathway involving Toll-like receptor 4 (TLR4) upon *Leishmania* spp. infection in macrophages, resulting in the production of IFN- β from these cells. While NE and IFN- β are detrimental to *L. major*, *L. donovani* requires NE activity and the resulting IFN- β for efficient growth in macrophages. We therefore asked whether *L. amazonensis* was, likewise, dependent on the components of this pathway by investigating parasite internalisation by macrophages (at 3 hours). We found that parasites were internalised significantly less by macrophages from NE-deficient mice than those from the genetic background strain. A similar profile was observed when TLR4 was inhibited prior to *L. amazonensis* infection. Taking this further, we investigated the involvement of another host serine peptidase of the same family, cathepsin G (CG), in the phagocytosis of *L. amazonensis*. As was seen in the absence of NE, when CG was inhibited prior to infection, *L. amazonensis* was internalised at a lower rate. Overall, we show that *L. amazonensis* requires both NE and CG for the initial stage of host cell infection, and that uptake of *L. amazonensis* is dependent on TLR4. **Supported by:** FAPERJ; CNPq **Keywords:** *Leishmania*; Elastase Neutrofílica; TLR4.

HP-35 - *Leishmania* spp. expressing red fluorescence (*tdTomato*) as model for screening of anti-*Leishmania* compounds

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The main challenges associated with leishmaniasis chemotherapy are drug toxicity, the possible emergence of resistant parasites, and a limited choice of therapeutic agents. Therefore, new drugs and assays to screen and detect novel active compounds against leishmaniasis are urgently needed. We thus validated *L. braziliensis* and *L. infantum* that constitutively express the tandem tomato red fluorescent protein (*tdTomato*) as a model for large-scale screens of anti-*Leishmania* compounds. Confocal microscopy of *Lb* and *Li::tdTomato* revealed red fluorescence distributed throughout the entire parasite, including the flagellum, and flow cytometry confirmed that the parasites emitted intense fluorescence. We evaluated the infectivity of cloned promastigotes and amastigotes constitutively expressing *tdTomato*, their growth profiles in THP-1 macrophages, and susceptibility to trivalent antimony, amphotericin (AmB), and miltefosine *in vitro*. The phenotypes of mutant and wild-type parasites were similar, indicating that the constitutive expression of *tdTomato* did not interfere with the evaluated parameters. We applied our validated model to a repositioning strategy and assessed the susceptibility of the parasites to eight commercially available drugs. We also used our new model to screen 32 natural plant and fungal extracts and 10 pure substances to reveal new active compounds. The mutant parasites expressing *tdTomato* could serve as a model for assays to evaluate the susceptibility of anti-*Leishmania* compounds *in vitro*. The infectivity of mutant and wild-type parasites in BALB/c mice were similar. Standardizing our methodology would offer more rapid, less expensive, and easier screens of compounds against *L. braziliensis* and *L. infantum in vitro* and *in vivo*. Our method could also enhance the discovery of active compounds for treating leishmaniasis.

Supported by: Programa INOVA FIOCRUZ - Fundação Oswaldo Cruz (VPPCB-007-FIO-18-2-94); Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG – APQ 02816-21), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq 304158/2019-4), and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - BRA (CAPES) - Finance Code 001.

Keywords:Leishmania;compound screening;chemotherapy.

HP-36 - Infection of non-phagocytic cells by promastigotes and amastigotes of *Leishmania amazonensis*: a comparative study of host cell invasion and the cellular mechanisms involved in cell entry

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Intracellular parasites are characterized by the need to invade and establish intracellular cycle within host cells. For *Leishmania* spp., the causative agents of Leishmaniasis, it is assumed that the parasites are phagocytosed and live inside professional phagocytic cells. However, the presence of *Leishmania* spp. amastigotes within non-phagocytic cells have been described for decades in animal and human lesions. Although the parasites of the genus *Leishmania* have been known for a long time, we know very little about how they can infect non-phagocytic cells and the possible role of these cells in the biology of the parasite. The objective of this work was to characterize and compare the mechanisms involved in the invasion of non-phagocytic cells by promastigotes and amastigotes of *Leishmania*, both highly infective to mammalian hosts. We developed an infection model using mouse embryonic fibroblasts (MEF) and amastigotes and promastigotes of *Leishmania amazonensis*. We performed time-course infection assays, labelled infected cells and parasites to visualize molecules, organelles and structures possibly involved in cell invasion. Our results show that while promastigotes can invade non-phagocytic cells independently of host cell cytoskeleton, amastigotes are highly dependent on F-actin polymerization, being able to strongly induce this process to promote cell invasion. By comparing the infection rates in time-course experiments it was also possible to conclude that amastigotes are more invasive for non-phagocytes than promastigotes. Thus, our results suggest that the two evolutive forms of *Leishmania* spp. have different mechanisms to invade non-phagocytic cells and that amastigotes, the form of the parasite responsible for infection amplification in the mammalian host, are particularly infective to non-phagocytes.

Supported by:CAPES **Keywords:**Leishmania spp;;Cell Invasion;Non-phagocytic Cells.

HP-37 - Assessing the infectivity of *Leishmania infantum* promastigotes to non-phagocytic cells using HeLa, C2C12 and Mouse Embryonic Fibroblasts as host cell models.

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Intracellular parasites are characterized by the need to invade and establish an intracellular cycle within host cells. For *Leishmania* spp., the causative agents of Leishmaniasis, it is assumed that the parasites are phagocytosed and live inside professional phagocytic cells, notably macrophages. However, the presence of *Leishmania* spp. amastigotes within non-phagocytic cells have been described for decades in animal and human lesions. The presence of *Leishmania* spp. amastigotes in non-phagocytic cells is an overlooked aspect of the biology of these parasites that may contain key elements to understand unsolved gaps of their biology such as parasite persistence and drug resistance, since parasites may be out of reach of drugs and immune effectors while living within these cells. For *Leishmania amazonensis*, the causative agent of human cutaneous leishmaniasis, we have recently demonstrated that promastigotes are able to infect and persist within fibroblasts in which they enter using host cell lysosomes, thus by a non-phagocytic route. Here our objective was to assess the infectiveness of *Leishmania infantum*, a causative agent of human visceral leishmaniasis, to three models of non-phagocytic cells: HeLa, C2C12 and Mouse Embryonic Fibroblasts (MEF). First, we produced GFP-expressing promastigotes of *L. infantum* and compared parasite growth to show that both WT and GFP-expressing promastigotes displayed the same growth pattern. In the sequence, we cultivated HeLa, C2C12 and MEF and performed infection assays using GFP-expressing *L. infantum* promastigotes. After infection, cells were labelled using phalloidin and anti-LAMP1 antibodies which showed that parasites were internalized and established themselves as intracellular amastigotes living within typical intracellular vacuoles rich in lysosomal markers, as expected for *Leishmania* spp. **Supported by:**FAPEMIG **Keywords:**Leishmania infantum;Parasite Life Cycle;Cell Invasion.

HP-38 - Infection of muscle, epithelial and connective cells by *Leishmania amazonensis*: assessing parasite viability and the potential role of non-phagocytic cells as parasite reservoir

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Intracellular parasites are pathogens that need to invade and exert at least part of their life cycles within host cells. For *Leishmania* spp. it is assumed that the parasite is phagocytosed and lives in professional phagocytic cells. However, several works have described in vivo the presence of intracellular stages *Leishmania* spp. in non-phagocytic cells such as muscle, epithelial and connective cells. The presence of *Leishmania* spp. amastigotes within non-phagocytic cells is an overlooked aspect of their biology that may provide important knowledge to understand unsolved gaps such as drug resistance, parasite persistence and late infection reactivation. Our objective with this work was to study the infection of muscle, epithelial and connective cells by *Leishmania amazonensis*, to assess the viability of the parasites inside these cells and evaluate their ability to function as parasite reservoir, ultimately leading to the infection of macrophages. We used light, conventional and confocal microscopies, immunobiochemical methods and flow cytometry to assess *L. amazonensis* infectivity in muscle fibers, epithelial cells and fibroblasts as long as the ability of these cells in producing leishmanicidal effectors such as nitric oxide (NO) and reactive oxygen species (ROS). Our results show that both amastigotes and promastigotes can invade and persist within these cells as typical intracellular amastigotes and that, unlike macrophages, they are unable to produce NO or ROS, thus facilitating parasite persistence. When macrophage-like cells (RAW cells) were co-cultured with the infected non-phagocytic cells, infection spread to the macrophages, demonstrating that parasites could be transferred from one cell to the other—similar to the previously preconized for neutrophils and named as the “Trojan Horse Hypothesis”. Our studies suggest that non-phagocytic cells can function as parasite reservoirs from which the amastigotes can reach the macrophages.

Supported by:CNPq **Keywords:**Leishmania spp.;Cell invasion;Non-phagocytic cells.

HP-39 - Characterization of Nfs, Isd11 and Mtu1: role in the mitochondrial thiolation pathway of tRNAs in *Trypanosoma cruzi*

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One of the key biological features of *Trypanosoma cruzi* is the presence of a single and branched mitochondrion, which harbors the mitochondrial genome (kDNA) in a structure called kinetoplast. The kDNA does not encode for tRNAs, which are imported from the cytoplasm in order to allow mitochondrial protein expression. tRNAs are extensively post-transcriptionally modified, and among those modifications identified so far, thiolation can be found at uridines at positions 33 and 34 of tRNAs. Remarkably, uridine thiolation at position 33 (sU³³) has only been identified in trypanosomatids. It has been shown that sU³³ negatively modulates tRNA^{Trp} editing in the mitochondria of *Trypanosoma brucei*, which directly affects mitochondrial gene expression. The mitochondrial thiolation of tRNAs requires the protein complex formed by the cysteine desulfurase (Nfs; EC:2.8.1.7), a tRNA-specific 2-thiouridylase (Mtu1; E.C.:2.8.1.14) and an accessory protein (Isd11), besides of using the amino acid cysteine as a thiol group donor. The *T. cruzi* genome encodes putative orthologs of genes involved in this pathway. Aiming to investigate the impact of these enzymes on the biology of *T. cruzi*, hemi- and total-knockout strains for the genes putatively involved in the mitochondrial thiolation pathway were generated using CRISPR-Cas9. Phenotypical characterization of Isd11^{-/+} and Mtu1^{-/-} cell lines demonstrated no impact in cell proliferation. However, in Isd11^{-/+} mutants, differentiation of epimastigotes to the metacyclic trypomastigote are 2.6 times more than the control. Additionally, the putative coding sequences of Nfs, Isd11 and Mtu1 were endogenously tagged with mNeonGreen and c-myc tags to allow subcellular localization of these proteins. So far, C-terminus tagging of Nfs showed mitochondrial localization. In *T. cruzi*, little is known about the functioning of the tRNA thiolation pathway, so this study may elucidate the link between amino acid metabolism and regulation of gene expression.

Supported by: FAPESP Proc n.o. 2022/04890-0, Welcome Trust Proc n.o. 222986/Z/21/Z

Keywords: *Trypanosoma cruzi*; CRISPR-Cas9; tRNA.

HP-40 - IN VITRO INFECTION OF PERITONEAL MACROPHAGES WITH *Leishmania braziliensis* PROMASTIGOTES OR AMASTIGOTES FROM IFN-GAMA KNOCKOUT MICE LESION

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INTRODUCTION: *Leishmania* parasites infect the host as promastigotes after sandfly bite, but internally, amastigotes maintain the infection of macrophages during disease development. **OBJECTIVE:** Compare the susceptibility of Thioglycolate-elicited peritoneal macrophages to stationary phase promastigotes or amastigotes from mice lesion. **METHODS:** *Leishmania braziliensis* (*Lb*) parasites were isolated from the hind paw of infected IFN γ Knockout mice or stationary phase promastigote from culture to infect peritoneal macrophages from thioglycolate-elicited BALB/c mice to evaluate parasite burden (PB) defined as percentage infected cells (%IC) parasites per infected macrophage (P/C) and infection index (II) 3h, 72h, 144h and 216h post infection (PI). **RESULTS:** There was a significant decrease in PB in infections with stationary phase promastigotes (SPP) from 3h to 144h PI (P/C = 3,5 \pm 0,3 to 2,3 \pm 0,1; II = 223,0 \pm 58,4 to 107,8 \pm 21,7 respectively). Differently, there was a maintenance of the PB in infections with amastigotes (LA) from 3h and throughout 72h and 144h (%IC = 76,2 \pm 5,4; 79,6 \pm 8,8; 79,0 \pm 5,4) (P/C = 4 \pm 0,8; 5 \pm 1,2; 5,4 \pm 1,1) (II = 315,7 \pm 91,3; 409,8 \pm 140,4; 431,9 \pm 117,8). The %IC was greater in infections with LA during 72h and 144h (%IC = 79,6 \pm 8,8; 79,0 \pm 5,4) when compared to infection with SPP (%IC = 50,8 \pm 5,3; 45,8 \pm 5,7). Interestingly there was a sudden decrease of infected macrophages in infection with LA from 144h to 216h (44,6 \pm 7,7%) matching the %CI with SPP at 216h PI (44,4 \pm 5%). **CONCLUSION:** LA from mice lesion are more adapted to survive inside BALB/c macrophage than SPP.

Supported by: INCT/MCTI/CNPQ/Universal 14/2014 **Keywords:** Leishmaniasis; mice; Parasite burden.

HP-41 - Removal of Complement Membrane Attack Complex by Plasma Membrane Repair in *Leishmania amazonensis*: an ancestral cellular mechanism of Eukaryotes promoting pathogen immune escape.

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Plasma membrane repair (PMR) is a key process present in eukaryotic cells that promotes the resealing of damages eventually inflicted to the plasma membrane (PM). Cells under constant mechanical stress, such as muscle cells, or cells attacked by bacterial pore-forming proteins (PFP) can use this mechanism to prevent lytic death. In the case of attack by PFPs, cells can trigger PMR to remove the pores from their PMs. PMR triggered by PFPs typically involves 1- Ca^{2+} influx; 2- lysosomal exocytosis and 3- pore removal by either endocytosis or membrane budding. The Complement System is one of the first shields of innate immunity of hosts against pathogens. Given that the Membrane Attack Complex (MAC), which is the result of the final activation of the Complement System, is a lytic transmembrane pore, we postulated that mammalian cells and eukaryotic pathogens can use PMR to avoid self-lysis and to evade the immune system, respectively. By using conventional, confocal and live imaging microscopies, immunobiochemical methods and flowcytometry we demonstrated that mammalian cells are able to repair MAC pores by triggering typical PMR responses. Furthermore, and given that the major function of MAC is to pierce the membrane of pathogens, we hypothesized that PMR could be used by eukaryotic parasites, such as *Leishmania* spp. to evade the Complement System by removing MAC pores eventually formed on their PMs. Using fresh human complement and *Leishmania amazonensis* promastigotes we were able to show that PMR is, indeed, conserved in this parasite and that, like mammalian cells, *L. amazonensis* promastigotes can resist complement killing by removing MAC lytic pores from their membranes. Thus, our results show that PMR is evolutionarily preserved in *Leishmania* spp. and that - this physiological mechanism can be used by parasites as an extra weapon to evade the final activation of the Complement System and to escape host's first line of defense. **Supported by:** CNPq, **Keywords:** *Leishmania amazonensis*; Plasma Membrane Repair; Immune evasion.

HP-42 - In silico virtual screening to discovery new natural compound for visceral leishmaniasis chemotherapy

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Treatment for leishmaniasis is still a challenge so is necessary to find orally compounds that are less toxic and more effective. Drug discovery has been relied on the assistance of computational tools, a promising approach to tackle this problem. In this context, the objective of this project was to select natural compounds for a new chemical entity using computational approach based on *L. infantum* trypanothione reductase (TrLi). First, a library with 67 natural products was created, and ADMET parameters was estimated by pkCSM server to identify safe compounds for oral administration. 4 of them fill this criteria and through virtual screening PyRX software, the ΔG value was calculated, ranging from -7.2 to -9.4 kcal/mol, indicating that they interact at the TrLi active site. NC3 presented ΔG values of -9.0 and -8.9 kcal/mol for the oxidized and reduced forms of TrLi, respectively. Calculations performed by Autodock Tools software estimated that NC3 showed an estimated ΔG and K_i values of -7.9 kcal/mol and 1.7 μM for oxidized LiTr, and -8.31 kcal/mol and 805 nM for reduced LiTr, and interacts with residues of the catalytic site. Next, *L. infantum* promastigotes were incubated with different concentrations of NC3 (0.97-2000 μM) for 72 hours, the viable cells were estimated fluorometrically by resazurin and ROS generation by H2DCFDA. NC3 inhibited 98% of the parasites in a dose-dependent manner with an IC_{50} of 194.3 μM and accumulated intracellular ROS level in 3.7 fold related with non-treated group at the highest concentration used (500 μM) with a linear correlation ($R^2 = 0.92$). Finally, a cytotoxicity assay demonstrated a CC_{50} of 483.4 μM and an IC_{50} of 2.6 μM in intracellular amastigote with a selectivity index of 186. Together our data demonstrate that the structure-based virtual screening is an excellent ally for drug discovery, and that compound NC3 is a potential competitive inhibitor of trypanothione reductase and candidate for the treatment of leishmaniasis. **Supported by:** FAPERJ; CNPQ; IOC/FIOCRUZ **Keywords:** Structure-based virtual screening; Trypanothione reductase; Natural products.

HP-43 - Resident and Stimulated Peritoneal Macrophages from mice control proliferation of *Toxoplasma gondii* strain ME-49 in iNOS+ and iNOS- populations

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Toxoplasma gondii is an obligate intracellular parasite of homeothermic vertebrates. This parasite infects nucleated cells including macrophages. Macrophages control proliferation of the parasite through the production of nitric oxide (NO) produced by inducible NO synthase (iNOS). Virulent *T. gondii* strains inhibit NO production and can modulate iNOS expression depending on the type of macrophage. The aim of this work was to evaluate whether *T. gondii* of the ME-49 strain (less virulent) inhibits NO production by modulating iNOS expression in resident peritoneal macrophages (ResMO) and stimulated peritoneal macrophages (StdMO) obtained after peritonitis caused by inoculum of *T. gondii* tachyzoites. Macrophages were obtained by peritoneal lavage, cultured and activated with interferon- γ and lipopolysaccharide for 24h. After activation, they were infected with a 5:1 parasite:macrophage ratio, the production of NO was evaluated using the Griess reagent and iNOS expression was quantitatively and qualitatively evaluated by fluorescence microscopy. ResMO produced around 17 μ M and StdMO produced about 35 μ M of NO. Difference of NO production was not detected after infection of both types of macrophages. By fluorescence microscopy it was observed that after 24h of interaction, iNOS+ infected ResMO increased from 30% to 48%. Infectivity index (IF) dropped from 78.9 to 3.9 in iNOS+ population while ResMO iNOS- dropped from 85.0 to 8.4. After 24h of interaction, StdMO infected iNOS+ changed from 93% to 52% and the IF of the iNOS+ population dropped from 167.8 to 22.8 and in the iNOS- population from 157.1 to 32.2. It is concluded that in 24 hours of interaction, ResMO and StdMO did not change the production of NO. iNOS expression increased in ResMO and decreased in StdMO, but both controlled parasite replication in iNOS+ and iNOS- populations, indicating that this control is independent of iNOS expression. **Supported by:**FAPERJ-UENF **Keywords:***Toxoplasma gondii*;Macrófagos;iNOS.

HP-44 - Modulation of infection by *Toxoplasma gondii* strain ME-49 in M1 macrophages cultivated in collagen I biofilm when compared to rigid substrate

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Conventional cell culture is performed on rigid substrates (SR). However, alternatives using three dimensional substrates are on the rise. Collagen I biofilm (COL I) is an alternative of great importance because collagen is an abundant extracellular matrix protein related to cell growth, motility and regulation. Macrophages are classified according to their activation profile. M2 macrophages induce humoral response and maintain tissue homeostasis. M1 macrophages are more microbicidal, capable of killing intracellular pathogens by high production of reactive oxygen/nitrogen species like nitric oxide (NO). NO acts on the site of infection and reduces the proliferation of parasites such as *Toxoplasma gondii*, which causes Toxoplasmosis, a disease spread worldwide. *T. gondii* grows in host cell in the form of tachyzoites inside parasitophorous vacuoles inducing the acute phase of the disease. We compared NO production and prevalence of infection with *T. gondii* of the ME-49 strain in activated M1 macrophages cultured in COL I and SR. COL I was obtained from rat tail. Raw 264.7 macrophages were cultured on COL I and SR, were M1 activated for 24 h with lipopolysaccharide and interferon- γ , and infected for 24 h with a 1:1 macrophage:tachyzoite ratio. NO was evaluated by the Griess reaction and infection measured by direct count of cells after Giemsa staining. M1 macrophages cultured in COL I produced less NO compared to M1 macrophages cultured in SR. After 24 h of infection, few tachyzoites were seen in the M1 macrophages cultured in COL I, but M1 macrophages cultured in SR controlled the infection, presenting vacuoles without tachyzoites. Thus, M1 macrophages cultivated in COL I are less microbicidal. COL I can be used as a key alternative substrate to provide a possible more realistic response of the immune system's functionality and may constitute an interesting culture substrate to study the interaction of host cells with pathogens.

Supported by:FAPERJ, CAPES **Keywords:**Collagen ;Macrophage;*Toxoplasma gondii*.

HP-45 - **ANTAGONIC ROLES OF THE NEUTROPHIL ELASTASE-TLR4 PATHWAY AND CATHEPSIN G IN EXPERIMENTAL INFECTION BY *L. braziliensis***

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Leishmania sp. have genes similar to bacterial ecotins, potent inhibitors of trypsin-type serine peptidases (SPs), which were thus named ISPs. ISP2 targets neutrophil elastase (NE) in macrophages preventing TLR4 activation during phagocytosis. The NE-TLR4 pathway results in the production of type I interferons, which are leishmanicidal for *L. major* but required for successful *L. donovani* infection. Here, we addressed the role of host SPs in the infection by *L. braziliensis*. Internalisation of *L. braziliensis* by murine macrophages was reduced by NE inhibition or in macrophages from NE-knockout mice (*ela2^{-/-}*). In mice, NE inhibition led to reduced plasma infiltration in the footpad 3 h post-infection, accompanied by reduced parasite burdens in the lymph nodes at week 2, as compared to untreated infected controls. Macrophage infection was reduced following the use of a TLR4 inhibitor or an antagonist to PAR2, a receptor that crosstalks with TLR4, while inhibition of TLR4 had no effect in the infection of *ela2^{-/-}* macrophages, indicating that NE acts through TLR4. BALB/c mice infected with *L. braziliensis* in the presence of the PAR2 antagonist showed reduced plasma infiltration in the footpad at 24 h, followed by reduced parasite loads in the lymph nodes at week 5. We further addressed the contribution of the trypsin-type SP, cathepsin G (CG). Infection of macrophages derived from CG-knockout mice or control macrophages treated with a CG inhibitor enhanced parasite uptake. CG inhibition augmented infection of *ela2^{-/-}* macrophages, suggesting that NE and CG modulate phagocytosis of *L. braziliensis* through opposite and independent pathways. CG-knockout mice displayed increased footpad lesions as compared to infected 129Sv mice, while parasite burdens were similar at week 5. Taken together, our findings indicate that the NE-TLR4 pathway acts positively in parasite uptake by macrophages and in the establishment of infection at distant sites, while CG exerts a protective role. **Supported by:**FAPERJ, CNPq and UK research and Innovation **Keywords:**Leishmania braziliensis;Serine Peptidases;ELA-TLR4 pathway.

HP-46 - **Immunosuppression post-heart transplantation promotes a decrease in miRNA-146b-5p levels in the blood of patients with Chagas disease**

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The most severe form of Chagas disease (CD) is chronic Chagas cardiomyopathy (CCC) which, in its last stage, has heart transplantation as its main outcome. Post-transplant CD reactivation occurs due to treatment with immunosuppressants, which are necessary to prevent graft rejection. Previously, miR-146b-5p was shown to be related to the inflammatory process triggered by *T. cruzi* and the production of cytokines, being a potential biomarker of tissue damage and cardiotoxicity in CCC. . There are few studies of biomarkers for monitoring CD reactivation. Thus, in this study, we analyzed miR-146b-5p levels in the blood of transplanted and immunosuppressed CD patients, with or without treatment with benznidazole (Bz). miR-146b-5p levels in the blood of transplanted and immunosuppressed CD patients with or without Bz treatment. The extraction of miRNAs was performed in samples from patients with positive serology for CD, positive or negative for the presence of *T. cruzi* in the blood (by qPCR), after undergoing heart transplantation and immunosuppression. As a control, samples from patients with negative serology for CD were used. Then, reverse transcription and gene expression analysis was performed for target miR-146b-5p and cel-miR-39-3p, as an exogenous internal control of RT-qPCR. The control group, transplanted patients, but with negative qPCR for *T. cruzi*, after treatment with bz), showed a significant increase in the expression of miR-146b-5p (2.5 times; p<0.05). Transplanted patients, but with positive qPCR for *T. cruzi*, showed a significant decrease in the expression of miR-146b-5p (6 times; p<0.01) compared to patients with negative qPCR for *T. cruzi*. This suggests that miR-146b-5p has the potential to be studied as a biomarker of reactivation by immunosuppression in CD, being important for early detection of reactivation and indication for treatment.

Keywords:Immunosuppression;Cardiac Transplantation;MicroRNAs.

HP-47 - Cellular and molecular mechanisms involved in the formation and detachment of the parasitophorous vacuole during infection of non-phagocytic cells by *Leishmania amazonensis*

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Intracellular parasites are characterized by their need to invade and exert at least part of their cycle within host cells. These pathogens present a series of strategies to overcome the barrier imposed by the plasma membrane and enter their host cells. For *Leishmania* spp. it is assumed that the parasite is phagocytosed and lives inside phagocytes. However, several studies have shown the presence of these parasites inside non-phagocytic cells. Despite being able to establish infection after capture by phagocytosis, it has been shown that promastigotes of *Leishmania amazonensis* are also capable of inducing their penetration into host cells via lysosome sequestration and subversion of the cellular process of plasma membrane repair. Regardless the form of penetration employed, a fundamental step during invasion is the fission of the plasma membrane at the invasion site, culminating with the detachment of the nascent vacuole and its release into the host cell. Despite efforts to discover the molecular effectors involved in the process and some evidence pointing to the membrane fission proteins dynamin and endophilin, the process is still unknown. In this work we investigate the molecular machinery involved in the initial stages of biogenesis of the parasitophorous vacuole of intracellular parasitic trypanosomatids using *L.amazonensis* promastigotes and mouse murine fibroblasts (MEFs) as a model. Our hypothesis is that proteins of the ESCRT complex (endosomal sorting complex required for transport), classically promoters of membrane scission, may be involved in cell invasion by these parasites. In this project we are silencing the expression of some ESCRT complex proteins in MEFs, proceeding infection assays using *L.amazonensis* promastigotes and quantifying infections. To verify if ESCRT complex proteins colocalize with the nascent parasitophore vacuole, we are proceeding time-course infections and performing immunofluorescence and fluorescence microscopy analyses. **Supported by:**FAPEMIG

Keywords:Leishmania spp;;Cell Invasion;Membrane scission.

HP-48 - Preservation of blood in guanidine-EDTA solution for the analysis of microRNAs in patients with Chagas disease

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Chagas disease (CD) is a neglected tropical disease caused by the flagellate protozoan *Trypanosoma cruzi*. The gold standard for evaluating the therapeutic efficacy of CD is the seroconversion of conventional serological tests, which can take years to be achieved. Thus, the development of biomarkers of therapeutic efficacy in Chagas disease is the subject of intense research. MicroRNAs (miRNAs) are a class of small, single-stranded, non-coding RNAs that act to regulate the expression of target messenger RNAs. In a previous study by our group, 641 miRNAs from mice infected with *T. cruzi*, treated or not with benznidazole in the chronic phase of the disease, were analyzed, highlighting 20 miRNAs that increased or decreased expression in infected animals, returning to normal levels after the treatment. Considering that many blood samples are preserved in a 6M guanidine hydrochloride-EDTA 0.2 M pH 8.0 solution, we evaluated the ability of this solution to preserve miRNAs when mixed with human blood in a 1:1 ratio. For this, blood samples were stored with and without guanidine, in parallel, and were artificially contaminated with two exogenous *C. elegans* miRNAs (cel-mir39 and cel-mir54). Expression analysis of exogenous miRNAs and human microRNA miR-146b-5p and small U6 RNA was performed by the comparative Ct method, using pre-validated TaqMan assays (Applied Biosystems). We observed that blood samples preserved in guanidine showed Ct values even lower than samples without the preservative agent, both for endogenous and exogenous miRNAs. This result paves the way for the use of many retrospective samples from clinical studies in Chagas disease, which are being preserved in guanidine-EDTA only for *T. cruzi* DNA analysis. In view of this, the analysis of miRNAs in blood with guanidine-EDTA can be promising in studies seeking biomarkers of pathogenesis and therapeutic response in Chagas disease.

Keywords:microRNAs;Guanidina-EDTA;Doença de Chagas.

HP-49 - Exploring the Shikimate Metabolic Pathway in *Plasmodium falciparum*: Uncovering Key Insights

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Malaria is a disease that's impacting the tropical regions, caused by protozoan parasites belonging to the phylum Apicomplexa, specifically the *Plasmodium* genus. Among those, *Plasmodium falciparum* stands out as the causative agent of severe cases. The emergence of drug resistance in different endemic regions demands the identification of novel targets for effective disease treatment, while minimizing host impact. The intriguing observation of glyphosate, a widely used herbicide, inhibiting the proliferation of *P. falciparum* raises questions regarding the presence of the shikimate pathway on those parasites. Although this metabolic pathway has been well-established in plants, bacteria, and fungi, its absence in mammals renders an appealing target for the development of new antimalarial drugs. The shikimate pathway comprises seven enzymatic steps leading to the synthesis of chorismate, a pivotal metabolite involved in diverse biochemical processes, including the biosynthesis of folates and prenylquinones. To explore the shikimate pathway in *P. falciparum*, we conducted experiments using parasite cultures or C3A cells (HepG2/C3A) cultivating them under compound concentration to determine the 50% inhibitory concentration, thereby assessing parasite proliferation. By investigating the impact of these compounds on parasite growth, our study aims to shed light on the potential of targeting the shikimate pathway for the development of novel antimalarial drugs. Inhibition tests showed a decrease in the proliferation of parasites against the compounds used, mainly chlorogenic acid and its derivatives, additionally, these compounds did not exhibit cytotoxicity in C3A cells (HepG2/C3A) in tested concentrations. After looking over the results obtained, we concluded the tested compounds are capable of inhibiting the proliferation of *P. falciparum* cultures, against to what was observed in mammalian cells. **Supported by:**FAPESP 2019/08637-5 **Keywords:**Malaria;*Plasmodium falciparum*;Shikimate pathway.

HP-50 - Host cell cholesterol and *T. cruzi* intracellular cell cycle

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Trypanosoma cruzi is a protozoan parasite and the etiological agent of Chagas disease, *T. cruzi* subverts a membrane repair mechanism, dependent on host cell lysosomes, for cell invasion. Data from our group has shown that cells lacking a lysosomal protein, LAMP (LAMP1/2^{-/-} fibroblasts), are less permissive to invasion, but more susceptible to parasite multiplication. Impaired invasion was shown to be due to cholesterol trafficking disruption, which leads to free cholesterol accumulation in the cell interior, compromising membrane repair process. Previous data from our group suggested host cell cholesterol was also important for *T. cruzi* intracellular multiplication. Treatment of wild type fibroblasts (WT) with atorvastatin was shown to increase intracellular parasite multiplication. Atorvastatin inhibits intracellular cholesterol synthesis. On the other hand, it is also known to enhance the expression levels of LDL receptor, leading to increased cholesterol uptake. In order to confirm the influence of cholesterol in *T. cruzi* intracellular life cycle, in the present work we evaluated the effects of serum deprivation in parasite intracellular multiplication. As for atorvastatin treatment, serum deprivation also leads to increase in intracellular cholesterol, but in this case by enhancing endogenous cholesterol synthesis. For this, we submitted WT and LAMP1/2^{-/-} fibroblasts to treatment with atorvastatin or serum deprivation for 6h, previous to infection with Dm28c strain of *T. cruzi*. After parasite exposure, cells were washed and the number of intracellular parasites, at 24, 48 and 72 hours post-infection was evaluated. As expected, serum reduction as atorvastatin treatment increased the number of intracellular parasites in WT cells. Both treatments, on the other hand, did not alter parasite multiplication in LAMP1/2^{-/-} cells. Likely due to the fact that the latter already have an increased content of intracellular cholesterol. **Supported by:**CAPES **Keywords:**T; cruzi;Cholesterol;LAMP.

HP-51 - Evaluation of the efficacy anti-*Leishmania amazonensis* the drug buparvaquone with a ubiquinone biosynthesis inhibitor using *in vitro* model.

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Leishmaniasis is an important problem in public health caused by protozoa of the genus *Leishmania*. These parasite presents two evolutionary forms: promastigote (extracellular) that is present in the insect vector and intracellular amastigote form that resides mainly in macrophages, present in the vertebrate host. The treatment of this disease is considered a major challenge, due to the limited therapeutic arsenal and high toxicity of drugs. To study the activity of a drug for this disease, it is necessary that it acts mainly in the intracellular amastigote form, and this makes the search for new drugs very difficult, since it requires macrophages to maintain the intracellular amastigote. So, it is necessary to present activity in intracellular amastigotes forms for the study to be more thorough and have greater relevance, thus being able to continue the study in the *in vivo* model. This parasite is distinguished from mammalian cells by having a unique and essential mitochondrion, which makes it an important therapeutic target. In the inner membrane of mitochondria, ubiquinone is found, an isoprenoid that plays an important role in the electron transport chain. Based on this information, the drug buparvaquone was chosen, which is an analogue of ubiquinone, and already has anti-*Leishmania* activity described in the literature. An inhibitor of ubiquinone biosynthesis, 4-iodobenzoate (4-IO) was chosen to be studied in association with buparvaquone, which showed activity only in the promastigotes forms (unpublished). The results of the therapeutic association showed an additive effect on the promastigotes forms. As for the intracellular amastigotes, the results showed that when testing buparvaquone with fixed and non-toxic concentrations of 4-IO, the effect of buparvaquone was potentiated two to three times. The results obtained so far encourage us to continue studies of the mechanism of action, as well as to carry out studies using the murine model. **Supported by:**Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) - número processo 2021/01877-0

Keywords:Leishmaniasis;therapeutic target;ubiquinone biosynthesis.

HP-52 - *In vitro* Chemotherapy against *Leishmania amazonensis* promastigotes using novel metallocomplex compounds (AP1022)

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Leishmaniasis are diseases with high incidence and wide geographical distribution. They also have a wide spectrum of clinical manifestations related to the species of parasite that causes the disease. Among these clinical manifestations are local skin lesions caused by the species *Leishmania amazonensis*. The therapy available for this disease is based on the use of Amphotericin B or pentavalent antimonial drugs, which have side effects and drug-resistant strains have been reported. Therefore, it is necessary to develop new therapies to treat the infectious process. Metallocomplexes are composed of transition metals, which can be coordinated with drugs, and present possibilities of coordination and geometries with the ligand. This coordination allows the interact with the specific molecular target, mainly with biological molecules. Metallocomplexes may be an interesting alternative for use in chemotherapy of *Leishmania spp. in vitro*, as it was recently found for a cobalt metallocomplex against *L. amazonensis in vitro*, altering the parasite's flagellar pocket, leading to death by autophagy. In this work, we evaluated the *in vitro* effect of the compound AP1022 on the growth of promastigotes of *L. amazonensis* (strain WHOM/ BR/ 75/ Josefa). The parasites were treated with the compound in concentrations that varied from 1 to 100 μ M and the growth after the treatment was quantified. To evaluate the effect of this metallocomplex cell viability of the LLC-MK2 host cell and an antiproliferative assay with promastigote of *L. amazonensis in vitro* were performed. The AP1022 compound showed an IC₅₀ value of 0.98 μ M to 1.80 μ M after 3 and 5 days of treatment of promastigotes; no toxicity was found to the host cells. The results open a gateway to innovation, as the IC₅₀ value indicated that the AP1022 complex as a drug capable of controlling the growth of the parasite in the promastigote form maintained *in vitro*. **Supported by:**CAPES

Keywords:Metallocomplexes;Leishmania amazonensis;Chemotherapy.

HP-53 - Leishmania amazonensis extracellular vesicles (EVs) induce neutrophil extracellular traps (NETs).

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Sandfly vectors inoculate *Leishmania* in a pool of blood together with promastigotes-derived extracellular vesicles (EVs). It has been shown that this co-injection induces inflammation and exacerbates leishmaniasis lesions. EVs are a heterogeneous group of particles released by cells that play a key role in intercellular communication, and carry proteins, RNA, DNA, lipids and metabolites, being easily transferred from one cell to another. Neutrophils, the major leukocyte in the blood, are one of the first cells to interact with the parasite. Upon interaction with *Leishmania*, these cells release neutrophil extracellular traps (NETs) that ensnare and are toxic to the parasite. Our investigation aimed to determine whether EVs can also induce NETs. Thus, we incubated neutrophils from healthy human blood with EVs secreted by promastigotes (150 nm by NTA) and measured the number of NETs produced by the picogreen assay. Our results showed that EVs induced NET release in a reactive oxygen species-dependent manner. NETs induced by EVs are inhibited by chloroamidine, BAPTA, inhibitors of myeloperoxidase (MPO), elastase, and gasdermin D. EVs stained by CA7AE and anti-GP63 are observed inside neutrophils and trapped in the NETs. Interestingly, EV-induced NET is specifically inhibited by phenanthroline. Together, our results ensure that ROS, elastase, MPO, peptidyl arginine deiminase, calcium and gasdermin D are involved in EVs-induced NET formation. **Supported by:**FAPERJ, CNPq, CAPES

Keywords:Leishmania;Extracellular Vesicles;Neutrophil Extracellular Traps (NETs).

HP-54 - Unveiling the potential role of lncRNAs in the Murine Model of Cutaneous Leishmaniasis

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Introduction: Cutaneous Leishmaniasis (CL) is characterized by ulcerative skin lesions that lead to significant and lasting scarring. Previous transcriptomics studies from human CL lesions caused by *Leishmania braziliensis* (*Lb*) have provided insights into critical aspects of immunopathology. To gain insights into the initial stages of CL infection, disease progression, and the healing process, we conducted comprehensive transcriptomic profiling of lesions and draining lymph nodes (dLNs) using a murine model that replicates *Lb*-induced CL in humans. **Methods:** BALB/C mice were infected with *Lb* in their ears, and ears and draining lymph nodes (dLN) were collected at the early (2, 6, and 48 hours, and 14 days) and late (35 and 77 days) phases post-infection. Three biological replicates per time-point were used for both tissues, and bulk RNA-seq was performed. CEMltool was used to identify gene modules (M) with significant co-expression during lesion development. **Results:** Gene co-expression analysis revealed dynamic changes shortly after the inoculation of *Lb* into the dermis, persisting until lesion healing. During the early phase, lesions exhibited the activation of modules associated with inflammation and wound healing. In the late phase, modules involved in Phagosome formation (M3) and LXR/RXR (M2) were significantly activated. Surprisingly, a distinct module (M4) containing 219 genes, including 63% unclassified genes and 23.3% long non-coding RNAs (lncRNAs), showed marked deactivated at Day 35. Correlations and enrichment analysis suggested the involvement of these lncRNAs in multiple pathways, particularly TCR and TLR receptor activation. **Conclusion:** The findings demonstrate a remarkable overlap of known pathways observed in both the murine model and human disease, emphasizing the translational relevance of the murine model. In addition, data presented in this study highlight new potential targets for therapeutic interventions in the treatment of *Lb* infections. **Supported by:**CAPES, PIAP/IGM/FIOCRUZ-BA (001/2017) **Keywords:**Cutaneous leishmaniasis;RNA seq;Inflammation.

HP-55 - Effects of Adipose Mesenchymal Stromal Cells Extracellular vesicles in experimental infection from *Leishmania amazonensis*

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Leishmaniasis is a neglected disease caused by protozoa of the genus *Leishmania*. One of its characteristics is the imbalance of the host's immune response to favor the survival of the parasite, generating inflammatory processes that do not promote parasite control and induce tissue injury. In this context, the use of mesenchymal stromal cells (MSCs) and some of their biological products such as their Extracellular Vesicles (MSC-EVs) may be a viable therapeutic strategy, given the already described immunomodulatory potential they present. Our group demonstrated that treatment with MSCs from adipose tissue contributes to lesion control in C57BL/6 mice infected with *Leishmania amazonensis*. As the paracrine effect is one of the main mechanisms of efficacy of treatments with MSCs, this project aims to investigate whether extracellular vesicles these cells secrete have the property of controlling the lesion similar to full cell treatment. With this, the project aims to understand the effects of treatment with MSC-EVs from adipose tissue of C57BL/6 mice in the model of experimental leishmaniasis induced by *L. amazonensis*. The first step it's the purification and expansion of MSC cells from adipose tissue and extraction and characterization of it's extracellular vesicles. Next, we evaluate the *in vitro* effect of extracellular vesicles. Infected C57BL/6 macrophages were treated with MSC-EVs and the parasite load of these macrophages were evaluated. Finally, we evaluate the ability of MSC-EVs to control injury *in vivo* in infected C57BL/6 animals. The size of the lesions was evaluated by pachymetry, the parasite load by limiting dilution technique and the immune response by RT-PCR and ELISA. As preliminary results, MSC-EVs reduced lesion size without affect parasite load. MSC-EVs could be an alternative therapy for the treatment of leishmaniasis lesion. **Supported by:** CAPES, 88887.695120/2022-00

Keywords: Cellular Therapy; Extracellular Vesicles; Alternative therapy.

HP-56 - Investigation of the role of the endosymbiont *Wolbachia pipientis* in the infection of the vector *Lutzomyia longipalpis* by the parasite *Leishmania infantum chagasi*

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The transmission of American visceral leishmaniasis (AVL), caused by *Leishmania infantum chagasi*, occurs through the bite of the female sand fly *Lutzomyia longipalpis*. Current methods for leishmaniasis control, as the use of insecticide, have been shown to be ineffective. This indicates the need to develop new strategies. The bacterium *Wolbachia pipientis* has been used to control arboviruses. Other studies point to a decrease in the infectivity of the protozoan *Plasmodium*, which causes malaria, in *Anopheles* mosquito in the presence of *Wolbachia*. A recent study of our group tested the infection susceptibility of *L. longipalpis* Lulo and LL-5 embryonic cell lines to the *Wolbachia* wMel and wMelPop-CLA strains. The study showed that the cells, when exposed to the bacterium, modulate genes belonging to the main immunological pathways Toll, IMD and Jak-Stat. To investigate the role of *Wolbachia* in adult insects we now performed artificial blood feeding of the insects with Lulo cells stably infected with wMelPop-CLA. We then evaluated the permanence time of the bacterium in the vector *Lutzomyia longipalpis*, as well as the possible effects of a coinfection with *L. i. chagasi*. Preliminary results showed that it was possible to detect *Wolbachia* in the insect at 0h, 24h, 48h, 72h and 144h post-infection times, which proves the oral route to be a promising way of introducing the endosymbiont into the sand fly. We also evaluated the *L. i. chagasi* load in the presence and absence of *Wolbachia*, and we observed no significant difference in relation to the control infected only with *Leishmania*. In addition, the modulation of some targets for insect immunity genes is being evaluated, and in our first tests, we observed that in the group containing the bacteria there is a slight tendency for reduction of the expression of some of these genes in relation to the group without *Wolbachia*. These experiments are being repeated in order to obtain a reliable number of replicates and proper statistical data. **Keywords:** Vector control; *Lutzomyia longipalpis*; *Wolbachia pipientis*.

HP-57 - Oral immunization using live bacteria expressing the LACK protein from *Leishmania amazonensis*.

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Leishmaniasis are neglected diseases caused by protozoans of the *Leishmania* genus, divided into Cutaneous – most common, Mucosal and Visceral forms. To date, no injectable vaccine aimed at boosting protective Th1 responses has shown to be sufficiently effective and safe for human use. An alternative strategy is a tolerogenic vaccine using predominantly Th2 antigens to prevent the swift expansion of Th2 responses following infection, to allow the manifestation of protective Th1 responses. Our group has previously shown that oral and nasal tolerogenic immunization with LaAg, which is predominantly Th2 response biasing, protects against leishmaniasis in different animal models of infection. An important Th2 component of LaAg is the LACK protein (*Leishmania* homologue of Activated C Kinase), highly conserved among *Leishmania* spp. In this work we produced and evaluated the potential use of non-pathogenic live bacteria expressing the LACK protein as an oral vaccine against Cutaneous Leishmaniasis (CL). To this end, the lack gene was successfully cloned into *Escherichia coli* DH5 α bacteria, as shown by PCR, SDS-PAGE and Sanger sequencing. Next, BALB/c mice pre-treated or not with a cocktail of antibiotics received two oral doses with an interval of seven days with *E. coli* expressing plasmid with the *lack* gene (pLACK+) or plasmid without *lack* (pLACK-). One week after the second vaccine dose, the animals were infected s.c. with *L. amazonensis* in the paw, and the lesion growth monitored periodically. We observed that animals pre-vaccinated with *E. coli* pLACK+ developed smaller lesions than those that received *E. coli* pLACK-. The pre-reduction of the intestinal microbiota with antibiotic therapy enhanced the vaccine effect of *E. coli* pLACK+ as confirmed by the parasite load measurement. Overall, these results show the viability of a live oral vaccine expressing the LACK protein gene against CL.

Supported by: CAPES **Keywords:** cutaneous leishmaniasis; live vaccine; LACK.

HP-59 - Evaluation of ATP-citrate lyase expression in macrophages during *Leishmania amazonensis* infection

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Leishmania spp. is an obligatory intracellular parasite that causes leishmaniasis, and is transmitted to vertebrate hosts by sandflies of the genus *Phlebotomus* and *Lutzomyia*. Even though it is estimated that about 1 billion people worldwide are at risk of acquiring leishmaniasis, it is still a neglected disease, and to this date, there is no available vaccine against leishmaniasis. Although there are available treatment options, they have problems such as adverse effects and high toxicity. Plus, there has been a rise in treatment failure and drug resistance reports. Thus, it is essential to investigate the parasite-host interaction to improve the treatment. Once inside the macrophages, parasites evade the microbicidal mechanisms and continue replicating and infecting other cells. They also induce alterations in host metabolism to favor their survival. It has been recently described that *Leishmania* spp. induce metabolic reprogramming and generate lipid droplets (LDs) in infected macrophages. Based on these findings, we investigate how the parasite modulates the host lipid metabolism. We hypothesized that during the infection, *L. amazonensis* induces an increase in lipids in macrophages and that this is due to the enzyme ATP-citrate lyase (ACLY) modulation. ACLY cleaves citrate in oxaloacetate and acetyl-CoA, and these two products can be used to produce inflammatory mediators like PGE₂, NO, and ROS and cause epigenetic alterations like acetylation and malonylation. Our results show that in *L. amazonensis*-infected macrophages, there is an increase in lipids detected by Nile Red. When macrophages were pre-treated with the ACLY inhibitor BMS-303141, there was a decrease in ROS production in the early stages of the infection, but after 48 h, no effect was observed. ACLY inhibition modulated NO production and diminished 41% of parasite survival. These results indicate that the enzyme may be involved in the modulation of macrophage response to *L. amazonensis*. **Supported by:** CNPq **Keywords:** *Leishmania amazonensis*; ATP-citrate Lyase; macrophages.

HP-60 - Leishmaniasis, Chagas disease and their vectors: representations and risk factors among Kariri-Xocó indigenous children

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Trypanosomatid-transmitted protozooses affect populations with limited access to education and sanitation, such as indigenous. Children within these communities exhibit high capillarity and crucial role for prevention that necessitate a comprehensive understanding of local knowledge and reduce environmental risks. Thus, we investigated representations of Kariri-Xocó children (Bahia State) about leishmaniasis, Chagas disease, vectors, and the local risk factors. Ethical approval was obtained, and semi-structured interviews with the visual stimulus (*Lutzomyia* sp. and *Triatoma infestans* specimens) were conducted. Additionally, non-participant observation was used. Out of 21 interviewed children, 52% associated the sandfly with *Aedes* sp., and 62% were unaware of its habitat, eating habits, and preventive measures. Only 9% mentioned practices like eliminating standing water and tire care. Misconceptions were observed, with 4% relating the transmitted disease to zika and 9% to yellow fever. Symptoms reported included "balls on the body", fever (9% each) and headache (4%). Regarding Chagas disease, 33% recognized the insect as a kissing bug, 9% as a cockroach, and 57% lacked knowledge about its appearance and its habit. A minority (38%) mentioned hematophagy and said that the bug can be found in trees (19%), stones, walls (9% each), mud houses and sheets (4% each). The children's understanding of the disease transmission (95%) and its symptoms (62%) was limited, with only 4% citing fever, swollen heart, and death as consequences. Environmental risk included direct contact with forests and rivers, livestock farming, the presence of dogs, and improper waste management. The findings highlight significant knowledge gaps, misconceptions about disease severity and vectorial transmission, and the environmental risk within the community. Therefore, it's require health education campaigns and community empowerment to mitigate risks and effectively preventing infectious diseases. **Supported by:**FAPESB

Keywords:Indigenous health;Environmental risks;Health education.

HP-61 - Evaluation of nitroreductase type I bioactivation and anti-amastigote activity of two nitroderivative chalcone-thiosemicarbazone hybrids in *Leishmania infantum*

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Recently, our group evaluated the anti-promastigote activity against *L. infantum* and the cytotoxicity against murine peritoneal macrophages of 13 chalcone-thiosemicarbazone hybrids, obtaining IC₅₀ values between 3.2 ± 0.9 and 23.3 ± 3.6 µM, and CC₅₀ between 38.6 ± 1.8 and 99.3 ± 4.9 µM, except CT-7 and CT-14, which presented, respectively, CC₅₀ equal to >512 and 143.9±12.6 µM. Interestingly, among the compounds tested, these were the only ones to present nitro groups in their structure. This led us to consider bioactivation through the nitroreductase type I (NTR1) enzyme as a possible mechanism of bioactivation. Thus, this work aimed to evaluate the bioactivation mechanism through NTR1 overexpression in *L. infantum*, and the anti-amastigote activity of CT-7 and CT-14. The mechanism of bioactivation was evaluated through the anti-promastigote activity in *Lin*fWT (WT), *Lin*fSPaNEOa (PSP) and *Lin*fSPaNEOaNTR1^{high} (NTR1^{high}) strains, using nifurtimox as a control, and the anti-amastigote activity was evaluated as a function of the reduction of the infection rate, in a concentration of 10 µM, concerning the infected control. As expected, nifurtimox showed a lower IC₅₀ for the NTR1^{high} strain than for the WT and PSP strains (1.1±0.6, 2.0 ± 0.8, and 2.1 ± 0.4 µM, respectively). In contrast, CT-7 and CT-14 showed less significant IC₅₀ reductions for the NTR1^{high} strain, when compared to the WT and PSP strains (CT-7: WT = 10.7 ± 2.5, PSP = 10.6 ± 1.2 and NTR1^{high} = 10.2±1.6 µM) (CT-14: WT = 9.0 ± 0.9, PSP = 8.7 ± 0.7 and NTR1^{high} = 6.6 ± 1.5 µM). In the anti-amastigote activity experiments, reductions in the infection rates for CT-7 and CT-14 equal 88.2% and 87.7%, respectively, were observed. These results suggest that NTR1 does not bioactivate CT-7 and CT-14. As a continuation of this study, investigations will be carried out about the mechanism of action, in addition to the determination of IC₅₀ and selectivity index of CT-7 and CT-14 against the amastigote form of *L. infantum*. **Supported by:**FAPERJ PROCESSOS E26-201.158/2022 E 26-210.157/2018 **Keywords:**Visceral Leishmaniasis;Leishmania infantum;Nitroreductases.

HP-62 -Building the *Neospora caninum* GRAome: Bioinformatic and Proteomic strategies for the assembly of a Dense Granule Proteins database

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Apicomplexa parasites require a parasitophorous vacuole (PV) for replication and infection maintenance. The PV is formed through protein secretion from organelles such as micronemes, rhoptries, and dense granules. Dense granule proteins (GRAs) maintain the PV structure and regulate host gene expression. Research has focused on GRAs of *Toxoplasma gondii*, but little is known about related *Neospora caninum*, which causes reproductive disorders in cattle and has a high impact on the livestock industry. In this study, we aimed to compare the known GRAs of *T. gondii* described in the literature and at the Toxoplasma Informatics Resources Database (ToxoDB), to *N. caninum* orthologues, in order to describe and annotate those genes and look for conserved or unique motifs. We initially found a total of 186 GRAs in *T. gondii* and, of these, 145 present orthologs in *N. caninum*, with a high level of synteny. The similarity between sequences was also analyzed through alignments and determined to be in the range of 40 to 80%. Gene expression of the GRAs were also analyzed in RNASeq experiments involving infections by these parasites in murine bone marrow-derived macrophages, where, a similar profile was observed in the gene expression of the GRAs in both parasites, with the exception of some genes. To validate the *in silico* strategy, we used proximity biotinylation to identify predicted targets in the *N. caninum* PV. As baits, we chose a known GRA with predicted good expression in tachyzoites (GRA48), and a knocked in *T. gondii* GRA without orthologue in *N. caninum* (GRA24). The BioID assays confirmed at least 57 predicted GRAs in *N. caninum*'s PV, including GRAs only predicted by HyperLOPIT and yet to be described in *T. gondii*. In conclusion, the union of bioinformatics and proteomics strategies is a successful method to describe the GRA content in the *N. caninum* infected cell, serving as a starting point for new experimental approaches regarding parasite biology and host interaction. **Supported by:**CNPq (313761/2020-5), FAPEMIG (RED-0313-16) **Keywords:**Toxoplasma gondii;RNASeq;BioID.

HP-63 - Identification of aneuploidy and copy number variation (CNV) events in the TcCh39 chromosome of *Trypanosoma cruzi* (clone CL Brener) by dual color FISH assay

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Aneuploidy appears to play a role in the protozoan parasite's adaptation to environmental changes, including drug resistance. Although *Trypanosoma cruzi* is considered a diploid organism, whole-genome sequencing and comparative genomic hybridization analyses have shown the occurrence of aneuploidy in this parasite. Here, we investigated the presence of aneuploidy in *T. cruzi* using dual-color fluorescence in situ hybridization (FISH), which allowed us to estimate the copy number of a given chromosome (somy) in individual cells. The single-copy genes H49 and JL8 were used as specific markers of chromosome TcCh39 of clone CL Brener. The central region of genes H49 and JL8 comprises an uninterrupted large array of tandem repeats that is a good DNA hybridization target. Hybridization signals for H49 and JL8 were revealed with anti-Digoxigenin and Alexa Fluor 488 and anti-Biotin and Alexa Fluor 594, respectively. Ploidy levels were also confirmed by confocal microscopy. Cells in mitosis were excluded from the analysis. We looked at changes in the chromosome TcChr39, specifically for chromosomal and local copy number variations. We identified the copy number chromosomal variation of TcCh39 in the same cell population: 55% of cells were disomic; 25.2%, monosomic; and 5.4%, trisomic for chromosome TcCh39. Trisomy or monosomy of TcChr39 could result from an error in chromosomal segregation in mitosis due to the non-disjunction of sister chromatids. Among disomic cells, some displayed local gene copy number variation. For instance, they had two copies of the H49 gene and just one copy of the JL8 gene. The lag in the replication of gene JL8 could explain this difference. The variable TcChr39 somy and copy number of genes among individual cells may have generated intra-strain heterogeneity and chromosomal mosaicism. We will compare the chromosome copy number changes estimated by FISH with data provided by next-generation sequencing of clone CL Brener. **Supported by:**Acknowledgement: FAPESP (PD 2019/05049-4) and Thematic Project (2016/15000-4), CAPES and CNPq. Elizabeth Naomi for help in handling the confocal microscopy (INFAR/UNIFESP).

Keywords:Trypanosoma cruzi;aneuploidy;copy number variation.

HP-64 - A genetically modified live attenuated *Leishmania major* as a potential vaccinal candidate for Leishmaniasis

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Leishmaniasis is a neglected tropical disease caused by more than 20 different species of parasites belonging to the genus *Leishmania*. The disease constitutes a global health problem, affecting approximately 1 million people annually worldwide. Brazil is an endemic country for leishmaniasis. Although treatment exists, it is generally highly toxic and costly. Furthermore, currently there are no vaccines approved for humans against this pathogen. In this study, we investigated the physiological characteristics of a genetically modified strain of *Leishmania major* in which a specific gene has been knocked out, and explored its potential application as a vaccine candidate. In culture, the KO promastigotes exhibited enhanced growth, reaching approximately 3.0×10^7 parasites/mL compared to 2.5×10^7 parasites/mL observed in the wild-type strain after 5 days of growth. Additionally, a lower percentage of metacyclic forms was observed in the KO strain (around 10%) compared to the wild-type strain (35%). Conversely, overexpression of this gene resulted in the opposite effect, with a higher percentage of metacyclic forms and reduced growth. Furthermore, the KO strain demonstrated attenuation during *in vitro* infection, exhibiting a lower percentage of amastigotes per macrophage. *In vivo*, subcutaneous infection with 2×10^6 parasites in C57BL/6 mice did not result in any lesions, unlike infection with the wild-type strain. Additionally, Balb/C and C57BL/6 IFN- γ -/- mice infected with 2×10^5 parasites did not develop lesions, while those infected with 2×10^6 parasites showed significantly smaller lesions compared to the wild-type strain. However, in both infections, it was possible to recover the KO strain from infected tissues. These data collectively demonstrate the high potential of this strain as a vaccine candidate. Currently, ongoing research is being conducted to evaluate whether these parasites can provide protection against infection by the wild-type strain. **Supported by:**FAPERJ / CNPQ **Keywords:**Leishmania;Leishmanization;Vaccine.

HP-65 - Analysis *in vitro* of RNA as a molecular marker of *Trypanosoma cruzi* viability

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Real-time polymerase chain reaction (qPCR) allows the detection and quantification of different pathogens and are widely employed in molecular diagnostic laboratories. However, DNA amplification does not differentiate between viable or dead parasites. Until now there are few studies comparing the application of molecular diagnostic tools that effectively differentiate between viable and nonviable parasites especially when it comes to *T. cruzi* evaluation. Herein, we considered *T. cruzi* RNA as a potential molecular marker of pathogen viability, due to RNA's half-life and lability when compared to the DNA. At first, we compared a qPCR with Reverse Transcription (RT-qPCR), targeting *T. cruzi* GAPDH mRNA (a housekeeping gene), and a qPCR, targeting *T. cruzi* satellite DNA. Both methodologies presented an improved performance with linearities ranging from 10^7 to 10^2 parasites equivalents for RT-qPCR and 10^6 a 10^{-1} parasites equivalents for qPCR and efficiencies of 100.3% and 102.6%, respectively. Then, we evaluated and compared RNA and DNA detection of live *T. cruzi* and heat-treated lysed *T. cruzi*, where was confirmed that RNA was faster degraded, no longer being detected at day 1 after parasite lysis, while DNA detection was stable with no decrease in parasite load over the days even after parasite lysis. Moreover, *in vitro* assays with rat cardiomyoblasts (H9C2) infected with *T. cruzi* (Dm28c) and under Benznidazole treatment, also showed a considerable decrease in RNA detection, which was no longer detectable at day 1 post-treatment. At the same time, parasite DNA detection remained constant with no significant decrease in its detection up to 4 days after treatment. In conclusion, these differences between DNA and RNA detection raises the possibility that RNA is a potential molecular marker of *T. cruzi* viability, which could contribute to understanding the dynamics of the parasite infection and explore new possibilities for trypanocidal drugs evaluation. **Supported by:**IOC-FIOCRUZ; CnPq; FAPERJ **Keywords:**Trypanosoma cruzi;Viability;qPCR, RT-qPCR.

HP-66 - Leishmania antigen 2 (Leish-Ag2) induced protection by intranasal route against *Leishmania amazonensis* infection

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Leishmaniasis is a neglected tropical disease caused by the infectious protozoa *Leishmania*. The various species of *Leishmania* cause cutaneous (CL) and visceral (VL) leishmaniasis. According to the Pan American Health Organization (PAHO), over 12 million people worldwide are infected, resulting in 20,000 to 30,000 deaths annually. Developing a vaccine for effective disease control is the most viable solution to address this health issue. However, there is currently no approved vaccine against leishmaniasis for human use. In the literature, several potential candidates have been studied to advance research in this field. In this study, we evaluated the efficacy of *Leishmania* antigen 2 (Leish-Ag2) administered via the intranasal route in a BALB/c mice model of experimental *Leishmania amazonensis* infection. Leish-Ag2 is an antigen that is well-preserved across different species of *Leishmania* and has recently been studied by our research team, with its findings being kept confidential. By itself, Leish-Ag2 was unable to induce protection in the tested model. However, when the antigen was combined with adjuvants such as MPLA, Leish-Ag2 demonstrated a significant reduction in lesion size and parasite load. This protective effect is likely linked to the adjuvants' ability to modulate a Th1 immune response in BALB/c mice, thereby enabling control of the infection. This is supported by data from our research group, indicating high levels of IFN- γ at the site of the lesion in animals vaccinated with Leish-Ag2 associated to MPLA. Taken together, our research group has developed a new antigen with the capacity to protect against cutaneous infection when administered via the intranasal route. Furthermore, there is potential for the mucosal route to induce protection against such an infection. **Supported by:**CNPq **Keywords:**Leishmaniasis;Vaccine;Intranasal.

HP-67 - A novel fatty acid binding protein in *Leishmania amazonensis*: characterization of the LeiFABP domain

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Leishmaniases are diseases caused by parasites of the genus *Leishmania*, of the Trypanosomatidae family. During their life cycle, *Leishmania* parasites multiply in vertebrate and invertebrate hosts, differentiating into distinct cell forms: promastigotes and amastigotes. These parasites have limited biosynthesis capacity, having mechanisms for sequestering nutrients from the host. The amastigote forms depend on fatty acids and lipids present inside the phagosome, which are the main sources of carbon used for production of molecules essential for their survival. Searches on TriTrypDB for hypothetical proteins similar to eukaryotic fatty acids binding proteins (FABP) led to the hypothetical protein encoded by *LmxM.34.3070*. Different protein structure prediction methods revealed a C-terminal domain structurally similar to FABPs, suggesting that *LmxM.34.3070* could be associated with the metabolism of lipids and fatty acids. Also, BLAST alignments showed that the *LmxM.34.3070* is strongly conserved among the *Leishmania* genus. Therefore, the main goal of this work was to obtain the recombinant C-terminal domain of *LmxM.34.3070* suitable for structural biology studies. The fragment *LmxM.34.3070*₆₃₈₋₈₂₂ (LeiFABP), was cloned, expressed, and purified. Circular dichroism spectroscopy data indicated that 32% of LeiFABP amino acids assume a β -type structure, while 15% assume an α -helix structure. The result of this analysis is consistent with the early *in silico* predictions. Multiangle light scattering showed that the LeiFABP domain is monomeric. The ¹H Nuclear magnetic resonance (NMR) spectrum showed good dispersion in the HN_s region and relatively narrow lines, suggesting that the LeiFABP construct is well folded and is suitable for structural analysis by NMR in solution or crystallography. Supported by CAPES and Fapesp.

Supported by:CAPES PROEX , PROCESSO: 88887.613399/2021-00

Keywords:fatty acid binding protein;circular dichroism spectroscopy;nuclear magnetic resonance.

HP-68 - Repositioning oral treatment for visceral leishmaniasis managed by molecular docking

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Molecular docking is a great tool for the drug repositioning strategy. Considering visceral leishmaniasis is a disease that leads to death in around 90% of non- treated patients, the quick development of an oral effective treatment would save thousands of lives. Some macromolecules that are key to *Leishmania* spp. survival and pathogen-specific have been described as good treatment targets. This work presents a target-directed virtual trial, to find repositionable oral drugs that are potent inhibitors for an enzyme involved in the viability of the parasite. Due to the possibility of the deposit of patents, sensitive data related to the invention was omitted. A library of around 4300 compounds was screened and ranked by the binding affinity with the specific *L. infantum* enzyme. The 3D structures are available in the ZINC database. The Raccoon software was used to input ligands charges. For the receptors, two physiological forms of the enzyme were obtained by RCBS-PDB. The protonation state in 7.4 pH was normalized at the APBS server. The molecular docking was calculated with PyRx software. Both states of the enzyme were tested by all the compounds binding in their active site, creating two rankings on the affinity. The first enzyme form had 1118 compounds in conformations with binding values equal to or under -8 Kcal/mol. The second form had 504 molecules at the same range, of which, 475 had conformations in this binding affinity with both forms. These compounds were analyzed by their ADMET properties, where 239 orally administered drugs were found, of which 54 were approved by the FDA and ANVISA. The molecular mechanism of action shows that many of those interactions occur with important residues in the catalytic site of the enzyme. Taken together, our data proposed that those drugs may be a key to improve the treatment of leishmaniasis by competitive inhibition to a relevant macromolecule for the parasite's metabolism as a part of the mechanism of action. **Supported by:** CAPES, CNPQ, IOC/Fiocruz, FAPERJ
Keywords: Structure-based virtual screening; visceral leishmaniasis; chemotherapy.

HP-69 - LAMP-CAS12 METHODOLOGY FOR MOLECULAR DIAGNOSIS OF INFECTIOUS DISEASES

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Infectious diseases are a global health problem that impact billions of people, causing an immense burden of disabilities and deaths, affecting the economic and social dynamics of all the continents. The detection of its etiologic agents is necessary both for treatment of the patients in a timely manner and prevention of spread of the pathogens. Many approaches based on nucleic acid detection have been used to enhance the sensibility and specificity of the tests, reducing limitations such as the use of high-cost equipment and the delay in delivering results. Among the strategies recently tested and approved by the U.S Food and Drug Administration (FDA), is the use of the LAMP (Loop-Mediated Isothermal Amplification) methodology, alone or combined with CRISPR/Cas12 technology. We established the LAMP-Cas12 methodology using as a model the detection of SARS-CoV-2. In addition, we developed in our laboratory all the steps for expression and purification of Cas12a enzymes, as well as the synthesis of their sgRNAs, comparing their efficiency in the detection of SARS-CoV-2 in in vitro assays. We also validated the assays that use the nonspecific activity of Cas12a after detection of the target of interest, coupled to a fluorescent molecule to confirm the diagnosis. Finally, we performed a proof of concept of this methodology, using samples from patients diagnosed with COVID-19 previously confirmed by qRT-PCR, and demonstrated that the system is able to detect with specificity different types of target. Now, we will use these established diagnostic platform to establish new protocols for the detection of other infectious disease agents, including protozoans, worms and bacteria. **Keywords:** Infectious disease; CRISPR; Diagnostic.

HP-70 - UNRAVELING THE FUNCTION OF GLYCOCONJUGATES IN LEISHMANIA AMAZONENSIS INFECTION THROUGH CRISPR/CAS9 EDITING OF THE LPG2 GENE

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Introduction: The *lpg2* gene encodes a GDP-mannose transporter involved in synthesizing phosphoglycan-containing molecules (PGs), which are critical for *Leishmania* infection of the mammalian host. This study investigated the role of these glycoconjugates in 2 strains of *L. amazonensis* (*La*) previously isolated from patients with localized (LCL BA125) or diffuse (DCL BA336) forms of the disease.

Methods: *La lpg2*KO strains were generated using CRISPR/CAS9 and clonal *lpg2*KO parasites were isolated. Neutrophils and BMDM were infected with WT or *lpg2*KO parasites, and parasite burden was determined at 4h or 48h post-infection. Cellular migration assays were performed with human dendritic cells (hDCs) infected with WT or *lpg2*KO parasites *In vivo*. Evaluation involved the intradermal inoculation of parasites in the ear of BALB/c mice, and lesion development was monitored using an analog caliper for up to 12 weeks. **Results:** The disruption of the *lpg2* gene was characterized by PCR, Sanger and next generation sequencing. Western blot analysis demonstrated the complete absence of LPG and PG expression. *In vitro* assays showed no differences in infection and replication rates between *lpg2*KO and WT parasites in BMDM or human neutrophils. However, both *La lpg2*KO strains exhibited reduced hDC migration. Surprisingly, *in vivo* infection of mice yielded contrasting outcomes between the two *lpg2*KO strains. The BA336 *lpg2*KO strain displayed minimal differences in lesion development, while the BA125 *lpg2*KO strain failed to induce any lesion development. **Conclusions:** Our findings demonstrate successful disruption of the *lpg2* gene in both strains of *La*. The absence of PGs does not appear to be essential for parasite virulence *in vitro*, although it impacts hDC migration. In contrast, PGs seem to play an essential role in lesion development of BA125. Further research is needed to understand the factors responsible for the divergent *in vivo* virulence observed between these strains. **Supported by:**FAPESB, CAPES

Keywords:Leishmania;CRISPR/CAS9;LPG.

HP-71 - Targeting different portions of trans-sialidase for the development of Chagas disease vaccine

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Trans-sialidases (TS) are proteins present on the surface of *Trypanosoma cruzi*, which only a subgroup of TS has catalytic activity, responsible for transferring sialic acid residues from host glycoconjugates to mucins on the parasite surface, a mechanism that is related to the parasite capacity to evade the host immune system. Some TS have a C-terminal domain containing 12 amino acids repeats known as SAPA (shed acute parasite antigen). In addition, the SAPA domain increases the stability of the enzyme in the bloodstream, which is considered a parasite virulence factor. To evaluate an active TS as vaccine candidate, three recombinant versions of the protein were produced in *E. coli*: full-length protein, TS without repeats, and only SAPA repeats. BALB/c mice were immunized with each protein and then challenged with a virulent strain of *T. cruzi*. Analyses of the cellular immune response showed that immunization with TS without SAPA resulted in higher levels of IFN- γ and lower levels of IL-10 produced by splenocytes from animals, compared to splenocytes from animals immunized with the other two antigens. Furthermore, after challenge, mice immunized with protein containing only SAPA repeats resulted in higher parasitemia and mortality compared to immunization with TS without SAPA. It is important to emphasize that tissues of animals immunized with TS without SAPA did not show inflammatory infiltrate or detectable levels of parasite DNA in the heart. Taken together these results indicated that immunization with TS antigen without SAPA induces the development of a protective Th1 response, essential for intracellular pathogen infection control, and that the presence of SAPA repeats results in the negative modulation of this protective response. Since RNA vaccines have several advantages and TS without SAPA is a promising antigen for a vaccine model against Chagas disease, the mRNA of this protein was produced and tested in a lipid formulation for animal immunization. **Supported by:**CAPES **Keywords:**Trans-sialidase (TS);SAPA repeats; T; cruzi.

HP-72 - THE ROLE OF ANNEXIN A1 IN AN EXPERIMENTAL *TRYPANOSOMA CRUZI* INFECTION

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Chagas disease (CD) is a neglected disease caused by the protozoan *Trypanosoma cruzi* (*Tc*), whose complications include cardiac, digestive and neurological dysfunction. The imbalanced inflammatory response is associated with the clinical forms development, thus Annexin A1 (ANXA1) emerges as an important protein with a central role in the inflammatory process resolution. Herein, the influence of ANXA1 on gut microbiota composition and in the development of *Tc*-induced pathogenesis were investigated. For *in vivo* analysis, female BALB/c (WT) and ANXA1 knockout (KO) mice, 8 to 9 weeks old, were infected ip with 10^3 trypomastigotes forms (Y strain). Parasitemia, body weight and survival were evaluated. We collected fresh stool samples at 10 and 20 day post-infection (dpi) for cultivable fecal microbiota analysis. For *in vitro* analysis, we used the primary myenteric neurons culture assays. The supernatant was collected at 24, 48 and 72 hpi with *Tc* Y strain (10:1) for lactate dehydrogenase (LDH), nitric oxide (NO) and ELISA for the cytokines IL-6, TNF- α , IFN- γ and IL-10. The results showed that the deficiency of ANXA1 resulted in higher parasitemia, weight loss, and mortality rate compared with WT. The cultivable fecal microbiota quantification showed that ANXA1 KO mice had less *Bacteroides*, *Staphylococcus* and *Enterococcus* at 10 and 20 dpi. *Staphylococcus* in WT mice decreased with *Tc* infection, whereas it increased in ANXA1 KO mice. *In vitro*, at 24 hpi, myenteric neurons from infected-ANXA1 KO mice showed higher levels of NO, IL-6, TNF- α and IFN- γ and lower levels of IL -10 production compared with WT. Collectively, these results suggest that ANXA1 is a vital regulator of the development of CD pathogenesis, which may be influenced by the intestinal microbiota affecting communication between gut/immune/central system. This helps portraying new insights into disease pathogenesis and may provide new potential therapeutic approaches. **Supported by:** CNPq, FAPEMIG e CAPES

Keywords: Trypanosoma cruzi; Annexin A1; Microbiota.