HP25 - **NEW MOLECULES (SCHIFF'S BASE) EXERT ANTI-*TRYPANOSOMA CRUZI* ACTIVITY INDEPENDENT OF THE MICROBICIDAL MACHINERY OF THE HOST CELL**

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INTRODUCTION: The treatment of Chagas disease(CD), caused by *Trypanosoma cruzi*(Tc), is inefficient, requiring new therapeutic approaches. Schiff's bases(BS) have antimicrobial activities, thus, we aimed to evaluate the anti-Tc activity of new BS-formulations. MATERIAL/METHODS: Macrophages(MO) from C57Bl/6(WT) mice and cardiomyocytes were infected/stimulated with BS:P8/P9/P10/P11. The uptake, amastigote replication and release of trypomastigotes were evaluated after 4, 24/48h, and from 3rd post-infection(dpi) to 8th dpi, respectively. In cultures was analyzed the production of nitric oxide(NO), cytokines(TNF/IL-6) and LDH(toxicity). MO deficient of NO(iNOS-/-) or reactive oxygen species-ROS(Phox-/-) were also infected/stimulated with P8/P10. Preincubation of trypomastigotes or MO with P8/P10 were performed. Epimastigotes WT or Topoisomerase3α-deficient(Topo3α-/-) were incubated with P8/P9/P10/P11 and parasite replication was analyzed in 48h. In vivo, Tc-infected mice were treated: P8 or P10[1mg/kg]. RESULTS: MO treated with P8 or P10 have reduced replication and release of Tc, independently of NO and/or ROS. P10, but not P8, reduced the parasite uptake. Moreover, in Tc-infected MO stimulated with P10 the production of TNF was potentiated. However, stimulation with P8 decreased the levels of IL-6. Further, P10 reduced the release of trypomastigotes by cardiomyocytes also independently of NO and TNF/IL-6. Infection of MO with trypomastigotes pre-incubated with P8/P10 or MO pre-stimulated with these molecules before infection reduced the parasite release. LDH-assay showed that P8/P10 are non-toxic for the host cells. P8/P10 has trypanocidal action on WT-epimastigotes and parasites Topo3α-/- were more susceptible to this action. Of great relevancy, P8 or P10 diminish parasitemia in Tc-infected mice. CONCLUSION: Collectively these data suggested that P8 and P10 are candidates for CD treatment presenting activity on both, host cells and parasite. **Supported by:**CNPq/FAPEMIG **Keywords:** Trypanosoma cruzi; treatment; schiff bases

HP26 - **PEPTIDES ISOLATE FROM *TITYUS SERRULATUS* (YELLOW SCORPION) VENOM IS ABLE TO CONTROL *TOXOPLASMA GONDII* INFECTION *IN VITRO* AND *IN VIVO***

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INTRODUCTION: The current treatment of toxoplasmosis, caused by *Toxoplasma gondii*(Tg), is ineffective, requiring therapeutic innovations. Inflammatory mediators produced during Tg infection is crucial for the parasite control. Since yellow scorpion (*Tityus serrulatus*) venom(TsV) can modulates the production of inflammatory mediators, we aimed to isolate/apply TsV-components against Tg. METHODS/RESULTS: The TsV induced nitric oxide(NO), cytokines(IL-12/TNF/IL-6) and decreased Tg replication in murine(C57Bl/6) macrophages(MO). In order to isolate effector molecules, TsV was separated(gel filtration chromatography) into 7 fractions(Ts1-Ts7). The Ts6 and Ts7 fractions induced IL-12/TNF/IL-6 and NO production, being greater the capacity of Ts6 to induce cytokines production by infected-MO. Ts6 was purified(cation exchange chromatography) and subfractions(toxins) were generated: Sub6-A and Sub6-B. MO was infected/treated with Sub6-A or Sub6-B and 48h after infection/stimulus the parasite replication was analyzed. The infected and Sub6-B stimulated-cells had lower parasitic load than the control group. Sub6-B had its partial sequence elucidated and by in silico assays three peptides were generated from the Sub6-B-original sequence: Pep1, Pep2a and Pep2b. MO were infected/stimulated with Pep1, Pep2a or Pep2b[100, 50 or 25ug/mL. In vivo, C57Bl/6 mice were infected/treated with Pep1, Pep2a or Pep2b[1mg/kg]. Development of brain cysts was analyzed at 30thday after infection(dpi). The Pep1 or Pep2a infected/stimulated-MO had decreased parasitic load, in contrast, the Pep2b infected/stimulated-cells presented increased parasitic load. The infected and Pep1 or Pep2a-treated mice had reduced numbers of brain cysts, whereas Pep2b-treated animals did not change this amount, when compared to the control group. CONCLUSION: Our data suggest that TsV molecules have therapeutic potential against Tg infection. **Supported by:**CNPq/FAPEMIG **Keywords:** Toxoplasma gondii; treatment; tityus serrulatus venom

HP27 - **LIPID METABOLISM OF RHODNIUS PROLIXUS INFECTED WITH TRYPANOSOMA RANGELI.**

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Trypanosoma rangeli is a protozoan of Trypanosomatidae family is found in the salivary gland of *Rhodnius prolixus*. In the life cycle, when a triatomine feeds on the infected host, it acquires the trypomastigote forms. Initially, in the insect's anterior gut the proliferative forms, epimastigotes, can be found. These forms pierce the wall of the intestine and occupy the hemolymph where they can invade the hemocyte and within these cells differentiate. Due to the excess of parasites the hemocytes ruptures releasing trypomastigotes, in which they can invade other hemocytes or the salivary gland. This protozoan does not have the complete lipid metabolism, so they need to capture them from a host, which could be the invertebrate, for its survival and proliferation. There by insect infection leads to various complications even death. Due to this characteristic, we can think of this parasite as a biological controller of Chagas disease, thus reducing the population of triatomines. Thus, the objective of this work is to study the lipid metabolism of *R. prolixus* infected with *T. rangeli.* There fore, 24 hours after fed, two groups of *R. prolixus* were injected with only PBS1x, control group, or PBS1X containg 10 7 of *T. rangeli,* infected group. Then 3 days of infection the dissection was performed and the following organs were removed: fat body, ovary, intestine and hemolymph of the both groups. The organs were homogenized and subjected to lipid extraction, the lipids classes were separated by the slim layer chromatography (TLC). Then the lipids classes of control is infected group were compared. As previous results was possible, to observe a decrease of triacylglycerols in the infected group in relation to the control group. Which *T. rangeli* may be able to modulate the composition of the lipids classes in *R. prolixus*. Probably this modulation is serving to supply the parasites with lipids essential for their metabolism. **Supported by:**FAPERJ **Keywords:** Trypanosoma rangeli; rhodnius prolixus; lipids

HP28 - **THE ROLE OF SMALL HEAT-SHOCK PROTEINS IN THE PHYSIOLOGY AND VECTOR COMPETENCE OF RHODNIUS PROLIXUS, AN INSECT VECTOR OF CHAGAS DISEASE**

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Rhodnius prolixus, a vector of Chagas disease, takes three times its own weight in a single blood-meal. The midgut homeostasis is achieved by preventing tissue damage upon potential stressors generated during blood digestion, the microbiota increasing or pathogen's infections. The transformation of non-infecting forms into infective forms of the *Trypanosoma cruzi* protozoan that occurs in the digestive system of its vector, is an important step in the life cycle of the parasite, and consequently for the transmission of Chagas disease. First stage nymphs gut transcriptomic analysis shows differential gene expression regarding the infection, including members of the Small heat shock proteins (sHSP) family. The sHSP are proteins involved in response to heat shock and other stress responses, and function primarily as chaperones avoiding protein missfold. The present study aims to investigate the role of sHSP in the physiology of *R. prolixus* and in vector competence. The transcriptomic analysis revealed five sHSP genes that were upregulated (50-200X) by blood feeding and downregulated by infection (3-20X). Phylogenetic analysis revealed a cluster formed by recent gene expansion. sHSP gene function were analyzed by qPCR, gene silencing and exposing insects to heat-shock treatment. Parasite infection of silenced insects were evaluated by qPCR. These sHSP are also overexpressed by heat shock (60-1000x), confirming their in silico identification as sHSP. Silencing of the sHSPs genes affects digestion and microbiota prevalence. In silico gene promoter analysis revealed putative binding sites for the transcription factor heat shock factor (HSF) and for ecdysone-responsive elements, which suggest a hormonal signaling regulation. Simultaneous knockdown of the five modulated sHSP caused an increase in the amount of parasites in the insect gut. We identified here a group of sHSPs that appears to be involved in the homeostatic response to blood ingestion and to *T. cruzi* infection. **Supported by:**CNPq **Keywords:** Small heat shock protein; trypanosoma cruzi; chagas disease

HP29 - **COMPOUNDS DERIVED FROM SCHIFF BASES EXERT ANTI-*TOXOPLASMA GONDII* ACTIVITY *IN VITRO* AND *IN VIVO*: ROLE OF CCR2 AND CCR5 RECEPTORS AND 5-LIPOXYGENASE ENZYME**

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Toxoplasmosis is disease of high global prevalence,requiring therapeutic innovations.Studies demonstrated antimicrobial activity of Schiff bases(BS).Macrophages(MOs)and glial cells are crucial in controlling this infection and CCR2/CCR5 chemokine receptors and 5-Lipoxygenase(5-LO)enzyme play an important role in this cellular type.Herein,the aim was investigate anti-*T.gondii* activity from the new BS(P8 or P10)and to verify the involvement of the CCR2/CCR5 and 5-LO receptors in this activity.C57Bl/6and SV129(WT),CCR2 knockout(KO),CCR5 KO and 5-LO KO mice and peritoneal MOs from them were infected or not(strain ME49/strain RH)respectively,and treated or not with BS.Moreover,glia cells were isolated and infected or not with the RH strain and treated or not with BS.We demonstrated,*in vitro*,that infected MOs and glial cells stimulated with BS,MOs infected by parasites pre-incubated decreased number of intracellular parasites when compared to their respective non-stimulated/preincubated controls.Cell viability demonstrated that BS are not toxic to MOs and glial cells.In addition,we observed that CCR2 KO MOs,but not CCR5 MOs partially lost anti-*T.gondii* activity when stimulated by BS.In the absence of 5-LO there was greater reduction of parasite replication in the infected/stimulated group BS.*In vivo*,we demonstrated that treatment with P8 contributed to the survival of infected C57BL/6 mice.In infected and treated P8-treated CCR5 KO and CCR2 KO mice the same was not able to assure survival,demonstrating the dependence of these receptors on the other hand, infected 5-LO KO mice treated with BS presented higher survival when compared to infected mice,demonstrating that BS acts better in the absence of this enzyme.In SV129 background mice for 5-LO KO treatment with P8 was not effective,but treatment with P10 promoted an increase in survival,as compared to untreated infected mice.Collectively,our data suggest that BS are promising compounds for the treatment of toxoplasmosis. **Supported by:**CNPq, Capes e Fapemig **Keywords:** Toxoplasma gondii; schiff base; macrophages

HP30 - **UNRAVELING INTERACTION BETWEEN TRYPANOSOMA CRUZI METACYCLIC TRYPOMASTIGOTE SURFACE MOLECULE GP82 AND ITS HOST CELL RECEPTOR LAMP2**

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Trypanosoma cruzi metacyclic trypomastigote (MT) invades host cell mediated by MT-specific surface molecule gp82, which binds to major lysosome membrane-associated protein 2 (LAMP2). This event induces an increase in cytosolic Ca^{2}++ concentration and lysosome spreading, followed by exocytosis that is required for parasitophorous vacuole formation. The gp82 sequence P4 (residues 254-273) has been previously identified as the main host cell binding site. Since LAMP2 was identified as the receptor for gp82, we aimed at investigating the structural basis of this interaction. Firstly, 3D models of LAMP2 and gp82 were generated in on-line servers Phyre2 or Swiss-model, energy were minimized in YASARA Server and the structures were validated using several on-line tools. Afterwards, the structures were used for protein-protein docking in on-line server ClusPro. The results showed two main regions of LAMP2 interacting with P4 of gp82. Then, 13 peptides based on these LAMP2 domains were synthetized, each comprising 20 residues, with an overlap of 10 amino acids. To evaluate the ability of these peptides to inhibit the binding of recombinant gp82 (r-gp82) to HeLa cells, r-gp82 was added to 96-well microplates coated with cells, in the presence or absence of individual peptides, followed by reaction with monoclonal antibody to gp82. One of these peptides (P5) inhibited the binding of r-gp82 binding by more than 85%. Peptide P5 was then tested for its capacity to inhibit MT (CL strain) entry into HeLa cells. It exhibited a dose-dependent inhibitory effect, reducing MT internalization by about 60% at the concentration of 100 µg/ml. Based on in vitro assays and docking analyses, attempts are currently under way to identify LAMP2 residues directly involved in interaction with gp82. **Supported by:**FAPESP, CNPq, CAPES **Keywords:** Gp82; lamp2; trypanosoma cruzi metacyclic trypomastigote

HP31 - **THE TOXIC EFFECT OF CHLOROTONIL A ON MATURE GAMETOCYTES OF PLASMODIUM FALCIPARUM IS ASSOCIATED WITH MITOCHONDRIAL DYSFUNCTION, ROS PRODUCTION AND PLASMA MEMBRANE DISRUPTION**

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Malaria, the most significant parasitic infectious disease, is caused by Plasmodium spp. This parasite has a complex life cycle including sexual and asexual stages in Anopheles mosquitoes and vertebrates (including humans) hosts. A large portion of the world`s population is at risk of contracting malaria, and the emergence of resistant parasites remains a big issue. Natural compounds have been served as a source of development of many pharmaceuticals current in use. Chlorotonil a, derived from Sorangium cellulosum, is a compound that was tested against all intraerythrocytic stages of P. falciparum in vitro. Sexual and asexual stages were maintained in culture to perform the drug assays (72 h of drug incubation). The fluorescence microscopies were performed with gametocytes after 5 and 24 h of treatment with the IC 50 and cellular events as mitochondrion functionality and distribution, acid compartment detection, nuclei and membrane integrity and ROS production demonstrated. In addition, the transmission electron microscopy (TEM) of the gametocytes was performed with 240 nM of chlorotonil a for 6 h. The chlorotonil a showed a IC 50 value to asexual and sexual stages around 30 nM. The fluorescence microscopies suggest that after 5 hours of treatment the mitochondrion lost its membrane potential and the plasm membrane was damaged. After 24 h, a weak cytoplasmic acidification occurred and the ROS production was visible but the nuclei had its typical morphology. The TEM showed the untreated parasites with their typical morphology, with well-structured nuclei, well-distributed cytoplasmic material and food vacuole containing hemozoin crystals, but after treatment the parasites presented loss of cytoplasmic material, disorganization of internal structures and vacuolization. The chlorotonil a is a promising compound because it was effective at nM concentrations against all blood stage P. falciparum. Further studies should be performed to investigate its mode of action. **Supported by:**the Deutsche Forschungsgemeinschaft and the Open Access Publishing Fund of the University of Tübinge **Keywords:** Malaria; clorotonil; plasmodium

HP32 - **ABSENCE OF FPR2 IMPROVES THE CONTROL OF PARASITEMIA AND PROTECTS AGAINST BRAIN DAMAGE DURING *PLASMODIUM BERGHEI* ANKA INFECTION**

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INTRODUCTION:The *Plasmodium berghei* ANKA(PbA) infection in mice closely recapitulates many aspects of human cerebral malaria(CM).N-formyl peptide receptor2(FPR2) is involved in the organism's response to infections and plays an important role in the anti-inflammatory pathway presenting high affinity for lipoxin A4(LXA4). LXA4 is a potent anti-inflammatory eicosanoid that is produced by the activation of the enzyme 5-lipoxygenase(5-LO) and Zileuton(Zil)is an inhibitor of 5-LO.The role of FPR2 as well the effect of Zil treatment in the development of CM is unknown and was the mainly aim in this study.METHODS: C57Bl/6(WT) and FPR2knockout(KO) mice were inoculated with PbA and 5days after infection(dpi) were treated daily every 12hours with Zil(50mg/kg) alone and/or in combination with chloroquine(Chq)(30mg/kg) once per day.Parasitemia, body weight, survival, clinical score, memory and brain intravital analyses were evaluated.RESULTS:Parasitemia was similar between WT and KO mice.However, PbA-infected WT mice treated with Zil showed reduction in the parasitemia and improved clinical score when compared to infected untreated WT mice. The benefic effect of Zil wasn´t observed in the absence of FPR2.Also, treatment with Zil didn´t protect against lost of cognition and no altered the levels of parasitism in brain tissue of both group. In addition, as expected, the treatment with Chq reduced the parasitemia but didn´t protect against brain damage in PbA-infected WT mice.In the other hand, PbA-infected KO mice treated with Chq presented lower parasitemia, better score and intact memory when compared with infected KO untreated mice.Interesting, the treatment with combination of Chq+Zil aborted the protection of the Chq against the lost of cognition found in KO mice.CONCLUSIONS:Together, our results suggested that during the PbA-infection the FPR2 contributes for the brain parasitism and damage to nervous system being a target for the development of new therapeutic approaches. **Supported by:**CNPq, CAPES, Fapemig **Keywords:** Malaria; n-formyl peptide receptor2; zileuton

HP33 - ***TRYPANOSOMA* SP. INFECTION IN *BOA CONSTRICTO*R SNAKES FROM NORTHEASTERN BRAZIL: HEMATOLOGICAL AND CLINICAL BIOCHEMISTRY CHANGES AND MOLECULAR CHARACTERIZATION**

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Among reptiles, the occurrence of trypanosomiasis in snakes is rarely described, with few reports restricted to venomous species. Therefore, the present study aimed to investigate the occurrence of *Trypanosoma* sp. infection in *Boa constrictor* from northeastern Brazil, to characterize the identified parasites and to evaluate the influence of the parasitism on the animal physiology. A total of 67 *Boa constrictor* were evaluated, 47 from Bahia and 14 from Sergipe states, from which blood samples were obtained for blood cell counts and clinical biochemistry analysis, genomic DNA obtaining and isolation of trypanosomatids. Nine (15%) animals with extracellular flagellate forms were identified in blood smears, 01 in a snake from Bahia and 08 in animals from Sergipe. The parasitemia ranged from 1% to 7%. The parasites had an exuberant undulating membrane and a small kinetoplast. Parasitized animals were debilitated, presenting opaque scales and remnants of ecdysis. The statistical comparison of hematological values between infected and uninfected animals was significant for globular volume (p <0.0005), hemoglobin (p <0.005), mean corpuscular volume (p <0.005) and mean corpuscular hemoglobin (p <0.05), with infected animals showing lower values than uninfected animals. In leukometry, there was a difference between eosinophil (p <0.00005) and heterophil (p <0.05) counts, always higher in parasitized animals. Slow-growing pleomorphic epimastigote forms were cultivated, with an average of 57.04 µm in length, 3.45 µm in width and 29.47 µm of free flagella. The sequencing of part of the 18S gene identified a 100% homology with *Trypanosoma cascavelli* and *Trypanosoma freitasi* . We concluded that the infection by *Trypanosoma* sp. leads to the development of pathological process in these animals, as expressed by the hematological and clinical biochemistry founds. Other molecular markers will be used for an accurate identification of the isolated trypanosomatids. **Supported by:**FAPEX / BA **Keywords:** Conservation medicine; reptiles; trypanosomiasis

HP34 - **RECOGNITION OF *LEISHMANIA INFANTUM*-DERIVED GLYCOINOSITOLPHOSPHOLIPIDS BY DOGS WITH DIFFERENT CLINICAL PRESENTATIONS OF VISCERAL LEISHMANIASIS**

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Canine visceral leishmaniasis (CVL) is a chronic zoonosis transmitted by Phlebotomine vectors, being caused by intracellular parasites of the genus Leishmania. One of the most prevalent molecules present in the parasite's membrane is the glycoinositolphospholipid (GIPL), a surface glycoconjugate that participates in the processes of adaptation of the parasite to its vector and in the pathogenesis of the disease. Commercially available immunodiagnosis assays fails in detect all positive animals, especially the asymptomatic ones. In face of the need of more sensitive and specific diagnostic tests for CVL detection, the present work aimed to develop an indirect ELISA using *Leishmania infantum* membrane GIPLs as antigens and to analyze the humoral immune response triggered by these molecules in dogs with different CVL clinical profiles. GIPLs were purified from *L. infantum* membrane and applied in an immunoenzimatic platform, where 68 positive and 57 negative serum samples were used as controls. Some positive animals were classified according to the CVL outcome. In addition, serum samples from dogs experimentally infected with *Trypanosoma cruzi* and naturally infected with *Leishmania braziliensis* were tested with the objective to observe possible cross-reactions. With the results obtained, it was possible to conclude that the GIPLs were recognized by sera of animals infected with *L. infantum*, but with a low antigenic recognition, a situation that compromised the sensitivity of the assay (73,5%). However, 100% (8/8) of the asymptomatic animals were positive in the assay. There were cross-reactions when the sera of animals infected with *T. cruzi* and *L. braziliensis* were tested. It can be concluded that GIPLs are recognized by animals infected with *L. infantum*, but are mostly recognized by sera from asymptomatic animals, and that cross-reactions with antibodies specific to glycoconjugates of other protozoa can occur. **Supported by:**FAPEX/BA, CNPq, FAPEMIG, FAPESB **Keywords:** Dogs; glycoconjugates; immunodiagnosis

HP35 - **EVALUATION OF IMMUNE RESPONSE AFTER CHEMOTHERAPY WITH LIPOSOME FORMULATION OF MEGLUMINE ANTIMONIATE IN SYMPTOMATIC DOGS NATURALLY INFECTED BY *LEISHMANIA INFANTUM***

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Canine visceral leishmaniasis (CVL) caused by L. infantum is a disease of great veterinary significance. Over the past decades, effort has been put towards developing novel and cost-effective strategies against CVL, since conventional chemotherapy has not been effective. A major advance occurred when it was found that liposome-encapsulated antimonial drugs were more effective than the free drugs against experimental VL based on parasite suppression in organs. Since then, much effort has been devoted to the search for efficacious liposomal formulations in CVL. An innovative conventional and pegylated liposomal formulation of antimoniate meglumine was recently developed that has significant advantages over conventional formulations. Thus, the aim of the study was to evaluate the immune response after chemotherapy with liposomal meglumine antimoniate (AMLip) in symptomatic dogs naturally infected by Leishmania infantum. For this, nine dogs received treatment with conventional and pegylated liposome formulation of meglumine antimoniate in six intravenous doses (6,5 mg Sb+5/kg) with four days interval/dose. Peripheral blood was collected before (T0) and 30, 90 and 180 (T30, T90, T180) days after treatment for further immunological analysis (ex vivo and in vitro analyses) by flow cytometer. In ex vivo analysis, treated dogs showed increased CD4+ and CD8+ subsets T lymphocytes and CD21+ in T30 and T90, increased of CD5-CD16+ in T90 in relation to T0 and T180 and increased CD14+ in T30 compared of T0. In the in vitro context, dogs developed a strong antigen-specific lymphoproliferation by TCD4+ cells in T90 compared to T0 and an increase of production of IFN-□ in T90 in relation to T0 and T30, besides increase of production of IL-4 in T90 in relation to T0. The results suggested that chemotherapy induced a change in immune response in the early times post treatment. So, further approaches aiming to demonstrate the potential of AMLIP as chemotherapy for CVL are required. **Supported by:**FAPEMIG, CAPES, CNPq, UFOP **Keywords:** Leishmania infantum; liposomal antimoniate meglumine; chemotherapy

HP36 - **TREATMENT OF VISCERAL LEISHMANIASIS WITH ALLOPURINOL ASSOCIATED WITH THE RECOMBINANT CYSTEINE PROTEINASE FROM LEISHMANIA (LEISHMANIA) INFANTUM CHAGASI (RLDCCYS1)**

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Allopurinol is one of the drugs used for treatment of canine visceral leishmaniasis (CVL). Previous data from our laboratory showed that the treatment of CVL with the recombinant cysteine proteinase from Leishmania (Leishmania) infantum chagasi, rLdccys1 and Propionibacterium acnes, led to protective immune responses and a significant reduction of the parasite burden, control of disease development and increase of survival in treated dogs. This study evaluated the efficacy of the treatment of visceral leishmaniasis with Allopurinol associated with rLdccys1 plus P. acnes as an adjuvant. In a first experiment the hamsters were infected intraperitoneally with 1x108 L. L. infantum chagasi amastigotes and 30 days after they were treated with Allopurinol (20 mg/kg by oral route for 30 days), rLdccys 1 (3 doses of 50 µg/hamster by subcutaneous route every 10 days) and Propionibacterium acnes (3 doses of 50 µg/hamster by subcutaneous route every 10 days). In the second experiment the hamsters were treated with Allopurinol, rLdccys 1 and Allopurinol+rLdccys 1 at the same doses previously used. The data from the first experiment showed a significant reduction of parasite burden by limiting dilution in the spleen of hamsters treated with Allopurinol and rLdccys 1 alone or associated.The results from the second experiment also showed a significant reduction of parasite burden in hamsters treated with Allopurinol and rLdccys1 alone or associated and a small increase of IFN-gama and IL-10 mRNA expression in the spleen of hamsters treated with rLdccys 1 plus Allopurinol, indicating a mixed Th1 and Th2 response in these animals. So far our data showed that the treatment with Allopurinol and rLdccys1 alone or in association resulted in a significant reduction of parasite burden in L. (L.) infantum chagasi-infected hamsters. **Supported by:**FAPESP **Keywords:** Leishmania ; allopurinol; rldccys 1

HP37 - **BIOACTIVE LIPIDS REGULATE TRYPANOSOMA CRUZI DEVELOPMENT**

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Trypanosoma cruzi is the etiological agent of Chagas disease. These parasites undergo dramatic morphological and physiological changes during their life cycle. The human-infective metacyclic trypomastigotes differentiate from epimastigotes inside the midgut of the Triatominae insect vector. Our group has shown that the saliva and feces of *Rhodnius prolixus* contains a lysophospholipid, lysophosphatidylcholine (LPC), which modulates several aspects of *T. cruzi* infection in macrophages. LPC hydrolysis by a specific lysophospholipase D, autotaxin (ATX), generates lysophosphatidic acid (LPA). These bioactive lysophospholids are multisignaling molecules and are found in human plasma ingested by the insect during blood feeding. Here we show the role of LPC and LPA in T. cruzi proliferation and differentiation. Both lysophospholipids are able to induce parasite proliferation. We observed an increase in parasite growth with different fatty acyl chains, such as C18:0-, C16:0-, or C18:1 LPC. The dynamics of LPC and LPA effect on parasite proliferation was evaluated in vivo through a time and space-dependent strategy in the vector gut. LPC but not LPA was also able to affect parasite metacyclogenesis. Finally, we determined LPA and LPC distribution in the parasite itself. Such bioactive lipids are associated with reservosomes of *T. cruzi*. To the best of our knowledge, this is the first study to suggest the role of surrounding bioactive lipids ingested during blood feeding in the control of parasite transmission. **Supported by:**CAPES **Keywords:** Trypanosoma cruzi; lysophosphatidylcholine; lysophosphatidic acid

HP38 - **IMPACT OF NUTRITIONAL STATUS ON INFECTION COURSE AND EXPERIMENTAL TREATMENT OF *LEISHMANIA INFANTUM*-INFECTED MICE**

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Malnutrition and visceral leishmaniasis are a major public health problem, since both are responsible for millions of deaths in many countries. The development and progression of leishmaniasis is associated with the host immune response. In this scenario, malnutrition can affect directly the course of leishmaniasis, once it impairs several components of the immune system. It has already been reported that the host nutritional status directly interferes with immunity and/ or susceptibility to parasitic diseases, including leishmaniasis. However, there are few information available regarding the effects of malnutrition on treatment response in *Leishmania*-infected hosts. In this context, the objective of this work is to evaluate the influence of nutritional status on treatment and in the immune response of mice subjected to experimental malnutrition and refeeding, infected by *Leishmania infantum*. Weaned BALB/c mice that received diet control or low diet were infected or not and treated or not with Glucantime. The mice's nutritional status was evaluated through murinometrics and biochemical parameters. The infection effects and immune response were evaluated through LDA assay and CBA assay, respectively. Our results showed that the low diet was able to induce an experimental malnutrition in mice, demonstrating a significant weight loss, when compared to eutrophic mice. Malnourished mice demonstrated slow body growth and low body mass index (BMI) values, while those that were refeeding, began to grow and recover the BMI. Malnourished and refeed mice presented low parasitic load in spleen. In the liver, both groups presented high parasitic load, and when were treated with Glucantime, they showed a smaller decrease in parasitic load, when compared to treated eutrophic mice. Malnourished and eutrophic mice showed differences on TNF and IFN-y production. Further studies are necessary to better understand the malnutrition/leishmaniasis relationship. **Supported by:**CAPES, CNPq **Keywords:** Visceral leishmaniasis; malnutrition; treatment

HP39 - **T. CRUZI INFECTION RATE IS INCREASED BY IMPAIRED HOST MITOCHONDRIAL FUSION**

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Chagas disease (CD) is caused by the protozoan Trypanosoma cruzi. Currently 8 million people are infected, mainly in Latin America (Endemic region). Only benznidazole and nifurtimox are available to treat CD, and they are partially effective in the acute phase and ineffective in the chronic phase of the disease. Therefore, identification and validation of new targets and therapies are needed.
Host mitochondria are affected in different phases of CD. Increased mitochondrial density and size were reported after T. cruzi infection. These changes are associated with parasite proliferation and parasite-host mitochondria co-localization. However, there is no study available showing causality between changes in mitochondrial morphology (or dynamics) and infection rate or parasite proliferation. Considering that T. cruzi has a single mitochondrion, we hypothesized that T. cruzi infection triggers changes in host-mitochondria dynamics and metabolism to ensuring that sufficient energy source is available to match T. cruzi metabolic demand during infection and proliferation. Therefore, targeting host-mitochondria metabolism may compromise T. cruzi infection. To address this question, we infected WT and knockout (Mfn1, Mfn2, Mfn1 / 2, Opa1, Fis1, Mff or Atg5) mouse embryonic fibroblasts (MEF) with T. cruzi (CL-14). Of interest, MEFs knockout for mitochondrial fusion-related proteins (Mfn2, Mfn1 /2 or Opa1) displayed increased infection rates) 3h post-infection compared to WT (ranging between 1,8 and 2,3 fold increase vs. WT. These preliminary findings suggest that host mitochondrial fusion-fission balance plays a role in T. cruzi infection rate. **Supported by:**FAPESP **Keywords:** Mitochondrial dynamics; fusion; host-parasite interaction

HP40 - **CHEMILUMINESCENT ELISA AND SYNTHETIC PEPTIDES FOR THE DIAGNOSIS OF CANINE VISCERAL LEISHMANIASIS.**

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Leishmaniasis is caused by protozoan from genus Leishmania and is a major public health problem. Visceral leishmaniasis (VL) is the most severe form of this disease and can be fatal without proper treatment. Nowadays, one of the control measures to zoonotic VL is the identification of infected dogs, as they are important parasite reservoir. Serologic methods have been widely used in the diagnosis of canine VL (CVL) due to its easy execution and affordable cost. Our research group have already demonstrated the potential of PQ20 multiepitope recombinant protein as a diagnostic tool to CVL through chemiluminescent ELISA, a technique that enhanced test performance in comparison to the conventional photometric assay. In this study, peptides that compose PQ20 were evaluated as antigens in chemiluminescent ELISA as diagnostic tools to CVL. Initially, assay conditions were standardized. Best results were: peptide concentration of 0,5 µL/mL, sera dilution at 1:100 and conjugated dilution at 1:4000. Among the 20 investigated antigens, peptide 5 (WSRKLGVSF) presented the best performance with 100% of sensibility, 82,02% of specificity, 0,93 of AUC and P value < 0,0001. Those results suggest the potential of this peptide as a diagnostic tool to CVL using chemiluminescent ELISA. **Supported by:**Capes **Keywords:** Chemiluminescent elisa; diagnosis; canine visceral leishmaniasis.

HP41 - **THE MIR-294, MIR-30E AND MIR-302D MODULATE MACROPHAGE NOS2 AND TNF EXPRESSION DURING LEISHMANIA AMAZONENSIS INFECTION**

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The microRNAs (miRNAs) are non-coding RNAs (21-24 nt), that can modulate the gene expression by binding with the 3'UTR of target mRNA, inducing the cleavage or traduction inhibition of the mRNA. The miRNAs are modulators of inflammatory mechanisms during immune response by pos-transcriptional regulation of genes involved in these pathways. The miRNAs profile of macrophages is altered during infections by bacteria e virus, as well as cancer cells. In this work, we determine whether the infection with Leishmania amazonensis can subvert the miRNAs profile of mouse macrophages and its implications in the regulation of parasite infectivity. L. amazonensis infection modulated host cytokine production such as IL-6, MCP-1/CCL2, and RANTES/CCL9. The TLR pathway also influences in the L-arginine metabolism leading to nitric oxide (NO) production kill parasite, via increase of Nitric Oxide Synthase 2 (Nos2) mRNA expression and cytokines, which was blocked in the absence of TLR2, TLR4 and MyD88. The infection of BALB/c-macrophages upregulated the miR-294 and miR-302d expression, targeting Tnf, Mcp-1/Ccl, and Nos2. Also, miR-30e-5p and miR-302d-3p were upregulated during infection and inhibition assay of these miRNAs increased Nos2 mRNA expression levels and NO production, reducing the infectivity. In conclusion, miRNA impact in the macrophage response during L. amazonensis infection. **Supported by:**FAPESP and CNPq. **Keywords:** Microrna; macrophages, nos2; leishmania

HP42 - **EXPRESSION, LOCALIZATION AND FUNCTION OF STAGE-SPECIFIC TCTASV-B PROTEIN BY MEANS OF TRANSGENIC TRYPANOSOMA CRUZI TCI PARASITES**

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TcTASV is a surface protein family of T. cruzi comprised of about 40 genes. The amino and carboxy terminal portions are conserved, and a variable central core allows dividing TcTASV in subfamilies A, B, C and W. All TcTASV are expressed in trypomastigotes and have no orthologs in other organisms, hence being potential candidates for vaccines or drug targets. Recently, we have been studying TcTASV-B, which has the peculiarity that it seems to be absent in T. cruzi strains belonging to DTU TcI. By cloning, sequencing and/or detailed in silico inspection we detected TcTASV-B in genomes of TcII, TcV and TcVI strains (no in TcI). By using polyclonal antisera we detected TcTASV-B expression in both trypomastigotes and amastigotes, localized at the surface as discrete points, although it is not secreted. As the function of TcTASV-B is unknown and taking advantage of the absence of TcTASV-B in TcI strains, we expressed a tagged TcTASV-B gene in TcI parasites (Dm28 strain), using a tetracycline inducible vector. Our final goal is to obtain transgenic trypomastigotes to interrogate TcTASV-B function both in vitro and in vivo. Expression of TcTASV-B was detectable in transfected epimastigotes after 4hs of induction and did not affected parasite growth rate. Indirect immunofluorescence with monoclonal anti HA antibody revealed that TcTASV-B localizes all along on the membrane surface. Metacyclic trypomastigotes were used to infect Vero cells to obtain transgenic Dm28 amastigotes and trypomastigotes. Although TcTASV-B expression was detected in transgenic amastigotes, cell-released trypomastigotes were scarce and tended to round up quickly. TcTASV-B was localized at the membrane surface with two characteristic labeling patterns (discret points or polarized), which were different to the observed in transgenic epimastigotes. We are currently optimizing TcTASV-B expression in transgenic trypomastigotes, to evaluate its infection and transmigration ability in 3D culture systems. **Supported by:** ANPCyT, PICT 2014-1151 and PICT 2016-0108. Argentina. **Keywords:** Tctasv-b; trypanosoma cruzi; transgenic parasite

HP43 - **PURINE ANALOGUES: PROMISING CANDIDATES AS ANTI-TRYPANO[illegible]IDS AGENTS**

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Among the Neglected Tropical Diseases – [illegible]s population and prevails in populations living in poverty [illegible] are three major human diseases caused by trypanosom[illegible]se (CD) and human African trypanosomiasis (HAT). No[illegible]d current drugs have significant drawbacks in terms of ef[illegible]stance, justifying the need for alternative chemotherapies. P[illegible]ids are purine auxotroph's, focused purine libraries can[illegible] as described for both T. cruzi and T. brucei in literatur[illegible] screened in vitro in the panel of trypanosomatid sp[illegible]gainst T. cruzi ranging nanomolar levels (IC50 < 0.3 µM). and one[illegible]oader effect against T. brucei and Leishmania infantum. Anti-T. cruzi[illegible]se analogues also have displayed promising data in vitro and in vivo mode[illegible]. brucei RNA interference (RNAi) library was performed using some of the most p[illegible]oside analogues and the findings revealed interesting targets, including the amino acids ph[illegible]orylation and evasion molecules involved in the mammalian host immune response. **Supported by:**Fiocruz, Ghent University, Antwerp University, CAPES and FAPERJ **Keywords:** Phenotypic-screening; mode-of-action; trypanosomatids

HP44 - **NEW REPETITIVE SEQUENCE FOUND ON 3' UTRS OF MULTIGENIC FAMILY GENES: POSSIBLE CIS-REGULATORY ELEMENT OF MRNAS ALONG *TRYPANOSOMA CRUZI* LIFE CYCLE.**

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Trypanosoma cruzi protozoan parasite presents a highly repetitive genome which represents 50% of it. These repetitive elements are comprised of multigenic family and non-coding repetitive elements, and most of these latter elements have no identified function so far. Here we used a new search approach on *T. cruzi* genome in order to identify new repetitive sequences not yet described. Using a sliding window of 150 nucleotides moving one nucleotide at a time over the genome, millions of sequences were generated. To clear up these data, the sequences were submitted to 4 filtering steps: Exclusion of sequences with at least 1 "N" nucleotide; Selection of sequences with at least 10 copies; Exclusion of sequences retrieved from multigenic family data base and exclusion of sequences retrieved from Repeat Masker. At the end, 67 sequences of 150 bp found in repetition on the genome were obtained. Once the sliding window generated sequences one nucleotide apart from each other, these 67 sequences were aligned and it was revealed that they were part of a longer repetitive sequence of 241 nucleotides. This new repetitive sequence, named 241-nt, is interspersed through the genome with a close relation with multigenic family of surface proteins. From the 326 repetitions found, 97% were located at the 3' UTR of trans-sialidase, MASP, Mucin, GP63 and 90 kDa surface protein genes. Also, the 241-nt is located within the predicted mRNA 3' UTR of these genes and mRNA binding protein motifs were found within the repetitive sequence. It is known that mRNA binding proteins and 3' UTR from mRNA are involved on mRNA stabilization and translational control in T. cruzi. In fact, 85% of the trans-sialidases related to the 241-nt sequence are stage-specific expressed on tripomastigote forms. So we propose that the new 241-nt repetitive element identified can potentially be a cis-regulatory element on 3'UTR of mRNAs, playing a role on post-transcriptional control on *T. cruzi.* **Supported by:**FAPESP **Keywords:** Multigenic family; post-transcriptional control; repetitive sequence

HP45 - ***IN VITRO* AND *IN VIVO* SUSCEPTIBILITY ASSESSMENT OF PYRIMETHAMINE IN THE CONTROL OF REPLICATION OF ATYPICAL STRAINS OF *TOXOPLASMA GONDII***

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The current treatment of toxoplasmosis consists in the combination of Sulfadiazine and Pyrimethamine (PYR). Considered the most effective drug against *Toxoplasma gondii*, PYR is a standard component of therapy, however, treatment failures have been reported and the possible natural resistance of *T. gondii* to antifolates has been widely discussed in the literature. Therefore, the objective of the present work was to evaluate, *in vitro* and *in vivo*, the susceptibility to different doses of PYR during infection with *T. gondii* strains TgWildBrMG2, TgWildBrMG3 and TgWildBrMG4 previously isolated from free-living wild birds from the state of Minas Gerais, Southeastern Brazil. For *in vitro* assays, primary cultures of Human Foreskin Fibroblast (HFF) were infected with tachyzoites of *T. gondii* strains under study and treated with PYR at 0.5 µM, 1.0 µM and 2.0 µM for 24 hours. Posteriorly, cells were fixed and stained with panotic in coverslips mounted on microscopy slides. The cells were examined under light microscopy to assess intracellular replication and infection index. For *in vivo* trials, Swiss mice were infected intraperitoneally with 10^4 tachyzoites of each strain. Treatment was started 48 hours after infection and continued for 10 days. The animals were divided into three groups: PYR3, PYR12 and PYR50 treated orally with 3.13; 12.5 and 50 mg/kg per day of PYR, respectively. In the *in vitro* results TgWildBrMG2 and TgWildBrMG4 strains showed a higher parasitic load compared to TgWildBrMG3 strain following treatment with PYR 1.0 µM. The *in vivo* results corroborated with our *in vitro* findings demonstrating the TgWildBrMG2 strain has lower susceptibility to treatment, while the TgWildBrMG3 strain has higher PYR sensitivity. TgWildBrMG4 strain has showed lower susceptibility using *in vitro* assay and high susceptibility using *in vivo* treatment. In conclusion, *in vitro* and *in vivo* results suggest the variability in susceptibility to PYR by atypical *T. gondii* strains. **Supported by:**FAPEMIG **Keywords:** Toxoplasma gondii; atypical strains; pyrimethamine

HP46 - **L-ARGININE AND PUTRESCINE AVAILABILITY MODULATE EXPRESSION OF GENES INVOLVED IN ITS METABOLIZATION IN MACROPHAGE DURING *L. AMAZONENSIS* INFECTION**

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L-arginine is an important amino acid for macrophage immunological response. During the *Leishmania amazonensis* infection, nitric oxide synthase 2 (NOS2) uses L-arginine to produce nitric oxide (NO) and this way, to kill the pathogen. On the other hand, this response can be subverted by the interaction between the parasite and the macrophage host, favoring the pathogen survival by supplying the polyamine pathway via enzymes arginase 1 and 2 (*Arg1* and *Arg2*). In this work we investigated gene expression of enzymes of L-arginine metabolism and NO production in murine BALB/c-macrophages infected with *L. amazonensis*, under L-arginine and putrescine deprivation or supplementation. Our data showed that infection of macrophage under L-arginine deprivation increased the amounts of mRNA of *Nos2* at 4 and 24 h and *Arg1* and *Cat2B* at 24h, when compared to the supplemented one. We also observed reduction of *Arg1* levels at 24h in uninfected macrophage subjected to L-arginine deprivation, compared to L-arginine supplemented one. Indeed, L-arginine and putrescine supplementation increased the expression levels of *Cat2B* at 24h in infected macrophages supplemented only with L-arginine. We also observed increased levels of *Arg1* (4h) and *Nos2* (4 and 24h) in infected macrophage subjected to putrescine supplementation under L-arginine deprivation, compared to L-arginine supplemented one. In uninfected macrophages, the supplementation with L-arginine and putrescine increased the expression level of *Arg2* and *Nos2* at 4h, and reduced *Cat2B* and *Nos2* at 24h, in relation to L-arginine supplementation. Our data showed that L-arginine deprivation reduced the number of infected macrophages at 4h and the amastigotes per infected macrophages at the 4 and 24h, when compared with the L-arginine supplementation. Our results indicate that L-arginine and putrescine availability to the host impact on the enzymes involved in macrophage metabolism during *L. amazonensis* infection. **Supported by:**Fapesp e CNPq **Keywords:** Leishmania; l-arginine; polyamine, nitric oxide

HP47 - **IMMUNIZATION WITH TCTASV ANTIGENS DELIVERED IN BACULOVIRUS GENERATES A ROBUST IMMUNE RESPONSE AND ELICITS PROTECTION AGAINST *TRYPANOSOMA CRUZI* INFECTION**

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TcTASV is a medium size multigenic family unique to *Trypanosoma cruzi* present in all strains of the parasite and expressed in the life cycle stages of the mammalian host. Subfamilies TcTASV-A and TcTASV-C are the most numerous, are in contact with the host immune system and show differential expression patterns: TcTASV-A is expressed intracellularly in amastigotes and trypomastigotes while TcTASV-C is expressed at trypomastigote surface and secreted (Garcia et al, 2010; Bernabó et al, 2013; Floridia et al, 2016). Previous vaccination assays with TcTASV-C resulted in delayed appearance of bloodstream trypomastigotes but impacted only slightly in mortality, after challenge with RA (TcVI), a highly virulent T. cruzi strain. The immune response was essentially humoral, with negligible cellular response (Caeiro et al, 2018). We hypothesized that a vaccination protocol with TcTASV could be improved by triggering also a cellular response against TcTASV-A (intracellular antigen). As heterologous antigen display at baculovirus (BV) capsid has been reported to induce cellular responses, we engineered a recombinant BV that accurate express TcTASV-A (BV-TcTASV-A) fused to VP39, the major nucleocapsid protein. Mice were first immunized with rTcTASV-C adjuvanted with aluminum hydroxide, followed by a boost with BV-TcTASV-A plus rTcTASV-C. This immunization scheme induced a strong anti-TcTASV-C humoral response along with CD8+/IFNγ+ (5,2%) and CD4+/IFNγ+ (0,7%) T cell populations after restimulation with TcTASV-A and TcTASV-C, respectively. When challenged with RA strain, BV-TcTASV immunized mice presented lower levels of circulating trypomastigotes and 100% survival (vs 50% or 75% in controls, in 1st or 2nd immunization assay). We conclude that this immunization protocol elicited a robust immune response against TcTASV family, which could be relevant in protection against *T. cruzi*. **Supported by:**ANPCyT PICT 2016-0108. Argentina. **Keywords:** Trypanosoma cruzi; baculovirus; immunology

HP48 - **EFFECT OF LYSOPHOSPHATIDYLCHOLINE ON THE MODULATION OF GROWTH, DIFFERENTIATION AND INFECTIVITY OF LEISHMANIA MEXICANA**

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Leishmaniasis is among the major emerging parasitic diseases that affect humans. These infections are caused by different species of the genus Leishmania. Among these, Leishmania mexicana causes cutaneous and eventually diffuse (anergic) lesions, occurring especially in Mexico and Central America. Lipid mediators, including lysophosphatidylcholine (LPC) and platelet activation (PAF), play an important role in cell differentiation and infectivity of some trypanosomatids. PAF triggers a signal transduction cascade that activates protein kinase CK2, through protein kinase C (PKC), in Herpetomonas muscarum muscarum, and stimulates the activity and expression of CK2 in Leishmania tropica. Recently, our group showed that Trypanosoma cruzi synthesizes a bioactive LPC (C18:1 LPC), which aggregates platelets and induces cell differentiation of the parasite. Based on these studies, the hypothesis formulated for this project was that C18:1 LPC would present a modulatory effect on several aspects of the physiology of L. mexicana. We analyzed the proliferation of promastigotes for seven days in the presence and in the absence of LPC and detected that the LPC-treated parasites grown three times more than the untreated ones. We have also tested if C18:1 LPC would trigger the differentiation of the parasites from promastigotes to amastigote-like in vitro. The parasites were then cultivated for 30 days in the presence and in the absence of LPC and we observed that the LPC-treated parasites presented 40% more amastigote-like forms than the untreated ones. Preliminary results from the interaction of promastigotes with BALB/c mice peritoneal macrophages showed an increase of about 10% in the association index of LPC-treated macrophages with L. mexicana, within 24 hours after the infection. Therefore, our results suggest a modulation of several important features of the life cycle of L. mexicana by C18:1 LPC. **Keywords:** Trypanosomatids; leishmania; lipids

HP49 - **INFLUENCE OF A LYSOPHOSPHATIDYLCHOLINE (LPC) AND PLATELET-ACTIVATING FACTOR (PAF) RECEPTOR IN CELL PROLIFERATION AND VIABILITY IN *TRYPANOSOMA CRUZI***

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Platelet-activating factor (PAF) and lysophosphatidylcholine (LPC) have several cellular functions in eukaryotes, including trypanosomatids, such as proliferation, cell differentiation and infectivity. Our group identified a putative receptor for PAF and LPC in T. cruzi and produced knockout and knockdown mutant phenotypes for the gene encoding this receptor to evaluate its relevance. Ultrastructural analysis reveals changes in the mitochondrial morphology of KD parasites when compared to the wild type cells (WT). Thus, the aim of the present work is to evaluate the influence of the receptor for PAF and LPC in T. cruzi on the proliferation and viability of these parasites. In addition, we aim to evaluate possible dysfunctions in the respiratory metabolism of mutant parasites, which may be associated with the mitochondrial alterations. Parasites (WT, KD and KO) were then count in hemocytometer for 7 days, in order to obtain their respective growth curves. In addition, cell viability was inferred from the ability to convert MTT to formazan, determined by spectrophotometry, and by labeling with propidium iodide as determined by cell cytometry. A decrease of 83.15% in proliferation was observed in KO parasites on day 5 and viability analysis showed that mutant parasites are less viable (12.14% for KD and 37.33% for KO) when compared to wild type. These results suggest the importance of LPC and PAF receptor in T. cruzi survival mechanisms and that the receptor removal culminates in a reduction in proliferation, in addition to turn cells less viable, which can be exploited as a potential target for experimental chemotherapy against Chagas disease. **Supported by:**CNPq, CAPES, FAPERJ, INCTEM **Keywords:** Trypanosoma cruzi; bioactive lipids; mitochondria

HP50 - **CHARACTERIZATION OF THE TH17 IMMUNE RESPONSE IN HUMAN CUTANEOUS LESION CAUSED BY *LEISHMANIA (VIANNIA) PANAMENSIS* IN PANAMA, CENTRAL AMERICA.**

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Cutaneous leishmaniasis (CL) caused by *Leishmania (Viannia)* parasites is an endemic disease in Panama, which is characterized mainly by ulcerated skin lesions. It has been described that Th17 cells has an important role in eliminating pathogens that are not properly destroyed by Th1 cells. However, Th17 could be an ambiguous role in the development of disease, helping the elimination of the parasites in some cases; and contributing in the pathogenesis by exacerbate the skin lesions. Despite the increasing knowledge of immunopathological mechanisms that contribute to disease progression, the role of Th17 during *L. (V.) panamensis* infection remains unclear. In this study we characterize the Th17 immune response in skin lesions of patients with CL caused by *L. (V.) panamensis* to better understand the pathogenesis caused by this specie that is endemic in Panama. Biopsies (n = 46) from panamanian patients with localized CL were collected and processed by usual histological techniques. *L. (V.) panamensis* infection was proven by in vitro isolation and characterization of the parasites by HSP70-RFLP. In situ Th17 immune response was evaluated by immunohistochemistry using anti-CD4, anti-RoRγt, anti-IL17, anti-IL6, anti-IL23 and anti-TGF-β antibodies. Quantitative morphometric analysis showed that the density of CD4+ cells were 914.5±51.76 cells/mm2, RoRγt+ 229,20±13,49 cells/mm2, IL-17+ 859.8±70.66 cells/mm2, IL-6+ 273.2±15.89 cells/mm2, IL-23+ 669.8±34.73 cells/mm2 and TGF-β+ 132.2±9.50 cells/mm2. Positive correlation was observed among these markers. Although not significant, an inverse correlation was observed between the amastigote forms and the number of RoRγt+ and IL-17+ cells. The results suggest that Th17 cells may help the elimination of parasites through the action of IL17, but their role in the progression of the disease pathology caused by *L. (V.) panamensis* could not be disconsidered. **Supported by:**FAPESP, CNPq and LIM50 HC-FMUSP. **Keywords:** Leishmania (v.) panamensis ; th17 immune response; immunohistochemistry

HP51 - **TGF-β INHIBITOR THERAPY DECREASES FIBROSIS AND STIMULATES CARDIAC IMPROVEMENT IN A PRE-CLINICAL STUDY OF CHRONIC CHAGAS' HEART DISEASE**

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Chronic chagasic cardiomyopathy (CCC) is the most important and frequent clinical manifestation of chronic Chagas' disease. During CCC, the parasite remains inside the cardiac cells, leading tissue damage, involving extensive inflammatory processes and irregular fibrosis. Some molecules act in the fibrosis formation, but one in particular plays a key role in the fibrogenic process inducing extracellular matrix synthesis: TGF- β. The aim of this study is to investigate the effect of 1D11 and GW788388 treatment during the chronic experimental model of Chagas disease. To this end, animals C57Bl/6 were infected with T. cruzi colombian strain and treated after 120 days post–infection (dpi) with: 1D11 in two different schemes: single dose or once a week and; GW788388 in three different schemes: single dose; once a week or three times a week during 30 days. Functional analysis (electrocardiogram and echocardiogram), molecular (ELISA, RT-qPCR and Western blot) and histopathological analyses (immunofluorescence and immunohistochemistry) were performed before and after 1D11 and GW788388 treatment in control and infected animals. Our data suggested that the chronic model has 100% cardiac damage after 120 dpi and that GW1x and GW3x treatment schemes are more efficient than 1D11 treatment. GW788388 treatment: reestablished the electrocardiographic profile of the infected animals: reduced bradycardia, PR interval and P wave duration; restored the left ventricular ejection fraction, decreased during infection; reversed the higher levels of circulating TGF-β1; SMAD2/3 proteins, fibronectin and collagen type I and collagen deposition in the heart of infected animals; increased MMP-9 and Sca-1, reduced TIMP-1/TIMP-2/TIMP-4, and partially restored GATA-6 and Tbox-5 transcription, supporting cardiac regeneration. The therapeutic effects of GW788388 are promising and suggest a new possibility to treat cardiac fibrosis in the chronic phase of Chagas' heart disease by TGF-β inhibitors.

Supported by:INSERM / FIOCRUZ / CNPq / FAPERJ / DECIT **Keywords:** Chagas disease; fibrosis; tgf-beta

HP52 - **TGF-β NEUTRALIZATION IMPROVES CARDIAC FUNCTION DURING THE CHRONIC PHASE OF CHAGAS' DISEASE IN A MURINE EXPERIMENTAL MODEL**

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Cardiac commitment is the most severe and frequent manifestation of chronic Chagas' disease. TGF-β is involved in the development of chronic chagasic cardiopathy with increased serum levels of this cytokine and activation of its signaling pathway in the cardiac tissue, thus resulting in increased expression of extracellular matrix proteins, which characterizes fibrosis. Inhibition of TGF-β signaling pathway significantly attenuates T. cruzi infection, preventing the development of cardiac damage during the acute phase of Chagas disease in an experimental model. Thus, the main goal of this study is to evaluate the effect of a neutralizing antibody anti TGF-β (1D11) during the chronic phase of Chagas' disease in a murine experimental model. Female mice were infected with trypomastigotes of the Colombian strain of T. cruzi and were monitored electrocardiographically to observe the evolution cardiac function. After 120 dpi, treatment with anti TGF-β (10mg/kg) was initiated in two different schemes: single dose and once a week up to 150 dpi. We observed that the infection altered the cardiac electrical conduction: decreasing the heart rate, increasing the PR interval and the duration of the P wave. The treatment with anti TGF-β reversed this process. Moreover, the treatment with anti-TGF-β reversed collagen deposition in the heart of infected animals. The therapeutic effects of 1D11 are promising and suggest a new possibility to treat cardiac fibrosis in the chronic phase of Chagas' heart disease by TGF-β inhibitors. **Supported by:**INSERM / FIOCRUZ / CNPq / FAPERJ / DECIT **Keywords:** Chagas disease; fibrosis; tgf-beta

HP53 - **MEMBRANE PROTEOME COMPARISON MAY EXPLAIN DIFFERENCES IN PHAGOCYTOSIS AND COMPLEMENT RESISTANCE OF *L. AMAZONENSIS* LV79 AND PH8 STRAINS**

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Human diseases caused by *Leishmania* are classified according to their clinical manifestations into visceral or cutaneous leishmaniasis (CL). Promastigote forms of the parasite are transmitted to humans during the blood meal of infected female sand flies. In the human host, promastigotes are phagocytosed by macrophages, where they reside in parasitophorous vacuoles and become amastigotes. To successfully establish an infection *Leishmania* has several virulence factors, whose abundance vary according to the parasite species, strain and developmental form. Recently, our group compared two strains of *Leishmania amazonensis*, a species frequently associated to CL in Brazil, demonstrating that PH8 strain caused greater lesions *in vivo* than LV79 strain. In addition, PH8 promastigotes adhere and infect more macrophages *in vitro*, indicating differences in their membrane composition.
The aim of this study was to perform a comparative analysis of LV79 and PH8 promastigotes´ membrane proteomes to identify factors that account to the differences between these strains. We also compared complement sensitivity.
Main findings: In murine complement lysis sensitivity assay we found that PH8 promastigotes are more resistant to lysis, again indicating a difference in membrane molecules. The comparative analysis of promastigotes´ membrane proteomes identified 1659 proteins, 37 of which were detected exclusively in LV79 and 65 in PH8. In addition, 96 proteins were differentially abundant, indicating that each strain has a characteristic proteomic profile. Virulence and metacyclogenesis related proteins such as enolase and putative ABC transporter of subfamily G member 1 were identified as more abundant in the PH8 strain, while GP63 was more abundant in the LV79 strain.
We believe the comparative analysis of promastigotes´ membrane proteome may help identify proteins responsible for the higher virulence of the PH8 strain, as well as its higher resistance to complement lysis.
Supported by:FAPESP **Keywords:** Leishmania ; proteome; virulence

HP54 - **FOLLOWING LEUCINE CATABOLIC PATHWAY IN *TRYPANOSOMA CRUZI*: CHARACTERIZATION OF AN ISOVALERYL-COA DEHYDROGENASE**

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Chagas disease caused by *T. cruzi* is one of the most important neglected diseases in the Americas. Amino acids are relevant metabolites having a myriad of functions beyond its obvious participation in protein synthesis. Our laboratory is interested in describing how *T. cruzi* can metabolize leucine (Leu) and the contribution of its metabolic intermediates during the development of the parasite life cycle. In *T. cruzi* genome were found putative sequences that encodes for enzymes associated with BCAAs catabolism. The product of Leu transamination is catabolized to isovaleryl-CoA by the branched-chain α- keto acid dehydrogenase complex (BCKDH). Then, this product becomes a substrate of the enzyme Isovaleryl-CoA dehydrogenase (TcIVDH). Leu catabolic products could have two main destinations: they can become an energy source, producing acetyl-CoA to feed the tricarboxylic acid cycle, and they can donate carbons into the sterol biosynthesis pathway. Here, we propose the hypothesis that TcIVDH constitutes an important decision point for metabolic control of the flux in the Leu degradation pathway. At the moment, we evaluated the activity of TcIVDH in total epimastigote extracts and the coding sequence for the mature form of the enzyme was successfully expressed in *Escherichia coli* pGRO7. The K_{Mapp} of 12 µM for the recombinant enzyme was determined spectrophotometrically for isovaleryl-CoA and its V_{max} was around 0.28 µM/min/µg. Additionally, we obtained a specific polyclonal antibody against the recombinant enzyme and determined that this enzyme is located inside the mitochondrion of epimastigote form of the parasite. We are currently selecting knock out mutants obtained by the CRISPR-Cas9 methodology for both copies of the TcIVDH gene to evaluate the influence of this enzyme on the parasite phenotype. The study of the importance of TcIVDH will allow us to better understand the factors that make Leu as an energetic source or anabolic precursor. **Supported by:**FAPESP **Keywords:** Trypanosoma cruzi; leucine; enzyme characterization

HP55 - **SERINE ACETYLTRANSFERASE AND CYSTEINE SYNTHASE: TWO ENZYMES CATALYZING THE SERINE-CYSTEIN INTERCONVERSION IN *TRYPANOSOMA CRUZI*.**

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Trypanosoma cruzi can use amino acids as energy sources and to support several biological processes such as differentiation, resistance to stress conditions and host-cell invasion. Metabolites containing -SH groups (such glutathione, trypanothion, cysteine, and some of its intermediates such as cystathionine) are relevant to buffer the redox state of the different sub-cellular compartments of this organism. The *de novo* cysteine biosynthesis pathway is comprised of serine O-acetyltransferase (SAT) and cysteine synthase (CS) enzymes which sequentially mediate two consecutive steps of cysteine biosynthesis, and are absent in mammalian host. However, despite the apparent dependency of redox metabolism on cysteine biosynthesis pathway, the role of SAT and CS in redox homeostasis has been unexplored in *T. cruzi* parasite. Herein, we have characterized SAT and CS to investigate their interaction and relative abundance of these proteins in *T. cruzi*. We also identified the putative genes encoding TcSAT and TcCS enzymes, which were cloned, expressed, and affinity-purified. The recombinant TcSAT and TcCS (which contains PLP as cofactor), showed the predicted molecular mass 38 kDa and 36 KDa, respectively. The biochemical characterization showed that TcSAT catalyzes the synthesis of O-acetylserine using serine and Acetyl-CoA. The K_M 1.7 mM for the recombinant protein was determined for serine and its V_{max} was around 0.33 µM/min/µg. Additionally, we obtained specific polyclonal antisera against both recombinant enzymes. Preliminary results obtained by Indirect Immunofluorescence showed that both enzymes are located in the cytosolic compartment of epimastigotes. At present, we are selecting knock out mutants obtained by the CRISPR-Cas9 methodology for both genes to evaluate the influence of this enzymes on the parasite phenotype and determined the important role that this pathway have in the redox state. **Supported by:**FAPESP **Keywords:** Trypanosoma cruzi; cystein biosynthesis; sat, cs

HP56 - **CLASSICAL AND EARLY/RAPID RELEASE OF NEUTROPHIL EXTRACELLULAR TRAPS IN RESPONSE TO TOXOPLASMA GONDII**

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Neutrophil extracellular traps (NETs) are composed of DNA fibers associated with histone, antimicrobial peptides and enzymes from granules, and are produced as a defense mechanism against pathogens. NETs can be released either in an early/rapid way, while maintaining
membrane integrity and allowing neutrophils to play other mechanisms of defense, or in a classical way where cell membrane selective permeability is lost, leading to cell death (NETosis). Little is known about the kinetics of NETs release in response to protozoan and the
mechanisms and role of NETs induced by the causative agent of toxoplasmosis, *Toxoplasma gondii*. Therefore, the aim of this study was to quantify the production of NETs at several time points in response to *T. gondii* and to evaluate the infection of host cells by the parasite in the
presence of NETs. Human neutrophils isolated from peripheral blood of healthy donors were incubated for different time intervals with two parasite:cell ratios (1:1 and 5:1) of two different strains of *T. gondii* (RH and ME49). dsDNA on the supernatants was quantified using the
PicoGreen assay. We found that the production of NETs increased over time and with ratio in a parasite strain dependent manner. Production of NETs as early as 15 min and its increase over time suggests the presence of both classical and early/rapid release of NETs in response to *T. gondii*. To evaluate the infectivity of host cells by *T. gondii* in the presence of NETs we treated parasites with conditioned media (CM) containing NETs and infected Vero cells for 3h. Afterwards cells were washed, incubated for 21h, fixed and stained with Giemsa. Preliminary results show that treatment of parasites with CM did not affect infectivity of cells. We are now working on the characterization of the components involved in both classical and early/rapid production of NETs induced by *T. gondii*, and also on the reasons whereby infectivity of Vero was not affected by parasite treatment with NETs. **Supported by:**FIOCRUZ, FAPERJ, CNPQ **Keywords:** Toxoplasma gondii; classical nets release; early/rapid nets release

HP57 - **MOLECULAR AND ULTRASTRUCTURAL ASPECTS OF NEUTROPHIL EXTRACELLULAR TRAPS RELEASED IN RESPONSE TO TOXOPLASMA GONDII**

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Production of neutrophil extracellular traps (NETs) is a mechanism of innate immune response capable of trapping and killing microorganisms thereby preventing infection. NETs are composed of decondensed chromatin associated with cytoplasmic granules, and its production in response to several protozoan is documented, including the parasite *Toxoplasma gondii*. However, the molecular mechanisms and structural components involved in the production and release of NETs deserve exploitation. Therefore, this study aims to witness the production of NETs in response to *T. gondii* and to analyse the ultrastructure and some of the molecular components of NETs. Neutrophils were purified from the blood of healthy donors by density gradient and incubated with *T. gondii* of either RH or ME49 strains, or with phorbol myristate acetate (PMA) for 180 or 240 minutes. Afterwards cells were fixed and processed for both optical and electron microscopy. Detection of histone H1 and myeloperoxidase (MPO) in released NETs was performed by immunofluorescence microscopy, after staining neutrophils with anti-H1 and anti-MPO antibodies. DNA was stained with dihydrochloride (DAPI). NETs components were seen in close proximity to parasites, which led us to better investigate the traps associated with *T. gondii*. By transmission electron microscopy we evaluated nuclear and cytoplasmic morphological changes during NETs release. We found that non-stimulated neutrophils present preserved lobulated nucleus and intact plasma membrane, while stimulated neutrophils show loss of nuclear membrane integrity and several vesicles containing DNA in the extracellular medium. By scanning electron microscopy we observed fibers of chromatin clearly trapping the parasites, possibly leading to their death. We show that neutrophils efficiently produce NETs with its classical components in response to *T. gondii*. We are now investigating whether the NETs production occurs in vivo in a murine model of toxoplasmosis. **Supported by:**Fiocruz, FAPERJ and CNPq **Keywords:** Toxoplasma gondii; neutrophil extracellular traps; ultrastructure

HP58 - **INVOLVEMENT OF CD100/SEMA4D IN *LEISHMANIA* SPP. INFECTION.**

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Leishmaniasis is a tropical and subtropical disease caused by protozoa of the genus Leishmania, which may present cutaneous (CL) or visceral (VL) clinical manifestations. In humans and other mammals, parasites preferentially infect macrophages. Factors such as Leishmania species and host immune response influence the clinical form and severity of the disease. CD100 or Sema4D is a membrane or soluble glycoprotein of the semaphorin family involved in several arms of the immune response. Our group was the first to show that soluble CD100 (sCD100) augments infection of macrophages by Leishmania amazonensis (LV79 strain), an effect dependent on macrophage CD72 receptor. We recently demonstrated that sCD100 also increases murine macrophage infection by two other Leishmania species often associated with CL and VL in Brazil. In order to understand the mechanisms involved in increasing Leishmania spp. infection, we compared proteomes of murine macrophages treated or not with sCD100 and identified 100 differentially abundant proteins. Some of phagocytosis-related proteins such as epiplakin, dynamin, myoferlin and synaptotagmin have been selected for proteome validation. To verify the combined effect of CD100 in Leishmania infection in vivo, wild-type and CD100 knockout (KO) C57BL/6 mice were infected with Leishmania amazonenis (LV79 and PH8 strains). Analysis of the area under the curve showed that lesions caused by the less virulent LV79 strain in CD100 KO animals are smaller at the 9th and 13th weeks post-infection when compared to the wild-type group. However, lesions of wild-type and CD100 KO mice infected by strain PH8 were statistically similar. We believe that this study will help elucidate the mechanisms by which CD100 increases the infection of macrophages by Leishmania and the role of this molecule in leishmaniasis. **Supported by:**Fapesp **Keywords:** Semaphorin; proteome; in vivo infection

HP59 - **ROLE OF SAPA REPEATS PRESENT IN TRYPANOSOME CRUZI TRANS-SIALIDASES**

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Trans-sialidases (TS) belong to a large family of Trypanosoma cruzi surface proteins encoded by around 1400 genes. Only a sub-family of TSs belonging to group I contains active trans-sialidases whose function is to transfer sialic acid from host glycoconjugates to mucins at the surface of the parasite. Active TSs also possess a 12 amino acid repeat named SAPA at its C-terminal with a variable number of copies. Besides being highly immunogenic, the SAPA domain increases the stability of the enzyme in the bloodstream, suggesting that the repeat domain may contribute to parasite virulence. To investigate the role of the SAPA domain, we immunized mice with three versions of recombinant TS that were generated after cloning into the E. coli pET expression vector (i) the complete coding region of a TS from group I, (ii) a fragment corresponding only to the catalytic, N-terminal domain and (iii) a fragment corresponding only to the SAPA domain containing 19 SAPA repeats. Three groups of mice were immunized with the recombinant antigens together with alum and CpG and challenged with trypomastigotes of the Y strain. Parasitemia data demonstrated that, similarly to studies describing the protective effect of several T. cruzi antigens, all three antigens conferred some protection against the challenge with a virulent strain. However, only mice immunized with the antigen containing the catalytic domain without the SAPA repeats were fully protected. Importantly, ELISA performed with sera collected before challenge demonstrated the induction of high levels of IgG in mice immunized with the complete antigen and with the version containing only the SAPA domain. The observation that mice immunized with the TS protein without SAPA resulted in higher protection levels, without inducing strong IgG production, not only corroborates the role of SAPA as a virulence factor but also highlights the importance of cellular immune response to control T. cruzi infection. **Supported by:**CNPq, FAPEMIG, INCTV **Keywords:** Trans-sialidase; sapa repeats; immune response

HP60 - **CHARACTERIZATION OF BIOLOGICAL FUNTION OF ASPARAGINE AND ITS TRANSPORT IN *TRYPANOSOMA CRUZI***

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The etiological agent of Chagas disease, *Trypanosoma cruzi*, is well adapted to the consumption of a diversity of carbon and energy sources available in their hosts. In the absence of glucose, amino acids such as glutamate, proline, histidine, alanine and aspartate are relevant energy and carbon sources. Other amino acids such as asparagine (Asn) seem to participate of these processes as well. However, their role in the parasite biology has not been elucidated yet. Asn is a precursor of aspartate through its biosynthesis by the enzyme asparaginase [EC 3.5.1.1], which involved several critical processes in the biology of the parasite. It participates of the cell differentiation from epimastigotes to metacyclic trypomastigotes (metacyclogenesis) in the insect vector, in the ammonium detoxification and in the synthesis de novo of pyrimidins and purins. The aim of this study is to understand how Asn is taken up and metabolized by the parasite, and to unveil its possible biological roles. We kinetically characterized the system responsible for Asn uptake in epimastigotes (K_M^{app} = 0.818 [0.591 … 1.045] mM, V_{max}^{app}= 0.706 [0.640…0.772] nmol Asn $2*10^{-7}$ $cell^{-1}$ min^{-1}, α = 0.05), and we heterologously expressed two hypothetical asparaginases of *T. cruzi* (Q4DZN4 and Q4D990 in UniProt database). Additionally, we confirmed the Asn participation in the mitochondrial bioenergetics of the parasite since it was able to trigger mitochondrial O_2 consumption. In addition, we demonstrated that Asn is able to sustain cell viability and ATP levels of *T. cruzi* during a metabolic stress (24 and 48h). **Supported by:**FAPESP **Keywords:** Asparagine; trypanosoma cruzi; bioenergetics

HP61 - **OBTAINING AND CHARACTERIZATION OF δ1-PYRROLINE-5-CARBOXYLATE SYNTHETASE AND ITS DOMAINS INVOLVED IN PROLINE BIOSYNTHESIS IN *TRYPANOSOMA CRUZI***

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Trypanosoma cruzi, the etiological agent of Chagas disease, colonizes different environments throughout their life cycle, facing several nutrient availability and conditions of stress. To succeed in surviving these challenges, the parasite is able to metabolize different amino acids, L-proline among them. Many processes have been widely described in which L-proline is involved, such as ATP production, osmotic control, protection against to nutritional, oxidative and thermal stress, cellular differentiations and cellular invasion. In *T. cruzi* L-proline can be obtained through its uptake from the extracellular environment and through its biosynthesis from L-glutamate. This biosynthesis pathway is catalysed by two cytosolic enzymes. The first one is a bifunctional Δ^1-pyrroline-5-carboxylate synthetase (P5CS), a single enzyme catalysing two enzymatic steps: i. the phosphorilation of L-glutamate forming γ-glutamyl phosphate (corresponding the glutamil kinase domain, GK) and ii. the reduction of γ-glutamyl phosphate to glutamate-γ-semialdehyde (GSA) (corresponding the glutamil phosphate reductase domain, GPR), which happens with the concomitant oxidation of NADPH to $NADP^+$. GSA is spontaneously converted into Δ^1-pyrroline-5-carboxylate (P5C) which is reduced into L-proline by a second enzyme P5C reductase. The P5CS and both domains were separately cloned and expressed in Escherichia coli BL21 pLysS. The obtained proteins were purified by affinity chromatography using NTA-Ni^{2+} resin. Kinetic analysis of TcGPR was performed measuring the reverse reaction (using GSA and $NADP^+$ as substrates). The obtained kinetic parameters for GSA and $NADP^+$ were as follow respectively: $K_{M,GSA}^{app}$ = 0.25 [0.06 … 0.45] mM and $V_{max(GSA)}^{app}$= 0.20 [0.14…0.26] μmol NADPH min^{-1} protein mg^{-1}; $K_{M,NADP+}^{app}$ = 0.36 [0.19 … 0.54] mM, and $V_{max(NADP+)}^{app}$= 0.29 [0.24…0.33] μmol NADPH min^{-1} protein mg^{-1}, α = 0.05). This is the first characterization of a P5CS in trypanosomatids. **Supported by:**FAPESP **Keywords:** Proline biosynthesis; Δ 1-pyrroline-5-carboxylate synthetase

HP62 - **EVALUATION OF MACROPHAGE BEHAVIOR OF *LEISHMANIA* (*V.*) *BRAZILIENSIS* INFECTED BALB/C MICE.**

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Leishmania sp. infection produces a wide spectrum of clinical manifestations and studies using animal models are valuable in helping to understand the relationship parasite-host. The BALB/c mice are susceptible to almost *Leishmania* species, however, they are considered resistant to *Leishmania braziliensis*. When infected, these animals have small lesions that heal spontaneously. The macrophage is the main host cell of the parasite and also the final effector cell, able to control the infection. The objective of this study was to evaluate the behavior of macrophages from BALB/c mice infected with *L. braziliensis*. Peritoneal macrophages were obtained from the BALB/c mice at three different times of infection: 7 days (infection onset), 15 days (near the peak of the lesion) and 21 days (lesion resolved). These macrophages were tested for microbicidal activity against in vitro infection with *L. braziliensis*; nitric oxide production by the Griess method; phagocytic capacity using fluorescent latex beads; and cytokine production by the Cytometric Bed Array method. The results showed that BALB/c macrophages with 7 days of infection have increased phagocytic capacity a significant increase in nitric oxide production. However, when infected in vitro with *L*. *braziliensis*, macrophages from infected animals showed no difference in the amount of amastigotes when compared to macrophages from control animals. Macrophages infected or under stimulation of *L. braziliensis* antigen showed an increase of proinflammatory cytokines production TNF and MCP-1, mainly in the 7 days post-infection. Regarding IL-10, these macrophages also presented differentiated modulation, showing lower production when exposed to specific antigen and higher production when stimulated with LPS. The TNF/IL-10 ratio showed a more proinflammatory profile. These results show that there are some changes in the behavior of macrophages from *L. braziliensis* infected BALB/c mice, especially in the 7 days post-infection. **Supported by:**CAPES **Keywords:** Leishmania(v.) braziliensis; balb/c; macrophages

HP63 - **TRANSCRIPTOME PROFILE OF HUMAN FIBROBLAST IN RESPONSE TO INFECTION WITH VIRULENT AND NON-VIRULENT TRYPANOSOMA CRUZI STRAINS REVEALED DIFFERENCES IN FIBROBLAST CAPACITY OF RECRUITMENT AND ACTIVATION OF NEUTROPHILS**

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Chagas disease is caused by Trypanosoma cruzi, a protozoan parasite that presents a highly heterogeneous population, composed of a pool of strains with distinct characteristics including distinct levels of virulence. Transcriptome analyses of human fibroblasts infected with a virulent cloned strain (CL Brener) or with a non-virulent clone (CL-14), revealed a reduced or delayed expression of genes encoding parasite surface proteins in CL-14 compared to CL Brener during the final steps of the intracellular differentiation from amastigotes to trypomastigotes. Here we compared changes in the expression of human genes in response to the infection with both strains at 60 and 96 hours post-infection (hpi). Differential expression analyses showed similar transcriptome profiles in cells at 60 hpi with both strains when compared to uninfected samples. In contrast, at 96 hpi, significant differences were observed both in the numbers and expression levels of several genes, particularly those involved with immune response, cytoskeleton organization and transcription factors. By analyzing the expression levels of long non-coding RNAs followed by the construction of co-expression networks with coding genes, we were able to identify enriched pathways associated with cell survival, cell proliferation and transcription in response to infection with both strains. However, the cytokine/chemokine signaling pathway was found to be enriched only in response to CL Brener infection, an observation that was validated by ELISA, which confirmed that several cytokines and chemokines involved in the recruitment and activation of neutrophils were up-regulated only during CL Brener infection. These findings suggest that infection with the virulent CL Brener strain induces a more robust host immune response than with the non-virulent CL-14 strain and also expose an unexplored role of fibroblasts as sentinel cells that play a role in recruiting neutrophils to the initial site of infection. **Supported by:**CAPES, CNPQ, INCT **Keywords:** Trypanosoma cruzi; gene expression; immune response

HP64 - ***PLASMODIUM CHABAUDI*** **MEROZOITES INHIBIT NITRIC OXIDE PRODUCTION OF MACROPHAGES BY APOPTOTIC MIMICRY**

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Malaria is one of the most prolific parasitic diseases. Mice infected by *Plasmodium chabaudi* is one of the closest models to the human disease caused by *P. falciparum*. Studies showed that *Plasmodium* spp. can survive and multiply in macrophages. Some intracellular parasites expose phosphatidylserine (PS) generating anti-inflammatory responses, such as lower production of nitric oxide (NO) by macrophages, a concept known as “apoptotic mimicry”. We verified whether *P. chabaudi* merozoites expose PS and reduce NO production by macrophages. Merozoites were obtained from infected erythrocytes and were filtered. Filtration was able to remove mice blood cells from the merozoite preparation and resulted in a better flow cytometry analysis. Merozoites were able to infect and differentiate into ring forms in erythrocytes in an *in vitro* assay. PS exposure and viability of merozoites were assayed by flow cytometry. Activated murine peritoneal macrophages were infected with merozoites to analyze parasite development and NO production. We produced an anti-merozoite mice polyclonal antibody to detect merozoites inside macrophages, evaluated the expression of inducible nitric oxide synthase (iNOS) and verified lysosomal fusion to vacuoles containing parasites by labeling lysosomal-associated membrane protein 1. About 50% of the *P. chabaudi* merozoites exposed PS. Merozoites were capable of infecting about 80% of the macrophages after 2 h of interaction and this infection remained after 24 and 48 h. Macrophages infected with merozoites for 24 and 48 h produced less NO. This result was confirmed by lower iNOS expression in 24 h infected macrophages by immunofluorescence microscopy. In addition, low lysosomal fusion to vacuoles containing merozoites was observed after 6 h of macrophage infection. Thus, *P. chabaudi* merozoites perform “apoptotic mimicry”, reinforcing the hypothesis that such evasive mechanism is common to parasitic protozoa that interact with macrophages. **Supported by:**FAPERJ **Keywords:** Macrophage; plasmodium chabaudi; phosphatidylserine

HP65 - **THE BALANCE OF HOST NOS2 AND *LEISHMANIA AMAZONENSIS* ARGINASE IS IMPORTANT TO REGULATE THE INFLAMMATORY RESPONSES MEDIATED BY MICRORNAS IN INFECTED MURINE MACROPHAGES**

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Leishmania amazonensis infection causes a several range of modifications on macrophage metabolism and inflammatory response. In this context, the L-arginine availability plays a pivotal role due to the competition between Nitric Oxide Synthase (NOS2) that produces Nitric Oxide (NO), killing the parasite, and Arginase 1 (ARG1), which produces L-ornithine, an initial step of polyamine production allowing the parasite survival. MicroRNAs are involved in the regulation of inflammatory response via post-transcriptional mechanisms, impacting in the infection diseases. We infected macrophages from C57BL/6J wild type mice (B6-BMDM) or NOS2 knockout (NOS2$^{-/-}$-BMDM) with *L. amazonensis* wild type (*La*-WT) or with the arginase knockout (*La*-arg^{-}) and evaluated the impact of both enzymes to infectivity, miRNA expression and the number of transcripts of *Nos2, Arg1, Cat1 and Cat2b* (cationic amino acid transporters 1 and 2B – L-arginine transporters). The absence of host-NOS2 increased the infectivity of *La*-WT; but, the absence of parasite arginase reduced the infectivity on both macrophages. The *Nos2* transcript amount increased only after 4h of infection on B6-BMDM infected with *La*-WT. In a similar way, B6-BMDM infected with *La*-arg^{-} increased the amount of *Nos2*, but lower than in *La*-WT infection. However, the amount of *Cat2b* increased only after 24h, but *Arg1* and *Cat1* transcripts remained unchanged in all tested groups. The functional inhibition of miR-294, miRNA up-regulated in the infection of B6-BMDM with both *Leishmania* strains, increased the number of transcripts of *Cat2b*. The *Cat1* mRNA is a predicted target for the miR-410, up-regulated in *La*-arg^{-} infection and its inhibition increased the amount of *Cat1* transcripts after 4h of infection. Our data suggest that these miRNAs are involved in the regulation of L-arginine metabolism targeting the inflammatory response against *L. amazonensis*. **Supported by:**FAPESP and CNPq **Keywords:** Arginine metabolism; m1/m2 balance; inflammatory response

HP66 - **CHARACTERIZATION OF *TRYPANOSOMA RANGELI* MUCIN-LIKE GLYCOPROTEINS**

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Trypanosoma rangeli is a hemoflagellate parasite of mammals transmitted primarily via triatomine bites. Although avirulent to these hosts, in which the parasite life cycle is unknown, *T. rangeli* is pathogenic to triatomine vectors. *T. rangeli* is phylogenetically closer to *Trypanosoma cruzi* and these species share several antigenic epitopes that leads to serological cross-reactivity. Notwithstanding, only a few mucin-like were described for *T. rangeli* and the function of these glycoproteins on *T. rangeli* is unknown. For that, we propose the overexpression of homologous and heterologous (T. cruzi) mucins by *T. rangeli* to assess phenotypic changes. Using the up-to-date *T. rangeli* genome assembly, 14 new mucin sequences were found and classified in two groups: *Tr*MUCg and *Tr*MUCp. Considering the mature protein size, tandem repeats pattern, glycosylation distribution and amino acids composition, *Tr*MUCg shares common characteristics with *Tc*MUCI, but keep *T. rangeli*-specific sequences. While *Tr*MUCp reveals to be similar to *Tc*MUC II, this group carry a hypervariable region with hydrophobic amino acids, absent in *T.cruzi* mucins. Three *T. rangeli* mucins (*Tr*MUCg1, TrMUCg3 and *Tr*MUCp35) and *Tc*MUC II were selected for overexpression fused to a 3xFlagTag, mNeonGreen and a homologous GPI anchor sequence. Overexpression of the fused mucins was achieved in the surface of *T. rangeli* epimastigotes and trypomastigotes. WB assay unveils the expression of *Tc*MUCII by *T.rangeli* as a heavily glycosylated protein, showing a similar molecular weight as observed in *T. cruzi.* However, *T. rangeli* mucins are little or non-glycosylated, which might influence their ability to act as sialic acid acceptors. Besides that, is already know the *T.rangeli* lacks an active trans-sialidase. Therefore, despite the common features, this set of molecules could be involved in a different function in *T.rangeli* from those described for *T.cruzi*. **Supported by:**UFSC, FINEP, CAPES, CNPq. **Keywords:** Glycoproteins; surface proteins; sialic acid

HP67 - **MOLECULAR DETECTION OF TRYPANOSOMA MINASENSE IN ALOUATTA PALLIATA (HOWLER MONKEY) FROM PANAMA**

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Background: Howler monkeys (Aluotta sp) often have natural infections with trypanosomatid parasites, this as result of frequent contact with vectors in a primarily forest environment. The identification and characterization of these hemoparasites is essential to understand its eco-biology and to adopt coherent measures of surveillance/prevention of potential zoonotic diseases.
Objective: To molecular detect blood trypanosomatids infecting Aluotta palliata in the eastern region of Panama.
Methods: The total DNA of 11 blood samples, taken from anesthetized howler monkeys, captured in the eastern region of the isthmus of Panama, were analyzed with the generic trypanosome 18S rDNA PCR primers (TRY927F and TRY927R; SSU561F and SSU561R) in a nested PCR protocol. Samples were also screened for trypanosomes using Tcmit-10 / Tcmit-21 primers set that amplify subunit 2 of Cytochrome C Oxidase (COII). For phylogenetic analyses, nucleotide sequences of both amplicons were sequenced.
Results: The generic trypanosome nested PCR was positive (550-bp products) in the 11 evaluated samples. These products were purified and sequenced in both directions. BLAST analysis of the sequences showed highest identities with Trypanosoma minasense. In addition, Tcmit-10/Tcmit-21 primers also succeeded in amplifying 400-bp products in all samples. The sequence products of this PCR analysis differed from the published trypanosome sequences for this gen.
Conclusions: T. minasense infection is confirmed in howler monkeys captured in Panama. COII gene sequences of this trypanosome is reported for the first time. These results help to evaluate the role of A. palliata as a reservoir of trypanosomatid parasites in the eastern region of Panama. **Supported by:**INSTITUTO GORGAS. SNI SENACYT Panamá **Keywords:** Trypanosoma minasense; aluoatta palliata; 18s rdna

HP68 - **EVALUATION OF THE INTERACTION BETWEEN LEISHMANIA BRAZILIENSIS ISOLATES AND HUMAN MACROPHAGES: ROLE OF PARASITE ENTRY AND INTRACELLULAR ESTABLISHMENT ON THE COURSE OF INFECTION**

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In Brazil, cutaneous leishmaniasis (CL) transmitted by Leishmania braziliensis (Lb) is highly prevalent. This parasite species is responsible for different clinical manifestations of CL, including localized CL (LCL) and disseminated leishmaniasis (DL). Few studies have been conducted to assess the mechanisms involved in the initial Leishmania-MΦ interaction that may contribute to infection outcome. We hypothesized that the mechanisms involved in the early stages of the interaction between Lb isolated from patients with LCL (Lb-LCL) or DL (Lb-DL) with MΦ are different and determinant of the establishment and course of infection. To compare the mechanisms involved in Lb-LCL and Lb-DL phagocytosis, human PBMC-derived MΦ (hMΦ) were incubated with U73122 or GSK 690693, AKT and PLC inhibitors, respectively, and infected with the isolates of Lb. Inhibition of AKT decreases the phagocytosis of both Lb-LCL and Lb-DL, but not the binding, by hMΦ of healthy individuals in comparison to untreated control cells, while, PLC inhibition did not alter binding nor phagocytosis of Lb isolates. Interestingly, AKT inhibition favors Lb-LCL intracellular viability in hMΦ, at 4 and 12h after infection. In Lb-DL infected cells, AKT inhibition favors parasite survival only at 24h after infection. The differential effect of AKT inhibition in intracellular survival of Lb-LCL and Lb-DL suggests the influence of NADPH oxidase assembly in the infection outcome. Next, we assessed whether the activation of the autophagic pathway in infected macrophages may account for the differential course of infection by these two parasite isolates. We found that Lb-DL induces autophagy to a greater extent than Lb-LCL, although both similarly recruit the main autophagy marker, LC3, to parasitophorous vacuoles, In sum, our findings suggest that Lb-LCL and Lb-DL differentially activates the phagocytic pathway, which may account for distinct infection outcomes. **Supported by:**FIOCRUZ, UFBA, CNPq, CAPES, FAPESB, INCT-DT **Keywords:** Leishmania; phagocytosis; autophagy

HP69 - **EFFECT OF SERUM LIMITATION IN THE EXTRACELLULAR HYDROLYSIS OF NUCLEOTIDES AND IN THE CYTOTOXICITY OF TRICHOMONAS VAGINALIS AGAINST VAGINAL AND PROSTATE EPITHELIAL CELLS**

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Trichomonas vaginalis is a flagellated protozoan that causes trichomoniasis, the most common non-viral sexually transmitted infection (STI) in the world. There is only two FDA-approved drugs for the treatment of trichomoniasis, metronidazole and tinidazole. Given the drug resistance and failures in the treatment, the search of new agents is essential. The purinergic signaling occurs when nucleotides and nucleosides, regulated by enzymes called ectonucleotidases, bind to specific receptors called purinoceptors. Ectonucleotidases are the enzymes involved in the hydrolysis of extracellular nucleotides and include NTPDase, ecto-5'-nucleotidase, and adenosine deaminase. These activities have been characterized in *T. vaginalis* by our group. The aim of this study was to investigate the effect of heat inactivated bovine serum (HIBS) limitation as adenosine source restriction in the cytotoxicity involved in the pathogenesis of trichomoniasis. The *T. vaginalis* isolates were cultivated in TYM medium at 37ºC supplemented with 10% HIBS and treated parasites were grown with 1.0% HIBS based on a kinetic growth assay. The cytotoxicity of *T. vaginalis* was measured by the release of lactate dehydrogenase (LDH) by vaginal epithelial cells (HMVII) and by prostate epithelial cells (DU145). The NTPDase activity was tested using ATP and ADP as substrates by malachite green colorimetric method. All isolates tested were cytotoxic against both lineages and the highest level of LDH release by cells occured at 5 h. All 1.0% HIBS-treated isolates showed lower numbers of trophozoites in relation to control up to 48 h. The NTPDase activity strongly increased in 1.0% HIBS-treated parasites (three-fold for ATP). Assays to determinate the effect of serum limitation in ecto-5'-nucleotidase and adenosine deaminase activities, as well as in the cytotoxicity exerted by *T. vaginalis* against host cells are in progress. **Supported by:**CAPES e CNPq **Keywords:** Trichomonas vaginalis; serum limitation; extracellular hydrolysis

HP70 - **ANTI-TRICHOMONAS VAGINALIS ACTIVITY OF 1,10-PHENANTROLIN-5,6-DIONA DERIVATIVES AND THE EFFECT ON OXIDATIVE METABOLISM**

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Trichomonas vaginalis causes trichomoniasis, the most common non-viral STI in world, facilitating HIV acquisition and associated with complications such as cervical and prostate cancer. The FDA-approved treatment is based on drugs of the 5-nitroimidazole class, metronidazole and tinidazole; however 10% are resistant to therapy. Phenanthrene derivatives such as 1,10-phenanthroline-5,6-dione (phendione) and metal complexes [Ag (phendione)2] ClO4, (Ag-phendione), and [Cu (phendione)3] (ClO4)2 (Cu - phendiona) have been investigated for their antimicrobial activities. The objectives of this study were to determine the anti-T. vaginalis activity and to investigate the death mechanism generated by phendione and its metal complexes, Ag-phendione, and Cu-phendione. MIC, IC50 and CC50 values of the compounds were determined against T. vaginalis isolates, vaginal epithelial cells, fibroblasts, and human erythrocytes. Alterations in oxidative metabolism were analyzed by flow cytometry quantification of reactive oxygen species (ROS), as well as gene expression analysis of enzymes involved in this process by qRT-PCR. Death type assessment was performed by flow cytometry after incubation with FITC-AnnexinV/Propidium Iodide. The results revealed potent trichomonicidal activity of tested compounds and low levels of toxicity against cell lines. Cu-phendione presented the lowest IC50 value (<1.0 μM) and high selectivity index (SI≥11), revealing selective activity to the parasite. Compounds increased ROS production by reducing gene expression of antioxidant enzymes responsible for detoxification, leading to damage and death of parasite. Furthermore, when investigating the type of cell death, characteristics suggesting apoptosis-like were observed. Considering the drug-resistance observed in the treatment of T. vaginalis and the necessity for new therapies, these compounds arises as molecules of pharmaceutical interest. **Supported by:**CNPq **Keywords:** Trichomonas vaginalis; 1,10-phenantrolin-5,6-diona ; ros

HP71 - **TRYPANOSOMA CRUZI DISCRETE TYPING UNITS DETECTED IN WILD AND DOMESTIC MAMMALS FROM A CHAGAS DISEASE ENDEMIC REGION IN PANAMA**

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Background: Trypanosoma cruzi infects a large number of wild and domestic mammals. These reservoirs represent a critical epidemiological factor that must be considered during the surveillance of Chagas disease. Equally important, is the study of discrete typing units (DTUs) of T. cruzi populations that circulate in these reservoirs.
Objective: To identify T. cruzi DTUs infecting wild and domestic mammals from a Chagas disease endemic region in the Province of Panama Oeste.
Methods: DNA was extracted from animal' blood samples and analized by two conventional PCR methods (kDNA/SatDNA) for detection of T. cruzi. The positive samples were genotyped (DTUs) by the amplification of mini-exon gene. In addition, the genotyping was performed by a real-time PCR using two nuclear (SL-IR and 18S rDNA) and two mitochondrial (COII and ND1) genes.
Results: A total of 526 samples from mammalian reservoirs were analyzed; (62 D. marsupialis, 14 P. opossum, 5 M. nudicaudatus, 164 P. semispinosus, 1 M. robinsoni, 73 C. hoffmanni, 27 B. variegatus and 180 C. familiaris) 74 resulted positive to the infection with T. cruzi. The initially genotyping analysis detected 15 D. marsupialis infected with TcI and 1 with TcII/V/VI; 1 P. opossum with TcI and 1 with TcII/V/VI; 6 P. semispinosus with TcI and 2 with TcII/V/VI. Real-time PCR genotyping was successful in 19 D. marsupialis samples characterized as TcI and 1 as TcII/V/VI; 2 P. opossum as TcI; 1 M. nudicaudatus as TcI; 5 P. semispinosus as TcI and 2 as TcIII; 2 C. hoffmanni as TcI and 1 as TcII and 1 C. familiaris as TcI.
Conclusions: The infection with T. cruzi was frequent in the evaluated reservoirs (14%, 74/526), especially in the didelphidae group (46.3%, 38/82) and less common between spine rats (7.3 %, 12/164), sloths (14%, 14/100,) and dogs (3.3%, 6/180). TcI is confirmed as the main circulating DTU in these mammals (39%, 29/74). In addition, the presence of other DTUs is reported for the first time in Panama. **Supported by:**INSTITUTO GORGAS, SENACYT PANAMA **Keywords:** Trypanosoma cruzi; reservoir; discrete typing unit

HP72 - **GENERATING P21 GENE KNOCKOUT TRYPANOSOMA CRUZI BY CRISPR/CAS9 SYSTEM**

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In the process of interaction of *Trypanosoma cruzi* with the host cell, highly specific molecules of both organisms are involved. One of these molecules, P21, has been described as a protein involved in the invasion process of *T. cruzi*. More recent studies show that P21 is involved in the chronicity process of Chagas disease. Mice infected with *T. cruzi* and treated with P21 showed increased collagen deposition and reduced angiogenesis in heart tissues damaged by the parasites. Also, P21 regulated intracellular replication *in vivo* and *in vitro* by modulating the parasite cell cycle. From this, what would be the effect of the absence of protein P21 on the parasite and in the course of infection? Thus, as a first step, this study aimed to produce P21 knockout parasites using the CRISPR/Cas9 technique. P21 is coded by a single copy gene located at chromosome TcChr22 of clone CL Brener. Two P21-specific guides were designed from different regions of P21 gene. Constructs containing the Cas9-EGFP gene and P21-specific single guides were produced and electroporated into epimastigotes of *T. cruzi* (Y strain). Transfected parasites were selected with G418 antibiotic, cloned by cell sorting, and kept in culture for 2 months. Following growth of stable Cas9-single guide strains, parasites were electroporated with a donor DNA containing homology arms and a second selection antibiotic (blasticidin) marker. Antibiotic concentration was previously determined using WT parasites by viability and dose-response assays. Transfected parasites with P21-single guides showed extremely impaired growth when compared to scrambled control. Besides, after electroporation with donor DNA, more than 90% of parasites died in the presence of blasticidin after one week. Our data suggest that P21 plays key role in epimastigote replication or P21 is an essential gene that makes parasite survival impossible. Other approaches used CRISPR/Cas9 are being developed to elucidate our findings. **Supported by:**CNPq, CAPES, FAPESP **Keywords:** P21 protein; growth impairment; crispr/cas9

HP73 - **MODULATION OF THE GAP JUNCTION IN THE MACROPHAGUE LINE CELL INFECTED WITH *TOXOPLASMA GONDII* AND INFLAMMATION CONDICTION IN IMMUNE SYSTEM**

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Toxoplasma gondii is a protozoan parasite responsible for toxoplasmosis. In immunocompromised individuals can cause several problems. Some of these complications are associated with the communication mediated by Gap Junctions. But, there are still systems that aren't fully characterized regarding the junctional communication, including the innate immune system, represented by Macrophages. In view of this, the aim of this study is to assess the structural and functional modulation of gap junctions formed by Connexin 43 (Cx43) in macrophage lines after infection with *T. gondii*, and treatments with pro-immune-inflammatory factors. The methodology used is: (1) J774-G8 macrophage cell line culture; (2) Western Blot Assays; (3) Immunofluorescence assays and analysis by confocal microscopy; and (4) Intracellular dye microinjection (functional evaluation). The cell cultures are activated with TNF-α and IFN-γ or infected with Toxoplasma in its tachyzoite form. The results revealed that J774-G8 cells showed significant changes in their profile junctional communication dye injection experiments, when subjected to microenvironments with the factors combined in incubations 48 hours. The Cx43 and Phalloidin proteins interact in the plasma membrane of the J774-G8, and they undergo a reduction in the membrane after 72 hours of infection. The evaluation of the Cx43 protein expression has been shown to be altered (elevated) in J774-G8 infected with the parasite 24 and 48 hours compared to uninfected cells. And in the micrographs in phase contrast using J774-G8 infected and TNF-α+IFN-γ trated it was observed were a substantial modification of the structured growth of macrophage cells which may be associated with junctional coupling, once the cells are growing into lumps in all treatment which the factors are combined. Additionally, we can infer that there is a relationship between Cx43 and the cytoskeleton in macrophages, which may be changed in the microenvironment of infection.
Supported by:CAPES, CNPq and FAPERJ. **Keywords:** Macrophages; gap junction; toxoplasma gondii

HP74 - **INTERFERENCE OF CHITIN SYNTHASE GENE SILENCING IN *RHODNIUS PROLIXUS* VECTOR COMPETENCE: *TRYPANOSOMA CRUZI* CYCLE AND ROS PRODUCTION**

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Rhodnius prolixus is a Chagas disease insect vector. Chitin is an essential biopolymer of insect structures synthesized by the chitin synthase enzymes (CHSs). In insects, the chitin can be found in structures such as the cuticle, ovary and peritrophic matrix (PM). In some insects' groups the PM is replaced by an analogous structure called perimicrovilar membrane. *R. prolixus* adult females and 5th stage nymphs were fed in rabbit and infected (or not) with *T. cruzi*. The aim of this work is to investigate the CHS gene role in the *R. prolixus*: evaluating the CHS gene silencing effects in ROS production and in the *T. cruzi* life cycle. The CHS clone contain a catalytic domain gene sequence was used as template for the dsRNA synthesis specific for CHS (dsRNACHS), which was injected (1µg) into the metathoracic cavity 3 days before feeding. The gene silencing phenotype was evaluated in infected insects, in control groups (unrelated gene dsRNA injected/ without injection) and in dsRNACHS-treated groups by observation and analysis. It was observed that dsRNACHS- treated insect gut was very fragile, with fewer trachea, stunted ovaries and reduced oviposition. Growth and parasitic differentiation in the *R. prolixus* gut was monitored on the 5th and 11th days after parasite ingestion, cells were counted using Neubauer chamber. In the dsRNACHS-treated guts occurred a decrease in the percentage of trypomastigotes and an increase in epimastigote forms, compared with the control group. The oxidative stress level in CHS gene silenced insects was measured by lipid peroxidation assays. In the present study, higher oxidative stress level was observed in gut of insects injected with dsRNACHS, which showed MDA/mg levels of tissue 21 times higher than the control guts, suggesting an increase in the oxidative stress level in CHS silenced. These results indicate that CHS gene silencing in R. prolixus affects blood digestion, increasing oxidative stress and interfere with *T. cruzi* life cycle. **Supported by:**Cnpq/Faperj/INCT **Keywords:** Rhodnius prolixus; trypanosoma cruzi; chitin sythase

HP75 - **COMPARATIVE *IN SILICO* SECRETOME AND INTERACTOME OF *TRYPANOSOMA CRUZI* AND *TRYPANOSOMA RANGELI* REVEALS SPECIES-SPECIFIC MOLECULES THAT MODULATE IMMUNE RESPONSE**

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Trypanosoma cruzi and *T. rangeli* are evolutionarily related protozoa species. Whereas *T. cruzi* causes serious illness in humans, *T. rangeli* does not sustain the infection being promptly eliminated by the host. Differences in the secreted proteins by each protozoa are related to how each parasite is able to subvert host immune system. We have previously performed a comparative computational analysis of the secretome profile from both trypanosomes. We identified 463 exclusively secreted proteins in *T. cruzi* Sylvio strain and 202 in *T. rangeli*. We then selected proteins to perform a computational interactome with the human host based on the following criteria: possible immune system modulators; degree of identity with human proteins; protein secreted in only one of the species. DNAJ protein, heat-shock HslVU, ATPase subunit HslU were selected from *T. cruzi* and 4-nitrophenyl phosphatase from *T. rangeli*. Ingenuity Pathways Analysis (IPA) *software* was used for network and functional analysis. Two different gene networks were built: T. cruzi network showed some central nodes such as IL-17A, RORα and BCL2 associated to some canonical pathways, such as PI3K/AKT and TGF-beta signaling and, interestingly, *T. rangeli* was associated to IL-15 signaling. We can hypothesize that *T. cruzi* secretes DNAJ triggering the decrease of inflammatory interleukin IL-17 by inhibiting RORα, one of the key signal transducer of IL-17 production, ultimately favoring its homeostasis inside human host. This might indicate that *T. cruzi* maintenance depends on host ineffective IL-17 mediated immune activation. In contrast, *T. rangeli* would indirectly induce IL-15, an inflammatory interleukin leading to a IL-15 mediated parasite blood elimination. These hypotheses have yet to be confirmed experimentally. Interactome analysis has shed light on new possible mechanisms of modulation of the immune system performed by the parasites. **Supported by:**CNPQ, FAPEMIG, CAPES and FAPESP **Keywords:** Trypanosoma cruzi; trypanosoma rangeli; interactome of secreted proteins

HP76 - **ANALYSES OF MOLECULAR MARKERS IN CLINICAL ISOLATES AND PATIENT SAMPLES FROM A RECURRENT CASE OF VISCERAL LEISHMANIASIS WITH MULTIPLE RELAPSES**

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Leishmania infantum is the causative species of a neglected tropical disease known as Visceral Leishmaniasis (VL), responsible for infecting spleen, liver and bone marrow and is lethal when untreated. Characterization of the species and clinical investigation during the infection period are important for appropriate diagnostic and treatments. Here, our aim was to perform analyses of two molecular markers, the small subunit rRNA (SSU/rRNA) and glycosomal Glyceraldehyde 3-phosphate dehydrogenase (gGAPDH) DNA sequences from samples of a 40-year-old male patient in VL remission state. The patient was admitted for clinical follow-up at the University Hospital of the Federal University of Sergipe at 2005 and he has presented multiple relapses dated from 2003. He underwent splenectomy in 2011 and currently has a palpable accessory spleen on the left costal margin. Parasite isolation was performed from bone marrow (BM) aspirates during three hospital admissions, in 2009, 2010 and 2013. Genomic DNA of promastigotes from these three clinical isolates and BM and blood samples (referring to 2009 admission) were extracted and used for SSU/rRNA and gGAPDH amplification by PCR. Positive PCR amplicons were sequenced by Sanger dideoxy method. SSU/rRNA sequences (561bp) were analysed by BLASTN, while GAPDH sequences (998bp) were analysed by BLASTX in NCBI) and TriTryDB. Phylogenetics analyses also were performed including sequences from other trypanosomatids to build the trees. The results showed that BM and blood sequences are more similar to Leishmania genus, whereas clinical isolates resemble other members of Leishmaniinae, such as Crithidia genus. Continuous analyses will be performed, including the whole-genome sequencing. Morphological characterization and in vitro infection with clonal colonies are in progress to better elucidate these parasites and to understand this recurrent case of VL. **Supported by:**FAPESP **Keywords:** Molecular markers; human visceral leishmaniasis; recurrent cases

HP77 - ***IN VITRO*** **INFECTIVITY ASSESMENT AND CELL GROWTH OF CLONAL STRAINS FROM CLINICAL ISOLATES OF ATYPICAL VISCERAL LEISHMANIASIS**

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Corresponding to one of the most prevalent neglected diseases throughout the tropical regions, leishmaniasis is a serious vector-borne infection transmitted by sandflies. Visceral Leishmaniasis (VL) is lethal when untreated or treatment fails caused by protozoa from genus *Leishmania*. Here, our aim was to analyze two clinical isolates obtained from an atypical VL case, who the patient died after disease's complication. They were isolated from bone marrow aspirate (BM) and skin lesion (SL). Previous genomic analysis of them compared to Trypanosomatidae reference genomes revealed that BM/SL isolates shared 92% identity with *Crithidia fasciculata*, which interestingly is a monoxenous parasite exclusive from mosquitoes and non-infective to humans. To further evaluation, we obtained clonal colonies from cryopreserved stocks to perform *in vitro* infectivity and cell growth analyses. *In vitro* infection of BM/SL with original and clonal strains using human macrophages differentiated from THP-1 cells were performed and compared to *L.infantum* reference strains. The macrophages were stained and microscopically analysed to confirm the infection. Cell growth was measured daily, seeded at a cell density of 2×106 and incubated at 25°C and 35° C, which simulated vector and human hosts, respectively, in order to verify parasites thermal tolerance. Through microscopical visualization, we observed that BM/SL strains were able to infect the macrophages. Cell growth showed no significant statistical differences among the strains. Parasites at 25°C were elongated with long flagella matching the promastigote form in the vector host. Parasites strains were able to survive at 35°C and were shorter with small flagella and slender motile. *In vitro* infection and cell growth analyses demonstrated that these *Crithidia*-like strains were able to infect macrophages and also were thermo tolerant and potentially viable in a vertebrate host temperature. **Supported by:**FAPESP **Keywords:** Visceral leishmaniasis; in vitro infection; crithidia-like

HP78 - **SEVERE FORMS OF VISCERAL LEISHMANIASIS: A CASE STUDY FOCUSING ON PHYLOGENETIC AND PHENOTYPIC ANALYSES**

ROGERIO, L.A.[*1]; TAKAHASHI, T.Y.[1]; TAKAMIYA, N.T.[1]; SANTANA, A.K.M.[2]; JESUS, A.[3]; ALMEIDA, R.P.[3]; RIBEIRO, J.M.[4]; SILVA, J.S.[5]; MARUYAMA, S.R.[1]

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Leishmaniases are a group of infectious diseases caused by protozoan parasites from more than 20 *Leishmania* species and transmitted to humans by infected female phlebotomine sandfly bites. Visceral Leishmaniasis (VL), caused by *L.infantum*, is the second most lethal parasitic disease and is prevalent throughout underdeveloped and tropical regions of the world. A 9-year-old male patient admitted at the University Hospital of the Federal University of Sergipe was diagnosed with VL presenting severe manifestations. Patient did not respond well to treatment, had several recurrences and at his last hospital admission underwent splenectomy. Two clinical isolates were obtained from bone marrow (BM) and spleen (Sp) of this patient. Preliminary results showed that clinical isolates of VL obtained from this endemic region do not belong to genus *Leishmania* and are phylogenetically related to genus *Crithidia*. To evaluate these clinical isolates, we characterize these parasites phenotypically and phylogenetically. Thus, we obtained clonal strains of both BM/Sp clinical isolates and promastigote cultures were analyzed for morphology, cell growth and phylogenetic analysis of conserved nucleotide regions (SSUrRNA and GAPDH). The morphology of the studied strains has shown rounded body with short flagella when compared to *L.infantum* reference strains. Phylogenetic analyzes of SSUrRNA and GAPDH gene showed that the isolates BM/Sp and their clones clustered with the monoxenous *C.fasciculata*, which parasitize exclusively insects, and apart from *Leishmania* clade. Cell growth at 25 °C showed similarity between BM/Sp strains with Leishmania strains. Despite their genotypic similarity with *Crithidia*, cell growth at 35 °C showed that BM/Sp strains were able to survive in higher temperatures (vertebrate host temperatures). Besides, whole genome sequencing analysis and *in vitro* infectivity assessment of these isolates will be performed to compare these strains to other trypanosomatid species. **Supported by:**FAPESP **Keywords:** Cell growth; visceral leishmaniasis; crithidia-like

HP79 - **UPREGULATION OF THE GAP JUNCTIONS IN FIBROBLASTOID CELL LINE BY *TOXOPLASMA GONDII* INFECTION**

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Toxoplasma gondii is a mandatory intracellular parasite that affects all kinds of animals and the pathology is believed to affect about one third of the world's population. In immunocompromised individuals, toxoplasmosis can affect many different tissue types. It is described in the literature that Connexin 43 (Cx43) modulation directly affects the wound-healing process and this phenomenon is closely associated with fibroblasts. In view of this, the aim of the study is to evaluate the modulation of Gap Junctions, more specifically, by Cx43 in a fibroblast cell line, represented by the HFF-1 cell line, comparing its morphological aspect before and after *Toxoplasma Gondii* infection. The techniques used for the methodology of this study were: (1) culture of the HFF-1 cell line; (2) phase-contrast microscopy; (3) immunofluorescence assays; (4) Western Blot assays. Cultured cells were infected by strain RH of the *Toxoplasma Gondii* in its tachyzoite form during 24, 48 and 72 hours. It's reported that in fibroblasts, Cx43 is mostly localized inside the intracellular environment under physiological conditions, which is confirmed by controls used in immunofluorescence assays. During infection, there was an increase in Cx43 labeling during the three infection periods, a subtle increase of the positioning of Cx43 at the membrane during 24 hours of infection, a more significant increase during 48 hours of infection, and at 72 hours, the labeling of Cx43 couldn't be seen in its morfofunctional place and the phalloidin marking shows that the cytoskeleton was also disorganized this low connexin positioning may be associated with membrane cytoskeleton disruption caused by parasitemia. The results suggests that either the cell may be generating or positioning new connexins on the cell membrane to signal to the immune system the presence of the protozoan or that the parasite may be inducing Cx43 positioning to increase their susceptibility of infection by chemical signaling. **Supported by:**CNPq, CAPES and FAPERJ **Keywords:** Gap junctions; fibroblasts; toxoplasma gondii

HP80 - **STUDY OF HUMAN PERIPHERAL BLOOD FIBROCYTES INTERACTION WITH LEISHMANIA (L.) AMAZOMENSIS (LA) – AN IN VITRO ANALYSIS**

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Leishmaniasis is an infectious disease caused by protozoan of the genus Leishmania. After parasite transmission, resident and inflammatory cells are attracted to the site of infection. Among these cells are fibrocytes, express CD45 (pan-leukocyte protein) and produce extracellular matrix proteins. Studies have showed the fibrocytes participation in different pathological processes. However, there are no studies on the role of human fibrocytes in leishmaniasis. We analyses, in vitro, the interaction of human fibrocytes with L.a promastigote. Primary cultures were established and from the 15th day, fibrocytes were infected and analyzed. Phenotypic fibrocytes characterization was performed by fluorescence microscopy through two assays using the identification of three main markers: CD45, collagen type I and HSP-47 (heat shock protein involved in the synthesis of collagen type I). In ultrastructural analysis by scanning and transmission electron microscopy, we observed that the fibrocytes emit cytoplasmic projections that rolled up the parasites favoring phagocytosis. After 15 days of interaction, fibrocytes were susceptible to infection by L. amazonensis, as we observed the differentiation of promastigotes into amastigotes, as well as their multiplication within parasitophorous vacuoles. From the analysis of the inflammatory chemical mediators, we verified that fibrocytes respond to the infection with the production of pro and anti-inflammatory cytokines during the times established in this study, making favorable the conditions for the establishment of the infection. These results allow us to suggest that human fibrocytes may serve as host cells in the initial response and the development of cutaneous leishmaniasis because parasites of the Leishmania genus modulates the response of human fibrocytes, multiplies, breaks the cell, and is released to infect other types cells or the fibrocyte itself. **Supported by:** Instituto Oswaldo Cruz/Fiocruz **Keywords:** Leishmania (l.) amazomensi; fibrocytes; cytokines

HP81 - ***TOXOPLASMA GONDII* IMPAIRS MYOGENESIS *IN VITRO***

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Toxoplasma gondii is an obligate intracellular parasite and causative agent of toxoplasmosis. The parasite preferentially form tissue cysts in neural and muscle cells. Myositis caused by *T. gondii* infection has been demonstrated even in immunocompetent hosts. Previous studies of our group demonstrated alteration in muscle development in a murine model of congenital toxoplasmosis. However, the mechanisms by which the infection affects this development was not addressed yet. This work aims to investigate the molecular process involved in myogenic damage during *T. gondii* infection. To this purpose, the murine myoblast C2C12 were infected with *T. gondii* and then myogenic differentiation was induced. At 24 or 120 hours after induction, Myogenic Regulatory Factors (MRF) expression was assessed by qRT-PCR. MyoD and myogenin immunostaining were also performed. At the same time points, the conditioned medium was collected for cytokine/chemokine analysis profiling using Cytokine Bead Array and ELISA. To evaluate the role of IL-6 in myogenic loss, cultures were treated with Tocilizumabe (TCZ), an inhibitor of the IL-6 receptor. After 120 hours, the differentiation index and maturation of myotubes were accessed by MyHC immunostaining. Our results show that *T. gondii* infection can alter MRF expression: MyoD and myogenin expression was downregulated and Myf5 expression was upregulated in infected cultures. The infection also increases proliferation rates. The pro-inflammatory IL-6 and MCP-1 cytokines were highly increased in infected cultures and the anti-inflamatory TGF-β secretion was decreased. TCZ treatment wasn't able to restore the levels of myogenesis. This data indicate that *T. gondii* leads SkMC to a pro-inflammatory phenotype, leaving cells unresponsive to activation of the differentiation program. Such deregulation may suggest similar molecular mechanisms to those involved in myositis observed in human patients, although IL-6 pathway might not be involved. **Supported by:**Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, CNPq and FAPERJ. **Keywords:** Toxoplasma gondii; myogenesis; toxoplasmosis

HP82 - **INTERACTION OF HUMAN NEUTROPHILS WITH LEISHMANIA INFANTUM PROMASTIGOTES DEFECTIVE IN THE BIOSYNTHESIS OF LIPOPHOSPHOGLYCAN 1**

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Lipophosphoglycan (LPG), present only in promastigote forms of Leishmania, is associated with the establishment of the parasite in the vertebrate host. However, few studies demonstrate the LPG-neutrophil relationship in the initial moments of infection. To explore this context, human neutrophils were infected with wild-type L. infantum (WT), LPG-deficient L. infantum (∆lpg1) or LPG-restored L. infantum (∆lpg1+LPG1). The ∆lpg1 forms showed higher infection rate and amastigotes/neutrophils when compared to parasites that had the LPG molecule, WT and ∆lpg1+LPG1. However, ∆lpg1 parasites showed a lower viability rate compared to L. infantum WT or ∆lpg1+LPG1 forms. The expression of activation molecules on the surface of neutrophils was not significant among infected groups. Only metalloproteinase-8 (MMP-8) increased due to infection with L. infantum ∆lpg1 compared to WT form, while myeloperoxidase (MPO), neutrophil elastase (NE) and metalloproteinase 9 (MMP-9) activity, was not altered among infected neutrophils. Flow cytometric analysis revealed significant production of reactive oxygen species (ROS) during neutrophil infection with the ∆lpg1 parasites and inhibition of the oxidative pathway could restore the intracellular viability of these Leishmanias without LPG. We also analyzed the release of deoxyribonucleic acid (DNA) and lactate dehydrogenase (LDH) in the culture supernatant and noted that cell damage was higher when neutrophils were infected with L. infantum ∆lpg1. This study opens new perspectives for the understanding between LPG and activation innate immunity mechanisms triggered in the Leishmania-neutrophil interplay. **Supported by:**CNPQ, FAPESB E FIOCRUZ **Keywords:** Leishmania infantum; lipophosphoglycan; neutrophils

HP83 - **IN SILICO ANALYSIS OF GP63 AND PGFS PROTEIN SEQUENCES AND PGFS GENE EXPRESSION DURING GROWTH OF DIFFERENT LEISHMANIA SPECIES OF NEW WORLD**

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Introduction: Eicosanoid metabolism in Leishmania is still poorly studied. Nevertheless, some enzymes essential for prostaglandin production have already been described in trypanosomatides such as of human-like cyclooxygenase-2 (GP63) and prostaglandin synthase (PGFS) enzyme. Methodology: We performed in silico comparison between Leishmania species of GP63 and PGFS enzymes. NCBI identified protein sequences and similarity analysis was performed by BLASTp. The sequences were aligned and the phylogenetic tree construted by MEGA software using neighbor-joininng method. In addition, we evaluated PGFS protein expression in axenic culture extracts in the logarithmic and stationary phases of Leishmania infantum, that causes the visceral leishmaniasis and L. amazonensis and L. brasiliensis, etiological agents of tegumentary leishmaniasis. Results: In silico data indicated the presence of polymorphism between species, with minor variations among the phylogeneticly closer Leishmania species in the logarithmic phase, L. infantum expressed more PGFS when compared to L. amazonensis and L. brasiliensis, while in the stationary phase there was an increased expression of this enzyme for the three Leishmania species. Conclusion: GP63 and PGFS protein sequences showed variations between the species that cause visceralization and in tegumentary form of the illness, grouping them into different clades. Together the data suggest that eicosanoid metabolism in distinct Leishmania species of New World can be associated to pathogenicity and clinical manifestations leishmaniasis. **Supported by:**CNPQ **Keywords:** Leishmania; prostaglandin synthase (pgfs; cyclooxygenase-2

HP84 - ***LEISHMANIA* ARGINASE ACTIVITY IMPACTS BOTH HOST AND PARASITE GENE EXPRESSION MODULATION**

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The fate of *Leishmania amazonensis* infection is strongly influenced by the host genetic background. Based on RNA-seq data, we analyzed both host and parasite gene expression modulation of BALB/c and C57BL/6 macrophages, after 4 h of infection with *L. amazonensis* wild type (La-WT) or arginase knockout (La-arg-). We identified 12,641 host transcripts and 8,282 parasite transcripts through alignment to *Mus musculus* and *L. mexicana* genomes, respectively. The host transcriptomic data in the comparison of BALB/c_La-arg- vs. BALB/c_La-WT pointed to 39 downregulated and 194 upregulated genes. Among these differentially expressed genes (DEGs), we highlight the upregulation of amino acids transporters and of genes related to immune response, such as cytokines, chemokines, Toll-like receptors and transcription factors. On the other hand, the comparison C57BL/6_La-arg- vs. C57BL/6_La-WT pointed to only 3 downregulated and 27 upregulated genes. Among these DEGs, we highlight the downregulation of genes related to calcium binding and rRNA processing, as well as the modulation of genes related to immune response such as some cytokines and MHC antigen profile. According to parasite DEGs profile, in the comparison La-arg-_BALB/c vs. La-WT_BALB/c we identified 66 downregulated and 28 upregulated genes, including the modulation of 14 amastins and 26 hypothetical proteins. In the comparison La-arg-_C57BL/6 vs. La-WT_C57BL/6 we identified 29 downregulated and 16 upregulated genes, including the modulation of 1 amastin and 7 hypothetical proteins. These results indicated that the absence of parasite arginase can impact amastigote survival and replication in both BALB/c and C57BL/6 macrophages and also pointed several hypothetical proteins that need to be characterized. Altogether, our data show how Leishmania is able to coordinate gene expression modulation to survive in macrophages in the absence of the parasite arginase indicating a mixed M1-M2 immune response modulation. **Supported by:**FAPESP, CAPES, CNPq, SiU **Keywords:** Leishmania amazonensis; rna_seq; arginase knockout

HP85 - **WHAT HAPPENS TO THE PARASITE INSIDE THE VECTOR: *LEISHMANIA INFANTUM CHAGASI* GENE EXPRESSION WITHIN *LUTZOMYIA LONGIPALPIS*, THE MAIN VECTOR OF VISCERAL LEISHMANIASIS IN BRAZIL**

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Leishmania infantum chagasi is the causative agent and *Lutzomyia longipalpis* is the main vector of visceral leishmaniasis in Brazil. Understanding the interaction between parasite and vector is instrumental for the development of VL control strategies. We analyzed *Leishmania* genes modulated during infections of *L. longipalpis*. Among these are genes involved with sugar and amino acids metabolism. Glucose transporters and amino acid permease 3 are more expressed at 1h, 6h and 72h post infection (PI) when there is higher parasites proliferation inside the insect, requiring more nutrient intake. Polyubiquitin, with a role on protein degradation, has higher expression at 144h PI, when parasite morphological changes take place. Other genes studied were SHERP protein, important in metacyclogenesis, and GP63, with a role in invasion of macrophages. Both have higher expression in late infection, indicating that inside the insect the parasite prepares for the contact with the vertebrate host. Elongation-factor 1-alpha is an important vertebrate host immunomodulator, more expressed in the initial hours of vector infection, indicating that this gene may be involved in establishment of infection in the vector. Xanthine phosphoribosyltransferase, important in the purine salvage pathway, has reduced expression at 168h PI. Purines are relevant for cellular and metabolic processes, although in late infection the parasite might decrease purine acquisition since metacyclic forms do not replicate. Our findings show *Leishmania* genes modulated during the establishment of the infection, suggesting that the gene expression of the parasite can be affected by midgut microenvironment within sandfly. In order to confirm the role of these *Leishmania* genes in vector infection, we are using the CRISPR-CAS9 approach to generate mutants. We have already produced *L. i. chagasi* mutants for EF1-alpha and GTs genes, which are being used to infect *L. longipalpis*. Other mutants are also being produced. **Supported by:**CAPES, IOC-FIOCRUZ **Keywords:** Leishmania infantum chagasi; gene expression; crispr-cas9

HP86 - **ANTI-PHOSPHATIDYLSERINE ANTIBODIES REDUCE THE ACTIVE PENETRATION PROCESS OF *TOXOPLASMA GONDII* IN LLC-MK2 CELLS AND RAW 264.7 MACROPHAGES**

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Toxoplasma gondii is the etiological agent of toxoplasmosis. About 40% of the tachyzoite population of RH strain exposes phosphatidylserine (PS) on the outer leaflet of the plasma membrane, mimicking apoptotic cells. PS exposure induces anti-inflammatory response in macrophages. The subpopulation that exposes PS actively penetrates macrophages and inhibits nitric oxide (NO) production. The subpopulation that does not expose PS is phagocytosed by macrophages. The aim of this study was to verify the importance of PS exposure by *T. gondii* in the active penetration process into non-phagocytic and phagocytic host cells through the use of anti-PS antibodies. For this, LLC-MK2 cells and activated RAW 264.7 macrophages were infected with tachyzoites untreated or treated with anti-PS antibodies (Fab portion, entire antibody or isotype control) and annexin-V. The entry process in both host cells was analyzed by light microscopy after 1 h of infection. Culture supernatants of RAW 264.7 macrophages were collected after 24 h of infection and NO was quantified by the Griess reagent. In LLC-MK2 cells, the treatment of tachyzoites with the Fab portion, entire antibody and annexin-V reduced the percentage of infected cells and infection index. Treatment with the Fab portion and entire antibody increased the mean number of adhered parasites and reduced the mean number of internalized parasites. In RAW 264.7 macrophages, Fab portion treatment increased the percentage of infected cells. Entire antibody treatment reduced the percentage of infected cells and infection index. Treatment with the entire antibody and annexin-V increased the mean number of adhered parasites and reduced the mean number of internalized parasites. Preliminary results showed that anti-PS antibodies treatment does not block the capacity of *T. gondii* to inhibit NO production. The possible PS blockade with anti-PS antibodies suggests the importance of this phospholipid in the active penetration process of *T. gondii*. **Supported by:**FAPERJ, CAPES, CNPq, UENF, UFRJ **Keywords:** Toxoplasma gondii; active penetration; phosphatidylserine

HP87 - **CROTOXIN DERIVED FROM CROTALUS DURISSUS TERRIFICUS STIMULATES LIPID DROPLETS FORMATION IN MACROPHAGES DURING LEISHMANIA (LEISHMANIA) AMAZONENSIS INFECTION.**

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American Tegumentary Leishmaniasis is a disease caused by several species of Leishmania genus. They are obligatory intracellular parasite and it targets macrophage as its host. Eukariotic cells have organelles known as lipid bodies that store lipids used for the production of eicosanoids such as prostaglandin E2 (PGE2), a pro-inflammatory cytokine to combat protozoan infection.Thereby reducing the production of prostaglandin E2, reduces pro-inflammatory response, which favors Leishmania proliferation. Some natural substances are able to prevent parasite immune system evasion. Crotoxin (CTX) is the main component of Crotalus durissus terrificus venom and has several biological effects range from anti-inflammatory to anti-tumoral activities. Thus, this study had the objective to evaluate CTX activity on lipid body formation during macrophage – Leishmania infection. It was observed by BODIPY® 493/503 staining that infected macrophages treated with 4,8 µg/mL of CTX presented significantly increase the number of lipid bodies compared to non-treated infected cells. Ultrastructural analysis evidenced high amount of electron dense lipid droplets in treated macrophages. Elisa assays showed a PGE2 level increase in infected macrophages and treated with 4.8 µg/mL of CTX by 48 hours. In addition, cytometry analysis showed that CTX also induces an increase of lipid bodies in infected and non-infected macrophages after 24 hours of treatment with 4.8 µg/mL. In conclusion, the results obtained in this study show for the first time that the CTX induces the formation of lipid bodies in non-infected and infected macrophages. However, to our knowledge, this is the first report correlating formation of lipid bodies during macrophage-Leishmania interaction after treatment with crotoxin. Therefore, these results provides new insights for research on the lipid metabolism and the role of Crotoxin during Leishmania infection.**Supported by:**CAPES, CNPq, UFPA, INBEB/CNPQ **Keywords:** Crotoxin; leishmania (l.) amazonensis; lipid droplets

HP88 - **INVESTIGATING THE ROLE OF LEISHMANIA INFANTUM CHAGASI IRON TRANSPORTER (LIT1) DURING THE ESTABLISHMENT OF INFECTION IN LUTZOMYIA LONGIPALPIS, THE MAIN VECTOR OF VISCERAL LEISHMANIASIS IN BRAZIL**

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Leishmania infantum chagasi is the etiological agent and Lutzomyia longipalpis the main vector of visceral leishmaniasis in the Americas. Leishmania has a digenetic life cycle. Amastigote parasites are acquired when the female sand fly feeds on an infected host. The ingested parasites must differentiate into promastigotes in order to survive in the insect gut. In the early hours of blood digestion intense proteolytic activity occurs, as well as oxidative stress caused by the production of heme derived from hemoglobin digestion. Heme contains a molecule of iron that can be taken by the parasite. At the end of blood digestion the parasites migrate to the anterior part of the insect gut and differentiate into infective metacyclic forms. Leishmania iron transporter (LIT1) is a ferrous transporter important during Leishmania amastigotes differentiation in the mammalian host. Our objective is to investigate LIT1 expression during the establishment of Leishmania infection in Lutzomyia longipalpis. In infections initiated with procyclic forms we observed a higher expression of LIT1 at 72h and 144h post-infection. At these times the blood digestion finished and so a lower availability of iron is expected. Previous studies in mammals showed that LIT1 is up-regulated when lower levels of iron are available. Our findings show that LIT1 expression increases in late times within sandfly and could be important for metacyclic form development. **Supported by:**CNPq, FIOCRUZ **Keywords:** Leishmania infantum chagasi; lutzomya longipalpis; lit1

HP89 - **CHARACTERIZATION OF COMPONENTS OF THE ENDOSOMAL SORTING IN *TRYPANOSOMA CRUZI***

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Trypanosoma cruzi (Tc) sheds extracellular vesicles (EVs) in the extracellular milieu that modulate the communication and interaction with the host. It is accepted that exosomes originate from multivesicular bodies (MVBs), which is dependent of endosomal sorting complex required for transport (ESCRT). Several studies have also shown that ubiquitylation is a signal required for degradative receptor sorting into MVBs and consequent lysosomal processing. Nevertheless, ubiquitin (Ub) modification can be reversed by the action of deubiquitinases (DUBs) that recycle Ub to the cytoplasmic pool and receptors to plasma membrane. Since ESCRT pathway recognizes ubiquitinated proteins and sorts them into intraluminal vesicles (ILVs), we hypothesized that DUBs and ESCRT could regulate the endocytic process and EVs release in *T. cruzi*. Therefore, we performed gene knockouts (KO) of DUBs (TcUsp7 and TcVdu) and the ESCRT *T. cruzi* proteins TcVps4, 23, and 36 by using CRISPR/Cas9 in the DM28c strain. We additionally induced the overexpression of DUBs (TcUsp7::GFP and TcVdu::GFP). To date, it was not possible to obtain the KO of Usp7, Vps4, and Vps36, suggesting that these are essential genes for parasite survival. In contrast, TcVps23 and TcVdu KOs and TcVdu overexpressors epimastigotes were obtained and showed normal epimastigotes growth phenotype. TcVsp23 and TcVdu overexpressors presented reduced transferrin or BSA endocytosis. Of note, TcVdu::GFP decreased cell infectivity and delayed parasite egress from the host cell and TcVps23KO was unable to differentiate into metacyclic-trypomastigotes. In the opposite direction, TcVduKO augmented trans-sialidase secretion and EV secretion by tissue culture-derived trypomastigotes. These findings support that DUB and ESCRT proteins regulate differentially the endocytic and exocytic pathways in the distinct stages of *T. cruzi.*
Supported by:FAPESP **Keywords:** Endosomal sorting ; deubiquitinases; endocytic and exocytic pathways

HP90 - **POLYMERIC MICELLES CONTAINING MONOTERPENE: *IN VITRO* ACTIVITY AGAINST *LEISHMANIA AMAZONENSIS***

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Leishmaniasis are diseases that have high incidence and high morbidity in the population. Medicines for the treatment of leishmaniasis have several limitations of use. The 3-carene (3C) is a monoterpene that presented activity in vitro against some leishmania species. Poloxamers micelles have been used to solubilize poorly soluble drugs in aqueous medium in order to improve its effectiveness. This study aimed to evaluate in vitro the action of poloxamers micelles containing 3C in *L. amazonensis*. Micelles with poloxamer P407 alone or combined to poloxamer L81 in final concentration of 5,15% of polymers were utilized. Inert micelles and micelles containing 1000 µg/mL of 3C were evaluated. The antipromastigote activity was determined after 48 h of treatment by using resazurin method. Also, the cytotoxicity of formulations was determined in lineage cells L929 and J774 through MTT method. Plasma membrane changes after 12 h of treatment were investigated by flow cytometry using propidium iodide. In the results 3C alone presented half maximal inhibitory concentration (IC_{50}) of 64,20 µg/mL. P407 micelles containing 3C showed IC_{50} of 76,23 µg/mL and inert micelles showed no activity. P407-L81-3C micelles had an IC_{50} of 4.35 µg/mL and inert micelles had IC_{50} of 3.98 µg/mL. In the evaluation in L929 cells 3C at concentrations below 250 µg/mL and all micelles presented viability superior to 70%. In J774 cells the 3C showed half maximal cytotoxicity concentration (CC_{50}) of 120 µg/mL and the micelles showed CC_{50} which ranged from 123.60 to 352.45 µg/mL. The lowest IC_{50} values were obtained by combining poloxamer P407 and L81; However, inert micelles also showed activity. In preliminary results, promastigotes after 12 h of treatment with 3C or P407-L81 micelles did not show cell membrane permeabilization. More studies will be needed to identify the mechanisms involved in this biological action in promastigotes and which effect of formulations in intracellular amastigotes. **Supported by:**FAPITEC-SE/CAPES **Keywords:** Terpenes; triblock copolymer; neglected disease

HP91 - **BRADYKININ INCREASES ALBUMIN ENDOCYTOSIS IN HUMAN BRAIN MICROVASCULAR ENDOTHELIAL CELLS DURING MALARIA INFECTION**

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INTRODUCTION: Cerebral malaria development is characterized by disruption of blood-brain barrier, BBB, integrity. It has been proposed, in different neuroinflammatory diseases, that protein transcytosis through endothelial cells precedes BBB disruption. Recent data from our group have shown that bradykinin, BK, released from Plasmodium falciparum-infected red blood cells, Pf-iRBC, mediates endothelial dysfunction. OBJECTIVE: The aim of this work is to investigate the role of BK and protein transcellular transport in the BBB disruption observed in cerebral malaria. METHODS: HBMECs were maintained in medium 199. When indicated, conditioned medium, CM, from Pf-iRBC or non-infected red blood cells, RBC, at a concentration of 20%, were used. The culture of Plasmodium falciparum in human RBC was previously approved (CEP-HUCFF, Document 074/10). Albumin uptake by HBMECs was analyzed by BSA-FITC endocytosis. Cell death was measured using Annexin V / PI through flow cytometry analysis. RESULTS: We observed that HBMECs have low endocytosis capacity compared with proximal tubule renal cells, LLC-PK1, as detected by confocal microscopy. Interestingly, BSA-FITC uptake was increased by 2,5 fold when cells were incubated with CM from Pf-iRBC. This effect is mimicked by BK 10-7M or when cells were serum deprived for 12 hours. CM from Pf-iRBC induced HBMECs apoptosis, observed through increased Annexin V staining. This effect was completely blocked by HOE-140, a bradykinin receptor 2, B2R, antagonist. Similarly, CM from Pf-iRBC increased transcellular permeability to albumin, and this effect was also blocked by HOE-140. CM from RBC had no effect on BSA-FITC uptake, HBMECs apoptosis or transcellular permeability. CONCLUSION: These results indicate that bradykinin mediates BBB disruption induced by Plasmodium falciparum infection of red blood cells, highlighting new perspectives to understanding the pathogenesis of cerebral malaria. **Supported by:**FAPERJ, CNPq e CAPES **Keywords:** Cerebral malaria; bradykinin; endothelial cells

HP92 - **IDENTIFICATION OF PEPTIDES TARGETING *TRYPANOSOMA CRUZI* CELL SURFACE THROUGH PHAGE DISPLAY TECHNOLOGY**

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Trypanosoma cruzi trypomastigotes have a complex cell surface composition. Despite its antigenic diversity, the identification and characterization of parasite ligand molecules can help in the development of new anti-chagasic therapies. In this work, we performed phage display approach incubating tissue cultered derived trypomastigotes with a random library encoding X15 peptides (where X corresponds to any amino acid) fused to capsid protein. To perform the biopanning rounds, 10^7 live trypomastigotes forms were incubated with 10^9 bacteriophages for 1 hour, and the non-specific phages were washed out. The recovered phages were titrated, amplified, and used in a new round of biopanning. Four rounds of biopanning were performed. Twelve individual clones were sequenced, and subjected to BLAST analysis against human proteome. Nine non-redundant clones presented homology to mammalian host proteins, most of them encodes immunoglobulins. Our results suggest that these identified peptides may mimic potential host-parasite interactions. To confirm their binding capacity, 10 clones were amplified and evaluated by Cell-ELISA assay. 4 out of 10 clones showed strong interactions with the parasite, and tested on invasion assay. Preliminary results show that these 4 clones impair the ability of trypomastigotes forms to invade LLC-MK2 cell monolayers. Additional experiments using synthetic peptides are needed to better characterize their binding capacity and its capacity to interfere in parasite infection. **Supported by: Keywords:** Phage display; trypanosoma cruzi; binding peptides

HP93 - **BK/B2R AXIS INDUCES ADHESION OF MONOCYTES TO HUMAN BRAIN ENDOTHELIAL CELLS DURING INFECTION WITH PLASMODIUM FALCIPARUM: POSSIBLE ROLE OF PKC AND MTOR PATHWAYS**

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INTRODUCTION: Malaria is a parasitic disease caused by protozoa of the genus Plasmodium and is responsible for the deaths of thousands of people. The most severe form of malaria is caused by Plasmodium falciparum leading to different pathologies including blood-brain barrier (BBB) injury. Sequestration of P. falciparum-infected red blood cells (iRBC) to the endothelium is seen as a crucial event that leads to inflammation and disruption of BBB. Earlier data from our group showed that bradykinin (BK), generated in the P. falciparum culture supernatant, increases iRBC adhesion to human brain endothelial cells, BMEC.
OBJECTIVES: Since adhesion of leukocytes to the vasculature represents a common event during inflammatory response, the aim of this work is to describe the role of BK in monocyte adhesion to BMEC during malaria infection.
METHODS: Health A-type human RBCs infected with P. falciparum W2 strain were used to obtain the Pf conditioned medium (PfCM). THP-1 monocytes and BMEC were used in adhesion assays. The protocol performed was approved (CEP-HUCFF, 074/10).
RESULTS: It was observed that CM-iRBC increased monocyte adhesion to BMEC by 300%, with the maximum effect obtained at 20% concentration ($n = 9$, $p < 0.05$). Incubation of monocytes with CM-RBC in the same condition had no effect. Interestingly, the effect of CM-iRBC on monocyte adhesion was mimicked by 10-7 M BK and was completely prevented by 10-7M BK receptor antagonist HOE140 ($n = 6$, $p < 0.05$). In addition, 10-6M calphostin C, a PKC inhibitor, as well as 10-9M rapamycin and 10-7 M WYE, mTOR inhibitors, blocked BK-induced monocyte adhesion ($n = 3$, $p < 0.05$). Incubation of monocytes with 10-7M BK increased ICAM expression by 34.2% ($n = 2$).
CONCLUSION: Our results indicate that BK through the B2 receptor mediates the effect of CM-iRBC in increasing monocyte adhesion to BMEC. These results open new perspectives for understanding the mechanisms involved in the BBB breakdown observed in cerebral malaria. **Supported by:**CAPES CNPq FAPERJ **Keywords:** Malaria; bradykin; monocytes

HP94 - **EFFECT OF IN VITRO TREATMENT WITH CRUDE EXTRACTS OF ENDOPHYTIC FUNGI ON RAW 264.7 MACROPHAGES AND *LEISHMANIA (VIANNIA) BRAZILIENSIS***

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Leishmaniasis are endemic infectious diseases in Brazil of chronic evolution. The treatment for leishmaniasis has been based on pentavalent antimonials, which present high cytotoxicity to those infected. In the search of new therapies, several compounds produced by endophytic fungi revealed leishmanicidal properties. This study aimed to evaluate the effect of in vitro treatment with crude extracts of endophytic fungi *Diaporthe oxe* and *Diaporthe infecunda*. For that purpose, cytotoxicity against a strain of promastigote form of *L. braziliensis* and non-infected and infected RAW 264.7 macrophages was evaluated by MTT assay, as well as nitric oxide (NO) and reactive oxygen species (ROS) production after 24, 48 and 72 hours of treatment. The treatment was cytotoxic for promastigote form of *L. braziliensis* (<30% parasite viability) and presented low cytotoxic for non-infected and infected RAW 264.7 macrophages. The crude extract by *D. oxe* increased NO production in non-infected and infected RAW 264.7 macrophages compared to untreated cells, while crude extract by *D. infecunda* produced a low decrease of NO production. The ROS production was increased in non-infected and infected RAW 264.7 macrophages after 24 and 48 hours of treatment with crude extract by both species of endophytic fungi, while the highest concentration of treatment with crude extract by *D. oxe* increased ROS production after 72 hours of treatment. Taken together, the compounds brought significant changes in macrophage physiology, raising the idea of interfering the modulation of the immune response in *Leishmania* infection. Experiments are under way to evaluate the infection rate and the activity against amastigote form of *L. braziliensis*, as well as cytokines production by non-infected and infected macrophages after treatment. **Supported by: Keywords:** Leishmania braziliensis; endophytic fungi; immune response

HP95 - **USE OF NEW METALLODRUGS DIRECTED TO THE TREATMENT OF LEISHMANIASIS**

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The treatment of leishmaniasis has become increasingly difficult and challenging over the years due to enhancing cases of antimonial resistance cases. Also, the occurrence of side effects from highly toxic drugs calls the attention to develop new drugs for the treatment of leishmaniasis. The study of parasites' transport proteins is a promising field in the search for new chemotherapy targets due to the possibility of using selective inhibitors that can act directly against the parasite. Aquaporins, already reported in Leishmania, are responsible for transporting water, glycerol and other metabolites and were described transporting trivalent metalloids such as arsenic and antimony. Additionally, recent studies demonstrated that gold compounds can inhibit some human aquaporins activities such as glycerol transport and water permeability on cell surface. Based on this, our project aims to use metallocompounds with potential aquaporin selective inhibition that can interfere on the permeability of the parasite's membrane. Eleven compounds were synthesized and tested on promastigote forms of Leishmania (L.) amazonensis in 96-well plates at increasing concentrations from 0 to 100 µM and their efficacy evaluated by the MTT method, as well as on murine cells (primary macrophages and fibroblasts L929) for cytotoxic evaluation. The most efficient compounds were chosen based on their EC50% (from 6.18 to 35.26 µM) and low mammalian citotoxicity obtained after the initial screenings. Our most promising compounds are being tested against axenic and intracellular amastigotes in a new amastigote-promastigote differentiation protocol to assess Leishmania viability.
Supported by: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) **Keywords:** Leishmania; metallodrugs; aquaporins

HP96 - **LEISHMANIA (LEISHMANIA) INFANTUM CHAGASI INDUCES THE HOST LIGAND CD200 IN MURINE MACROPHAGE**

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To guarantee the successes of the infection, *Leishmania* has to manipulate the immunological response of the host. Many studies have been published in the last years, but little is still known about how Leishmania can manipulate the activation of macrophages for their benefit. It was previously shown that *L. (L.) amazonensis*, species associated with the cutaneous form of the disease, induce the expression of CD200 in macrophages, a molecule involved in the inhibition of iNOS-Nitric oxide mechanism. In this study, we examined the involvement of CD200 in *L. (L.) infantum chagasi* infection, which is associated with the visceral form of the disease. Bone marrow macrophages (BMM) from C57BL/6 mice were infected with amastigotes or promastigotes of *L. (L.) infantum chagasi*. Intracellular parasite proliferation and CD200 levels were determined during different time points after infection by immunofluorescence and immunoprecipitation /qPCR, respectively. In another set of experiments, macrophages were infected with amastigotes and incubated in the presence of CD200-Fc or iNOS inhibitor, L-NAME. Our results demonstrated that parasites were unable to proliferate inside of BMM, but macrophages do not eliminate parasites. Quantification of CD200 in different time points of infection showed that Leishmania modulates the levels starting at 48h. Treatment of BMMs with L-NAME or CD200-Fc do not favor intracellular replication. Our results suggest that *L. (L.) infantum chagasi* induces CD200 in later time points of infection, an event that could be necessary during visceral infections by *Leishmania*. **Supported by:**CAPES; CNPq; FAPESP **Keywords:** Cd200; leishmania chagasi; macrophage

HP97 - **RELEASE OF MAST CELLS EXTRACELLULAR TRAPS INDUCED BY *LEISHMANIA***

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During the infection by *Leishmania amazonensis*, agent of the cutaneous and disseminated leishmaniasis, the insect vector inoculates promastigotes in a blood pool, whereby the parasite interacts with different cells of the innate immune system. Mast cells are resident granulocytes that modulate the immune response by releasing different mediators. Recently, it has been demonstrated that mast cells release, by the etosis mechanism, extracellular traps composed of DNA, histones and granular proteins (MCETs), and also can phagocyte promastigotes of *Leishmania*, killing them. Although different studies imply that the mast cells are the first line of defense against *Leishmania*, little is known about the importance of the MCETs. The objective of this study is to evaluate if the promastigotes of *L. amazonensis* induces the release of MCETs and its role in the leishmaniasis infection. In this study we used the human mast cell lineage HMC-1, cultivated in DMEM supplemented with 20% fetal bovine serum, penicillin-streptomycin at 37oC, 5% CO2. For the MCETs release assay, HMC-1 were incubated in RPMI without serum, with promastigotes and the release of MCETs was quantified in the culture supernatants by Quanti-it™PicoGreen®. Our results shown that promastigotes are capable of inducing the MCETs release in HMC-1. To analyze the toxicity of the MCETs in Leishmania, the parasite were incubated or not with different concentrations of MCETs. After 90 min at 35oC, the viability of the parasites was tested with propidium iodite and the samples were analyzed at FACScalibur. Our results show that, in the tested concentrations, the MCETs did not show any leishmanicidal activity. Next, to investigate the role of MCETs during the macrophage infection by *L. amazonensis*, human monocytes lineage THP-1, differentiated into macrophages with PMA, were infected with promastigotes in the presence or not of MCETs, and we analyzed the infection index and the number of amastigotes/cell. **Supported by:**CAPES FAPERJ CNPQ
Keywords: Leishmania amazonensis; mast cells; macrophages

HP98 - **ASSESSMENT OF THE GP63 EXPRESSION PROFILE DURING THE GROWTH OF LEISHMANIA BRAZILIENSIS PROMASTIGOTES**

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Among the main pathogenic species of Leishmania, L. braziliensis is characterized by having the largest number of genes encoding the virulence protein GP63. This metalloprotease is known to be involved in mechanisms by which the parasite evades the host defense systems. Multiple GP63 genes in L. infantum and L. major can be grouped based on differences in their 3' UTRs and on the C-terminuses of the encoded proteins. Transcriptomic analyses of these genes suggest that they may be differentially expressed during the parasite's life cycle. The larger set of L. braziliensis genes, however, are diverged in sequence and encode groups not found in other species. An expression analysis of the GP63 genes in L. braziliensis is of great importance to understand their roles during the parasite's life cycle and host infection. This study then aimed to evaluate the expression of defined sets of GP63 genes in L. braziliensis. First, RNASeq analyses were made of logarithmic and stationary phase promastigotes, with transcripts corresponding to all the annotated genes found in both life-cycle phases. A single transcript (LbrM.10.0590), however, was found to be represented by a much higher number of copies than all the others, with those remaining split into moderately abundant and very rare transcripts. Next, to evaluate differential protein expression, four different anti-GP63 sera were generated, raised against peptides specific to proteins classified in different groups. These were used in western blotting (WB) assays with whole cell extracts derived from L. braziliensis and L. infantum. Two of those sera recognized proteins constitutively expressed in promastigotes, although with different molecular weights, indicating that a minor set of the GP63 genes can be expressed simultaneously, presumably encoding functionally distinct proteins. These results suggest a preferential expression of selected genes which might be involved in activities within the invertebrate host. **Keywords:** Gp63; leishmania braziliensis; rnaseq

HP99 - **DORMANCY AND SYNCHRONICITY OF REPLICATION OF TWO *TRYPANOSOMA CRUZI* STRAINS**

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Trypanosoma cruzi is a protozoan parasite that may cause a severe illness in humans, called Chagas disease. *T. cruzi* is naturally transmitted to the mammalian host through the feces of triatomine bugs. However, other forms of transmission, such as oral, blood transfusion and congenital, have been increasingly growing and becoming important transmission routes. Despite host immune response, *T. cruzi* displays several mechanisms of endurance on its host. Dormancy has recently been described as one of them. The goal of this work was to evaluate dormancy rates during intracellular development of two *T. cruzi* DTUs, I and II. For this, purified trypomastigotes of Sylvio X10/4 (TcI) and Y strain (TcII) were used for infection in an epithelial cell lineage (LLC-MK_2), at an MOI of 50 for 1h. In order to evaluate the rate of proliferation/dormancy, parasites were previously labelled with CellTrace® CFSE. Decay in CFSE fluorescence would indicate proliferation, while high CFSE fluorescence would indicate either slow rate of proliferation or a stop in the cell cycle, characterizing dormancy. After parasite exposure, cells were washed and fixed at 24, 48, 72 and 96h, labeled with anti-*T. cruzi* polyclonal antibody (to exclude extracellular parasites) and DAPI to detect host cell and parasite DNA, then analyzed in a fluorescence microscope. Data obtained showed that the number of intracellular parasites per infected cell along time was similar between TcI and TcII parasites. However, the number of intracellular CFSE positive amastigotes for the two DTUs was not. The rate of CFSE positive parasites at 48h was higher for TcI, when compared to TcII (40 and 10%, respectively). At 72h, the rate of CFSE positive parasites was identical for both DTUs, while at 96h it was slightly slower for TcI. Data aforementioned suggests an asynchronous *T. cruzi* intracellular amastigote proliferation pattern within an infected cell and that dormancy rates may vary between parasite populations. **Supported by:**CAPES, CNPQ, FAPEMIG **Keywords:** T. cruzi; dormancy; intracellular multiplication

HP100 - **THE CHARACTERIZATION OF SIRTUIN FAMILY IN *RHODNIUS PROLIXUS***

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Rhodnius prolixus is a mandatory hematophagous triatomine found in tropical regions as Central and South America. It is one of the main vectors of Chagas disease caused by the protozoan Trypanosoma cruzi. After a blood-meal on an infected vertebrate host, the insect ingests trypomastigotes and the whole development of the parasite in the insect is limited to the intestinal environment. *R. prolixus* metabolism during T. cruzi infection is poorly understood, and classic energetic sensors have not yet been described in this model. Sirtuins are a family of histone deacylases dependent of the availability of NAD+ regulating different metabolic pathways. In mammals 7 sirtuins (SIRT1-7) have been described with different cell localizations (nucleus, cytoplasm and mitochondria). Here we characterize the expression of *R. prolixus* sirtuins and show how they are modulated during *T. cruzi* infection. In silico characterization has been performed through Blast and HMMER tools using protein databases revealing 4 sirtuins orthologs to mammal SIRT1, SIRT5, SIRT6 and SIRT7. qPCR analysis using infected and non-infected male insects verified sirtuins tissue specific expression. In testis a high expression of SIRT1, SIRT6 and SIRT7 was observed, which are not altered by the infection. The infection with T. cruzi increased the expression of SIRT1, SIRT5 and SIRT7 in the hindgut, while in the fat body there was a higher expression of SIRT1 and SIRT5 compared to non-infected insects. The fat body doesn't contact the parasite directly, suggesting it can modulate sirtuins expression indirectly through infection. Our group showed a reduction in triacylglycerol and lipid droplets in the infected fat body, implicating SIRT1 and SIRT5 as putative modulators of lipid metabolism. Our project shows for the first time the characterization of sirtuins in vector insects contributing to a better metabolism understanding enabling new targets for vector control. **Supported by:**CNPq **Keywords:** Rhodnius prolixus; sirtuins; bionformatics

HP101 - **THE VIRULENCE OF DIFFERENT SPECIES OF THE GENUS *LEISHMANIA* IS MODULATED BY LYSOPHOSPHATYDILCHOLINE (LPC)**

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Lipid mediators, including lysophosphatidylcholine(LPC)and platelet-activating factor(PAF) have been described as presenting a key role in the infection of some parasitic protozoa.We demonstrated that PAF stimulates cell differentiation of *H.m.muscarum* and *T.cruzi*, in addition to modulate infection of mouse peritoneal macrophages by *L.amazonensis*.We have also shown that *T. cruzi* synthesizes a C18:1-LPC,with the ability of aggregating platelets, similarly to PAF.In the present study, we demonstrate the effects of LPC on *L.infantum, L.amazonensis* and *L.mexicana* proliferation and differentiation, as well as in the interaction of these parasites with mouse peritoneal macrophages.We observed a 32% increase in proliferation of *L.infantum*, *L.amazonensis* and *L.mexicana* on the 5th day of growth, when the parasites were treated with C18:1-LPC,as compared to the control.Pre-treatment of the parasites with WEB 2086(PAF-receptor antagonist) reversed the C18:1-LPC effects.Also,the number of differentiated forms(intermediates) exceeded the number of promastigotes on the 8th day after induction of differentiation in the presence of C18:1-LPC, as compared to the control parasites, which present the phenomena on the 13th and 16th day for *L.amazonensis* and *L.infantum* respectively.We also tested the effects of C18:1-LPC on the infection of mouse peritoneal macrophages when parasites were pre-treated for 4 hours with this lipid.Preliminary results indicate an enhancement of the infection when C18:1-LPC-treated parasites were used in the interactions.We have also built a three-dimensional model of a putative *Leishmania spp* PAF receptor(PAFR) and a molecular docking study was performed to predict the interactions between the PAFR model and PAF or LPC.The docking data suggested that C18:1-LPC is predicted to interact with the PAFR model in a fashion similar to PAF.These results suggest that C18:1-LPC modulates the virulence of Leishmania spp via a putative PAF receptor in these parasitaes. **Supported by:**CNPq, CAPES, FAPERJ and INCT-EM **Keywords:** leishmania spp; c18:1-lpc; molecular docking

HP102 - **INTRACELLULAR *TRYPANOSOMA CRUZI* MODULATES HOST SPLICING MACHINERY DURING INFECTION**

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Morphological and metabolic changes resulting from the interactions between the host cell and Trypanosoma cruzi parasite, the etiological agent of Chagas disease, still are targets of studies due to health and economic damage caused by the lack of effective treatments. During infection, the parasite can modulate host cellular and immunological responses. In our recent data using LLC-MK2 cells infected with T. cruzi, we observed at different times through confocal microscopy that the parasites are able to change the dynamics of nuclear organelles involved in transcriptional and splicing events in non-professional phagocytic cells. The host nuclear bodies involved in transcription and splicing are affected by the parasite cell cycle and host ribonucleoproteins are also down-regulated in a time-dependent manner. In parallel, disruption of parasitophorous vacuole occurs and is an essential event in the parasite development. In addition, T. cruzi infection acts as a critical key in RNA processing observed using a splicing reporter minigene assay. These changes in the host transcription and splicing machineries are strong evidence that Trypanosoma cruzi could interfere in the host cell nucleus favoring the infection progress. **Supported by:**Fapesp **Keywords:** Trypansosoma cruzi; splicing; transcription

HP103 - **RADIAL GLIA CELLS INFECTION BY TOXOPLASMA GONDII DISRUPTS BRAIN MICROVASCULAR ENDOTHELIAL CELLS INTEGRITY**

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BACKGROUND: Congenital toxoplasmosis is a parasitic disease that occurs due vertical transmission of the protozoan Toxoplasma gondii during pregnancy. The parasite crosses the placental barrier and reaches the developing brain, infecting progenitor, glial, neuronal and vascular cell types. Here we studied the role of T. gondii infection on RG cells function and its interaction with endothelial cells. METHODS: Isolated RG cells were infected with T. gondii tachyzoites for 24 h. Cells were fixed and conditioned medium (CM) was collected for treatments and cytokine analyses. Cells were immunostained for specific neural markers and proliferation; CM was used on mouse brain microvascular endothelial cell line (bEnd.3). Alternatively, CM was used for TGF-b ELISA and cytokine profiling with cytokine bead array (CBA). bEnd.3 cells were plated on coverslips for ZO-1 immunostaining or transwell inserts for transendothelial electrical resistance (TEER) measurements. After 24 h of treatment with CM or infection with T. gondii cells were analyzed. RESULTS: We observed reduced cell proliferation and neurogenesis without affecting gliogenesis levels. CM from RG control cultures increased ZO-1 and b-catenin protein levels and organization on endothelial bEnd.3 cells membranes, which was completely impaired by CM from infected RG or by direct infection. These events were followed by altered TEER. CBA and ELISA assays revealed increased levels of the pro-inflammatory cytokine IL-6 and reduced levels of anti-inflammatory cytokine TGF-b1 in CM from T. gondii-infected RG cells.CONCLUSIONS: Our results suggest that infection of RG cells by T. gondii modulate the secretion of cytokines that might contribute to endothelial loss of barrier properties, thus contributing to impairment of neurovascular interactions establishment. **Supported by:**CNPQ, FAPERJ E CAPES **Keywords:** Congenital toxoplasmosis; radial glia; endothelial cells

HP104 - **CONTROL OF INTRACELLULAR DRUG RESISTANT LEISHMANIA INFANTUM ASSOCIATED WITH IMMUNE RESPONSE ACTIVATION IN MACROPHAGE**

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Visceral Leishmaniasis (VL) is a severe infectious disease more prevalent in neglected people and caused by Leishmania protozoa. The control of infection and parasite dissemination essentially executed by lymphocytes and phagocytes. However, when the immune system fails and the disease is stablished, the chemotherapy is the main form of control of the VL, however in the last years have been shown increasing cases of treatment failure and reports of Leishmania parasites naturally resistant to drug. Methods and Results: we collected mononuclear cell of peripheral blood of healthy donors and differentiate into macrophages (n = 7). After this, we infected the macrophages with different strains of Leishmania infantum: two from relapse patients to antimoniate meglumine treatment and one isolated from responsive patient and drug sensitive parasite. Moreover, we treated the infected macrophages with different immune modulators: IFNγ+LPS; recombinant sCD40L; blocking of Interleukin-10 action; inhibition of Nitric Oxide (NO) production by Aminoguanidine. Also, we used meglumine antimoniate treatment. The infected macrophages were counted by optical microscopy. From this, our results show failure of macrophages in control the resistant parasite infection at 24h when compared to initial infection (02h). In addition, the use of pentavalent antimonial is unable to revert this situation. Interestingly, we observed a significative reduction at 24h in the number of infected macrophages when we treated the macrophages with immune activators or make the co-activation, with IFN + LPS or recombinant sCD40L. Furthermore, when we inhibited the NO production we observed an increase in the number of amastigotes only in resistant parasites. Conclusion: the phenotype of drug resistance in L. infantum isolates is related to different pattern in macrophage infection and the modulation of immune response of infected macrophages can control the parasite dissemination. **Supported by:**FAPITEC; CNPq; CAPES; Introduction: Visceral Leishmaniasis (VL) is a severe infectious disease more **Keywords:** Leishmania infantum; drug resistant; macrophage

HP105 - **EVALUATION OF THE PROTECTIVE CAPACITY OF AMASTINS PROTEINS IN *LEISHMANIA BRAZILLIENSIS* INFECTION**

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Leishmaniasis encompass a complex group of parasitic diseases with a clinical spectrum that ranges from cutaneous ulcers to visceral infections, caused by diferent species of Leishmania. The study of the proteins of the parasite associated to the differentiation of promastigotes/amastigotes and intracelular parasite survival are fundamental to the understanding of such a complex group of diseases. Amastins are surface glycoproteins, mostly expressed in the amastigotes forms of Leishmania identified as highly imunogenic antigens whose function is still widely unknown. The objective of this project was to assess the protective capacity of amastins proteins in the infection by Leishmania braziliensis in a mice model. Aiming to achieve such goal, it was made the cloning and expression of γ-amastin related to the first extracelular loop. Subsequently, BALB/c mice were immunized with the referred protein associated with POLY (I:C) adjuvant. The mice were challenged with L. brazilliensis promastigotes and after euthanasia, multiple analyses were done to assess the size of the lesion, tissue parasitism, antibody title and cytokines quantification. It was possible to observe that the mice immunized with the protein plus adjuvant showed less tissue lesion when compared to the other control groups, although there was no apparent difference regarding the observed parasitism,showed in qPCR assays and limitant dilution. The higher IgG I titles and increasement of IL-10 levels showed that our protein associated with POLY(I:C) adjuvant leeds the imune response to a regulatory/ anti-inflammatory profile. **Supported by:**CNPq **Keywords:** Leishamnia braziliensis; amastin; vaccine

HP106 - **PLAYING WITH *TRYPANOSOMA CRUZ*I FLAGELLUM**

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Parasite flagellum is key structure for virulence, since recent reports have shown that not only for parasite swimming, the flagellum plays role on interaction with host structures/organelles, social motility, and cytokinesis. To identify conserved flagellar components among Trypanosomatids shared by Trypanosoma cruzi we performed in silico analysis using TriTrypDB data. Flagellar proteomic data from Trypanosoma brucei were used to identify orthologous genes in T. cruzi. For these genes, we analyzed RNAseq data to access its mRNA expression profile through amastigote to trypomastigote transition during infection. The genes that were conserved and RNAseq data compatible with flagellum size were selected for functional characterization, since they could be potential candidates for drug development or to attenuate parasite virulence. To this end, we are developing tools and strategies to dissect T. cruzi flagellum, such as gene editing, conditional knock out and protein tagging. CRISPR/Cas9 was applied to edit two conserved flagellar proteins Kharon1 and Trypanin, which were previously characterized in two related species, T. brucei and Leishmania mexicana. Edited parasites show interesting phenotypes, Kharon1 disrupted population show morphology alterations, and reduced growth, while Trypanin depleted has decreased motility. To confirm their function we are developing strategies to tag these proteins using easy-to-clone plasmid and conditional complementation using CRE recombinase system. We plan to use these strategies to dissect T. cruzi flagellum, which is poorly studied. **Supported by:**CNPQ, FUNDAÇÃO ARAUCARIA, CAPES. **Keywords:** Trypanosoma cruzi ; crispr/cas9; flagellum

HP107 - **THE ROLE OF IL-1β IN THE CHRONIC CHAGASIC CARDIOMYOPATHY**

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Trypanosoma cruzi, the causative agent of Chagas disease, infects cardiomyocytes leading to pro-inflammatory environment and in some cases to the development chronic chagasic cardiomyopathy (CCC). T. cruzi induces IL-1β secretion that contributes to control the parasitic load during the acute phase infection. Importantly, IL-1β acts as an arrhythmogenic agent in different cardiovascular diseases, but the contribution of IL-1β to the pathogenesis of CCC is currently unknown. Here we test the hypothesis that IL-1β is involved in the cardiac electrical and mechanical dysfunction in a mouse model of CCC. In order to test this hypothesis, we infected Wild-type (WT) and IL-1r-/- C57BL/6J mice with 50-100 parasites of Colombian strain for more than 200 days. The follow experimental groups were performed: WT, WT-infected (WTc), WT treated with 10 mg/kg/30 days of recombinant IL-1 receptor antagonist (IL-1ra - ®Kineret) (WTA), WT-infected treated with IL-1ra (WTcA). Other set of experiment was performed comparing IL-1r-/- infected. Electrocardiogram (ECG) and echocardiogram (ECO) were performed to evaluate cardiac function. Our results indicate that no difference was found in ECG recording of infected IL-1r-/- when compared to WT mice upon infection. Additionally, Anakinra did not reverse the electric disturbs such as first-degree atrioventricular block (AV block) while increased the susceptible to second-degree AV block development. The CCC induced lower ejection fraction , stroke volume and increase of right-ventricle area among all groups of WT and IL-1r-/- infected mice when compared to non-infected mice. The treatment with IL-1ra was not able to revert the mechanical damage induced by CCC. Taken together, the results obtained here indicate that either the genetic ablation of IL-1r or the treatment with IL-1ra did not improve cardiac electrocardiographic and mechanical impairment induced by T. cruzi infection in mice.
Supported by:CAPES **Keywords:** il-1β chronic chagasic cardiomyopathy

HP108 - **EVALUATION OF THE ROLE OF CHOLESTEROL IN T. CRUZI INTRACELULAR DEVELOPMENT**

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*Trypanosoma cruz*i host cell invasion involves lysosome recruitment and fusion with plasma membrane for the formation of a mature parasitophorous vacuole. *T. cruzi* resides temporarily in this lysosome derived vacuole before escaping to host cell cytoplasm. Data from our group shows that LAMP-1 and 2 (two lysosomal membrane proteins) are important for parasite infection. LAMPs participate in the transport of cholesterol from the intracellular mllieu to the cell plasma membrane. LAMP deficiency (LAMP-KO) decreases cholesterol content at cell surface, compromising *T. cruzi* invasion. However, once inside LAMP-KO cells, parasites show a higher replication rate when compared to infections in wild type (WT) cells. The reason for the latter is not yet understood. In the absence of LAMPs, the low plasma membrane cholesterol content is directly related to the free cholesterol accumulation in lysosomes. It has been shown in the literature that intracellular cholesterol levels can modulate some Apicomplexa parasites survival and multiplication rates. For *T. cruzi,* infection may alter cholesterol metabolism in host cells. Also, it has been shown that epimastigotes endocyte exogenous cholesterol. Thus it is possible that cholesterol accumulation inside LAMP-KO cells may be boosting *T. cruzi* intracellular multiplication in these cells. In order to test this hypothesis, we used *T. cruzi* Y strain trypomastigotes to infect WT and LAMP-KO cells pre-treated or not with atorvastatin, an inhibitor of endogenous cholesterol synthesis. It is well known that atorvastatin treatment leads to an increase in LDL receptor expression and uptake, likely increasing intracellular cholesterol content. Atorvastatin treatment did not affect parasite multiplication rates in LAMP-KO cells, but enhanced parasite multiplication in WT cells. Therefore we believe that drug rebound effect, leading to an increase in intracellular cholesterol, is responsible to augment parasite multiplication in WT cells. **Supported by:** CAPES, CNPq, FAPEMIG **Keywords:** Trypanosoma cruzi ; cholesterol metabolism; lamp

HP109 - **LEISHMANIA CALCINEURIN IN MACROPHAGE INFECTION**

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Leishmania (Leishmania) amazonensis grows in a unique and enormous parasitophorous vacuole (PV) inside of macrophages. To generate this tightly controlled structure, *Leishmania* metacyclic promastigotes or amastigote forms need to performed different strategies to efficiently create the conditions to survive, proliferate in such a harsh environment and modulates the immunological response of the host cell. Calcineurin is a Ca2+ dependent phosphatase involved in different cellular processes related to thermotolerance and adaptation to oxidative stress, but the function in the immunobiology of the host infection by the two infective forms of *Leishmania* remains unknown. In this study, we examined the role of calcineurin from the infective forms of *Leishmania* in the presence of calcineurin specific inhibitors during macrophage infection. Here we showed that both infective forms present different biological phenotypes in the presence of calcineurin inhibitors. Metacyclic promastigotes pretreated with CsA can proliferate intracellularly at the same levels than untreated parasites showing an increased number of parasites contained in enlarged PVs at 96 h of infection. When pretreated with FK506, a slight effect was observed in the intracellular proliferation and the size of the Leishmania-PV. Interestingly, a drastic effect on the macrophage infection is visualized when amastigotes are pretreated with CsA but not with FK506. CsA affects the capacity to proliferate inside of macrophages since a decreased number of parasites contained in small and sometimes individual PVs are detected. Thus, the present data describes the parasite Calcineurin as a crucial molecule participating in the evasion mechanism by *Leishmania* amastigotes in host infection. **Supported by:**CAPES; CNPq; FAPESP **Keywords:** Calcineurin; leishmania; macrophages

HP110 - **EVALUATION OF IMMUNOGENICITY OF TWO DIFFERENT DOSE CHIMERIC VACCINES AGAINST EXPERIMENTAL VISCERAL LEISHMANIASIS**

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Thus far, vaccines used to prevent visceral leishmaniasis (VL), both human and canine, are scarce. Most of them were developed using traditional vaccinology methods, which were based on the empirical screening of some candidates according to known characteristics of the pathogen. Given the need for more rational vaccine discovery, bioinformatics emerges as a computational tool that allows the prediction of immunogenic epitopes essential for vaccine development. Two chimeras were designed from the mapping of T-cell epitopes belonging to Leishmania infantum proteins described in the literature as vaccine candidates. The aim of the study was to evaluate the immunogenicity of two chimeric vaccines at different doses against VL in BALB/c mice. The animals were divided into seven groups Saline and chimeras A and B at the doses of 5, 10 and 20 µg. These were immunized with 3 doses and challenged with 1x10^7 L. infantum promastigotes. Were evaluated the proliferative activity of T lymphocyte subpopulations (total T, CD4+ and CD8+) and the production of intracytoplasmic cytokines (IFN, TNF, IL-2, IL-4 and IL-10) after stimulation with a soluble antigen of L. infantum by flow cytometry. Both chimeras at a dose of 20 µg showed higher T lymphocyte proliferation and increased the production of IFN, TNF and IL-2 cytokines when compared to the Saline group. A reduction in IL-4 and IL-10 production was observed by TCD4+ and TCD8+ lymphocytes in both chimeras, A and B, at the lowest dose. The results obtained demonstrate that even at low doses, the chimeras were able to elicit a satisfactory immune response. An excellent strategy to enable the use of these chimeras at low doses would eventually be to combine them with adjuvants to further enhance immunogenicity. **Supported by:**CAPES, CNPq, FAPEMIG, UFOP **Keywords:** Visceral leishmaniasis; bioinformatics; chimeric vaccines

HP111 - **LEISHMANIA AMAZONENSIS PORE-FORMING ACTIVITY MAY BE MEDIATED BY LYSOPHOSPHOSPHATIDYLCHOLINS**

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L. amazonensis promastigotes have a pore-forming cytolysin able to damage macrophages in vitro at pH 5.5. We have thus postulated that it could be involved in phagolysosome and plasma membrane rupture, contributing to infection amplification. We previously described that L. amazonensis promastigotes lysophosphatidylcholine (LPC) is hemolytic. To investigate whether L. amazonensis total lipids expressed lytic activity, they were incubated with erythrocytes. We verified that the lipids, totally devoid of protein, retained nearly all hemolytic activity present in the extracts, suggesting that they are the major responsible for the hemolytic activity of L. amazonensis previously described. Since in previous studies we have determined that promastigote total extracts damage macrophages by pore formation on plasma membrane, we performed an osmotic protection experiment with the lipid fraction. Colloid-osmotic lysis mediated by pore-forming molecules can be prevented by macromolecules added to the extracellular milieu, compensating osmotic imbalance. Our results showed that polyethylene glycol (PEG) completely inhibited hemolysis mediated by promastigotes lipidic fraction. This result suggests the colloid-osmotic nature of lysis, indicative of pore formation on the target cell membranes. Inhibition was not due to inactivation of the lytic protein by PEG, since lysis was restored after erythrocytes were washed and resuspended in PEG-free solution. This result also suggests that lipids bind to the target cell membrane before causing lysis, corroborating previous results. Since LPCs are produced by the action of phospholipases A, we also investigated the hemolytic activity of promastigotes grown in the presence of a phospholipase A2 inhibitor. We found that these parasites totally lost their hemolytic activity. Together, our results indicate that L. amazonensis hemolytic activity is due to phospholipase A2-derived LPCs that can directly lyse cells, probably by pore formation. **Supported by:**CNPq, FAPEMIG, CAPES **Keywords:** Leishmania; lipides; cytolisinas

HP112 - **IN VITRO POTENTIAL OF TRITERPENES MASLINIC ACID, URSOLIC ACID, BETULIN AND LUPEOL IN THE TREATMENT OF VISCERAL LEISHMANIASIS**

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Leishmaniasis is a disease caused by flagellated protozoa, affecting millions of people worldwide. Difficulties in treating patients with this disease include the limited efficacy and many side effects of drugs currently available. Prospecting natural plant-isolated compounds may be a promising alternative for treating leishmaniasis. Thus, the present study compared the in vitro effects of triterpenes maslinic acid (MA), ursolic acid (UA), betulin (Be) and lupeol (Lu), as well as their possible cellular targets in L. (L.) chagasi. These compounds were obtained commercially and their structure as well as the degree of purity reconfirmed by HPLC and magnetic resonance. The triterpenoids MA, UA and Lu were active against promastigote and amastigote forms as well as miltefosine. Be showed no significant leishmanicidal effect against amastigote forms. Parasites were incubated with the effective concentrations 50% (EC50) of the respective triterpenes for 24h, and ultrastructural analyzes were performed. The main alterations were related to the formation of vesicular compartments with or without myelin figures or rest of membranes, as well as intracellular organelles disruption, especially parasite mitochondria, showing swelling and fragmented, additionally its membrane potential was altered after 15 minutes of incubation with the triterpenes. Parasite chromatin was also fragmented, suggesting that triterpenes induce programmed cell death. The results indicate that these triterpenes may be interesting targets for the development of new classes of drugs against visceral leishmaniasis, since their in vitro effects were comparable to miltefosine, showed low cytotoxicity and high selectivity toward L. (L.) chagasi. **Supported by:**Fundação de Amparo a Pesquisa do Estado de São Paulo - FAPESP **Keywords:** Trirtepenes; natural compounds; treatement

HP113 - **EFFICACY OF URSOLIC ACID AND AMPHOTERICIN B AND GLUCANTIME COMBINED THERAPY FOR VISCERAL AND CUTANEOUS LEISHMANIASIS**

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Leishmaniasis is endemic in 98 countries and is caused by protozoa of the genus Leishmania sp. The disease may have clinical forms ranging from single lesions to visceral forms that can lead to death if left untreated. Combination therapy is considered an important strategy for increasing efficacy and prevent parasitic resistance in infectious diseases. In this context, the efficacy of ursolic acid (UA) alone and in combination with amphotericin B; glucantime and miltefosine was evaluated in vitro and in vivo. Drug interactions were evaluated in vitro by a modified isobologram method, whereas fractional inhibitory concentration index (FICI) determination was calculated. The therapeutic potential was verified in L. (L.) chagasi infected hamsters and *L. (L.) amazonensis* infected BALB/c mice. Relatively lower values of EC50 were observed when UA was associated with standard drugs (amphotericin B or miltefosine) used in the treatment of cutaneous and visceral leishmaniasis. In vitro results indicated that the interaction between UA and amphotericin B or miltefosine was additive, allowing to analyze the in vivo efficacy of the association between standard drugs and UA. The association of UA and amphotericin B in visceral leishmaniasis demonstrated that the therapeutic combination was not effective in reducing parasitism. However, the therapeutic combination between UA and glucantime was effective in reducing parasitism in animals infected with *L. (L.) amazonensis* in comparison to the treatment with single treatment demonstrating that the association of these molecules enhances efficacy of both drugs. The results presented here indicate that combination therapy may be an interesting strategy towards the treatment of leishmaniasis. **Supported by:**Fundação de Amparo a Pesquisa do Estado de São Paulo - FAPESP
Keywords: Trirtepenes; combination therapy; treatement

HP114 - ***TOLL-LIKE* RECEPTORS 2, 4, AND 9 EXPRESSIONS AND CYTOKINES PROFILE CLINICAL AND IMMUNOPATHOLOGICAL SPECTRUM OF AMERICAN CUTANEOUS LEISHMANIASIS**

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Leishmania (V.)braziliensis and Leishmania(L.)amazonensis a[illegible].can Cutaneous Leishmaniasis in Brazil, causing a wide [illegible]nological manifestations, including: localized cutaneous l[illegible]d cutaneous leishmaniasis (BDCL), anergic diffuse cutaneo[illegible]eishmaniasis (ML). It has recently been demonstrated, ho[illegible]ows a clear potential to advance the infection from central l[illegible]orm) towards ML (the highest T-cell hypersensitivity pole), l[illegible]ne opposite direction to ADCL (the lowest T-cell hypersensiti[illegible]mohistochemistry the expression of Toll-like receptors (TLRs[illegible]NF-α, IFN-γ, IL-10 and TGF-β cytokines and iNOS enzima[illegible]skin and mucosal lesions from 33 patients were examined[illegible]ed by L. (L.) amazonensis; as well as 10 cases of LCL and [illegible]NF-α+, IFN-γ+ and iNOS+ showed increased expression from [illegible]howed increased expression in the opposite direction, from ML to A[illegible]trongly evidence the role of L. (V.) braziliensis and L. (L.) amazonensis in [illegible]or effect of the inflammatory (TNF-α+) and anti-inflammatory (IL-10+ and TGF-β+) [illegible]cell immune response of ACL. With regards to TLR2, 4, and 9 expressions, strong inter[illegible]LR2 and 4 with clinical forms associated with L. (V.) braziliensis were observed, while TLR9, [illegible]ntrast, showed a strong interaction with clinical forms linked to L.(L.) amazonensis, strongly suggest the ability of L. (V.) braziliensis and L. (L.) amazonensis to interact with those TLRs to promote a dichotomous T-cell immune response in ACL. **Supported by: Keywords:** Leishmania (v.) braziliensis; toll-like 2, 4 and 9; leishmania(l.) amazonensis

HP115 - **IDENTIFICATION OF PROTECTIVE B CELL ANTIGENS DERIVED FROM BLOOD FORMS OF *PLASMODIUM* PARASITES THROUGH MONOCLONAL ANTIBODIES ISOLATED OF IMMUNE MICE**

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In 2017, it was estimated 219 million cases of malaria infections and approximately 435 thousand deaths. Although residents of malaria endemic areas are continuously exposed to parasites, they develop protective immunity. However, it is induced years later upon multiple parasite exposures. Antibodies specific to Plasmodium blood form antigens are pivotal for protection. Either P. falciparum and P. vivax antigens have already been identified as targets for this response in the last decades. None of them has shown great protection in clinical trials though. Thus, our main goal is to identify novel Plasmodium blood form antigens able to elicit protective monoclonal antibodies (mAbs) in immune mice. To generate immune mice, we infected C57Bl/6 mice with 5 P. berghei ANKA-infected red blood cells (RBCs). Whenever the parasitemia reached 0.5-1% of total RBCs, the mice received a chloroquine sub-curative treatment. A single treatment decreased the parasitemia levels, but did not clear the infection. In contrast, 4-6 rounds of treatment inhibited the parasitemia to bounce back, and mice were considered immune. To isolate protective mAbs in this model, we defined day 10th after the parasite re-challenge as the time point that antibody-secreting cells (ASCs) had their highest frequency in the spleen of immune mice through ELISPOT. Then, we sorted a total of 840 single ASCs (F4/80- CD3- B220+ CD138+) from 4 mice to perform immunoglobulin (Ig) V(D)J gene cloning and mAb production. From the 168 sorted ASCs already analyzed, we detected 47 cognate pairs of Ig heavy-light chain amplicons. Twenty-two out of those 47 pairs of amplicons were sequenced and 16 displayed productive sequences to be cloned out. After cloning, these antibodies will be expressed, purified and tested for specificity and capacity to neutralize RBC invasion by merozoites. The positive mAbs will have their targeted antigens solved by mass spectometry.
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HP116 - ***PLASMODIUM VIVAX* INFECTION DISTURBS MITOCHONDRIAL METABOLISM**

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Introduction: The most frequent and widely causative agent of malaria *Plasmodium vivax* is one of the five malaria parasites capable to cause the disease in humans. In Brazil, *P. vivax* is responsible for more than 80% of malaria cases in Amazon region. To achieve the complete control of the infection is required both innate and adaptive immune responses. Our group showed that monocyte subsets are differently activated during *P. vivax* infection. Interestingly, they also have distinct ability to produce mitochondrial oxygen reactive species (mROS). In this context, it is important to highlight that many studies have been shown the importance of mitochondrion in immune response regulation. Based on that, the aim of this study was to evaluate if those differences in mROS production are due malaria infection or are intrinsic characteristics of monocyte subsets. Methods: To address this question blood samples were collected from *P. vivax*-infected patients and healthy donors in Porto Velho, Rondônia. After separation of PBMC, monocyte subsets were sorted based on CD14 and CD16 expression using the FACSAria II. We analyzed mitochondrial content in monocytes using MitoTracker Green and Red probes. We also evaluate the role of mitochondrion in monocyte subsets immune response by assessed their recruitment to phagolysosome using ImageStream Mark II Imaging Flow Cytometer. In addition, we assessed the expression of genes involved in mitochondrial metabolism using Nanostring nCounter and evaluated the production of ATP, NAD/NADH and FAD/FADH2 using commercial kits. Results: We demonstrate that the infection with *P. vivax* causes a reprogramming in mitochondrial function in monocyte subsets, where it decreased ATP production and increased ROS production. Our results also indicate a role for mROS in *P. vivax* control by monocyte subsets. Conclusion: Our data suggesting that *P. vivax* infection disturbs mitochondrial metabolism in monocyte subpopulations. **Supported by:**FIOCRUZ, FAPEMIG and CNPq **Keywords:** Monocytes; mitochondrion; malaria