HP-04 - **Cytokine analysis associated with bioluminescent imaging to the follow up of mice infected with Leishmania amazonensis.**

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The outcome of leishmaniasis caused by *Leishmania amazonensis,* depends on the dynamic modulation of chemokines and cytokines expression, by cells involved in the cellular response. Thus, the protozoan establish the infection and the lesion progresses. Here we report data on cytokine identification in the serum and lesion of mice infected with *L. amazonensis* wild type (LamaWT) and genetically modified parasites (GM),expressing fluorescent proteins (LamaEGFP and LamaCherry) and firefly luciferase (LamaLUC). The infection was performed, at the base of the tail (10^8 promastigotes), which displays a protuberant lesion that stands out under the skin, facilitating the evaluation of lesion progression. In the lesion, TNF-α, IFN-ɣ and MCP-1 could be detected by flow cytometry at the 20 and 50 dpi. Mice infected with LamaWT displayed at least two fold higher MCP-1 levels than mice infected with LamaLUC, LamaEGFP and LamaCherry. MCP-1 levels dropped off only in the LamaWT at the 50dpi, and IFN-ɣ dropped off in the LamaWT and LamaLuc strains. In opposition, IL-6 levels in the blood of mice infected with LamaWT and LamaLUC, increased from 20 dpi to 50 dpi. TNF-α levels in mice infected with LamaWT, LamaEGFP and LamaCherry strains at the 20 dpi were significantly lower than the levels in the lesion of mice infected with LamaLUC strain. TNF-α increased significantly from the 20 dpi to the 50 dpi, in the LamaWT strain. The follow up of infection caused by LamaLUC in mice, was evaluated by bioluminescent imaging. The photons emitted by the bioluminescent LamaLUC, inside the lesion, at the top of the tail, are promptly available, which could give us in real time information about the *Leishmania* growth and lesion progression. The association of bioluminescent imaging with cytokine evaluation, in the blood and in the lesion by flow cytometry, is an improvement in the follow up of disease/ lesion progression and for pre-clinical studies. **Keywords:**Leishmania amazonensis;luciferase;cytokines.

HP-07 - **Acquired Toxoplasma gondii infection leads to retinal microvascular abnormalities in mice.**

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Toxoplasmosis affects one third of the world population and its etiologic agent is the protozoan *Toxoplasma gondii*. *T. gondii* has a tropism for 3 specific organs (muscle, brain and eyes) and ocular toxoplasmosis (OT) is well described in the literature. Retinochoroiditis is identified as the main manifestation of OT, but vascular occlusion can occur, even away from active lesions, which may result in hemorrhages. In addition, cases of retinal detachment and subretinal neovascularization have been reported. Our group demonstrated microcirculatory alterations and reduced angiogenesis in mouse brains after acquired *T. gondii* infection, suggesting that such alterations may also occur in OT. This work aims to analyze the effects of acquired *T. gondii in vivo* infection on the retina and its vasculature. For acquired ocular toxoplasmosis model, C57Bl6 mice were inoculated intragastrically with 2 ME49 strain cysts and analyzed at 10, 20 and 30 days post infection (dpi). We observed that infection led to body weight loss and reduced food consumption at all times studied. Cytometric bead array analysis showed a transient increase in serum TNF-α, IFN-g and IL-6 levels at 10 dpi. Confocal microscopy analysis of retinal flat mounts revealed a reduction in the interaction between endothelial cells and astrocytes in infected mice at 10 dpi. At 20 and 30 dpi, we observed an influx of immune cells in the superficial plexus of the retina, which is not seen at 10 dpi, in addition to an apparent astrogliosis. Expression of pro-inflammatory- and angiogenesis-related genes by RT-qPCR revealed an increase in CX3CR1, marker of microglial cells and a reduction in DLL4-NOTCH1 genes, part of angiogenic signaling cascade. Our data suggest that *T. gondii* infection induces an increase in retinal inflammatory profile that would lead to damage to the retinal vasculature, thus contributing to visual impairment in toxoplasmosis. **Supported by:**INOVA no. 3231984391; CNPq 401772/2015-2 - 444478/2014-0;INCT/INNT 465346/2014-6 for KC ,FAPERJ no. E-26/010-001199/2015; Projetos Temáticos no. E26/010.101037/201; Sediadas grant no. E-26/010.001493/2019 for KC). **Keywords:**Ocular Toxoplasmosis;Vasculature;Retina.

HP-12 - **Skeletal muscle pathology and atrophy in mice infected with *Toxoplasma gondii***

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Skeletal muscle tissue (SkM) is critical for the transmission of *Toxoplasma gondii* where it survives as tissue cysts and causes myositis in patients. However, little is known about how the infection can affect the SkM and its physiology. The aim of this work is to study SkM physiology and the impact of *T. gondii* infection in muscle fibers. For this purpose, Swiss Webster mice were inoculated intragastrically with *T. gondii* Me49 strain cysts and evaluated up to 40 days post infection (dpi). At 10 dpi, a decrease in the body weight was identified and, although no differences were observed in weightlifting and Kondziella's tests, functional grip test showed decreased time of applied force for infected animals. The oxidative muscle soleus (SO) and glycolytic tibialis anterior (TA) were collected for analysis. Parasites were identified in SkM fibers by histochemistry and RT-PCR. Reduced myofibers and inflammatory infiltrates were found in TA both at 10 and 40 dpi. Fiber distribution of the muscle showed a predominance of smaller fibers at 10, but not at 40 dpi. Interestingly, increased expression of atrophy-related genes *Murf1* and *Atrogin1* were only identified in infected TA. at 40 dpi. Regeneration was observed by the presence of myofibers with centralized nuclei, which were 12% of total fibers in TA. Pax7 expression, however, was reduced at 10 dpi and did not change after 40 dpi in TA. In SO, Pax7 was increased after 40 dpi indicating a late regenerative process. Mitochondrial fusion (Opa1) and fission (Fis1) genes were increased only in SO at 40 dpi. Infection induced a systemic increase of IL-6, IFN-γ and TNF-α at 10 dpi, which was normalized at 40 dpi. Because IL-6 signaling pathway is relevant for muscle atrophy, we evaluated the expression of IL-6 receptor (*IL6ra*), and *stat3*. *IL6ra* and *stat3* had increased expression at 40 dpi only in SO. *T. gondii* differentially affects muscle fibers according to metabolic features leading to functional defects. **Supported by:**INOVA Fiocruz, IOC, CNPq, Edital PAPES VII, Faperj **Keywords:**Toxoplasma gondii;Skeletal muscle;Muscle atrophy.

HP-14 - **Impact of nutritional status on the infection course and experimental treatment of *Leishmania infantum*-infected mice**

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Remoto Poster Session

Malnutrition and visceral leishmaniasis (LV) are major public health problems since both are responsible for millions of deaths in many countries. The development and progression of leishmaniasis is associated with the host immune response type. In this scenario, malnutrition can directly affect the course of leishmaniases, as it impairs several components of the immune system. It has already been reported that malnutrition directly interferes with immunity and/or susceptibility to LV. However, there is little information about the effects of malnutrition on the LV treatment response in infected hosts. In this context, the objective of this work was to evaluate the influence of nutritional status on the treatment of mice subjected to experimental malnutrition and refeeding and infected with *Leishmania infantum*. Weaned BALB/c mice that received the control diet or restricted diet were infected or not and treated or not with glucantime. The nutritional status of the mice was evaluated through phenotypic markers and hematological and biochemical parameters. The infection effects were evaluated through an LDA assay. Our results showed that the restricted diet was able to induce mild malnutrition. Malnourished mice demonstrated significant weight loss, slow body growth and low body mass index (BMI) values, while those that were refeeding began to grow and recovered their body weight and BMI. Malnourished and refed mice presented a low parasitic load in the spleen. In the liver, both groups presented a high parasitic load, and when the malnourished BALB/c mice were treated with glucantime, they showed a smaller decrease in parasitic load than treated control mice. In conclusion, a restricted diet was able to generate mild malnutrition and cause changes similar to those observed in marasmic malnutrition in the nutritional status of mice. During LV, data suggest that malnutrition impaired the treatment, and the refeeding process was not able to fully reverse this effect. **Supported by:**CAPES, CNPq and FAPERJ **Keywords:**Visceral leishmaniasis;Marasmatic malnutrition ;treatment.

HP-19 - **Interleukin-10 enhances parasite burden in *Leishmania (L.) major* infected macrophages *in vitro*, but not in *Leishmania (V.) braziliensis*.**

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INTRODUCTION: Leishmaniasis is a disease with high incidence in tropical countries and is an important concern for public health agencies. *Leishmania (V.) braziliensis* (Lb) is the main species causing cutaneous leishmaniasis in Brazil and *Leishmania (L.) major* (Lm) is the best-described species causing cutaneous leishmaniasis in murine models. Macrophages are the main cells parasitized by the protozoan and classically activated macrophages (cMo) by TH1 stimuli are related to the control of the parasite load. Alternatively activated macrophages (AAMo), stimulated by IL-4 and/or IL-10 are susceptible to Lm parasite, but there is no information about susceptibility of Lb *in vitro*. OBJECTIVE: Compare Lb and Lm survival *in vitro* in murine macrophages stimulated with IL-4 and/ or IL-10. METHODS: Macrophages from BALB/c mice elicited with thioglycolate were stimulated or not with IL-4 or IL-10 for 24h and infected with Lb or Lm. Presence of AAMo was evaluated by arginase activity assay and parasite load was determinated by counting amastigotes in light microscopy after 3, 24, 72 and 120 hours post-infection and by the recovery of promastigote forms in culture assay. RESULTS: There was a significant decrease in the parasite load after 72h in Lb infected macrophages in all unstimulated, IL-4 and IL-10-stimulated macrophages assayed by microscopy or promastigote recovery. This phenomenon contrasted with infections with Lm, which had an increase in parasite load after 72h in infections in unstimulated macrophages and in macrophages stimulated with IL-4 or IL-10 with an increase in parasite load in infections of cells stimulated with IL-10 in relation to the unstimulated. CONCLUSION: Control Lb occurs even in IL-4 or IL-10 stimulated macrophages. On the other hand, macrophages were susceptible to infection by Lm, alternative activation favored an increase in the parasite load. **Supported by:**FAPEG, CAPES, CNPq, INCT-IPH. **Keywords:**Murine macrophage;cutaneous leishmaniasis;Alternative activation.

HP-20 - **Nutritional and oxidative stress induce AMPK phosphorilation in Trypanosoma cruzi**

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Trypanosoma cruzi demonstrates great evolutionary and adaptive capabilities in its biological cycle according to the host due to variable nutritional and oxidative conditions. Therefore, it needs several metabolic pathways and mechanisms that allow it to develop and maintain its cellular stability. Adenosine monophosphate-activated protein kinase (AMPK) is an enzyme that acts as a central regulator of energy homeostasis in many organisms. AMPK is a heterotrimer formed by two regulatory subunits (β and γ) and a catalytic subunit (α). It is mainly regulated by changes in AMP in cells and in the active state, a conserved loop in the α chain is phosphorylated. Therefore, we investigated in which conditions AMPK is phosphorylated in *T. cruzi*. We found that AMPK was rapidly phosphorylated under nutritional and oxidative stresses, generating two different products recognized by a monoclonal anti-phospho-threonine 172, an epitope conserved in the α1 chain of the parasite kinase. In the first case, the phosphorylation occurred in a protein that migrate faster in SDS-PAGE and follows an increase in the AMP/ATP levels. In the second there was no change the AMP/ATP ratio and the product of phosphorylation migrate slower. We concluded that different stress types caused variable changes in the AMPK phosphorylation and possibly function, which might be relevant for parasite adaptation. **Supported by:**FAPESP 2021/12527-0 **Keywords:**AMPK;Stress response;Kinase.

HP-21 - **Serum antibodies blocked by glycan antigens in canine visceral leishmaniasis serology are mostly IgA immune complexes**

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Immune complexes (ICs) are found in canine visceral leishmaniasis (CVL) and interfere with the serum detection of antibodies. Dissociation of these monovalent complexes by dissociative enzyme-linked immunosorbent assay (ELISA) removes false-negative results and allows some characterization of antibodies and antigens. We studied the serology of dogs with suspected CVL in an endemic area, testing two Leishmania (Leishmania) [L. (L.)] infantum antigens. We analysed the presence of immunoglobulin G (IgG), immunoglobulin A (IgA) and immunoglobulin M (IgM) antibodies specific to promastigote soluble extract (PSE) and low-molecular weight glycans (glycan–bovine serum albumin (BSA) complex – GBC) by conventional and dissociative ELISA. Our results showed a significant fraction of IgA ICs (46.5% for PSE and 47.6% for GBC), followed by IgG ICs (10% for PSE and 23.5% for GBC). IgM ICs were more frequent for PSE (22.7%). Hypergammaglobulinaemia in CVL would be related to the presence of IgA and IgG ICs, resulting in deficient elimination of these antibodies. Our data confirmed the presence of ICs that can generate false-negative results in conventional serology. The production of IgA antibodies and the high frequency of blockade by glycan antigens suggest the active participation of this immunoglobulin and its ICs in the immunopathology of CVL, indicating a new path for further Research **Supported by:**FAPESP - PROCESSO 2017/14675-0 **Keywords:**Immune complexes;Antibodies;Glycan.

HP-22 - ***In vitro*** **evaluation of aldimine derivatives as drugs for the treatment of visceral leishmaniasis**

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Visceral leishmaniasis (VL) is the most serious form of leishmaniasis, which if not diagnosed and treated in time leads to death. Today, the available restrict treatment arsenal consist of few drugs, such as pentavalent antimonials, paramomycin, pentamidine, miltefosine, amphotericin B deoxycholate and liposomal. But due to some limitations, such as the adverse effects observed in the treatment and that there is still no ideal therapy, the World Health Organization (WHO) recommends and supports the investigation of new drugs and treatment strategies against the disease, such as the study of new compounds in order to broaden perspectives for the development of more effective therapies. In this sense, synthetic compounds such as aldimines, emerge as possible alternative. The main objective was to test the leishmanicidal/leishmaniostatic action of aldimine derivatives 3H8 and 3D7, compounds with known antimicrobial action, in *L. infantum* infected murine cells (RAW 264.7 lineage). For this study, the OP46 strain of *L. infantum* genetically modified with a fluorescent gene (OP46 GFP^+) was used. We evaluated the cytotoxicity of different compounds by the colorimetric method of 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl tetrazolium bromide (MTT) in murine macrophages, with a lower toxicity observed at 3H8 when compared to the compound 3D7. For the *in vitro* evaluation of leishmanicidal activity in amastigotes, RAW 264.7 murine macrophages infected with OP46 GFP^+ promastigotes were used and treated with different chemical compounds for 24, 48 and 72 h, being evaluated by flow cytometry. Our results showed a decrease in the parasite load in relation to the control infected group, regardless of the time of infection, with both compounds. The dataset obtained suggests that the aldimine compounds evaluated have leishmanicidal potential and deserve to be evaluated in experimental *in vivo* chemotherapy studies against VL. Acknowledgments: UFOP, PROPPI, CAPES, CNPq, FAPEMIG, INCT-DT. **Supported by:**FAPEMIG APQ-02256-18 CAPES finance code 001 **Keywords:**Visceral Leishmaniasis;Treatment;Aldimines.

HP-23 - **Unraveling the mechanisms of cholesterol scavenging by intracellular amastigotes of Trypanosoma cruzi**

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Trypanosoma cruzi amastigotes develop intracellularly in the vertebrate host. These forms live in the cytosol of the host cells where they have full access to host cytosolic macromolecules and organelles. Although amastigotes have a complete and functional endocytic pathway, data about which macromolecules they can endocytose and how they interact with host organelles are scarce. Cholesterol performs most of the sterol found in intracellular amastigotes suggesting that they can scavenge cholesterol from the host cell. However, the mechanisms utilized by amastigotes to capture cholesterol while inside the host cell cytosol are still to be determined. In this work, we investigate cholesterol traffic in infected host cells by using fluorescent cholesterol (TFChol) tracer, confocal and super-resolution microscopy analysis, and high-resolution electron microscopy. For this, infected host cells (24hpi) were incubated with TFChol added directly to the culture medium or preloaded in LDL particles. By confocal microscopy, TfChol were found in punctual structures at the anterior region of the amastigotes, close to the flagellar pocket/cytostome . The appearance of these structures inside the parasite was earlier when the TfChol was added directly to the culture medium, suggesting that the traffic from host membrane constitutes a faster cholesterol route of acquisition by the parasites. Antibody labeling of host cell organelle molecular markers showed participation of the endoplasmic reticulum in this fast route of cholesterol acquisition. For analysis at ultrastructural level we used an anti-Bodipy antibody, to label TFChol, and fluoronanogold particles as secondary antibodies to correlate localization of TfChol labelling in light microscopy with amastigotes intracellular structures using transmission electron microscopy. This analysis will give us more insights into the cholesterol traffic from the host to the amastigotes, unraveling a possible transfer via contact-sites. **Supported by:**Faperj, CNPq **Keywords:**amastigotes;cholesterol traffic;endocytosis.

HP-24 - **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 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. Since fungal IPC synthase (IPCS) null mutants are not viable and IPCS activity has been described in all parasite forms of *T. cruzi*, this enzyme has been considered an ideal target for the development of new and more effective drugs to treat CD. IPCS is an integral membrane protein conserved among 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 showed that the lack of IPCS activity does not affect epimastigote proliferation or its susceptibility to drugs 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, proliferation of intracellular amastigotes as well as 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 were unable to cause infection *in vivo*, even in a highly susceptible animal model of infection. **Supported by:**CNPq - Processo: 380642/2022-0 **Keywords:** Trypanosoma cruzi ;Sphingolipids; phosphorylceramide synthase.

HP-25 - **Ribozyme mediated knockdown of Jumonji-like C2 histone demethylase (PfJmjC2) decreases steady-state variant gene transcripts in the malaria parasite *Plasmodium falciparum***

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The persistence of the malaria parasite *Plasmodium falciparum* depends strongly on its capacity to evade the human host's immune response and this is mediated by the deposition of large parasite encoded proteins on the infected erythrocytes surface. These antigens were termed *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1) and are encoded by the *var* gene family. Due to the fact that these proteins become targets of the acquired immune response, their expression is controlled in a tight manner. This means that only one or two PfEMP1 per infected red blood cell is expressed - from a repertoire of up to 90 different *var* alleles present in the genome. The control of the PfEMP1-encoding *var* genes occurs at the transcriptional level and depends on multiple factors including chromatin writers, readers and erasers. In order to elucidate participating factors, we created a *P. falciparum* parasite line where the histone code modifier PfJmjC2 can be controlled by the action of a 3' inserted ribozyme. Upon knockdown of the PfJmjC2 transcript, less transcript was detected by qPCR, as expected. The knockdown led to no discernible growth phenotype, although only a fraction of HA-tagged PfJmjC2 could be detected compared with controls. When monitoring *var* transcripts in parasites phenotype-selected for the expression of determined *var* genes, we observed the downregulation of some - but not all - of the actively transcribed *var* genes. Global RNAseq analysis revealed that upon PfJmjC2 depletion, the transcripts of many regulatory proteins including PfAP2 proteins and other known histone modifiers (SET, SIR2A) were decreased. The same was true for invasion-related protein-encoding genes. We conclude that JmjC2 is an important regulator in a network of pathways, however, its function is probably redundant or timely compensated for due to the fact that knockout is possible and no growth phenotype under knockdown was observed. **Supported by:**FAPESP 2017/24267-7 **Keywords:**Plasmodium;chromatin modifier;transcript knockdown.

HP-26 - **Evaluation of immunogenicity and efficacy potential of a polyepitope T-cell vaccine candidate against visceral leishmaniasis in a murine model**

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The development of an immunogenic, effective, and safe vaccine is essential as an alternative for disease control. The present study aimed to evaluate the immunogenicity and efficacy potential of a polyepitope T-cell antigen candidate against visceral leishmaniasis in a murine model. BALB/c mice were immunized with three doses subcutaneously with Poly-T Leish alone or adjuvanted with Saponin plus Monophosphoryl lipid A, with 15-day intervals between doses, and challenged with 10^7 stationary-phase *Leishmania infantum* promastigotes. Immunogenicity and parasitism in spleen and liver of immunized mice were evaluated 45 days post-challenge. Our results revealed that the Poly-T Leish and Poly-T Leish/SM increases the percentage of specific T ($CD4^+$ and $CD8^+$) lymphocytes proliferation. Also, Poly-T Leish and Poly-T Leish/SM induced a high percentage of T cells producing IFN-γ and TNF-α, meanwhile, the Poly-T Leish/SM group also showed an increased percentage of multifunctional T cells producing double ($IFN\text{-}\gamma^+TNF\text{-}\alpha^+$, $TNF\text{-}\alpha^+IL\text{-}2^+$, $IFN\text{-}\gamma^+IL\text{-}2^+$) and triple-positive ($IFN\text{-}\gamma^+TNF\text{-}\alpha^+IL\text{-}2^+$) cytokines. Poly-T Leish and Poly-T Leish/SM stimulated a decreased IL-4 and IL-10 compared to the Saline and adjuvant group. Poly-T Leish/SM immunized mice exhibit a noteworthy reduction in the parasite burden (spleen and liver) through real-time PCR (96%) compared to the Saline group. Moreover, we observed higher nitrite secretion in antigen stimulated-culture supernatant. Spearman's *r* test analyses showed a strong negative correlation between splenic parasite burden and the percentage of T cells producing IFN-γ and TNF-α. We demonstrated that the Poly-T Leish/SM was potentially immunogenic, providing enhancement of protective immune mechanisms, and conferred protection reducing parasitism. Our candidate was considered potential against visceral leishmaniasis, and eventually, could be tested in phase I and II clinical trials in dogs. Acknowledgments: UFOP, PROPPI, CAPES, CNPq, and INCT-DT. **Supported by:**CNPq (MCTI/CNPq/CTBIOTEC nº 27/2013, 301526/2015-0, 486618/2013-7, 310104/2018-1 and 435224/2018-2); CAPES (Finance Code 001) **Keywords:**Visceral leishmaniasis;polyepitope vaccine;immunogenicity.

HP-27 - **INFECTION WITH *Leishmania (L.) amazonensis* IS AUGMENTED BY HOST SURFACE PROTEIN DISULFIDE ISOMERASE**

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Leishmaniases comprise a spectrum of diseases caused by protozoan parasites of the genus *Leishmania* spp., transmitted by the bite of infected female phlebotomines. Infections by different *Leishmania* species may lead to tegumentary or visceral complications in humans. Chaperones present on macrophage surface play a fundamental role in the regulation of cellular homeostasis and may affect survival and infectivity of *Leishmania*. Protein disulfide isomerase (PDI) is one of the 20 most abundant chaperones of the endoplasmic reticulum (ER). The canonical role of PDI is to assist in the isomerization of disulfide bonds in nascent ER proteins, controlling the protein folding processes. The presence of PDI on the macrophage surface was associated with increased infection by *Leishmania (L.) chagasi*, a species associated with visceral leishmaniasis. The present study aimed to evaluate PDI role on the infection by *L. (L.) amazonensis*, a species responsible for cutaneous leishmaniasis. Flow cytometry was carried out to confirm the presence of PDI on macrophage surface. Bone marrow derived-macrophages (BMDM) were then blocked with anti-PDI polyclonal antibody and infected with promastigotes of *L. (L.) amazonensis*. BMDM from transgenic mice overexpressing PDI and wild type macrophages were also *in vitro* infected with *L. (L.) amazonensis*. *In vivo* imaging using M2269 *La*-LUC infection was done to compare lesion swelling and parasite load in transgenic and wild type mice. The results of flow cytometry indicated a low abundance of PDI on macrophage surface. Infection of macrophages blocked with anti-PDI was lower compared with infection in the presence of isotype antibody. Accordingly, infection of macrophages overexpressing PDI was higher than of the wild type counterparts. The *in vivo* results visually showed higher lesion swelling and parasite load in the PDI overexpressing group in comparison with the wild type. **Supported by:**(CAPES) 88887.360800/2019-00 **Keywords:** Leishmania (L;) amazonensis; PDI; Macrophage infection.

HP-28 - **The infection of mammalian cells and insect vectors with Trans-sialidase knockout Trypanosoma cruzi.**

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Trans-sialidases (TS) are encoded by the largest gene family in the *Typanosoma cruz*i genome. Enzymatically active TS (aTS) correspond to 12 sequences. aTS are responsible for transferring sialic acid from host glycoconjugates to mucins present on the parasite surface. Using CRISPR/Cas9 technology, our group generated aTS knockout (aTSKO) parasites with undetectable levels of TS activity. Disruption of aTS genes did 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, aTSKO were unable to establish infection even in the highly susceptible IFN-g knockout mice. To investigate aTS roles, we performed *in vitro* infection assays with wild type (WT) and aTSKO parasites in two cell models: HeLa (non-phagocytic) and THP1 (phagocytic) cells. Our results confirmed that the lack of aTS did not impair parasite internalization and AMA multiplication in both cell types, but drastically affected TCT release. Given that several immune response genes, such as type I IFN, 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 and CXCL8. Contrarily to the aTSKO inability to infect mice, *in vivo* infection in *Rhodnius prolixus* insects was not affected by the lack of TS activity. After feeding fourth instar nymphs with blood containing WT and aTSKO epimastigotes no significant differences in total parasite numbers obtained from the digestive tract or in the urine were observed. We hypothesized that the infection success of *T. cruzi* in mammalian hosts are strictly dependent on the presence of aTS, thus aTSKO may serve as a target for vaccine designing in order to neutralize Chagas disease. **Supported by:** CNPq, CAPES **Keywords:**Active trans-sialidases (aTS);intracellular development;invertebrate infection.

HP-29 - *In vitro* and *in vivo* effects of zileuton against *Leishmania major*

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Cutaneous leishmaniasis is a public health problem causing a range of diseases from self-healing infections to chronic disfiguring disease. Currently, there is no vaccine for leishmaniasis, and drug therapy is often ineffective. 5-lipoxygenase (5-LO) is an enzyme required for production of leukotrienes and lipoxins and interferes with parasitic infections. Parasite as *Toxoplasma gondii* carries an enzyme like lipoxygenase activity that can interact with host biosynthetic circuits for endogenous negative signals that divert the host immune response and limite acute inflammation. Here, our aim was to evaluate the effect of zileuton (5-LO inhibitor) treatment during *Leishmania major* infection *in vivo*, and mainly to investigate if *L. major* itself wielding its own lipoxygenase. For this, promastigotes form of *L. major* was incubated with 5-LO inhibitor at 100, 33, 10, 3 and 1 micromolar or dimethyl sulfoxide (DMSO; used as a vehicle/solvent). *In vivo*, our preliminary data, demonstrated that treatment of *L. major*-infected C57BL/6 with zileuton reduced the parasite titer in the ear at 3 and 4 week after infection when compared with infected untreated mice. *In vitro*, zileuton induced a dose-dependent reduction of parasite numbers in an axenic culture. Collectively, these results suggest that zileuton treatment reduce the number of parasite *in vivo* and *in vitro* (axenic culture), mainly indicating that *L. major* promastigotes may carry a lipoxygenase-like activity that itself could respond to zileuton treatment. **Supported by:**: NPq, CAPES and FAPEMIG **Keywords:**L; major;Zileuton;5-lipoxygenase.

HP-30 - *Trypanosoma cruzi* induces DNA double-strand breaks and activation of the DNA damage response pathway in non-phagocytic cells

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Intracellular pathogens such as viruses, bacteria, fungi, and protozoa often activate the DNA damage response (DDR) pathway in host cells. *Trypanosoma cruzi*, the etiological agent of Chagas disease, alters many signaling pathways in the host cells, including apoptosis inhibition, senescence induction, inflammatory response, and transcriptional alteration. Recently, we demonstrated that *T. cruzi* infection modulates the transcription and the splicing machinery in the host cell causing alterations in the nuclear compartments. We wondering whether these modulations could have been done in answer to the DNA damage caused by the presence of the parasite. The aim of this work was to investigate the existence of DNA damage and analyze the activation of the DNA damage response pathway in cells infected by *T. cruzi*. Our results showed that LLC-MK2 infected by *T. cruzi* analysed by comet assay present a high number of DNA breaks in host cells at 12hpi ($p<0.001$). On the other hand, maximal phosphorylation of H2AX is observed between 2 and 4 hpi analysed by immunofluorescence and western blotting ($p<0.001$). Throughout the infection process, γ-53BP1 and γ-ATM kinases remained active ($p<0.001$), while the γ-DNA-PKcs kinase had maximum activation at 12hpi ($p<0.001$). In contrast, γ-ATR and γ-Rad51 kinases were not altered during the evaluated period. Our data demonstrate that *T. cruzi* infection induces the activation of the DDR pathway in the host cell and shows the phosphorylation dynamics that the main proteins of the DDR pathway follow throughout the first 24 hours of infection. Our data also suggests that the breaks induced by *T. cruzi* infection are of the double-strand break (DSB) type and that the non-homologous end-joining (NHEJ) repair pathway may be activated. **Supported by:**FAPESP: 2018/03677-5; CAPES: Finance Code 001 (Project 88882.461733/2019-01); FAEPA / FMRP / USP **Keywords:**DNA damage response pathway;Trypanosoma cruzi infection;host cell nucleus.

HP-31 - **EVIDENCE OF ANEUPLOIDY IN *TRYPANOSOMA CRUZI* BY FISH (FLUORESCENCE *IN SITU* HYBRIDIZATION) USING SPLICE LEADER GENE AS SPECIFIC DNA PROBE**

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T. cruzi presents a high degree of intraspecific genetic variability, with possible implications for its adaptation in different hosts. Several studies have shown aneuploidy in *T. cruzi*, even though it´s considered a diploid organism. Whole genomic approaches revealed chromosomal ploidy within a same *T. cruzi* population. Here we identified aneuploid events in *T. cruzi* by FISH that allows the evaluation of ploidy at the individual cell level. We used the Spliced Leader sequence (SL) as a chromosome-specific marker. SL gene encodes an essential RNA for translation, which is transcribed from a tandemly arranged repeated sequence. Epimastigotes (CL Brener) were grown in axenic cultures and cells that bear more than one flagellum were disregarded, excluding parasites in mitosis. Ploidy was estimated from 600 labeled cells for each probe by epifluorescence microscopy, by 3 independent observers in biological replicates. Ploidy frequency was as follows, 77.7% of cells were monosomic, 16.3% disomic, 1.8% trisomic and 0.5% polysomic. No statistically significant differences were found between the counts ($p<0.05$). Aneuploidy events were validated after cell cycle synchronisation by hydroxyurea (HU) by confocal microscopy. To investigate if there's a difference in the ploidy during cell cycle, epimastigotes were treated with HU for 18 h and samples collected in G1 (2h) and G2 (14h) for flow cytometry and FISH analyses. No statistically significant differences were found between treated and control groups, confirming the aneuploidy within the population. The high proportion of monosomic cells could be explained by a mistake in chromosomal segregation due to nondisjunction of sister chromatids during mitosis. A potential consequence is the change in the relative dosage of products from genes located on the missegregated chromosomes. To confirm the occurrence of chromosome aneuploidy, we will test markers (single copy genes) from the same homologue where the SL genes were mapped. **Supported by:** FAPESP 2016/15000-4, CAPES, CNPq **Keywords:** TRYPANOSOMA CRUZI;FLUORESCENCE IN SITU HYBRIDIZATION; ANEUPLOIDY.

HP-32 - **CHARACTERIZATION OF *Leishmania (L.) amazonensis* OLIGOPEPTIDASE B AND ITS ROLE IN MACROPHAGE INFECTION**

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Leishmania spp. are parasitic protozoa that cause leishmaniasis, a disease endemic in 98 countries. *Leishmania* promastigotes are transmitted by the vector and differentiate into amastigotes within phagocytic cells of the vertebrate host. To survive in multiple and hostile environments, the parasite has several virulence factors. Oligopeptidase B (OPB) is a serine peptidase present in prokaryotes, some eukaryotes and some higher plants. It has been considered a virulence factor in trypanosomatids, but only a few studies, performed with Old World species, analyzed its role in *Leishmania* virulence or infectivity. *L. (L.) amazonensis* is an important agent of cutaneous leishmaniasis in Brazil. The *L. (L.) amazonensis* OPB encoding gene has been sequenced and analyzed in silico but has never been expressed. In this work, we produced recombinant *L. (L.) amazonensis* OPB and showed that its pH preferences, Km and inhibition patterns are similar to those reported for *L. (L.) major* and *L. (L.) donovani* OPBs. Since *Leishmania* is known to secrete OPB, we performed in vitro infection assays using the recombinant enzyme. Our results showed that active OPB increased in vitro infection by *L. (L.) amazonensis* when present before and throughout infection. Our findings suggest that OPB is relevant to *L. (L.) amazonensis* infection, and that potential drugs acting through OPB will probably be effective for Old and New World Leishmania species. OPB inhibitors may eventually be explored for leishmaniasis chemotherapy. **Supported by:**FAPESP (Processo: 2019/02391-3) / CAPES **Keywords:**L; (L;) amazonensis;Oligopeptidase B;Macrophage infection.

HP-33 - **Study of the regulation of iron and heme transporters in *Leishmania (L.) amazonensis***

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Leishmaniases, a spectrum of diseases caused by protozoan parasites of the genus *Leishmania*, affects millions of people around the world. During infection, nutrient availability within the phagolysosome is known to have significant effects on parasite replication and virulence. These processes require the acquisition of essential nutrients such as iron and heme from the host since *Leishmania* does not have iron storage proteins and a complete heme biosynthesis pathway. *Leishmania*, therefore, must acquire heme and iron to survive in a hostile environment that restricts the availability of nutrients to the pathogen, a process called nutritional immunity. Identification of several proteins that participate in the transport of iron and heme was crucial for understanding these metabolic pathways in *Leishmania*, and the loss of those transporters causes serious defects in the differentiation and/or multiplication of these parasites in the host. Hence, our goal is to investigate the cross-regulation between iron and heme transporters of *Leishmania*. Thereupon, we will evaluate parasites genetically modified to overexpress LIT1 in LHR1 knockout or LHR1 in LIT1 knockout parasites. During the validation of previously obtained LIT1 knockout mutants via homologous recombination, we discovered that LIT1´s chromosome (30) is tetrasomic in *L. amazonensis* PH8 strain. Therefore, we are generating LIT1 knockout mutants using the CRISPR/Cas9 genome editing approach. In parallel, we obtained parasites overexpressing LIT1 tagged with GFP in the LHR1 single knockout background. These mutant parasites are being evaluated regarding replication and expression of genes and proteins essential for iron and heme metabolism. Characterization of the regulation of the pathways related to iron and heme transport is critical for a better understanding of *Leishmania* physiology and the host-pathogen interaction.
Supported by:2021/03355-0 **Keywords:**Leishmaniasis;Transition Metals;Host-Parasite Interaction.

HP-34 - **First isolation, genomic and biological characterization of a *N. caninum* strain isolated from free-ranging chicken**

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Neospora caninum induces abortions in cattle and consequent economic impact, since there are no treatments nor vaccines against the disease. It is known that birds are reservoirs for *N. caninum*, but the actual relevance of neosporosis in birds is not clear. In the present work we aimed to identify whether free-range chickens would be natural hosts of *N. caninum*. First, we analysed whether chickens sampled in Brazil had specific antibodies against the parasite. We then performed bioassays in mice with brain samples of positive animals. That led us to isolate a new strain of *N. caninum* (Nc-UDI4), with identity confirmed by positive parasite-specific Nc5 genomic sequence, further confirmed by the detection of IgG antibodies in the serum of infected mice. NGS of Nc-UDI4 showed coverage of 87.6% of the reference genome (Nc-Liv) and a series of SNPs and InDels in genes related to adhesion, invasion and survival of the parasite. Accordingly, Nc-UDI4 had lower invasion/replication rates compared to Nc-Liv. Microsatellite analysis showed that Nc-UDI4 is closely related to the genotypes found in Argentinian strains and Nc-1, and relatively distant from Nc-Liv. Also, we observed that that Nc-UDI4 and Nc-1 are less virulent than Nc-Liv in infected C57BL/6 mice, due to higher survival rates, reduced parasite burden and inflammation and a more prominent Th1-biased cytokine profile. In conclusion, we determined that chickens may naturally host *N. caninum*, and the strain we were able to isolate had common features to other known strains of the parasite. **Supported by:**CNPq 313761/2020-5; FAPEMIG PPM-00547-17, APQ-01313-14, REDE-00313-16 **Keywords:**Neospora caninum;Chicken;Isolation.

HP-35 - **Influence of multiple inoculum of *L. (L.) infantum* promastigotes in an experimental model in BALB/c mice**

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Visceral leishmaniasis (VL) is endemic in Brazil and affects 3,500 individuals/year, with a proportion of 1 patient for 18 infected. It is caused by the parasite *Leishmania (Leishmania) infantum* and transmitted by sandflies *Lutzomyia longipalpis* species. Mice are usually resistant to infection, similar to human self-limiting disease. In experimental models, a single inoculum is used to promote infection. However, for canine reservoirs, the host may receive multiple infective doses of different sand flies, and it can affect the development of the disease. To clarify this aspect, we intend to simulate multiple inocula in a mice model and relate it to the progression of the disease. BALB/c mice were infected intraperitoneally with 1 inoculum (Group 1) or with 4 weekly inoculums (Group 2) of $1X10^7$ promastigotes. The infection was monitored by measuring the production of IgG antibodies, detected by conventional ELISA (ELISAc) and dissociative ELISA (ELISAd). The animals were euthanized at 30 days of infection, and spleen and liver fragments were removed to determine the parasite load by real-time PCR, and paraffin embedding for conventional HE histology and immunohistochemistry. For the parasite load, the protein concentration of the organ homogenate was expressed as the number of parasites/mg of protein. Statistical analysis was performed using the Kruskal Wallis method with Dunn's post-test. The experimental group 1 showed increasing levels of IgG antibodies and the presence of circulating monovalent immune complexes (CIC) in the last post-infection periods (21st and 28th days). In group 1, the mean parasite load in the spleen was $2X10^5$ and in the liver $2X10^4$ parasites/mg of protein, higher than in group 2, mean of $1X10^4$ in the spleen ($p<0.01$) and $4X10^3$ in the liver ($p<0.001$). With this study, we observed that qPCR showed higher parasite load in animals that received a single dose. In addition, that the challenge with multiple inocula can protect the host from disease. **Keywords:**Experimental Infection;BALB/c mice;Visceral Leishmaniasis.

HP-36 - **Characterization of extracellular vesicles released by neutrophils stimulated with Leishmania amazonensis**

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Neutrophils are the most abundant leukocytes in the blood and play an extremely important role in the inflammatory process and the body's defense against pathogens. In the context of *Leishmania* infection, neutrophils are the first cells to migrate to the affected tissue, where they can either promote the control of the parasite or exacerbate the infection, as reported in the literature. In addition to their microbicidal mechanisms, neutrophils also interact with macrophages, the parasite's final host cells. In response to different inflammatory stimuli, neutrophils release extracellular vesicles (EVs), which are capable of regulating numerous physiological processes and contribute to tissue homeostasis or propagation of infectious agents. These particles have immunomodulatory properties and potentiate neutrophil migration. The participation of neutrophil EVs during *Leishmania amazonensis* (L.a) infection has not been explored. Our aim in this work is to characterize EVs released by neutrophils stimulated by this pathogen. For that, human neutrophils were isolated from peripheral blood of healthy individuals and stimulated or not with *L. amazonensis* promastigotes for 1h at 37ºC. To obtain the fractions enriched in exosomes and microvesicles, we chose to use ultracentrifugation, a widely technique used for vesicle purification. Our preliminary results indicate that neutrophils stimulated with parasites release more exosomes and fewer microvesicles than non-stimulated neutrophils, as observed by nanoparticle tracking analysis. Stimulation with L.a did not affect the size distribution profile of the particles. EVs showed spherical and membrane-delimited morphologies, as observed by negative contrast transmission electron microscopy. We are currently characterizing the content of these vesicles and performing functional experiments to understand how these microparticles can influence the macrophage response and infection by the parasite. **Supported by:**CAPES/FAPERJ/CNPq **Keywords:**Extracellular Vesicles;Neutrophils;Leishmania amazonensis.

HP-37 - **Sand fly yellow salivary proteins modulate neutrophil response to Leishmania parasites**

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Leishmaniasis is a group of neglected diseases caused by parasites of the genus *Leishmania,* which are transmitted by sandfly bites. Factors derived from both the insect and the protozoan, such as microbiota, saliva and proteophosphoglycans, are inoculated into the skin of the vertebrate host at the time of the bite and can modulate the immune response in favor of the infection. After the sand fly bite, circulating neutrophils are the first cells recruited to the site of infection and the first cells to be infected. Our group recently reported that proteins from the insect's saliva, the yellow family of proteins, act as chemoattractants for neutrophils as well as exacerbate the infection *in vivo*. In this study, we investigated the role of these proteins in the infection of human neutrophils with *Leishmania* parasites and consequences that arise from this initial interaction *in vitro*. Our results show that yellow proteins modulate the responses of infected neutrophils. Treatment of neutrophils infected by *Leishmania major* and *Leishmania infantum* with yellow proteins induced an increase in parasite survival, reduction of NETs release and a reduction in the percentage of neutrophils in direct contact with the parasite. *Leishmania major*-infected neutrophils treated with yellow proteins showed a higher elastase release. Furthermore, cultures of macrophages infected with *L. major* and treated with supernatant of yellow protein-treated neutrophils showed an increase in parasite survival. Our data reveal an immunomodulatory role for the yellow family of proteins on infected neutrophils and indicate their possible relevance on human infection. **Supported by:**CNPq, FAPERJ, CAPES **Keywords:**Neutrophils;Leishmania;yellow proteins.

HP-38 - **Identification of potential Leishmania infantum sirtuin inhibitors**

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Different species of Leishmania cause Leishmaniasis in 90 countries. It has a clinical spectrum ranging from cutaneous and mucocutaneous to visceral lesions. Its cycle life shifts between invertebrate and vertebrate hosts, facing different environmental changes, which require rapid adaptations to survival. Post-translational modifications, such as acetylation, has been implicated in the regulation of diverse cellular processes in eukaryotic organisms. We described the acetylated proteins of procyclic, metacyclic and amastigote forms of L. mexicana and found a differential acetylation profile among them, suggesting that might be important for parasite differentiation. Protein's acetylation are regulated by two families of enzymes: lysine acetyltransferases and lysine deacetylases (KDACs). Among the KDACs, sirtuins are involved in the regulation of several biological processes and have been explored as drug targets in different human diseases. Considering the importance of protein acetylation in Leishmania, this work explored the potential of sirtuins from L. infantum as drug target for the identification of inhibitors for future treatment. We screened a library of 80 sirtuin inhibitors using in vitro infection assays to identify molecules with inhibitory activity, three were chosen for further biochemical validation. These assays focused on testing the inhibitory deacetylation activity of the recombinant L. infantum sirtuins. The 3 genes (LiSir2rp1-3) were cloned in pNIC28 vector, sequenced and transformed into bacteria for heterologous expression. The expression of these proteins were confirmed and established the purification protocol for LiSir2rp1 and rp3. We confirmed the in vitro deacetylation activity of the enzyme LiSir2rp3 by biochemical characterization assays. One of 3 previously chosen inhibitors against LiSir2rp3 performed inhibitory activity greater than 30%. New experiments will test these inhibitors against LiSir2rp1-2. **Supported by:**FAPESP 2020/14754-0 **Keywords:**Leishmania;sirtuins;inhibitors.

HP-40 - **Characterization of manganese transport mediated by the iron transporter LIR1 in *Leishmania***

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Leishmaniases are a group of diseases caused by protozoan parasites of the genus *Leishmania*. These parasites alternate between insect and mammalian hosts, going through dramatic changes with shifts in temperature, pH, and availability of nutrients. Among these nutrients, iron is an important cofactor of several enzymes, but can also be highly toxic when free and at high concentrations in the cytoplasm. *Leishmania Iron Regulator 1* (*LIR1*) was identified and characterized in *Leishmania* as a plasma membrane transporter essential for iron efflux and regulation of the intracellular concentration of iron and other transition metals, such as manganese. LIR1 deficiency enhances the toxic effect of excess iron and manganese during promastigote replication. Like iron, manganese is also a cofactor of several *Leishmania* enzymes, in particular arginase, which is essential for parasite replication and infection establishment in mammalian hosts. Considering the significance of manganese for *Leishmania* and the evidence that LIR1 also modulates the intracellular levels of this metal, our main goal is to characterize the manganese transport mediated by LIR1 in *L. amazonensis*. Inductively coupled plasma mass spectrometry (ICP-MS) analysis revealed that LIR1 prevents the accumulation of manganese in *Leishmania.* Moreover, *Leishmania*'s arginase expression and enzymatic activity is modulated by LIR1 expression. In parallel, we observed that promastigotes overexpressing LIR1 present a delayed *in vitro* growth, which was also exacerbated by manganese supplementation. However, manganese intracellular accumulation was not affected by LIR1 overexpression. We anticipate that further ICP-MS analysis of manganese transport kinetics in these mutants will uncover the direct involvement of LIR1 in the transport of manganese across promastigotes' plasma membrane. **Supported by:**FAPESP - 2019/09715-9 **Keywords:**Leishmania;Manganese;Arginase.

HP-41 - **Removal of Complement Membrane Attack Complex (MAC) Pores by Plasma Membrane Repair: an ancestral mechanism of Eukaryotes enabling pathogen immune evasion.**

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Plasma membrane repair (PMR) is a crucial physiological mechanism of eukaryotic cells that promotes the resealing of damages eventually made on 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 are able to 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, respectivelly. By using conventional, confocal and live imaging microscopies, immunobiochemical methods and flow cytometry 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, similar to mammalian cells, *L.amazonensis* promastigotes are able to 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 provide the parasites with 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:**Plasma Membrane Repair;Complement System;Immune Evasion.

HP-42 - **Genomic Analysis of Leishmaniinae Parasites Isolated from Human Visceral Leishmaniasis**

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Leishmaniases are a group of neglected diseases caused by a variety of Leishmania species. In Brazil, Visceral Leishmaniasis (VL) is caused by L. infantum transmitted by sandfly vectors. It is the most severe form of the disease. Recently, we identified clinical isolates from VL cases that do not belong to the Leishmania genus and phylogenetic analysis showed a close relationship with Crithidia fasciculata, a monoxenous trypanosomatid considered non-pathogenic to humans. We called them Crithidia-like. Here, we characterized 47 clinical isolates from VL patients (Sergipe, Brazil) typed as Crithidia-like or L. infantum using an in-house PCR/qPCR assay. Whole-Genome Sequencing (WGS) of these samples were performed in Illumina platform (150 bp paired-end reads, average of 2 GB per isolate) and analyzed using bioinformatic pipelines in Comparative Genomics. Forty two isolates were identified as Crithidia-like and 5 as L. infantum. WGS reads were mapped to Crithidia-like LVH60aC1 assembly (36 pseudochromosomes/contigs). Genetic variations among them were analyzed. Overall, the coverage depth by pseudochromosomes was 40X. Somy values were characterized based on the median of median read depth coverage of all pseudochromosomes, calculated by the SAMtools program and visualized by ggplot2 package on the R studio program. Pseudochromosomes 12, 20 were classified as trisomic and pseudochromosome 36 as tetrasomic. Variant calling was done by SnpEff program and detected an average of 410.000 per isolate in which approximately 276.500 are single nucleotide polymorphisms (SNP) and 45.585 are insertions or deletions (INDELS). Roughly 30,5% of the variants are missense (causes a change on codon and produces a different amino acid), 0,3% are nonsense (causes a change on codon and and results in a stop codon) and 69,2% are silent (causes a change on codon but produces the same amino acid). Further, copy number variations (CNV) and mitochondrial DNA content (kDNA) will be analyzed. **Supported by:**FAPESP 2016/20258-0 **Keywords:**Visceral Leishmaniasis;Whole-Genome Sequencing;Crithidia-like.

HP-43 - **Clinical and parasitological aspects of the treatment of canine visceral leishmaniasis with miltefosine and associations in Florianópolis, Santa Catarina State, Brazil**

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Leishmaniases are zoonotic infectious diseases caused by protozoan parasites of the genus *Leishmania*. Visceral Leishmaniasis (VL) caused by *Leishmania infantum* is the most serious form of the disease, having the domestic dogs as the main reservoirs in the urban transmission cycle. Canine visceral leishmaniasis (CVL) was reported in southern Brazil in 2008, following the occurrence of human cases. In Florianópolis (SC State), CVL was reported in 2010, following the detection of human cases in 2017. Miltefosine has been used for CVL treatment since 2016 in Brazil, but few studies investigated the effectiveness of the treatment of naturally infected dogs. We have assessed clinical, parasitological and histopathological aspects of dogs naturally infected with *L. infantum*, before and after the treatment with miltefosine and associations. Skin and lymph node samples were collected from 24 dogs from Florianópolis before (T0) and after treatment, as follows: 31 days after the first dose of miltefosine (T1) and 6 months after the end of treatment (T2). A significant decrease ($p<0.05$) in the average parasite load on the skin was detected by qPCR on T1, along with a general clinical improvement. Linear regression analysis revealed a positive correlation between the reduction of the parasite load, the clinical improvement of the animal, and the reduction of the inflammatory infiltrates on the skin. In addition, the parasite skin load of dogs submitted to therapeutic regimen 1 (miltefosine+allopurinol+domperidone) was significantly lower ($p<0.05$) than animals treated with regimen 2 (miltefosine or miltefosine+allopurinol or miltefosine+immunotherapy). These results indicate that, despite distinct drugs used in association, the treatment of CVL based on miltefosine contributed to the reduction of the parasite load in the skin and the overall clinical improvement of the animals, but not leading to parasitological cure. **Supported by:**CAPES, FAPESC, FINEP, CNPq **Keywords:**Leishmania infantum;public health;Canine Visceral Leishmaniasis.

HP-44 - **Intracellular views of *Toxoplasma gondii* in cleaved cells by HR-SEM**

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Toxoplasma gondii, an obligate intracellular protozoan, infects up to one-third of the world population. Although most infections are asymptomatic, this protozoan may cause retinal lesions and, in immunocompromised individuals or when contracted congenitally, can lead to life-threatening infections involving the central nervous system. *T. gondii* has evolved several strategies for successful invasion and intracellular survival and multiplication. It includes the apical complex, composed of specialized secretory organelles, namely micronemes and rhoptries, and cytoskeletal elements, such as the polar rings and the conoid. Electron microscopy is a powerful tool to study many aspects of *T. gondii* development inside host cells. For many years, intracellular *T. gondii* could not be observed by Scanning Electron Microscopy (SEM). Field Emission Scanning Electron Microscopy enhanced the resolution power of SEM to a level comparable to TEM. We have applied the method proposed by in 1986 [1] to expose the cytoplasm and organelles of free cells to observe LLC-MK2 cells infected with the RH strain of *T. gondii* with the High-resolution SEM Auriga 40-ZEISS. The method consists in packing free cells infected with *T. gondii* together in a matrix of gelatin and chitosan, freezing and cleaving the assembly. With this method, we obtained a large number of cleaved host cells with parasites inside, so aspects of both the host cell and the parasite could be observed. Rhoptries, micronemes, the tubules of the intravacuolar network, aspects of parasites undergoing endodiogeny and the cytoskeleton around the parasitophorous vacuole could be observed. We believe that this is a simple and reliable method that could be applied not only to *T. gondii*, but other intracellular parasites to reveal new features of their biology. **Supported by:**Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro - E-26/200.135/2020 **Keywords:**Cell Ultrastructure;Scanning Electronic Microscope ;Fracturing method.

HP-45 - **Role of SAPA repeats present in Trans sialidases during *Trypanosoma cruzi* infection**

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Proteins with amino acid repeats have been identified in different protozoa. Among proteins that contain repetitive amino acid sequences in *Trypanosoma cruzi*, are trans-sialidases (TS), which are responsible for transferring sialic acid from host glycoconjugates to mucins on the parasite surface. TS activity is part of a mechanism related to the parasite evasion of the host imune system. Present in more than 1000 copies in the parasite genome, only 16 TS sequences encode proteins with catalytic activity and these proteins have a C-terminal domain containing variable numbers of amino acids repeats known as SAPA (shed acute parasite antigen). In addition, SAPA domain increases the stability of the enzyme in the bloodstream, suggesting that it may contribute to the parasite virulence. To investigate the role of SAPA repeats and their influence on the host immune system, three recombinant versions of an active TS were produced: full-length protein, TS without repeats, and only SAPA repeats. BALB/c mice were immunized with proteins and then challenged with a virulent strain of *T. cruzi*. Both the full-length protein and the protein without the SAPA domain protected animals after challenge but the protein without SAPA provided better protection. Importantly, immunization with the portion containing only SAPA repeats exacerbated the infection. ELISA assays showed that all proteins induced IgG production and its subclasses and that the antibodies induced by the full-length TS protein were directed to the SAPA repeats indicating its immunodominance. Analysis of the cellular response showed higher levels of IFN-γ produced by splenocytes from animals immunized with the TS protein without SAPA repeats, compared to splenocytes from mice immunized with the other two antigens. Taken together these results indicated that immunization with TS antigen without SAPA repeats induces the development of a protective Th1 response, essential for intracellular pathogen infection control. **Supported by:**Capes **Keywords:**Trans-sialidase (TS);SAPA repeats;T; cruzi.

HP-46 - **Identification and characterization of iron transporters candidates in Leishmania amazonensis glycosomes**

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The protozoan parasites of the genus *Leishmania* are responsible for the diseases known as leishmaniases that affect millions of people worldwide. *Leishmania* life cycle includes invertebrate and vertebrate hosts. In the vertebrate host, the parasites survive and replicate inside macrophages, despite the defense arsenal of these cells. One of the critical conditions found by *Leishmania* in the macrophage is the lack of various nutrients, such as iron, an essential cofactor of several enzymes. The identification and study of parasite genes involved in iron metabolism and transport revealed that the availability of iron plays a central role in virulence. Besides, it was also shown that iron deprivation modulates the expression of a series of genes whose function is still unknown. Among these, we found conserved genes encoding proteins addressed to the glycosome, which are trypanosomatids unique organelles. Therefore, our goal is to identify and characterize genes involved in the transport of iron into the *Leishmania* glycosome. *In silico* analysis of the *Leishmania* transcripts modulated by iron deprivation revealed 11 putative genes containing predicted glycosomal addressing signals (PTS1 or PTS2) and transmembrane domains. By immunofluorescence, we confirmed the glycosomal promastigotes compartmentalization of the proteins encoded by 2 of these genes. Moreover, the overexpression of 5 of these genes impacted promastigotes *in vitro* growth. Further characterization of these overexpressors and knockout parasite strains will contribute to elucidate the mechanisms involved in the metabolism and transport of iron into the *Leishmania* glycosome and may indicate new targets for the development of chemotherapeutic agents for the treatment of leishmaniases. **Supported by:**FAPESP 2017/23933-3 **Keywords:**Transfection; Immunofluorescence ; Gene Expression.

HP-47 - **EVALUATION OF SPECIES-SPECIFIC GENES OF Leishmania infantum AS POTENTIAL TARGETS FOR DIAGNOSIS OF VISCERAL LEISHMANIASIS**

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Leishmaniasis is a public health problem found in many countries and caused by several *Leishmania* species. Efficient and fast diagnosis is essential to assure the effectiveness of the treatment, thus, the search for new antigens capable of stimulating the immune response will help to develop more accurate serological tests for diagnosis. Our aim was searching for species-specific genes of *L. infantum* through *in silico* analyses of *Leishmania* genomes using Tritryp database by the following criteria: i) Orthology (excluding other Leishmania species and other trypanosomatids); ii) genes encoding proteins; iii) presence of signal peptide and/or transmembrane domain; iv) absence of post-translational modification. *L. infantum* candidate genes were analysed with Bepipred tool (threshold: 0.500) to predict B cell epitopes. Codon analysis was performed with GenScript Rare Codon Analysis Tool to assess the viability of heterologous recombinant protein expression. Genomic DNA extraction was performed using the Kit Wizard® SV Genomic DNA Purification System (Promega). Primers was designed using Ape and SnapGene programs and analysed with OligoAnalyzerTM tool. PCR reactions were performed and the analysis of the products was performed by agarose gel electrophoresis. A total of 24 genes was found in TriTryp search. Where, 17 have a signal peptide, 4 have a transmembrane domain and 3 have both. In *in silico* analysis we selected six genes, from chromosomes 7, 15, 18, 22 and two from 31. They presented average values of prediction of linear epitopes of B cells in 0.521 and 0.619. Codon usage analysis of these genes showed an adequate codon frequency and adaptability (codon adaptation index in 0.63 and 0.73), with the percentage of low frequency codons ranging between 8% to 18%, indicating a viable expression in *E. coli*. Out of six selected genes, three were successfully amplified by PCR using gDNA from *L. infantum* as template and will be used to perform cloning in experiments. **Supported by:**CAPES: 88887.486526/2020-00 / FAPESP: 2016/20258-0 **Keywords:**Visceral leishmaniasis; Diagnosis; bioinformatics.

HP-48 - **Dual RNA-seq mapping in visceral leishmaniasis: dataset of parasite transcripts in human blood transcriptome upon Leishmania infantum infection.**

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Visceral Leishmaniasis (VL) is a neglected disease caused by Leishmania infantum parasites in Brazil and can be lethal if untreated. Blood transcriptome of infected patients is valuable to reveal molecular mechanisms associated to development of the disease. When transcriptome of host is obtained through RNA-seq, the gene expression of parasite can also be ascertained. Here, our aim was determining a survey of parasite transcripts in blood transcriptome data of patients using dual RNA-seq mapping strategy. For this, fastq files were trimmed using Trimmomatic and high quality reads of groups A (asymptomatic individuals), PD0 (patient with active infection, before treatment), PD180 (cured patients, six months after treatment) from RNA-seq experiment E-MTAB-11047 (Maruyama et al. 2022) were aligned to the concatenated genome (parasite + host) built with Leishmania infantum (GCA_900500625.1) and Homo sapiens GRCh38.p13 genomes obtained from ENSEMBL database, using STAR aligner. A read count table was retrieved for each RNA-seq sample. Although parasitemia is not a feature of L. infantum infection, we were able to identify reads from parasites in blood samples of the three groups, being the PD0 samples those with highest number of representative transcripts totaling 525 mapped genes across the group with an average of 103 mapped genes per sample. For PD180 and A group, an average of 33 and 30 genes were mapped per sample, respectively. Gene ID searches on TriTrypDB revealed that most genes coding for hypothetical conserved proteins, amino acid metabolism and 40S ribosomal protein synthesis. Functional annotation of L. infantum mapped genes are being done on TriTrypDB to identify related biological pathways. Interestingly, parasite transcripts were detectable in cured patients and asymptomatic individuals, suggesting parasite persistence scenario even with absence of clinical signs of VL. **Supported by:**Fundação de Amparo à Pesquisa do Estado de São Paulo FAPESP - JP 2016/20258-0; Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES); FAPESP/ TT3 - 2022/01525-9 **Keywords:**Visceral leishmaniasis;RNA-seq;dual transcriptome mapping.

HP-49 - **THE INHIBITOR OF SERINE PEPTIDASES, ISP2, OF *Trypanosoma cruzi* PREVENTS TMPRSS2-MEDIATED PAR2-TLR4 CROSSTALK AND MODULATES INFECTION AND INFLAMMATION**

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Ecotin is a potent inhibitor of family S1A serine peptidases (SPs) and was first isolated from the periplasm of *E. coli*. Ecotin-like inhibitors were identified in Trypanosomatids and have been denominated **I**nhibitor of **S**erine **P**eptidase (ISP). Trypanosomatids apparently lack SPs from S1A family. In *L. major*, ISP2 is required for parasites successful infection since prevents the activation of the TLR4-NE pathway in macrophages and was also associated with reduction of iNOS expressing monocytes in lesions in mice. Here, we investigated the role of ISP2 in *T. cruzi* Dm28c by generating *isp*2 null-mutants (Δ*isp*2) using CRISPR/Cas9. Δ*isp*2 was more susceptible to lysis by human serum than the parental line or transgenic lines re-expressing ISP2. Tissue culture trypomastigotes Δ*isp*2 are more infective to human muscle cells *in vitro*, which was reverted by aprotinin or by camostate mesylate, suggesting that epitheliasin (TMPRSS2) is the target of ISP2. Pre-treatment of host cells with an antagonist to the PAR2 or with an inhibitor of TLR4 selectively reversed increased host cell invasion displayed by Δ*isp*2, while it did not affect invasion by the parental or re-expressor lines. Target gene silencing of PAR2, TLR4 or TMPRSS2 in muscle cells by siRNA prevented increased host cell invasion by Δ*isp*2 in comparison to cells transfected with control siRNA primers. BALB/c infection in the footpad revealed that Δ*isp*2 induced increased tissue edema after 3h, but reduced at 24h, as compared to the parental or re-expressor lines. Additionally, the spleen of mice infected with Δ*isp*2 displayed increased population of $Ly6C^{+}Ly6G^{+}$ cells, accompanied by higher levels of KC, MCP-1, TNFα and IFNγ. We propose that that ISP2 contributes to protect *T. cruzi* from the anti-microbial effects of human serum and to prevent the engagement of inflammatory receptors in host cells, resulting in the modulation of host cell invasion, and contributing to decrease inflammation during acute infection. **Supported by:**Medical Research Council - United Kingdom (MRC-UK), CNPq, FAPERJ **Keywords:**Trypanosoma cruzi;Inibidor de Serino Proteases;CRISPR/Cas9.

HP-50 - **In silico analysis of Toxoplama gondii Calcium-dependent protein kinase 1 (TgCDPK) as potential molecular target for etiological chemotherapy**

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Toxoplasmosis is a disease with worldwide distribution, caused by Toxoplasma gondii. Despite the high prevalence, few treatments options are available for the global population, often related to side effects. Therefore, the search of new drugs is need for the context of toxoplasmosis. The aim of this work is to identify new therapeutic molecules against Calcium-Dependent Protein Kinase 1 from T. gondii (TgCDPK1) using computer-based drug design techniques. TgCDPK1 plays a key role in calcium-dependent exocytosis and its inhibition results in blockage of essential functions including parasite motility, host-cell invasion and egress. Although several studies have targeted TgCDPK1 in experimental models, no molecules have yet been approved. Initially, a local alignment algorithm, BLASTp, was employed, using the SwissProt database and the TgCDPK1 sequence from ToxoDB, to identify possible human homologous enzymes. We identified 11 human enzymes that had some degree of similarity with TgCDPK1, which were then aligned using CLUSTAL, a global alignment algorithm, that revealed several matching regions along the sequence, indicating proximity between them. However, in the gatekeeper residue present in the ATP binding site, we found Phe, Leu and Met residues in this position in the human sequences, whereas in TgCDPK1 we found Gly. The three-dimensional structures of the human and T. gondii enzymes were then aligned using PyMol. Through the alignment, we found structural similarities between the proteins, in addition to similar ATP binding sites. While human enzymes have bulky nonpolar residues in the gatekeeper position, TgCDPK1 has a small residue. These results may indicate structural differences in TgCDPK1 in relation to human counterparts, which could be used for the rational design of selective molecules. **Supported by:**Fiocruz, Programa INOVA, Faperj **Keywords:**Toxoplasma gondii;Molecular target;TgCDPK1.

HP-51 - **Functional characterization of *Leshmania mexicana* N-acetyltransferase NAT10 using the S. *cerevisia*e model**

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Leishmaniasis is a group of diseases that in humans is caused by more than 20 species of *Leishmania*. During its life cycle, the parasite transits between invertebrate and vertebrate hosts. To survive the environmental changes, the parasite promotes alterations in the regulation of gene expression, protein synthesis and metabolism. The gene expression in *Leishmania* is regulated mainly at post-transcriptional level, through mechanisms of mRNA stabilization/degradation and translation regulation. Several chemical modifications have been identified in mRNAs, such as N6-methyladenosine (m6A), 5-methylcytosine (m5C) and N4-acetylcytidine (ac4) and implicated in all steps of RNA lifetime. The ac4C is added to RNA by the N-acetyltransferase, NAT10, recently described as promoting an increase in the mRNAs stability and translation efficiency. Considering the crucial role of post-transcriptional mechanisms in the *Leishmania* gene expression regulation, in this work we plan to characterize functionally the L. *mexicana* NAT10 using Saccharomyces cerevisiae as study model. L. mexicana has a NAT10 homologue with all essential domains for acetyltransferase activity and RNA interaction. Thus, to investigate the parasite protein function we obtained S. cerevisiae NAT10 knockout strains using homologous recombination, replacing the NAT10 gene with the URA3 selection marker. Also, we obtained a NAT10 mutated strain that affects yeast growth at higher temperatures. Both strains will be complemented with L. *mexicana* NAT10, native and mutated at position G647 to A647. This mutation inactivates the acetyltransferase activity of NAT10. In parallel, we are generating constructs to heterologous protein expression of L. *mexicana* NAT10 to be used in further *in vitro* activity assays. The results from this work will complete ongoing studies in the parasite and will contribute to characterize the role of ac4C in *Leishmania* biology, increasing our understanding in how it regulates gene expression. **Supported by:**FAPESP - 2021/13714-8 **Keywords:**Leishmania;Acetilation;NAT10.

HP-52 - **Invasion of non-phagocytic cells by Leishmania amazonensis amastigotes: a comprehensive study of the cellular mechanisms involved in cell entry**

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Intracellular parasites are characterized by the need to invade and live within host cells. For Leishmania spp. it is commonly assumed that the parasite must be phagocytosed by phagocytic cells, notably macrophages. However, several studies have described the presence of Leishmania spp. amastigotes within non-phagocytic cells. Since cell entry is a key process in infections by intracellular parasites we decided to investigate the mechanisms that allow Leishmania amazonensis amastigotes to invade cells lacking the ability to perform classical phagocytosis. Here we used different cell biology techniques to study the cell invasion process of non-phagocytic cells by L. amazonensis amastigotes in mouse embryonic fibroblasts (MEF). We performed time-course infection assays, labelled infected cells and parasites to visualize molecules, organelles and structures possibly involved in cell invasion and quantified infections. Our results show that living, but not PFA-fixed amastigotes, are rapidly internalized in MEFs, where they survive and persist in typical vacuoles rich in lysosomal markers. By labeling host cells with F-actin probes and using F-actin inhibitors we demonstrated that invasion is dependent on host cell cytoskeleton. Confocal microscopies of early moments of infection indicate that the parasite is able to induce an extremely localized re-arrangement of host cell cytoskeleton to induce invasion. Since we had previously demonstrated that promastigotes use host cell lysosomes and not F-actin to invade MEFs we now propose that cell invasion of non-phagocytic cells by L. amazonensis is mechanistically different for each infective form of the parasite. The presence of Leishmania spp. amastigotes within non-phagocytic cells is an overlooked aspect of their biology. Studies on these new infection routes and on the role of non-phagocytic cells in the course of infection may provide important information about the biology of these parasites and the diseases they cause.
Supported by:CAPES **Keywords:**Leishmania amazonensis ;Amastigotes ;Cell invasion .

HP-53 - **A potential beneficial influence of Selenium treatment on cardiac function in Chagas heart disease: Results from the STCC randomized Trial**

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For over 60 years, Selenium (Se) is known as an essential microelement for many biological functions, including cardiovascular homeostasis. Experimental and clinical data indicate that Se may be used as a complementary therapy to prevent and improve heart failure. We have previously shown that Se levels in chronic chagasic cardiomyopathy (CCC) decrease in severe cases. We performed the first randomized, placebo-controlled, double-blinded, clinical trial designed to estimate the efficacy and safety of Se treatment in CCC. 66 patients with CCC stages B1 (n = 54) or B2 (n = 12) were randomly assigned to receive 100 mcg/day sodium selenite (n = 32) or placebo (n = 34) for one year. LVEF changes over time and adverse effects were investigated. Trial registration number: NCT00875173. No significant differences between the two groups were observed for the primary outcome. In a subgroup analysis, statistically significant longitudinal changes were observed for mean LVEF in the stage B2 subgroup (b= +10.1; p = 0.02 for Se [n = 4] vs Pla [n = 8]). Se treatment was safe for CCC patients, and the few adverse effects observed were similarly distributed across the two groups. We showed a potential beneficial effect of Se treatment in a subset of patients with CCC with ventricular dysfunction (LVEF <45%). Despite trial limitations, this is a novel therapeutic option for mild CCC. Additionally, Se may decrease the speed of worsening cardiac function and may even improve cardiac function in patients with CCC stage B2. Se treatment did not improve cardiac function (evaluated from LVEF) in CCC. However, in the subgroup of patients at B2 stage, a potential beneficial influence of Se was observed. In conclusion, the present study elucidated that Se treatment was safe and showed a potentially beneficial effect. This new pharmaceutical/nutritional approach deserves further studies to clarify its potential use as adjuvant therapy in CCC. **Supported by:**Brazilian Ministry of Health, Fiocruz, CNPq, FAPERJ. **Keywords:**Selenium ;Chagas disease;Treatment.

HP-54 - **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 characterized by the need to invade and exert at least part of their life cycles inside host cells. For *Leishmania* spp. it is assumed that, upon inoculation by the sandfly vector, the parasite is phagocytosed and lives inside professional phagocytic cells, notably the macrophages. However, several works have showed the presence of these parasites also in non-phagocytic cells. Muscle cells are among the non-phagocytic cell types already described as harboring intracellular amastigotes. Similarly, several epithelial and connective cells have also been found infected *in vivo*. 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. Therefore, our objective of is to study the infection of muscle, epithelial and connective cells by *Leishmania amazonensis*, to assess the viability of the parasites inside these cell types as long as their possible roles asa reservoir, ultimately leading to macrophages infection. To this end, we used light, conventional and confocal microscopies, immunobiochemical methods and flow cytometry to assess *L. amazonensis* infectivity in muscle fibers, epithelial cells and fibroblasts. Here we show that both amastigotes and promastigotes are able to invade and persist within these cells as typical intracellular amastigotes. When macrophage-like cells (RAW cells) were co-cultured with infected fibroblasts, infection rapidly spread to the macrophages, demonstrating that the parasites could be transferred from one cell to the other. Our results suggest that non-phagocytic cells may serve as a parasite reservoir that may eventually lead to macrophage infection. Future experiments will be focused on *in vivo* infection experiments using infected non-phagocytic cells as parasite donators to validate this hypothesis. **Supported by:**CNPq **Keywords:**Leishmania spp;;Cell Invasion;Non-phagocytic Cells.

HP-55 - **Transforming growth factor beta neutralization reduces Trypanosoma cruzi infection and improves the cardiac performance**

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Chronic Chagasic cardiomyopathy (CCC) is the most prominent clinical form of Chagas disease, culminating in heart failure and high rates of sudden death. During CCC, the parasite remains inside the cardiac cells, leading tissue damage, involving extensive inflammatory response and irregular fibrosis. Some molecules act in the fibrosis development, but one in particular plays a key role in the fibrogenic process inducing extracellular matrix synthesis: the transforming growth factor-β (TGF-β). TGF-β is also involved in the development of CCC with increased serum levels of this cytokine and activation of its signaling pathway in the cardiac tissue, resulting in increased expression of extracellular matrix proteins, which characterizes the fibrosis. The aim of this study was to investigate the effect of 1D11, a neutralizing antibody to all three isoforms of TGF-beta, on T. cruzi infection: in vitro and in vivo. To this end, cardiomyocytes were seeded for 24h, incubated with trypomastigotes and treated with 1D11. Murine models of acute and chronic Chagas disease were also treated with 1D11 with different schemes. In the present study, we show that the addition of 1D11 greatly reduces cardiomyocyte invasion by T. cruzi, in vitro. Further, the treatment significantly reduces the number of parasites per infected cell. In murine experimental models, the T. cruzi infection altered the cardiac electrical conduction: decreasing the heart rate, increasing the PR interval and the P wave duration. The treatment with 1D11 reversed this process, improving the cardiac performance and reducing the fibrosis of the cardiac tissue. Taken together, these data further confirm the major role of the TGF-β signaling pathway in both T. cruzi-infection, in vitro and in vivo. The therapeutic effects of 1D11 are promising and suggest a new possibility to treat cardiac fibrosis in the Chagas heart disease by TGF-β neutralization. **Supported by:**CAPES; CNPq; FAPERJ and Fiocruz-RJ. **Keywords:**Chagas disease;TGF-beta;1D11.

HP-56 - **TLR4-PAR2 crosstalk influences macrophage infection by *Leishmania braziliensis.***

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The host serine peptidase neutrophil elastase (NE) has been shown to control *Leishmania major* infection both in macrophages *in vitro* and in *in vivo* animal models. However, *L. major* possesses an inhibitor of S1A serine peptidases, known as ISP2, which inhibits the activity of NE, downregulating phagocytosis during macrophage interaction but promoting intracellular growth via the blockade of a pathway associated with Toll-like receptor 4 (TLR4). In contrast to *L. major*, we show that the internalization of *L. braziliensis* is negatively affected when NE is inhibited by the addition of exogenous NE inhibitor prior to macrophage infection or when NE is absent, as in the case of macrophages from NE-knockout mice (*ela2*$^{-/-}$). A similar profile was seen when TLR4 was inhibited with a commercial inhibitor (TAK-242). Furthermore, proteinase-activated receptor 2 (PAR-2) has previously been shown to interact with TLR4. Likewise, the uptake of parasites was decreased when macrophages were pre-incubated with a commercial antagonist of PAR-2 (AZ3451). When the TLR4 inhibitor and PAR-2 antagonist were used together the rate of internalization was the same as when either was used alone suggesting that they act in the same pathway. Interesting, macrophages derived from transgenic mice lacking another S1A serine peptidase, cathepsin G, exhibited a higher rate of uptake of *L. braziliensis* and the infection was sustained until 72 h. Together, our data indicates that Leishmania braziliensis engages a neutrophil elastase-dependent TLR4-PAR2 crosstalk to infect macrophages. **Supported by:**CNPq, Faperj, CAPES **Keywords:**Leishmania braziliensis;Inibidor de Serino Proteases;Infection .

HP-57 - **Replication control in *Trypanosoma cruzi***

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MCM2-7 is a heterohexamer complex with a ring shape that can encircle DNA and unwind it during replication. This complex along with other replication factors must be activated in S phase, so DNA replication can be initiated. Also, MCMs are involved in DNA replication control by being exported from nucleus or degraded when DNA replication is no longer happening inside the nucleus. During *Trypanosoma cruzi* life cycle there are lifeforms capable of replicating itself and lifeforms that does not replicate. Epimastigotes are replicating forms responsible for *T. cruzi* expansion inside the insect vector. This lifeform must control its replication to generate two daughter cells with high copy fidelity and when transforming to tripomastigotes metacyclics. So, this works aims to understand how MCMs are involved in replication control in *T. cruzi*. Five subunits of the mcm2-7 complex where tagged with neon green protein using CRISPR/Cas9 genetic editing tool. Fluorescence images showed that Mcm 2,3,4,6 and 7 are found in the nucleus of epimastigote forms and colocalized with EdU, a DNA replication marker. This nuclear pattern was observed in all cell cycle phases of epimastigotes for the five proteins tagged. For further characterization knockouts of MCM 6 and 7 were generated by CRISPR/Cas9. While a double KO of MCM7 was obtained, only single KO of MCM6 were generated. These cell lines showed impairment in cell cycle growth and the double KO of MCM7 presented a population of cell with DNA content greater then G2 population identified by flow cytometry. Single KO of MCM6 and 7 did not presented changes in DNA content. These data indicates that the genes identified as MCMs in *T. cruzi* genome are indeed involved in DNA replication, are essential for *T. cruzi* replication and are involved in genomic stability. However, until now MCMs do not seem to participate in DNA replication control once they are expressed in all cell cycle phases and are always confined to the nucleus. **Supported by:**FAPESP 202101013-5 **Keywords:**cell cycle;replication control;Trypanosoma cruzi.

HP-58 - **Evaluation of the role of ATP-citrate Lyase on macrophage polarization during *Leishmania amazonensis* infection**

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Leishmaniases are neglected tropical and subtropical diseases caused by *Leishmania* parasites. About 20 different species of the genus *Leishmania* are known to cause the disease, consisting of a spectrum of clinical forms. *Leishmania* infects macrophages, surviving the phagolysosomal microbicidal mechanisms. It is important to understand the changes caused in macrophages during *Leishmania* infection and how these changes favor parasite survival. Classically (M1) and alternatively (M2) activated macrophages undergo metabolic reprograming, but differ in some aspects. ATP citrate lyase (ACLY) is involved in macrophage polarization towards M1 and M2 profiles. It uses citrate to produce acetyl-CoA and oxaloacetate, which are used to produce inflammatory mediators and induce histone acetylation. Recent work has shown that *Leishmania* parasites can induce metabolic reprograming in macrophages. In *L. amazonensis* infected macrophages, there is a switch in the metabolism. In *Leishmania* infected bone marrow-derived macrophages (BMDMs) OXPHOS and glucose metabolism are increased. Based on these findings, we hypothesized that macrophages undergo metabolic reprogramming when infected with *L. amazonensis*, leading to an increase in metabolite citrate. ACLY then uses citrate to produce acetyl-CoA, which leads to histone acetylation and modulation of macrophage response to the infection. Here, we are testing the ACLY enzyme's activity and describing its function during *L. amazonensis* infection in macrophages. Our preliminary results show that pharmacological inhibition of ACLY reduces parasite survival in infected macrophages. The enzyme inhibition did not affect promastigotes viability, suggesting that the effect was due to modulation of macrophage response. Moreover, ACLY inhibition enhanced ROS production in infected BMDMs. Based on these results, we consider that ACLY may contribute to macrophage polarization towards the M2 profile and may be a target for leishmaniasis treatment. **Supported by:**CNPq, CAPES, FAPERJ **Keywords:**L; amazonensis;metabolic reprograming;ATP citrate lyase.

HP-59 - ***Prediction, production and evaluation of chimeric proteins as an antigen for serological diagnosis of Chagas' disease.***

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Chagas' disease (ChD) is a neglected disease endemic in 21 Latin American countries. According WHO and DND*i*, 6 to 7 million people are affected by ChD, with estimated 75 million people at risk of contracting the disease and an average of 14,000 deaths each year. ChD is caused by *Trypanosoma cruzi*, but less than 10% of carriers have access to diagnosis and less than 1% receive treatment. Recombinant proteins may improve diagnosis which is an essential task among the strategies to control and prevent this disease. However, diagnosis based on a single recombinant antigen may not achieve high sensitivity, given the antigenic diversity seen inside *T. Cruzi* lineages and strains. Here, we developed chimeric antigens for to improve sensitivity for serodiagnosis of ChD. Bioinformatics analyzes were performed for *T. cruzi* proteins previously described and already tested as single antigens, to identify linear epitope sequences of B cells. The nucleotide sequences obtained were joined into synthetic genes, inserted into pET-24a(+) vector with a histidine tag to transform *E. coli* bacterial strains. Using a different combination of epitopes, three chimeric proteins were proposed: QTc1 (28,27 kDa); QTc2 (26,73 kDa) e QTc3 (30,8 kDa), with Isoelectric Point 6,53; 9,38 e 5,18, respectively. Protein production conditions were evaluated, followed by purification by affinity chromatography. QTc1 and 2 were induced for 3 hours at 37°C and QTc3, for 18 hours at 20°C. All proteins proved to be soluble. A Western Blot test was performed, using sera from healthy and chagasic individuals. The results suggest that QTc1 and 2 are promising antigens. The purified proteins will be used as antigens in ELISA and TR with standard sera samples to determine parameters (sensitivity, specificity, among others). Then, selected conditions will be further explored to prepare prototype tests for validation as alternative for the diagnosis of Chagas' disease. **Keywords:**serodiagnosis;Chagas' disease;chimeric antigens.

HP-60 - **IMPACT OF TOXOPLASMA GONDII INFECTION ON SKELETAL MUSCLE MYOGENESIS IN A THREE-DIMENSIONAL CULTURE MODEL**

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Toxoplasma gondii is the etiological agent of toxoplasmosis and has the skeletal muscle tissue as one of the main niches where tissue cysts are formed. For this reason, the skeletal muscle cell-T. gondii interaction has been subject of many studies from our group. In vitro studies related to this infection were performed in monolayer cultures, and we described that T. gondii impairs myogenesis in mouse myoblast cultures. Three-dimensional (3D) cell culture models better mimic the cellular organization of the tissues in vivo and have been increasingly used in research for drug discovery, developmental biology and infectious diseases. In the present work, we generated 3D cultures (spheroids) of the C2C12 mouse myoblast cell line to evaluate the infectivity rates and myogenesis events after infection by T. gondii. Cells were plated in agarose-coated 96-well U-bottom plates, and after 24 hours were infected with T. gondii tachyzoites (ME49 strain) in a 1:1 ratio. After 24 hours of infection, half of the plate had its proliferation medium changed to a differentiation medium that induces the myogenic program. Five days after myogenesis induction (that corresponds to 6 days of infection) we measured the area of the spheroids and performed immunostainings for myosin heavy chain (MyHC, a marker of differentiated myocytes), desmin (marker of the muscle cell lineage) and SAG1 (for tachyzoites). After 24 hours of differentiation, no changes were observed regarding the spheroids size, but at 120 hours after differentiation, a significant increase in spheroids surface was noticed, in infected cultures kept both under proliferation and differentiation media. T. gondii infection was detected throughout the spheroids by immunostaining and was accompanied by the reduced immunoreactivity of MyHC and desmin. This new model will be of great relevance to further understanding the mechanisms involved in skeletal muscle pathology induced by T. gondii infection. **Supported by:**PIBIC, CNPq, Faperj, INOVA Fiocruz, PAPES VII Fiocruz **Keywords:**T;gondii;Muscle cells;spheroids.

HP-61 - **Lipophosphoglycan-3 protein from *Leishmania infantum chagasi* plus saponin adjuvant: A new promising vaccine against visceral leishmaniasis**

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Given the lack of a vaccine against leishmaniasis, this work contributes to the development of a vaccine against visceral leishmaniasis. In this study, BALB/c mice were immunized with *L. chagasi* LPG3 or rLPG3 proteins alone or associated with incomplete Freund's adjuvant (IFA) or saponin (SAP) and challenged with *L. chagasi*. The parasite load in the spleen and liver and the immune response of the groups were compared. Among the formulations tested, LPG3 + SAP reduced about 98% of the parasite load in the spleen and liver, increased the production of cytokines IL-2, IFN-γ, IL-6, IL-17 and IL-10 by stimulated spleen cells by LcAg and reduces the IgG1/IgG2a ratio. Vaccine with rLPG3 induced Th1/Th17 response, produced IL-10 and protected the spleen of mice infected with *L. chagasi*. A reduction in parasitism was observed in the spleen of mice vaccinated with rLPG3 and rLPG3+SAP. LPG3 has already been investigated to stimulate an immune response, and it has been found to be able to do so if it is combined with an adjuvant. Choosing the right adjuvant is crucial in producing a vaccine to induce an effective response. In our results, SAP was more effective than IFA in providing protective immunity through the use of native and recombinant LPG3. In fact, SAP has been used in leishmaniasis vaccine formulations to induce a Th1 immune response. The rLPG3 + SAP vaccine suggests safety, as it preserves liver architecture and function. It is also noteworthy that the production of rLPG3 is easier and cheaper compared to current processes for obtaining the native protein. Work approved by the Ethics Committee for Animal Use (CEUA/UFV – process 16/2016), financed by Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG – Grant No. 9553 – FAPEMIG CBB – APQ-00668-13), Coordination Improvement of Higher Education Personnel (CAPES) and National Council for Scientific and Technological Development (CNPq) MCTI/CNPq/MEC/Capes – Transversal Action 06/2011 – Casadinho/Procad. **Keywords:**LPG3;Saponin;Visceral leishmaniasis.

HP-62 - **Lipophosphoglycan-3 recombinant protein vaccine controls hepatic parasitism and prevents tissue damage in mice infected by *Leishmania infantum chagasi***

BASTOS, D.S.S.[1]; MIRANDA, B.M.[1]; MARTINS, T.V.F.[1]; ERVILHA, L.O.G.[1]; SOUZA, A.C.F.[2]; EMERICK, S.D.O.[1]; DA SILVA, A.C.[1]; RAIMUNDO, V.D.[1]; NOVAES, R.D.[3]; NEVES, M.M.[1]; SANTOS, E.C.[1]; DE OLIVEIRA, L.L.[1]; MARQUES-DA-SILVA, E.D.A.[1].
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In this work, immunization with recombinant LPG3 protein was tested on the parasitism and on the liver of infected BALB/c mice, because this is one of the target organs for the parasite. The vaccination protocol consisted of three doses for five groups: uninfected and treated with PBS (NI), group challenged and treated with PBS (NV), saponin (SAP), group immunized with rLPG3 and group immunized with rLPG3 + SAP. Mice were challenged with 1×10^7 *L. infantum chagasi* promastigotes and subsequently sacrificed. rLPG3 and rLPG3 + SAP reduced parasitism, 93.82% and 99.33% respectively, compared to NV. rLPG3, with or without SAP, reduced the ratio of IgG1/IgG2a antibodies compared to the NV group. Immunization with rLPG3 + SAP was more effective, with levels of liver damage markers similar to the NI group, increased antioxidant enzyme activity and preserved liver architecture. The lower ratio of IgG1/IgG2a antibodies suggests that immunization with rLPG3 + SAP promoted immunity with a Th1 profile, reducing the parasite load. This reduction is also due to the activation of macrophages by IFN-γ, which activates infected cells and induces the production of nitric oxide and free radicals that kill the parasites. Immunization with rLPG3 + SAP can reduce the proinflammatory response that could affect organ structure. Additional studies are needed to clarify the immune mechanism triggered by immunization with rLPG3 plus SAP. However, the results of this work highlight the importance of vaccination, not only as an antiparasitic mechanism, but also as a factor that acts to maintain the integrity of the infected organ. Work approved by the Ethics Committee for the Use of Animals (approved by the CEUA/UFV – Research Project – process: 16/2016). Support: Fundação de Amparo à Pesquisa do Estado de Minas Gerais [grant APQ-01211-17 to EAM] and Coordination for the Improvement of Higher Education Personnel [doctoral fellowship for DSSB]. **Keywords:**LPG3;Visceral leishmaniasis;Liver morphology.

HP-63 - **Histological analysis of the seminiferous tubules and intertubular area of mice infected by the Colombian strain of *Trypanosoma cruzi.***

DOS SANTOS, B.L.P.[1]; MENEZES, T.P.[2]; SILVA, V.L.T.E.[1]; DIAS, F.C.R.[3]; RIBEIRO, L.[1]; PINTO, K.M.D.C.[2]; DA SILVA, A.T.P.[2].
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Chagas' disease is a tropical neglected illness caused by *Trypanosoma cruzi* and remains one of the most significant causes of morbidity and mortality in South and Central Americas. The disease is caused by a moderate to intense and persistent inflammatory response characterized by local upregulated expression and production of inflammatory mediators (such as cytokines and chemokines) that favors the activation and recruitment of distinct cells of the immune system into different tissues to eliminate the parasites. Heart, muscular and tissues of the gastric system are mostly common to be studied as a regular tissue tropism of the *T. cruzi*, but the protozoan can infect and cause an inflammatory response in several systems on its host, such as the reproductive system. This research focused on the testicular investigation in mice infected by 10^3 trypomastigote forms of Colombian strain of *T. cruzi*. Its was evaluated histological parameters of the seminiferous tubules and the intertubular area of 14 animals grouped as: control (non-infected) and *T. cruzi*. The luminal diameter and area increased in the presence of the protozoan resulting in a different seminiferous tubules/epithelial relation between the control and the *T. cruzi* group. The percentage of the seminiferous tubules in the group infected by the protozoan are also higher than the control group, such as the percentage and volume of the tunica propria. In the intertubular area, the percentage of the intertubule was higher in the control group, such as the percentage and volume of Leydig cells, connective tissue, and macrophages. The knowledge that testicular structures are changed by *T. cruzi* brings to the light to an important issue about a neglected route infection of the Chagas' disease: the sexual transmission. **Supported by:**FAPEMIG **Keywords:**Trypanosoma cruzi; Spermatozoids; Seminiferous tubule.

HP-64 - ***Theracurmin* regulates parasite and inflammatory mediators in mice infected by Colombian strain of *Trypanosoma cruzi*.**

DOS SANTOS, B.L.P.[1]; SILVA, V.L.T.E.[1]; MENEZES, T.P.[2]; PINTO, K.M.D.C.[2]; DA SILVA, A.T.P.[2].
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Theracurmin is a curcumin's derived formulation of nanoparticles. Its anti-inflammatory properties make this bioactive compound a mitigating factor in pathological cases after an overwhelming inflammatory response. The *Trypanosoma cruzi*, the etiologic agent of Chagas' disease, triggers an acute inflammatory response characterized by the expression/release of inflammatory mediators that favors the activation and the recruitment of distinct immune cells into different tissues to eliminate the parasite. The present research evaluated the effectiveness of the *Theracurmin* (CurcuminRich®, Natural Factors, Canada) treatment over the inflammatory and parasitological response of 32 male mice, Swiss lineage, infected by 10^3 trypomastigote forms of the Colombian strain of *T. cruzi*. These animals were grouped as: uninfected, *T. cruzi*, *T. cruzi* + *Theracurmin*, and *Theracurmin*. The mice were treated with 30 mg/Kg of *Theracurmin* (by gavage) during the period of 30 days and parasitemia was evaluated daily. At the 30th day post infection animals were euthanized, and its testicles, heart, gastrocnemius fragments and 1mL of blood collected to morphological and immunological assays. The animals infected and treated with *Theracurmin* presented a reduction in the parasitemia and in the levels of IL-15 (testicles, heart, and gastrocnemius) CCL2 (heart) and IL-6 (testicles). Therefore, it is concluded that the *Theracurmin* acts on controlling blood parasitic and on the tissue production of inflammatory mediators (IL-15, CCL2, and IL-6) in distinct tissues. **Supported by:**Fapemig **Keywords:**Trypanosoma cruzi;Theracurmin;Inflammatory response.

HP-65 - **Annexin A1 regulates inflammatory response and development of toxoplasmosis**

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Toxoplasma gondii(*Tg*) is arguably the most successful parasite because, in part, of its ability to infect and persist in most warm-blooded animals. A unique characteristic of *Tg* is its ability to persist in the SNC of a variety of hosts, including humans and rodents. The treatment is ineffective, so studies with new therapeutic approaches are needed. The immune system is fundamental for the control and pathophysiology of toxoplasmosis. Annexin A1, a pro-resolving and anti-inflammatory protein is induced by glucocorticoid hormones that mediate several actions of this class of drugs. Macrophages(MOs) and glial cells are crucial in controlling this infection. Herein, the aim was to evaluate the role of ANXA1 protein during an *Tg* experimental infection, *in vitro* and *in vivo*. Peritoneal MOs and glial cells from Balb/c (WT) and ANXA1 knockout (KO) mice were infected with tachyzoites forms of *Tg* RH strain (ratio 1:1 - cell:parasite). WT and KO mice aged 8 to 9 weeks were infected or not with 20 cysts intraperitoneally. The body weight and survival of the mice was monitored, every 5 days after infection (dpi) and daily, respectively. On the 25 dpi, the mice were euthanized and the brain removed for cysts counting. Brain histological analyzes of WT and KO animals were performed at 25 dpi. *In vitro*, MOs and glial cells from KO mice were more vulnerable to parasite replication compared to WT cells.*In vivo*, the deficiency of ANXA1 resulted in increased susceptibility to *Tg* infection when compared to WT. Of note, the vast majority of infected KO mice death occurred at 10 dpi. The weight loss was similar between infected groups. Furthermore, KO mice presented a higher number of brain cysts and more severe lesion in the cortex compared to WT mice. Suggesting that the absence of ANXA1 in the brain cells facilitate the parasite infectivity, or those cells have less ability to control the parasite replication. Collectively, our data suggest the ANXA1 is a regulator of *Tg* infection. **Supported by:**CNPq, Capes, Fapemig **Keywords:**Toxoplasma gondii;annexin A1;macrophages.

HP-66 - **Improved efficacy of breathing and neuroprotection effects of Amido-Coumarin co-treatment during severe experimental malaria**

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*Plasmodium berghei*ANKA(PbA)infection in mice closely recapitulates many aspects of severe malaria in humans including cerebral malaria and acute respiratory distress syndrome.Coumarins are a class of secondary metabolites present in plants and exhibit several biochemical effects.We aimed investigate the potential therapeutic of a coumarin compound in treating of severe experimental malaria(SEM).C57Bl/6mice were inoculated with 10^5red cells parasitized with PbA, and3days after infection(dpi) orally treated daily with coumarin compound MJM alone and/or combined with chloroquine(CQ) once per day.Analyzed:parasitemia,body weight,survival,clinical score,memory, and immune cell in: spleen, brain and bronchoalveolar lavage(BAL) by flow cytometry;lung conditions was evaluated by spirometry.Our results showed that the treatment with MJM alone reduced the parasitemia at 6 and7dpi, and improved the clinical score from 8to12dpi when compared with infected untreated mice.The animals treated with MJM+CQ showed a reduction in parasitemia in all analyzed kinetics(3-47dpi).Treatments with MJM and MJM+CQ increased the survival of infected mice(28dpi and 47dpi).Treatment with MJM resulted in the protection of cognitive ability at 5dpi, and the combined treatment of MJM+CQ preserved cognition at 5dpi and 47dpi.Treatment with MJM+CQ resulted:in the brain,reduction in the numbers(nº) of macrophages,dendritic cells,CD4+and CD8+IFNγ+,CD4+IL17+and IL10+,and in the spleen, reduction in the nº of neutrophils,CD4+IFNγ+orIL17+,and increased nº of CD8+IFNγ+.Spirometry results showed that,compared with infected untreated mice, or mice treated with MJM or CQ alone, the animals treated with MJM+CQ improved their lung capacities/functions.Treatment with MJM+CQ significantly increased the nº of alveolar macrophages IL10+, and CD4+IL17+ in the BAL.Collectively, our results suggest that the compound MJM,has potentially beneficial effects on neuroprotection and respiratory capacity during SEM. **Supported by:**CNPq (474971/2013-9) **Keywords:**Cerebral malaria;neuroprotection;MA-ARDS.

HP-67 - **Kinetic characterization of enzymes Enoyl-CoA hydratase, Isovaleryl-CoA dehydrogenase and GPR domain of Pyrroline-5-carboxylate synthase in *Trypanosoma cruzi* using a machine learning algorithm applied to time-course curves**

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Amino acid metabolism pathways play a significant role in the biology of *T. cruzi* besides protein synthesis. As an example, proline has been shown to be involved in the energetic sustenance of the parasite, by transferring electrons to the Electron Transport Chain, and its steady state concentration levels vary widely between parasite stages. In order to better understand these pathways, our group sought to biochemically characterize enzymes for which there was little or no data available in the literature. In particular, this work focuses on TcECH and TcIVDH, from the branched chain amino acids degradation pathway, and on the GPR domain of the bifunctional TcP5CS, from the proline biosynthesis pathway. After cloning their correspondent genes, expressing and purifying the proteins, and measuring their biochemical activity under varying concentrations of substrates, we sought to obtain the kinetic parameters of the enzymes. To achieve this, we developed an algorithm that fully utilizes the whole time course curves, instead of just approximating the slope of the initial phase. In doing so, we not only take advantage of all available data for increased accuracy, but also reduced the experimentalist bias. Moreover, our algorithm is able to correct for slight protein quantification and pipetting errors. The basic strategy is to use either a gradient descent or a genetic algorithm to infer the parameters that minimize the quadratic error between simulated and experimental time courses, assuming that the Uni-Uni or Bi-Bi Reversible Hill Equation applies (though the user is able to provide any other equation with minimal adjustments). Failures of the algorithm are also informative, as they reveal where and why more data is needed, and can highlight situations where results appear to be consistent, but fail to be reproducible, requiring improvements on sample preparation and handling protocols. **Supported by:**FAPESP: 2020/04482-3 **Keywords:**Kinetic characterization;Amino acid metabolism;Time-course curves.

HP-68 - ***Trypanosoma cruzi* and betacoronavirus MHV3 co-infection: the role of SOCS2 in the systemic manifestation.**

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Patient with Chagas disease (CD), caused by *Trypanosoma cruzi* (TC) infection, has no symptoms or present gastrointestinal/myocardial dysfunction. However, coinfections can lead to CD reactivation and influence the overall prognoses. COVID-19, generated by Sars-cov2 can lead respiratory and systemic pathophysiological manifestations/death. Mice inoculated with betacoronavirus murine hepatitis coronavirus 3 (MHV3) develops severe disease and it is an animal model for studies regarding betacoronavirus respiratory infection and related diseases. The SOCS2 protein is regulator of the innate/adaptive response to different infections/homeostasis. Herein, we evaluate the role of SOCS2 at the chronic phase of TC infection, MHV3, and TC/MHV3 coinfection in mice. C57BL/6 (WT) and SOCS2 knockout (KO) animals were infected with TC Y strain and at the chronic phase (100 days after infection; dpi) we performed the MHV3 co-infection. A reduction of lymphocytes was observed in the blood of WT and SOCS2 KO, and the deficiency of SOCS2 resulted in decreased numbers of granulocytes when compared with WT at 100 dpi. During MHV3 infection, was observed a lymphopenia being more significative in SOCS2 KO at 3dpi, and the deficiency of SOCS2 also dramatically increased eosinophils and monocytes numbers. The co-infection (TC+MHV3) resulted in restauration of circulating lymphocytes numbers that was dependent of SOCS2. The histology analyses demonstrated that absence of SOCS2 during TC and MHV3 infection increased the development of inflammation and lesion in the liver and gut. Interesting, in the co-infection, SOCS2 KO mice was more resistant to inflammatory development in the lung and liver, but more susceptible in the gut, that was associate with an increased Enterobacteriaceae, when compared to WT. Altogether, the results demonstrated the critical role of SOCS2 regulating the inflammatory response and tissue protection during TC, MHV3 and TC+MHV3 co-infection process. **Supported by:**CAPES, CNPQ e FAPEMIG **Keywords:**SOCS2;Inflammatory;Co-infection.

HP-69 - **Effect of zileuton treatment *in vivo* and *in vitro* during *Trypanosoma cruzi* infection**

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Chagas disease (CD) is a neglected disease caused by the protozoan *Trypanosoma cruzi*. The main complication of CD includes cardiac, digestive and neurological dysfunction. Among the challenges for the treatment of disease, there is the inefficiency of drugs available for the chronic phase. There are studies demonstrating that, during the *T. cruzi* infection, the host immune response could be regulated by the action of eicosanoids, including Lipoxins (LXA) that induces the expression of the protein Suppressor of Cytokine Signaling 2, an important regulator of immune response. Moreover, pathogens can evade immune response producing/expressing mediators that mirror the mammalian and plant molecules, including enzyme-like lypoxigenase, for instance the lypoxigenase expressed by *Toxoplasma gondii*. Zileuton (Zi) is a selective inhibitor of 5-lipoxygenase (5-LO) enzyme which is involved in the intracellular pathways that induce leukotrienes and LXA production. Herein, we investigate the effects of Zi, *in vitro* on trypomastigote and epimastigote forms of the parasite, and during experimental *T. cruzi* infection *in vivo.* Female C57BL/6, SV129 and 5-LO knockouts (KO) mice, 6 to 8 weeks old, were infected intraperitoneally with 1000 forms of trypomastigotes Y strain and treated or not with Zi (30mg/kg) or benznidazole (Bz; 10mg/kg). The treatment started 8 hours after infection, and was conducted each 12 hours for 10 days. *In vitro***,** incubation of trypomastigotes and epimastigotes with Zi (at 100, 33, 10, 3 and 1µM), but not with dimethyl sulfoxide (DMSO; vehicle control), for 72h, reduced the number of parasites. *In vivo***,** treatment with Zi and Bz reduced significantly the parasitemia when compared to untreated animals. The control of parasitemia during Zi treatment was partially dependent of 5-LO. Collectively, these preliminary results suggest that zileuton has a potential protective effect against T. *cruzi* infection acting in both host and parasite cells. **Supported by:**CNPq, FAPEMIG, CAPES. **Keywords:**Trypanosoma cruzi;Zileuton;5-lipoxygenase.

HP-70 - **Effects of formyl peptide receptor 2 deficiency in the modulation of immune response and development of pathogeneses during Plasmodium berghei ANKA**

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Severe malaria caused by Plasmodium falciparum in humans can results in high morbidity and mortality. The Plasmodium berghei ANKA (PbA) infection in mice closely recapitulates many aspects of human severe malaria, including respiratory distress and cerebral malaria. Formyl peptide receptor 2 (FPR2) is involved in the organism response to infections and plays an important role in the anti-inflammatory/resolution pathway presenting high affinity for lipoxin A4 (LXA4). FPR2 has been associated with the pathogenesis of sterile and infectious inflammatory diseases. However, the role of FPR2 in malaria is unclear. C57BL/6 (WT) and FPR2 knockout (KO) C57Bl/6 mice were inoculated with 105 red cells parasitized with PbA. Despite similar parasitemia found between WT and FPR2 KO mice, deficiency of FPR2: increased the numbers of macrophages producing IL-10; but decreased the numbers of CD4+ T cell producing IL-10; and decreased the numbers of CD8+ T cells producing IFN-gamma in the bronchoalveolar lavage (BAL) at 5 days after infection when compared with WT counterparts. In the brain, absence of FPR2 increased the frequency of PbA-infected erythrocytes detected/adhered in the vein when compared with WT. Moreover, at 5 dpi, a dramatically decreased of the number of CD4+ and CD8+ T cells producing IL-17 and CD8+ T cells producing IFN-gamma was observed in PbA-infected FPR2 KO mice when compared with WT counterparts. Collectively, our results suggested that during the PbA infection the FPR2 receptor is important controlling brain parasitism and orchestrating the immune response development. **Supported by:**Financial support: CNPq CAPES, Fapemig **Keywords:**MALARIA;FPR2;LIPOXIN A4.