HP001 - CASPASE-11 IS ESSENTIAL TO MEDIATE HOST RESISTANCE AGAINST LEISHMANIA SPP INFECTION.

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Introduction: Leishmaniasis is a disease that affects millions of people worldwide. Although several studies have been performed in order to better understand its pathogenesis, more effective treatments and vaccines haven't been succesfully developed so far. The innate immune response against Leishmania spp. has been shown to be coordinated by pattern recognition receptors. Although NLRP3 interacts with an inflamatory caspase called caspase-1, the functions of another inflammatory caspase in its activation is yet obscure. Thus, the aim of this work is to evaluate the contribution of the non-canonical inflammasome, mediated by caspase-11, in the recognition and restriction of Leishmania infection.
Methods and results: Bone marrow-derived macrophages (BMDMs) from C57BL/6 (WT) and caspase-11-/- mice were used to perform in vitro studies with Leishmania spp. After 24h or 48h of infection, supernatant was collected and used for ELISA and Western Blotting, respectively. Caspase-11-/- BMDMs showed decreased secretion of IL-1β and cleavage of Caspase-1 compared to WT mice, and were less capable of killing L. major, amazonensis and braziliensis parasites, as shown by FACS and Giemsa staining. Pull-down assays revealed that different species of Leishmania spp. are capable of activating Caspase-11. Moreover, mice were infected in vivo in the ear and the lesion was followed by eight weeks. After that, parasite titer in their ear and lymph nodes was determined. Caspase-11-/- mice displayed larger lesion and higher parasite titers compared to WT mice.
Conclusion: Our results suggest that Leishmania spp. trigger Caspase-11 activation, an important effector caspase involved in the non-canonical inflammasome activation, which has only been implicated in the control of bacterial infections so far. Therefore, this work reveals that Caspase-11 is also an important mechanism for the restriction of parasites, with great implications in the course of Leishmaniasis. **Supported by:**FAPESP
Keywords: Leishmaniasis; inflammasome; caspase-11

HP002 - ***LEISHMANIA*** **INFECTION INHIBITS MACROPHAGE MOTILITY BY ALTERING ADHESION COMPLEXES AND F-ACTIN DYNAMICS**

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Leishmania is a protozoan intracellular parasite that causes a broad spectrum of clinical manifestations, ranging from self-healing skin lesions to fatal visceralizing disease. The outcome of infections depends on the parasite species, genetic background, and host immunological status. As the host cells of choice for all species of *Leishmania*, macrophages are critical for the establishment of infections. How macrophages contribute to parasite homing to specific tissues and how parasites modulate macrophage function are still poorly understood. In this study we show that *Leishmania* infection inhibits macrophage roaming motility. The reduction in macrophage velocity is not dependent on parasitophorous vacuole expansion, parasite load or factors secreted by the parasites, and is observed after infection with species causing cutaneous or visceral disease. *L. amazonensis*-infected macrophages also show reduced directional migration in response to the chemokine MCP-1. We found that infected macrophages have lower levels of total paxillin, phosphorylated paxillin and phosphorylated FAK when compared to non-infected macrophages, indicating abnormalities in the formation of signaling adhesion complexes that regulate motility. Analysis of the dynamics of actin polymerization at peripheral sites also revealed a markedly enhanced F-actin turnover frequency in *L. amazonensis*-infected macrophages. Thus, *Leishmania* infection inhibits macrophage motility by altering actin dynamics and impairing substrate attachment and signaling events necessary for propelling cells forward.
Supported by:NIH (R01 AI67979) and CNPq (Ciências sem Fronteiras fellowship)
Keywords: Leishmania; macrophage; migration

HP003 - **ROLE OF TRYPTOPHAN METABOLISM REGULATION BY THE HOST AND THE INTESTINAL MICROBIOTA DURING ORAL INFECTION WITH *TOXOPLASMA GONDII***

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Indoleamine 2,3-dioxygenase (IDO) is an enzyme that catabolizes the initial rate-limiting step of tryptophan (Trp) metabolism and is thought to actively regulate immune activation. It has been previously reported that IFN-γ suppresses the growth of the parasite *Toxoplasma gondii* via induction of IDO *in vitro*. However, the role of the immune regulation of IDO is still unclear and controversial i*n vivo*. Oral infection with *T. gondii* in certain strains of mice results in a Th1-type ileitis that is accompanied by dysbiosis of the gut microbiota. Recent evidences suggest that the microbiota may play a role in the regulation of tryptophan metabolism. Our aim was to evaluate the role of Trp metabolism by the host and the microbiota during infection with *T. gondii*. C57BL/6 mice were orally infected with 10 cysts of a ME-49 strain. Infected mice were orally treated with either 1MT (IDO inhibitor) or ITE (Trp metabolite-like) along the course of infection. Mice were treated with an antibiotic cocktail (Atb) from weaning age. Atb-treated mice infected with *T. gondii* also received either 1MT or ITE during the course of infection. After 8 days, lungs, gut, mesenteric lymph nodes and spleens were collected. Our results show that depletion of the microbiota with Atb led to decreased parasite loads and inflammation in all analyzed tissues. Treatment with ITE alone had no effect on parasitism but reduced the numbers of $CD11b^+$ cells in lymph nodes and gut. Mice treated with 1MT alone showed decreased parasitism and reduced inflammation in lungs, gut and lymph nodes. Mice depleted of microbiota and treated with either ITE or 1MT presented the same reduced parasite burden that was observed in mice treated only with Atb. Our data suggest that regulation of Trp metabolism is important for resistance while the microbiota contributes to the susceptibility against *T. gondii*. Further studies are necessary to address the involvement of different components of the Trp catabolism pathway. **Supported by:**CAPES, CNPq **Keywords:**Toxoplasma gondii; tryptophan; microbiota

HP004 - **THE ROLE OF IL-18 IN THE ACTIVATON OF CD4+ T CELL : THE MAINTENANCE OF CUTANEOUS LESIONS IN MODEL OF INFECTION WITH L. AMAZONENSIS**

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In infection by Leishmania amazonensis the classic model of polarization Th1 and Th2, found in the model of infection with Leishmania major, does not apply. What is described is a mixture of responses by changing the profiles of susceptibility and resistance in mice models. The IL-18 is a pro-inflammatory cytokine that has a dubious role, polarizes T lymphocytes to Th1 phenotype because it has the ability to induce the production of IFN-γ by NK cells and T lymphocytes, however, depending on the environment in which it finds, can induce a Th2 phenotype. Our work aims to identify the role of the IL-18 in the course of infection and development of skin lesions in mice infected by L. amazonensis. Our data showed that the wild-type animals (C57BL/6) have larger lesions than those of IL-18 KO since the beginning of the infection. In addition, the times analyzed, the two strains have the same parasite burden when infected with 10,000 promastigotes forms of L. amazonensis. On the cytokine profile, we found considerable levels of IL-12, TNF in both strains, but we found higher levels of IL-10 in the KO mice. Analyzing the cellular profile, we note that the wt lesions tends to have a larger population of important inflammatory cells. We have also seen that bone marrow-derived macrophages do not express significant levels of IL-18 receptor in front of various stimulations. However, during the course of infection we identified a massive percentage of T cells CD4+ expressing the receptor for IL-18 after the fourth week of infection. This coincides with the appearance of the lesion exacerbated in wt mice and can be associated with the peak production of IL-18 seen by ELISA. When we treat the wt mice with anti-CD4 we found the same lesion profile of the IL-18 KO mice. Our data suggests that the IL-18 is partially involved in susceptibility to infection by L. amazonensis. This is likely due to the interaction of IL-18 on T lymphocytes helping the maintenance of the lesion. **Supported by:**CAPES, FAPEMIG, CNPq **Keywords:**Leishmania amazonensis; il-18; cd4+ t cell

HP005 - **INFLAMMASOME ACTIVATION AND IL-1β PRODUCTION ARE A CONSEQUENCE OF CYTOTOXIC CD8 T CELLS IN *LEISHMANIA* BRAZILIENSIS INFECTION**

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The host immune response plays a critical role in protection from human leishmaniasis, but also promotes disease severity. We have described a pathological role for CD8 T cells in the skin lesions caused by *Leishmania b*raziliensis. Using experimental models of infection and skin samples from infected patients, we found that cytotoxicity induced by CD8 T cells is a major mediator of immunopathology in cutaneous leishmaniasis. However, the downstream mechanisms of cytolytic activity that cause disease severity remain unclear. We hypothesized that dying cells targeted by cytolytic CD8 T cells release damage associated molecular patterns that activate the inflammasome with the consequent release of IL-1β. Using experimental models of cutaneous leishmaniasis, we observed that cytotoxic CD8 T cells induce inflammasome activation as measured by IL-1β protein production and active caspase-1 expression in the skin of infected mice. The increase in IL-1β expression and immunopathology was dependent on commensal bacteria present in the skin, since germ-free mice did not develop severe disease and had significantly less IL-1β expression in the skin in comparison to conventional mice. We also found increased production of IL-1β by cells from the lesions of *L. b*raziliensis patients, and IL-1β levels correlated with granzyme B production. IL-1β blockade prevented the pathology induced by CD8 T cells in vivo. In addition, caspase 1/11 and NLRP3 knockout mice did not develop severe pathology induced by CD8 T cells. Inhibiting the NLRP3 inflammasome with pharmacological inhibitors prevented exacerbated pathology in mice and reduced IL-1β release from patients' lesion biopsies. In summary, our findings demonstrate that inflammasome activation and IL-1β release are a consequence of cytotoxicity induced by CD8 T cells and in cutaneous leishmaniasis leads to immunopathology in the skin and should therefore be considered as targets for immunotherapeutic strategies to lessen the severity of *L. b*raziliensis infections. **Supported by:**NIH **Keywords:**Leishmania braziliensis; inflammasome; cytotoxicity

HP006 - **PROTEASES TRYPTASE AND CHYMASE MODIFY THE IMMUNOMODULATION VIA VASOACTIVE INTESTINAL PEPTIDE IN HUMAN CHAGAS DISEASE**

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Chagas disease, caused by Trypanosoma cruzi, affects millions of people, especially in Latin America. Characteristics of parasite, hosts and their immune system contribute to the evolution of Chagas disease in clinically distinct forms. The interactions between the immune and nervous systems participate in the modulation of immune response, preventing tissue damage. For this purpose, the nervous system acts on the immune system releasing mediators such as neuropeptides, hormones and neurotransmitters. The vasoactive intestinal peptide (VIP) has achieved great prominence in recent times due to its proven immunomodulatory role with potent anti-inflammatory activity. VIP expression is lower in cardiac patients when compared to indeterminate ones. The VIP degrading enzymes such as tryptase and chymase, could also play an important role in the patient's condition once they would modulate VIP levels. Plasma samples from 54 individuals were used to proteases quantification by ELISA (Enzyme Linked Immuno Sorbent Assay). Statistical analysis showed that plasma levels of chymase are higher in individuals with cardiac form when compared with the group of healthy individuals. Individuals with the indeterminate form of the disease also present higher chymase plasma levels than uninfected individuals. With regard to plasma levels of tryptase, no statistical differences were observed between analysed groups. Positive correlation between tryptase and chymase levels were observed only in cardiac form of Chagas disease, indicating that the higher the chymase levels, the higher the tryptase levels in these individuals. Together, these results show that the lowest levels of VIP, observed in cardiac form of Chagas disease could be due to a superior production of the degrading enzyme chymase and/or due to the interaction between chymase-tryptase in cardiac patients, which could contribute to the maintenance of an exacerbated inflammatory response. **Supported by:**FAPEMIG **Keywords:**Chagas disease; vasoactive intestinal peptide; chymase

HP007 - **BONE MARROW MESENCHYMAL STEM CELL AS NEW HOST CELL FOR LEISHMANIA INFANTUM**

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Leishmaniasis is one of the most important protozoan diseases that cause latent infectious. Visceral Leishmaniasis is the most serious form of these disease and it is caused by protozoan parasites of the Leishmania donovani complex: L. donovani and L. archibaldi in the Old World (India and Southern Asia), and L. infantum chagasi (also known as L. chagasi) in Southern Europe, Africa, and South America. L. donovani complex organisms are intracellular pathogens transmitted by phlebotomine female sandflies that primarily infect the reticuloendothelial cells of spleen, liver and bone marrow (BM). It has been reported that L. infantum infects not only professional phagocytic cells but also other cell types such as human epithelial cells, and fibroblast cells. VL is endemic in 47 countries; and little is known about the mechanism by which L. donovani complex organisms persist in a “latent-like” stage within the host during subclinical conditions, asymptomatic disease as well as after “successful” clinical treatment. The aim of the present study was to investigate whether Leishmania would be able to infect mesenchymal stem cells from bone marrow (BM-MSCs). We report that L. infantum was able to infect BM-MSCs CD271 + Sca-1 + population in vitro. In murine model of LV we found that MSCs CD271 + CD45- from bone marrow and spleen were positive for Leishmania in animals after 15 days of infection, and some amastigote-like forms were found in different time points of infection. These results suggest that CD271+ BM-MSCs may provide an intracellular niche in the host in which L. infantum can persist in latent infection.**Supported by:**Capes, CNPQ, NIH
Keywords: Leishmania infantum chagasi; visceral leishmaniasis; mesenchymal stem cells

HP008 - **THE JOURNEY OF *LEISHMANIA DONOVANI*: FROM PERIPHERY TOWARDS THE CENTRAL NERVOUS SYSTEM**

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Visceral leishmaniasis is a zoonotic disease caused by the parasitic protozoans *Leishmania donovani* and *L. infantum*. In both humans and dogs an inflammatory state is observed in the central nervous system, nevertheless, the pathogenesis of the brain lesions remains poorly understood and parasites are rarely seen. Therefore, this study aimed to evaluate the dynamics of the infection and the inflammatory response in the brain. To this end, Balb/c mice were infected with a virulent strain of *L. donovani* expressing the gene of the firefly luciferase and analyzed during 120 days. Using real-time in vivo bioluminescence imaging, we detected and quantified parasites in different tissues. The liver and the spleen presented high parasite load throughout the infectious process, attesting parasite implantation and persistence in our model. From day 3 post-infection, significant bioluminescent signals revealed the presence of live parasites in the brain, confirmed by RT-qPCR and parasite culture. Moreover, as neutrophils and Ly6Chigh monocytes are elevated in the brain, blood and bone marrow within the first 14 days p.i., along with matrix metalloproteinases enzymes, these cells could carry the parasites into the brain, resembling the “Trojan horse” mechanism. Additionally, we noted a two-phased brain inflammation pattern: a first phase occurring from day 3 to day 14 p.i., with remarkable concomitant overexpression of IFN-γ and IL-10, and a broad range of chemokines and chemokine receptors; and a re-inflammatory phase starting at 90 days p.i., with a specific proinflammatory profile, including IL-1β and TNF-α. Altogether, these results provide new evidences on the dynamics of the inflammatory process during *Leishmania* infection and open up the perspective that these phenomena in the brain could trigger the occurrence of nervous symptoms during the disease. **Supported by:**FAPESP-BEPE-DR_2014/03078-3 - PTR 403 - Institut Pasteur
Keywords: Brain inflammation; molecular imaging; visceral leishmaniasis

HP009 - **INVOLVEMENT OF SOCS2 IN REGULATION OF DENDRITIC CELLS FUNCTION DURING TRYPANOSOMA CRUZI INFECTION**

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Introduction: The infection by *Trypanosoma cruz*i (Tc) induces an inflammatory reaction and the efficacy of the host immune response (IR) is important to persist or eliminate the infection. SOCS (supressor of cytokine signaling)2 is fundamental during Tc infection by modulating the T cells and heart function. The role of SOCS2 in dendritic cells (DCs) and induction/maintenance of IR during Tc infection is unknown and was investigated herein. Methods/Results: CD11cDTR transgenic mice (DCs depletion), wild type (WT) and SOCS2 (knockout$^{-/-}$) were infected with Y strain of Tc. Our results demonstrated an increased parasitemia in depleted DCs mice, emphasizing the crucial role of DCs in this infection. During innate IR, absence of SOCS2 decreased the frequency of DCs producing IL-12 and TNF, but not IL-10, without change the Toll-like receptor (TLR) 2 and 4 and MHCII expression. In contrast, decreased expression of CD80 costimulatory molecule was observed in DCs SOCS2$^{-/-}$. During adaptive IR, absence of SOCS2 in DCs resulted in increased levels of TLR2 and 4 expression and reduced frequency of DCs expressing MHCII. Adoptive transfer of DCs SOCS2$^{-/-}$ increased parasitemia and changes in IR profile against Tc infection, specifically: i) reducing frequency of cells NK IFN-γ^+ and IL-17$^+$; ii) reducing frequency of T cells CD8$^+$IFN-γ^+ and CD4$^+$IL-17$^+$, despite increasing T CD4$^+$IFN-γ^+; absence of SOCS2 in DCs also resulted in reduction of IL-10 by CD4$^+$ CD19$^+$ and Treg cells. SOCS2 is also important in apoptosis modulation during Tc infection, where absence of SOCS2 leads to increased apoptosis in neutrophils during innate IR, macrophages in innate and adaptive IR and lymphocytes in adaptive IR. Conclusion: Together, our results demonstrated that SOCS2 is crucial in the modulation of DCs' function during generation/regulation of innate and adaptive IR during Tc infection. Financial Supported by CNPq and FAPEMIG. **Supported by:**CNPQ E FAPEMIG
Keywords: Trypanosoma cruzi; dendritic cells; socs2

HP010 - **CIRCULATING BIOMARKERS OF IMMUNE ACTIVATION, OXIDATIVE STRESS AND INFLAMMATION CHARACTERIZE SEVERE CANINE VISCERAL LEISHMANIASIS**

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Clinical manifestations in canine visceral leishmaniasis (CVL) have not been clearly associated with immunological status or disease progression. We simultaneously assessed biomarkers of inflammation, immune activation, oxidative stress, and anti-sand fly saliva IgG concentrations in dog sera with different clinical manifestations to characterize a biosignature associated with CVL severity. In a cross-sectional exploratory study, a random population of 70 dogs from an endemic area in BRA was classified according to CVL clinical severity and parasitological evaluation. A panel of biomarkers and anti–sand fly saliva IgG were measured in canine sera. Assessment of protein expression of profile biomarkers identified a distinct biosignature that could cluster separately animal groups with different clinical scores. Increasing severity scores were associated with a gradual decrease of LTB4 and PGE2, and a gradual increase in CXCL1 and CCL2. Discriminant analyses revealed that combined assessment of LTB4, PGE2 and CXCL1 was able to distinguish dogs with different clinical scores. Dogs with the highest clinical score values also exhibited high parasite loads and higher concentrations of anti-saliva antibodies. Our findings suggest CVL clinical severity is tightly associated with a distinct inflammatory profile hallmarked by a differential expression of circulating eicosanoids and chemokines. **Supported by:**FAPESB, INCT
Keywords: Dog; visceral leishmaniasis; biomarker

HP011 - **PARTICIPATION OF ADAPTER MOLECULE TRIF IN RESISTANCE OF C57BL/6 MICE AGAINST *NEOSPORA CANINUM* INFECTION**

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Neospora caninum is an intracellular parasite that has the dog as its definitive host and other mammals, especially cattle, as intermediate hosts. Economically, neosporosis is an important disease in Veterinary medicine due to the induction of relevant clinical signs, as abortions in cattle and neuromuscular paralysis in dogs. The aim of this study was to evaluate the role of the TLR adaptor protein TRIF in the resistance against N. caninum infection. For this, in vitro experiments with bone marrow derived macrophages (BMDMs) from C57BL/6 wild-type (WT) and TRIF knockout (TRIF-/-) mice, stimulated by tachyzoites and in vivo infections, were performed in order to investigate the production of cytokines and antibodies, cellular and tissue parasitism, histological changes during different phases of infection and survival analysis. We observed that TRIF-/- BMDMs presented notable defects in inflammatory cytokine production in relation to WT macrophages. Additionally, we found that the concentration of NO, IL-12p40, IFN-y and TNF were decreased in peritoneal fluids and lungs of TRIF-/- mice, while IL-2, IFN-γ, TNF and IL-17 were reduced in sera of these animals compared to WT mice. Higher parasite burden was observed in peritoneal cells, lungs and brain during the acute and chronic phases of infection, which were associated with inflammatory changes in the analyzed tissues, while TRIF-/- mice survival rate decreased 2-fold compared to WT. In conclusion, our results show that TRIF is required for resistance against the infection induced by N. caninum, regulating the production of key Th1 cytokines and participating in the control of the tissue parasitism and inflammatory lesions induced against the parasite. **Supported by:**FAPEMIG
Keywords: Neospora caninum; trif; innate immunity

HP012 - **EVALUATION OF BALB/XID MICE IN INFECTION BY *LEISHMANIA AMAZONENSIS***

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Leishmaniasis is a neglected disease and Leishmania amazonensis is the etiological agent of diffuse cutaneous leishmaniosis in BRA. This work aims to study the role of B lymphocyte using Xid in infection by L. amazonensis. Xid mice have a mutation on Bruton's tyrosine kinase's coding gene, because of it those animals have lower frequency of B1 and B2 lymphocyte's than wild type mice, mostly B1 lymphocyte. The infection was performed inoculating stationary promastigotes form of L. amazonensis in the footpad's of mice. The lesion's growth was weekly checked using caliper. Mice were euthanized and we used the macerated infected footpad and the draining lymph node for the limiting dilution analysis to determine the parasite load and the cells from lymph nodes for analysis by flow cytometry. Our results demonstrated that Xid mice displayed smaller lesions in comparison to wild type. However, the parasite load obtained on both infected footpad and lymph node were very similar. Besides, we observed an increase in the frequency of T CD4+, TCD8+, regulatory TCD4+ cells on the Xid's lymph nodes but there was no differences in effector cells producing INF- γ. Cytokines' analysis in the infected footpad, draining lymph node and spleen showed a decrease of IL-10 production by Xid mice, what is probably related to the low level of B cells. Xid mice had lower levels of IgM and IgG1 in the serum when compared to wild type. We performed in vitro interaction using peritoneal B1 lymphocytes in the presence or absence of LPS and/or L. amazonensis. IL-10 levels' analysis were made and , in the presence of both stimulus, B1 lymphocytes were capable to produce higher levels of IL-10 when compared to the control, suggesting that the low level of this cytokine in vivo could be related to the low numbers of B1 lymphocytes on Xid mice. In conclusion, B cells are associated to pathogenesis in murine cutaneous leishmaniasis caused by L. amazonensis through production of IgG1 and IL-10.
Keywords: Leishmania; leishmania amazonensis; b lymphocyte

HP013 - ***TOXOPLASMA GONDII* IMPAIRS CEREBRAL MICROCIRCULATION DAMAGE AND INCREASES LEUKOCYTE-ENDOTHELIAL ADHESION IN MICE**

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Toxoplasmosis is a worldwide spread zoonosis caused by *Toxoplasma gondii*, an obligate intracellular protozoan that affects all warm-blooded animals, including humans. *T. gondii* persists during the chronic phase of the disease in the form of tissue cysts, mainly in brain and skeletal muscle. Cognitive and behavioral alterations were already reported in infected hosts. The brain has a highly regulated microenvironment, mainly maintained by the Blood-Brain Barrier (BBB), composed by endothelial cells, astrocytes and pericytes. Damage to BBB has been shown in various disease models, accompanied with inflammatory cells infiltration to the brain parenchyma and neurotoxicity. Increased expression of adhesion molecules have been described in brains of *T. gondii*-infected mice, suggesting an increase in leukocyte rolling/adhesion, resulting in neuroinflammation. The purpose of this study is to analyze the cerebral microcirculation during acute and chronic infection of mice by *T. gondii* (ME49 strain). Using Laser Speckle, we observed that infected animals had a significant reduction in cerebral blood flow after 10 and 40 days of infection, corresponding to acute and chronic stages of the disease, respectively. Intravital microscopy imaging revealed that infected animals showed a progressive cerebral capillary rarefaction, with 30 and 43% less spontaneously perfused capillaries, at 10 ($p<0.01$) and 40 dpi ($p<0.001$). Infection increased drastically the number of rolling and adhered leukocytes, especially at 40 dpi. Vascular response to Acetylcholine (Ach) was impaired in infected animals. While uninfected animals showed increase in arteriolar caliber, chronically infected animals showed vasoconstriction after Ach stimulus. Our data indicate that T. gondii infection induces capillary rarefaction and dysfunction of the vascular endothelium cells. These results will contribute to a better understanding of the mechanisms involved in the pathogenesis of cerebral toxoplasmosis. **Supported by:**CNPq (Edital Universal), PAPES VII/Fiocruz **Keywords:**Toxoplasmosis; neuroinflammation; microcirculation

HP014 - **INFESTATION BY RHIPICEPHALUS SANGUINEUS INTERFERE WITH THE COURSE OF THE DISEASE AND IMMUNOLOGICAL PARAMETERS IN RESISTANT MICE INFECTED WITH LEISHMANIA MAJOR.**

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Dogs are considered the main domestic reservoirs of visceral leishmaniasis in BRA and many regions of the world. In nature, a wide range of coinfections may take place in dogs, especially those caused by brown ticks. Tick's saliva has many properties aiming the vector to circumvent the clothing, inflammatory and perception mechanisms of the host. However, the role of the immunomodulation driven by ticks in the outcome of leishmaniasis is poorly known and is still a missing step of the host-parasite interface. Mice (C57BL/6 and BALB/c) were previously infested once by two adult females of *Rhipicephalus sanguineus* on the dorso-thoracic skin, for 7 to 10 days and infected with 10^5 metacyclic promastigotes of *Leishmania major* in the left hind paw as well as a non-infested group. As a control group for some experiments, mice infested by *R. sanguineus* were inoculated in the footpad with sterile PBS. Footpad thickness was monitored weekly. Parasitism and immunologic parameters were measured at five and eight weeks post infection. The titers of anti-*Leishmania* antibodies and IL-10 were measured in sera by ELISA and the parasite load in the skin and spleen was measured by qPCR. Infestation with *R. sanguineus* promoted a minor increase in the thickness of the infected footpad when compared to non-infested mice. However, the parasite load in the paw and spleen was slightly higher in infested groups. Moreover, no difference in footpad swelling was observed in BALB/c mice, but the parasite load in the footpad was slightly higher in the infested group. Interestingly, titers of anti-Leishmania IgG1 and IgG2 antibodies were lower in infested mice eight weeks after the end of infestation. Serum levels of IL-10 were higher in both groups infested by *R. sanguineus* five weeks after infection, suggesting a long term effect of tick's driven immunomodulation. Those data strongly suggests that ticks may affect the immunity and the course of the disease in co-infected mice harboring *Leishmania major*. **Supported by:**CAPES, CNPq, FAPEMIG **Keywords:**Rhipicephalus sanguineus; leishmania; immunomodulation

HP015 - **CHARACTERIZATION OF THE HOST CELL NUCLEAR COMPARTMENT DURING THE INFECTION WITH *TRYPANOSOMA CRUZI***

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Interaction between *Trypanosoma cruzi* parasite and the host cell still is a target of studies due to the number of people affected worldwide and the lack of effective treatments. During the *T. cruzi* infection, morphological changes are observed in the host cell cytoplasm immediately upon contact of parasite to the cell. It also has been reported that different types of pathogens modulate the expression of several proteins during their permanency in the host cell. Furthermore, it has been demonstrated using *Leishmania* infected cells alterations in nuclear proteins at the early hours of infection through proteomics analysis, suggesting that parasites were able to promote changes in the nuclear compartment. Thus, the aim of this study is to investigate the dynamic of the nuclear compartment of the host cell during the infection with *T. cruzi* using specific markers to the host nuclear compartments. LLC-MK2 cells were infected with *T. cruzi* in different times and confocal microscopy analysis showed that parasite is able to modulate the morphology and dynamics of the nuclear organelles involved in transcriptional events, such as Cajal bodies and Speckles. Immunofluorescence and Western Blot analyses revealed that the heterogeneous nuclear ribonucleoproteins involved in the regulation and transport of newly transcribed RNAs in host cell are also modulated during the intracellular parasite proliferation. These results are strong evidence that the parasite can control host transcriptional events in behalf of its own survival. This study will help to understand the parasite biology and its interaction with the host cell, contributing to the search for possible therapeutic targets. **Supported by:**CAPES E FAPESP
Keywords: Trypanosoma cruzi; host cell nucleus; nuclear compartments

HP016 - **EXPRESSION OF CTLA-4 AND TIM-3 IN SPLEEN OF NATURALLY INFECTED DOGS WITH VISCERAL LEISHMANIASIS**

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The spleen is one of the main affected organs in canine visceral leishmaniasis (CVL). The disorganization of splenic white pulp (SWP), immunosuppression and decrease of cytokines and chemokines expression have been observed in CVL, compatible findings with cellular exhaustion. As the spleen is considered a persistence parasitic organ, could cell exhaustion play a role in failure in parasite replication control? This study aims to evaluate the in situ expression of exhaustion markers and its relation to histopathology and parasite load. 41 dogs were grouped according to SWP organization as: 1-Organized to slightly disorganized (OR-SD, n=11); 2-Moderate to intense disorganization (MD-ID; n=30). The parasite quantification through qPCR using ssRNA target, histopathology and immunohistochemistry were done. CD4+, CD8+, IFN-γ+, IL-10+, TIM-3+ and CTLA-4+ cells were detected. The disorganization of SWP occurred with the gradative reduction of lymphoid follicles/mm2 ($p<0.05$), CD4+/mm2 ($p=0.033$) and disease worsening ($p=0.024$). The MD-ID animals with high parasite load showed reduction of CTLA-4 cell number when compared to OR-SD animals with low parasite load ($p=0.033$). Although frequent, TIM-3 expression was similar between groups (4.73 to 52.2%). There was a correlation between expression of TIM-3 and CTLA-4 ($r=0.444$; $p=0.004$). The data suggest that: 1. T cells exhaustion can occur in the early stages of infection, since OR-SD animals showed TIM-3 and CTLA-4 cells; 2. The reduction in the number of lymphoid follicles and CD4+ cells could be a result of exhaustion and cell death; 3. There was no change in TIM-3, probably because other cells might be expressing this molecule; 4. The blockage of the exhaustion process as therapeutic should consider TIM-3 pathway blocking; 5. In conclusion, the results suggest a potential association between exhaustion and splenic disorganization, CD4 lymphocyte number reduction, failure of parasite control and the worsening of the disease.
Supported by:PAEF-IOC/Fiotec, FAPERJ e CNPq
Keywords: Leishmaniasis; cellular exhaustion; spleen

PV001 - **THE MITOCHONDRIAL CALCIUM UNIPORTER B (MCUB) OF *TRYPANOSOMA CRUZI* IS REQUIRED FOR CA2+ UPTAKE AND IS ESSENTIAL FOR PARASITE GROWTH AND INFECTION**

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Trypanosoma cruzi possesses a mitochondrial calcium uniporter (TcMCU) with similar characteristics to that of mammalian mitochondria. Several components of the TcMCU complex have been identified in the *T. cruzi* genome, and therefore the complex is predicted to be constituted by a main pore subunit (MCU), and at least other 3 accessory proteins: MCUb, MICU1, MICU2. MCUb is a paralogue of MCU and exhibits two critical substitutions in negatively charged amino acid residues conforming the pore region of the channel. In human cells MCUb has been found to exert a dominant-negative effect reducing the mitochondrial Ca^{2+} increase evoked by agonist stimulation. We aimed at investigating the physiological role of the MCUb component of TcMCU by generating overexpressing (TcMCUb-OE) and knockout (TcMCUb-KO) *T. cruzi* cell lines. We report here that the overexpressed TcMCUb tagged with 3xHA displayed the predicted molecular weight (31 kDa) and mitochondrial localization in *T. cruzi* epimastigotes. Moreover, disruption of TcMCUb by CRISPR/Cas9 (TcMCUb-KO) resulted in mitochondria unable to transport Ca^{2+} but preserving the mitochondrial membrane potential in digitonin-permeabilized epimastigotes, whereas the overexpression of TcMCUb (TcMCUb-OE) caused a significant increase in the ability of mitochondria to accumulate Ca^{2+} and generated an increase in reactive oxygen species production. In contrast to our previous results obtained with TcMCU knockout cell line, TcMCUb-KO epimastigotes exhibited a significant growth defect. Moreover, they showed a lower in vitro differentiation rate (metacyclogenesis) and were unable to infect mammalian tissue-cultured cells. In conclusion our results indicate that TcMCUb is a key component of the TcMCU that does not exert a dominant-negative effect on this channel but is essential for parasite survival along its life cycle. Work funded by FAPESP N° 2013/50624-0, 2011/50400-0 and 2014/13148-9. **Supported by:**FAPESP
Keywords: Mitochondrial calcium uniporter; crispr/cas9; trypanosoma cruzi

PV002 - **COMPARATIVE TRANSCRIPTOME PROFILING OF VIRULENT AND NON-VIRULENT *TRYPANOSOMA CRUZI* UNDERLINES THE ROLE OF SURFACE PROTEINS DURING INFECTION**

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Trypanosoma cruzi, the protozoan that causes Chagas disease, has a complex life cycle involvingseveral morphologically and biochemically distinct forms that interact with various insect and mammalian hosts. It has also a heterogeneous population comprising strains that shows distinct properties includingvirulence, sensitivity to drugs, antigenic profile and tissue tropism. We present a comparative transcriptome analysis of two cloned *T. cruzi* strains that display contrasting virulence phenotypes in animal models of infection: CL Brener, a virulent strain and CL-14, a strain that is neither infective nor pathogenic inin vivo models of infection. RNA-seq analysis of CL Brener epimastigotes, trypomastigotes and intracellular amastigotes harvested at 60 and 96 hours post-infection (hpi) of human fibroblasts revealed large changes in gene expression that reflect the parasite's adaptation to distinct environments during infection of the insect vector and mammalian cells, including genes involved with energy metabolism, oxidative stress responses, cell cycle control and cell surface components. Whereas an extensive transcriptome remodeling was observed in CL Brener and CL-14 trypomastigotes compared to 60 hpi amastigotes, only minor differences were observed when 96 hpiamastigotes and trypomastigotes of CL Brener were compared. In contrast, the differentiation of 96 hpiamastigotes to trypomastigotes in the avirulent CL-14 was associated with large changes in gene expression, particularly regarding genes encoding surface proteins such as trans-sialidases and the mucin associated surface proteins (MASPs). We conclude that the avirulent phenotype of CL-14 may be due, at least in part, to a delayed expression of genes encoding surface proteins that are likely involved with the establishment of the infection in the mammalian host. **Supported by:**CAPES, FAPEMIG, CNPq
Keywords: Trypanosoma cruzi; transcriptome; virulence

PV003 - **CONDITIONAL DELETION OF *HUS1* REVEALS A DUAL ROLE FOR THE 9-1-1 CLAMP IN THE STABILITY OF THE *LEISHMANIA* GENOME.**

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The 9-1-1 complex (RAD9, RAD1 and HUS1) is a DNA clamp that is pivotal for genome stability and participates in many aspects of the DNA damage response in eukaryotes. In *Leishmania major*, the 9-1-1 complex is conserved but, surprisingly, subunits RAD9 and HUS1 are found in alternative complexes in vivo. To further elucidate the function of HUS1, we generated a cell line in which its expression can be conditionally abrogated by the excision of the *HUS1* locus upon induction of the diCre recombinase activity. Conditional deletion of *HUS1* resulted in increased DNA synthesis and in a higher proportion of cells with a DNA content <2N. Accordingly, when these cells were released from G1/S arrest, they showed defective S phase progression and G2/M transition, and also a decrease in DNA damage-related H2A phosphorylation levels. When *HUS1* null cells were released from mid-S phase arrest, they also presented defective S phase progression and G2/M transition, despite increased levels of H2A phosphorylation. These data indicate that *i*) consistent with its detection outside the 9-1-1 complex, HUS1 has evolved to have at least two functions during the cell cycle, namely in S phase and G2/M phase; *ii*) although these functions are apparently compartmentalized, they seem to be coordinated to guarantee the orchestration between genome duplication and mitosis onset; *iii*) checkpoint activation in G1/S or mid-S are determinant for the HUS1-dependent signaling outcome. The evaluation of genome wide effects of the abrogation of HUS1 expression is currently being performed using next generation sequencing. Copy number variation, SNPs frequency and ploidy changes will be determined to further establish how HUS1 activity regulates genome stability in this parasite. **Supported by:**FAPESP
Keywords: Dna damage repair ; genome plasticity; gene amplification

PV004 - **THE HIGH MOLECULAR WEIGHT PROTEIN FAZ10 IS REQUIRED FOR FLAGELLUM/FAZ STABILIZATION AND CLEAVAGE FURROW POSITIONING IN TRYPANOSOMA BRUCEI**

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Trypanosoma brucei, the causative agent of sleeping sickness, has been used as experimental model for cellular, biochemical and molecular studies. The flagellum, which is the most prominent organelle, harbors most of the cytoskeletal content of the cell. It also comprises the axoneme and paraflagellar rod and both are linked to the cell body via the Flagellar Attachment Zone (FAZ). The FAZ is a large and complex interconnected set of fibres, filaments and junctional complexes composed of several cytoskeletal proteins. In all genera of the Trypanosomatidae family, a novel class of High Molecular Weight Proteins (HMWPs; 500-3500 kDa) has been described, which may play a role in the organization and regulation of the cytoskeleton. Trypanosomatid cytoskeletons contain abundant HMWPs, but many of their biological functions are still unclear. Aiming to describe the cellular and molecular properties of these proteins, here we report the characterization of the giant FAZ protein FAZ10 in T. brucei. Detergent-extracted cytoskeletons were analyzed by gradient SDS-PAGE and the HMWP bands were sent to MS analysis. We obtained a list with several putative proteins and FAZ10 matched for the highest score for unique peptides. Immunofluorescence using polyclonal antibody against FAZ10 demonstrated its localization along the FAZ and electron microscopy showed its specific localization at the intermembrane staples. Next, we used a RNAi inducible system in the 2913 cell line to knockdown FAZ10 and characterize its functional role in procyclic and bloodstream forms of T. brucei. Our data showed that FAZ10 is an essential giant cytoskeletal protein in both procyclic and bloodstream parasite life cycle stages, since its depletion led to defects in cell morphogenesis, flagellum attachment and kinetoplast and nucleus positioning. Moreover, ablation of FAZ10 impaired the timing and placement of the cleavage furrow during cytokinesis, resulting in premature or asymmetrical cell division. **Supported by:**FAPESP, FAEPA, CAPES-PDSE, CNPq **Keywords:**Giant protein; cytoskeleton; faz10

PV005 - **UAP56 IS A CONSERVED CRUCIAL COMPONENT OF A DIVERGENT MRNA EXPORT PATHWAY IN TOXOPLASMA GONDII**

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Nucleo-cytoplasmic RNA export is an essential post-transcriptional step to control gene expression in eukaryotic cells and is poorly understood in apicomplexan parasites. With the exception of UAP56, a component of TREX (Transcription Export) complex, other components of mRNA export machinery are not well conserved in divergent supergroups. Here we use Toxoplasma gondii as a model system to functionally characterize TgUAP56 and its potential interaction factors. We demonstrate that TgUAP56 is crucial for mRNA export and that functional interference leads to significant accumulation of mRNA in the nucleus. It was necessary to employ bioinformatics and phylogenetic analysis to identify orthologs related to mRNA export, which show a remarkable low level of conservation in T. gondii. We adapted a conditional Cas9/CRISPR system to carry out a genetic screen to verify if these factors were involved in mRNA export in T. gondii. Only the disruption of TgRRM_1330 caused accumulation of mRNA in the nucleus as found with TgUAP56. This protein is potentially a divergent partner of TgUAP56, and our results provide insights into a divergent mRNA export pathway in apicomplexans. **Supported by:**Programa Estratégico de Apoio à Pesquisa em Saúde (PAPES/FIOCRUZ,) [407775/2012-9 –CNPq]; Fun
Keywords: Mrna export pathway; conditional cas9 system; toxoplasma gondii

PV006 - **INVOLVEMENT OF THE CAP-BINDING PROTEIN EIF4E1 IN TRANSLATION REPRESSION OF TRYPANOSOMATIDS**

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The cap binding protein eIF4E is a major player during protein synthesis and it is also involved in several cellular processes regarding mRNA metabolism and transport. During translation, eIF4E is part of the trimeric complex eIF4F (composed by eIF4E, eIF4A and eIF4G subunits) and functions by binding to the mRNA's 5' cap structure, facilitating the recruitment of the translation apparatus. In trypanosomatids there are six eIF4E homologues (EIF4E1 to 6) and recent studies have separated them in three groups. EIF4E1 and EIF4E2 are part of group 1, the least understood of these homologues. Recent data, however, has indicated that EIF4E1 might be a regulator of gene expression, since it is able to reduce expression of a reporter mRNA when tethered to its 3' untranslated region. Here we aimed to further characterize the molecular properties of EIF4E1 from *T. brucei.* Firstly, we evaluated its expression and binding partners, after transfection of procyclic forms with a PTP-tagged construct. Cell extracts of cultures at early, mid and late log phase were used in Western blot assays which confirmed a constitutive expression of EIF4E1. Immunoprecipitation assays were then performed followed by mass spectrometry analysis. EIF4E1 failed to interact with any eIF4G homologue, however it bound to the *T. brucei* orthologue of the EIF4E1 interacting protein (4E1-IP), previously described in *Leishmania* and whose functions remain unknown. In a second approach, we evaluated the impact of EIF4E1 overexpression on mRNA reporters harboring the GFP ORF. Our results shows that EIF4E1 overexpression impairs cell growth and, when tethered to the mRNA, EIF4E1 decreases GFP protein levels, with no changes in the respective transcript, indicating that the translation of this mRNA was inhibited. EIF4E1, and its partner 4E1-IP, emerge then as possible translation repressors, however, their roles on translation of endogenous mRNAs need to be investigated. **Supported by:**CNPq, FIOCRUZ, FACEPE
Keywords: Eif4e; translation; gfp reporter

PV007 - **ACTIN BINDING PROTEINS (ABPS) FROM *NEOSPORA CANINUM*: ROLE OF ACTIN-DEPOLYMERIZING FACTOR (NCADF) IN ACTIN DYNAMICS**

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Neospora caninum, an apicomplexan intracellular parasite, is one of the main causes of infectious abortion in cows. In apicomplexan organisms, the filamentous actin (F-actin) has a central role in the generation of invasion and gliding motility at the motor complex, although actin dynamics are unusual. In this context, ABPs emerge as being responsible for actin dynamic regulation. Biochemical characterisation of ABPs will enlarge the comprehension of these proteins and their role in the cytoskeleton function of *N. caninum*. In this study we are investigating recombinant NcADF (actin-depolymerising factor – ADF – from *N. caninum*; expressed in BL21-DE3 cells) function in actin dynamics employing rabbit skeletal muscle actin as an in vitro model. In co-sedimentation assay recombinant NcADF enhanced a pH-independent disassembly of F-actin. However, the affinity of NcADF for F-actin, although weak, was pH-dependent and observed only at low pH (pH 6.5). Evidences of severing were observed as the improvement of the polymerisation rate of actin and an acceleration of late stages of polymerisation followed by a drop on fluorescence when higher concentrations of NcADF were used (6 or 10 µM). The drop of fluorescence was inhibited by the addition of 25 mM phosphate buffer into the reaction. Another evidence was a dose-dependent reduction of relative viscosity of F-actin solution in presence of NcADF. Depolymerisation assays were performed using 1 and 0.1 µM in high and low salt conditions, respectively. Both conditions resulted in positive but nonlinear dependence of K_{obs} on NcADF concentration, indicating a cooperative binding of NcADF and rabbit actin. The affinity of Mg-ATP-G-actin and Mg-ADP-G-actin with NcADF suggested that NcADF had a higher affinity with ADP-actin. This study concluded that NcADF has a conserved mechanism of action, and the elucidation will be useful not exclusively to the cytoskeleton of *N. caninum* but also to the whole phylum Apicomplexa. **Supported by:**Fundação de Amparo à Pesquisa do Estado de São Paulo - FAPESP
Keywords: Actin depolymerising factor - adf; neospora caninum; actin-binding proteins - abps

PV008 - **USING *TRYPANOSOMA CRUZI* TO RE-WRITE THE HISTIDINE DEGRADATION PATHWAY**

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Amino acids, such as histidine (His), are critical as energy and carbon sources for *Trypanosoma cruzi*. His is able to keep the parasites viable under severe metabolic stress and can fuel H^+ to the respiratory chain, through its oxidation to CO_2. Furthermore, it's known that its uptake occurs in an ATP dependent manner via a single transport system. The information available in the *T. cruzi* genome databases suggests that this is able to metabolize His to glutamate (Glu) through the classically described 4-enzymatic-steps pathway. Yet, a previous work alerted us about the possible existence of a non-enzymatic branch which was studied using a chemical system. However, to date the study of a possible occurrence of this branch in living organisms was not tackled. In this work, we demonstrated this new configuration for His catabolism. We first cloned, expressed and characterized the putative genes for the firsts two enzymes of *T. cruzi* His metabolic pathway -histidine ammonium lyase (HAL) and urocanate hydratase (UH) - in *Escherichia coli.* The kinetic parameters were estimated: K_M=0,77±0,33 mM and V_{MAX}=4,03±1,87 µmols of uro/min*mg protein for HAL and K_M=19,07±2,09 µM and V_{MAX}=39,9±4,06 nmols of uro/min*mg protein for UH. Then, we expressed HAL and UH in *Saccharomyces cerevisiae* strain W303-1a, which lacks the His catabolic pathway and is unable to oxidize this amino acid to CO_2 through OxPHOS. Our results show that, when expressing HAL and UH (confirmed by the measurements of enzymatic activities in cell-free extracts), *S. cerevisiae* was able to oxidize His to CO_2 through OxPHOS, without the need of its conversion into Glu, as described up until now. Using U-^{14}C-His we could measure a global metabolic flux corresponding to the consumption of 0,43±0,08 nmols His/min*25x10^{6}cels. As a whole, this work demonstrates that His oxidation is fully operative when only the first two enzymatic steps are present, and opens a new perspective in the studies of His bioenergetics. **Supported by:**CAPES **Keywords:**Trypanosoma cruzi; histidine; bioenergetic

PV009 - **CRISPR/CAS9-MEDIATED ENDOGENOUS C-TERMINAL TAGGING OF *TRYPANOSOMA CRUZI* GENES REVEALS THE CELLULAR LOCALIZATION OF PROTEINS INVOLVED IN CALCIUM SIGNALING**

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Methods for genetic manipulation of *Trypanosoma cruzi*, the etiologic agent of Chagas disease, have been highly inefficient and no endogenous tagging of genes has been reported to date. In this work we aimed at using the CRISPR/Cas9 system for endogenously tagging genes in this parasite. The utility of the method was established by tagging genes encoding proteins of known localization such as the flagellar calcium binding protein (TcFCaBP), and the vacuolar proton pyrophosphatase (TcVP1), and three proteins of undefined or disputed localization, involved in calcium signaling, the pyruvate dehydrogenase phosphatase (TcPDP), the mitochondrial calcium uniporter (TcMCU), and the inositol-1,4,5-trisphosphate receptor ($TcIP_3R$). We confirmed the flagellar and acidocalcisome localization of TcFCaBP and TcVP1 by co-localization with antibodies to the flagellum and acidocalcisomes, respectively. As expected, TcPDP and TcMCU were co-localized with the voltage-dependent anion channel (VDAC) to the mitochondria. Finally, endogenously tagged $TcIP_3R$ showed co-localization with antibodies against VP1 to acidocalcisomes, in contrast to previous reports using overexpressed $TcIP_3R$. These results are also in agreement with our previous reports on the localization of this channel to acidocalcisomes of *T. brucei* and suggest that caution should be exercised when overexpression of tagged genes is done to localize proteins in *T. cruzi*. Work funded by: FAPESP N° 2013/50624-0, 2011/50400-0 and 2014/08995-4. **Supported by:**FAPESP
Keywords: Crispr/cas9; endogenous c-terminal tagging; trypanosoma cruzi

PV010 - **CHROMOSOMAL COPY NUMBER VARIATION REVEALS EXTENSIVE LEVELS OF GENOMIC PLASTICITY AMONG AND WITHIN *TRYPANOSOMA CRUZI* DTUS**

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The taxon *T. cruzi* is divided into six discrete typing units (DTUs), named TcI-TcVI. CL Brener, the reference strain of *T.cruzi* genome project belongs to the hybrid DTU TcVI, presenting 41 putative chromosomes. Chromosomal Copy Number Variation (CCNV) is a mechanism of gene expansion possibly related to rapid adaptation to new environments, and is already documented in yeast and several *Leishmania* species. Although studies point toward karyotype variability in *T. cruzi* strains, the extent of diversity in CCNV among and within DTUs based on read depth coverage (RDC) analysis has not been determined. To identify CCNV among *T. cruzi* DTUs, we sequenced genomes of strains from TcI, TcII and TcIII DTUs and estimated the ploidy based on RDC of single copy genes in each chromosome. TcI strains had few aneuploidies, while strains from TcII and TcIII DTUs presented several chromosomal expansions, which is in agreement with the average DNA mass per cell and genome plasticity in these DTUs. Chromosome 31, the only supernumerary chromosome in all samples evaluated, is enriched with genes related to glycosylation pathways, such as the enzyme UDP-GlcNAc-dependent glycosyltransferase, involved in the initial steps of mucin glycosylation. As the strains from the TcII DTU presented a divergent pattern of chromosomal expansions, we sequenced the genome of 7 *T. cruzi* TcII field isolates from Minas Gerais, **BRA**. These samples presented a complex pattern of chromosomal duplication/loss, which is not in agreement with the phylogeny based on single copy genes. Finally, we sequenced 3 clones of the TcII Y strain, which presented the same CCNVs as the non-cloned population, suggesting stability in the chromosomal expansions/loss pattern in the Y population. Increased gene copy number due to chromosome amplification may contribute to alterations in gene expression, representing a crucial strategy for parasites that mainly depend on post-transcriptional mechanisms to control gene expression. **Supported by:**CNPq, FAPEMIG, CAPES **Keywords:**Copy number variation; chromosomes; trypanosoma cruzi

PV011 - **EDITING THE TRYPANOSOMA CRUZI GENOME USING ZINC FINGER NUCLEASES**

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Gene function studies in T. cruzi have been hindered by the lack of efficient genetic manipulation protocols. In most organisms, insertion and deletion of DNA fragments in the genome are dependent on the generation of double-stranded DNA break (DSB) and DNA repair. By inducing a site-specific DSB, Zinc Finger Nucleases (ZFNs) have proven to be useful to enhance gene editing in many organisms. Using a pair of ZFNs targeted to the T. cruzi gp72 gene and a donor DNA fragment containing a resistance gene flanked by gp72 sequences, we were able to generate gp72 knockout (KO) parasites with improved efficiency compared to the conventional KO protocol. Importantly, wild type (WT) parasites transfected with the same donor sequence did not showed the characteristic gp72 KO phenotype, indicating that, in the absence of ZFNs, only one gp72 allele was disrupted. PCR analysis confirmed integration of the donor sequence in gp72 locus and also that WT parasites transfected with the donor sequence retained an intact gp72 allele. In contrast with recent reports that demonstrated gene disruption in T. cruzi using CRISPR/Cas9 in the absence of a donor sequence, parasites expressing ZFNs and which were not transfected with a donor DNA did not showed the gp72 KO phenotype. In agreement with that, sequence analysis of DNA extracted from six different clones expressing ZFNs, showed no insertions or deletions at the ZFNs cleavage site in the gp72 gene. Since western blot analysis revealed that parasites express only low levels of ZFNs, we hypothesized that ZFNs may be cleaving one gp72 allele at time and, in the absence of a donor sequence, the DSB was repaired by homologous recombination using the intact allele as template. Most importantly, by generating a T. cruzi cell line expressing ZFNs with a target sequence in its genome, we provide a new tool that can greatly improve genetic manipulation and also can be used to study DNA repair of DSBs in this highly divergent eukaryote. **Supported by:**CNPQ, Fapemig, INCTV **Keywords:**T. cruzi; zinc finger nucleases; genetic manipulation

PV012 - ***TRYPANOSOMA BRUCEI* ACETYLOME REVEALS THE EFFECT OF PROTEIN ACETYLATION IN THE REGULATION OF GLYCOLYTIC ENZYMES ACTIVITY**

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Lysine acetylation has emerged as a major post-translational modification regulating diverse cellular processes. The function of this modification has not been extensively explored in protozoan parasites. Here we describe the acetylome of procyclic (PCF) and bloodstream (BSF) stages of *Trypanosoma brucei*. We detected 319 lysine-acetylated sites in 244 proteins in PCF and 389 acetylated sites in 289 proteins in BSF. Remarkably, PCF and BSF had distinct acetylome profiles, likely reflecting differences in adaptive mechanisms in these life cycle stages. This was more evident in glycolytic enzymes. BSF, which use glycolysis as main energy source, presented less acetylation in these enzymes compared to PCF, which relies on oxidative phosphorylation for ATP production. Furthermore, PCF cultivated in the presence or absence of glucose showed distinct glycosomal protein acetylation levels and changed the specific activity of aldolase biophosphatase, one of the highly acetylated glycolytic enzymes. By one hand, aldolase specific activity was 3 fold reduced in PCF growing in medium lacking glucose and increased when extracts of parasites growing in low glucose medium were treated with a deacetylase. On the other hand, its activity decreased when parasite extracts were incubated with acetic anhydride. These results indicate that aldolase, and possible other glycolytic enzymes, is controlled by acetylation in response to environmental changes *T. brucei*. **Supported by:**FAPESP and NIH
Keywords: Lysine acetylation; glycolysis; trypanosoma brucei

PV013 - **ORIGIN IS THE NEW BLACK: ACTIVATION OF FEW REPLICATION ORIGINS COULD CONTRIBUTE TO GENETIC PLASTICITY IN *TRYPANOSOMA CRUZI*.**

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DNA replication starts at specific regions of the genome, named origins. From these origins emerge a pair of replication forks, which migrate in opposite directions to the end of the chromosome or until find a fork heading in their direction, thus ensuring the complete duplication of the genome. Eventually, a fork can stall and if not repaired leads to double strand breaks that must be repaired generating genomic alterations. Therefore, it is reasonable to assume that the greater the number of origins in the genome, the more active forks and thus a better chance to maintain genomic stability. Since Trypanosoma cruzi presents a high genetic variability; we are interested to verify possible relations between genetic instability and DNA replication features. *T. cruzi* epimastigotes were sorted in early S and G2/M phases and the DNA was extracted from each group and analyzed by MFAseq (Marker Frequency Analysis). After the initial analysis, only one origin was identified for each chromosome in this organism. Comparative analysis using the transcriptome database of *T. cruzi* showed that in many chromosomes the origin is located where the transcription units diverge, which indicate a strategy used by *T. cruzi* placing the origins of replication in sites of transcription divergence, avoiding a frontal collision (which is quite harmful) between the machineries of transcription and replication. To validate these MFAseq data and to investigate the fork speed rate in this strain, we did analyses by DNA combing. We found that the fork speed rate in *T. cruzi* is 6.6+0.3 Kb/min, being faster than other eukaryotes. DNA combing allowed visualizing some molecules being replicated for more than one origin. Howsoever, the frequency of replication origins in T. cruzi detected by single molecule analysis is lower than in *T. brucei*. Therefore, we purpose that MFAseq indicates origins that are fired very frequent, but there are also less frequent origins fired during *T. cruzi* DNA replication. **Supported by:**FAPESP
Keywords: Replication origin; genetic plasticity; t. cruzi

PV014 - **DELETION OF HISTONE DEACETYLASE 2 AFFECTS INVASION AND REPLICATION OF *TOXOPLASMA GONDII***

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Toxoplasma gondii is a member of the phylum Apicomplexa and the agent of toxoplasmosis. *T. gondii's* life cycle is complex and alternates between its final host, felines, and the intermediate hosts, warm blood animals, including humans. The different forms have different gene expression profiles, which is crucial for the virulence and adaptation to host immune mechanisms. In the last 15 years, epigenetic factors have been associated as an essential part of gene expression regulation in *Toxoplasma. T. gondii* has all canonical histones, and several chromatin-remodeling enzymes. One class of these proteins are the histone deacetylases (HDACs) that remove the acetyl group in lysines acetylated histones, which results in more condensed chromatin and gene silencing. *T. gondii* has 7 HDACs, among them HDAC-2 that is the aim of this study. Although it has the feature of a classic HDAC class I and similarities to other eukaryotes, the HDAC-2 has 2 nucleotide insertions within the HDAC domain, which make it unique to Apicomplexa, and whose function, remains unknown. In addition, data in *T. gondii* database showed that tghdac-2 expression increases during S phase of the cell cycle. Due to its particularity, our goal is to characterize HDAC-2 of *T. gondii* (TgHDAC-2). To this end, we have obtained the knockout of *tghdac-2*, which was replaced by homologous recombination, by *hxgprt* gene. So far, we observed a lower infectivity and proliferation rate of *tghdac-2(-)* parasites, suggesting a role during cell cycle progression, possibly during the S phase. Finally, western blot analysis of histones H3 and H4 acetylation showed an increase in the level of acetylation of both histones in the absence of HDAC-2, suggesting that TgHDAC-2 is responsible for H3 and H4 deacetylation. We are currently producing antibodies in order to observe the localization of the TgHDAC-2 within cellular compartments and identify partners associate to this protein. **Supported by:**CAPES, CNPQ E FIOCRUZ
Keywords: Toxoplasma; histone deacetylase; cell cycle

PV015 - **EXPRESSION AND ACTIVITY OF THE *TRYPANOSOMA RANGELI* ARGININE KINASE (AK) DURING THE PARASITE LIFE CYCLE**

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Arginine Kinase (AK) is one of the key enzymes involved on phosphotransfer activity required for ATP homeostasis in trypanosomatids, catalyzing the reversible phosphorylation between ADP and phosphoarginine. In this work, the AK expression and activity was monitored during the *in vitro* growth of *T. rangeli* epimastigotes, revealing the increase of AK levels during the first couple of days and then stabilizing overtime up to the ninth day. The enzymatic AK activity have increased up to the fifth day of *in vitro* culture and then gradually decreased during the subsequent days, allowing us to infer a possible role of AK in the cell growth and multiplication. Addition of antioxidants GSH and NAC in the culture medium increased the expression of AK, especially with NAC. While high levels of AK were found in epimastigotes submitted to oxidative stress with H_2O_2, low AK levels were detected in parasites exposed to H_2O_2 + NAC, pointing out a putative role of *T. rangeli* AK in the oxidative stress response. During the *in vitro* differentiation, epimastigote forms (day 0) showed the highest AK expression levels while the highest enzymatic activity was detected on the intermediate (day 4) forms. During the *in vitro* differentiation of bloodstream trypomastigotes to replicative forms, AK expression have increased up to the fifth day and remained stable up to the ninth day as also observed for the epimastigotes growth *in vitro.* During this process, the AK enzymatic activity have shown the same pattern as the parasite growth curve, increasing up to the peak of growth and declining afterwards. The AK activity revealed to be distinct among *T. rangeli* strains, being two times higher for SC58 strain when compared to Choachí strain, which revealed an AK activity comparable to *T. cruzi*. We conclude that AK activity might be related to *T. rangeli* cellular multiplication, differentiation and metabolic adaptation to adverse conditions, being influenced by the parasite's energetic demands. **Supported by:**CNPq, CAPES and FINEP
Keywords: Trypanosoma rangeli ; arginine kinase; phosphotransfer activity

PV016 - **DOES RNA INCORPORATION INTO THE DNA GENOME OF *TRYPANOSOMA BRUCEI* DRIVE DNA REPLICATION AND ANTIGENIC VARIATION?**

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During transcription, nascent mRNA is able to bind to the template strand of the unwound DNA duplex, forming stable R loops. Several proteins prevent and remove R loops, including ribonucleases H (RNases H) that digest hybridised RNA. Although threatening to genome stability, over the last decade evolutionarily conserved R loop-forming sequences have been documented as powerful regulators of DNA replication, gene expression and recombination. In the human genome, the most conserved and efficient origins of replication (ORIs) are localised to CpG island promotors. These sites form R loops upon transcription and bind the origin recognition complex, ORC. As a kinetoplastid, *T. brucei* co-transcribes genes from a small number of multigene clusters, with adjacent clusters separated by poorly characterised promoters and terminators at so called strand switch regions (SSRs). Interestingly, *T. brucei* transcription and replication appear to be linked processes as ORIs localise to select SSRs and ORC binds to potentially all SSRs. Given this information, we are investigating whether R loops that form in *T. brucei* are able to sequester ORC and direct DNA replication of the nuclear genome. In order to preserve R loops across the genome, we have generated mutants deficient in two nuclear RNase H genes and will describe the impact of these mutations on cell growth and DNA synthesis. We are also employing ChIP-seq technology to map R loops across the complete genome. In addition to their role in replication, R loops have been shown to facilitate class switching in activated B lymphocytes to generate antibody diversity. As class switching recombination may be analogous to variant surface glycoprotein (VSG) switching in *T. brucei*, we will also describe the effect of RNase H mutation on VSG expression and switching. Finally, we will describe a third RNase H that appears to be catalytically active and localises to the antipodal sites of the mitochondrial (kinetoplast) genome. **Supported by:**Biotechnology and Biological Sciences Research Council (BBSRC)
Keywords: Dna replication; r loops; variant surface gylcoprotein

TB001 - **EFFICACY OF A BINUCLEAR CYCLOPALLADATED COMPOUND THERAPY FOR CUTANEOUS LEISHMANIASIS IN THE BALB/C MURINE MODEL OF INFECTION WITH *LEISHMANIA AMAZONENSIS* AND ITS INHIBITORY EFFECT ON TOPOISOMERASE 1B**

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This study describes the antileishmanial activity of a binuclear cyclopalladated complex (CP2) containing Pd(II) and *N,N'*-dimethylbenzylamine (DMBA) as well as its inhibitory effect on the parasite DNA Topoisomerase 1B (Topo1B). CP2 exhibited good antileishmanial activity (IC_{50}=10µM) against *Leishmania amazonensis* intracellular forms. Although its potency was 2-times lower than amphotericin B (IC_{50}= 4.92µM), its Selectivity Index (SI) indicated that CP2 (SI=49.9) was 10 times more selectivity to the parasite than amphotericin B (SI=4.70). Regarding the infection index treatment with CP2 was as effective as amphotericin B, causing 68.5% reduction in the number of intracellular forms. These data compelled us to explore the efficacy of CP2 in an *in vivo* cutaneous leishmaniasis model. Treatment of *L. amazonensis* infected mice with CP2 led to 80% reduction in the parasite load in foot lesion; however, the dose concentration of CP2 (0.35mg/Kg/day) used was 5-times lower than the amphotericin B (2mg/Kg/day) and we did not observe neither severe toxic effects nor hepatic/renal toxicity followed by biochemical analysis (ALT/AST/creatinine). Some studies described the ability of different metal compounds to inhibit the Topo1B. Based on this information, we investigated if CP2 is able to inhibit this enzyme, one of the most important targets in trypanosomatids. Thus, the effect of CP2 on *Leishmania* topo1B was analyzed in relaxation and cleavage assays. Pre-incubation of CP2 with the enzyme before substrate addition increased eight times the efficiency of CP2 in inhibit the enzyme, followed by the enzyme incubation with a linearized partially duplex DNA labelled at 5'-end with γ^{32}-ATP in a time course experiment. The cleavage reaction was fast in the absence of CP2 while in the presence of 150µM, the cleavage complex bands were no longer visible, indicating that Leishmania topo1B was fully inhibited, which permits us classify CP2 as a catalytic inhibitor of *Leishmania* Topo 1B. **Supported by:**CAPES, CNPq and FAPESP **Keywords:**Cyclopalladated compounds; leishmaniasis; topoisomerase

TB002 - **DEVELOPMENT OF INNOVATIVE ORAL DRUG FOR LEISHMANIASIS TREATMENT: THE CASE OF 18-METHOXYCORONARIDINE (18-MC)**

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18-MC is an indole alkaloid investigated due anti-addictive properties and for its leishmanicide effect also. A successful partnership between pharmaceutical industry and academy deepened the pre-clinical studies in mice models. Moreover, the First Time in Human Phase 1 Clinical Trial was conducted in 2014. *In vivo* studies were conduct in mice infected with *L.amazonensis* promastigotes/30 days. Treatment started at D_{30} for 5 days using 20mg/Kg/day 18-MC aqueous solution orally. 99.7% parasite load inhibition in footpad and 99.9% in popliteal linfonode were obtained. Glucantime, used as control, was administrated 200mg/kg/day IP, reducing in 99 and 99.8% parasite load in footpad and linfonode respectively. Acute toxicity studies were done and LD_{50} were 150mg/kg and 300mg/kg for males and females, respectively, suggesting males are more susceptible to 18-MC. These doses were 15 and 30 times higher than effective dose. Furthermore, mice repeated doses treatment for 28 days revealed NOAEL of 100mg/kg, but no death were observed. The FTiH phase 1 clinical trial was carried out using 14 normal volunteers at Clinics Hospital of HFPR. In the first cohort, 7 subjects received an unique dose of 20mg of 18-MC in 10mL 5% dextrose solution. They were followed by 36hr while blood samples were collected for pK, hematological and biochemical analysis. New blood collections were done 7, 14 and 30 days after treatment. Second cohort, additional 7 volunteers received 4mg of 18-MC in same solution twice a day for one day. The experimental procedures were the same of cohort one. No SAE were observed among subjects, considering vital signs, ECG, EEG and blood analysis. 18-MC is well absorbed in human and seems to have multi-compartmental pk behavior. The T_{max} was 30 minutes and $T_{1/2}$ was 100 minutes. Taking together, our results reveal that 18-MC is high effective and safe both to animals and human and it is a total innovative drug for leishmaniasis treatment. **Supported by:**MACKPESQUISA, NIH
Keywords: Leishmaniasis; first time in human clinical trial; pharmacokinetics

TB003 - **TOWARDS A BETTER THERAPY AGAINST CHAGAS DISEASE: INNOVATIVE PRODRUGS TARGETING THE *TRYPANOSOMA CRUZI* PROLINE RACEMASE**

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The parasitic protozoan *Trypanosoma cruzi* is the causative agent of Chagas disease, a neglected tropical disease affecting about 6-7 million people worldwide. Currently, benznidazol is the preferred therapy, however, it often presents low efficacy in chronic cases, high toxicity and drug resistance. We have identified and characterized a *T. cruzi* enzyme, proline racemase (*Tc*PRAC), which has been shown to be a promising drug target considering that it is essential to the parasite life cycle and virulence. By means of Structural Bioinformatics analysis, we have explored conformational transition pathways between the active conformation of *Tc*PRAC ("opened") and its conformation in complex with its natural competitive inhibitor pyrrole-2-carboxylic acid ("closed"), generating several intermediate structures of the catalytic site of the enzyme. Afterwards, we identified and tested numerous compounds, and biochemical assays established that some of them were able to covalently bind to *Tc*PRAC and to specifically inhibit its racemase activity *in vitro*. Using state-of-the-art bioluminescent and fluorescent *T. cruzi* parasites, bioluminescent assays, high-throughput screening and preclinical imaging systems, we validated different approaches to monitor intra- and extra-cellular parasite development. Moreover, in order to increase membrane permeability of these innovative *Tc*PRAC inhibitors, we produced analogous ester prodrugs, which presented higher trypanocid/trypanostatic activity when compared to benznidazol. These ester prodrugs were effective in killing epimastigotes and axenic amastigotes, and to reduce the parasite load in infected cells *in vitro*. Moreover, they presented quite low toxicity to host cells. Following these encouraging *in vitro* results, tests are in progress to evaluate the toxicity of these prodrugs *in vivo* and to assess their efficacy in both acute and chronic phases of the disease. **Supported by:**Agence Nationale de la Recherche (ANR; grant number 14-CE16-0001) - Institut Pasteur

Keywords: Chagas disease; drug discovery; drug screening

TB004 - **IN VITRO EVALUATION OF PROMISING EPITOPES SELECTED USING BIOINFORMATICS TOOLS IN PBMC FROM LEISHMANIA NATURALLY INFECTED DOGS**

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Canine visceral leishmaniasis (CVL) is a zoonosis in Latin America and dogs play a central role in the urban cycle of Leishmania infantum. In BRA, the Current strategies to control CVL are inefficient and there are no vaccines that could be employed in public health control program. In this context, rational vaccinology appears as a strong approach that will allow the discovery of potential antigens. In previous studies, our group, has identified 38 peptides with immunogenic potential from five L. infantum proteins, using bioinformatics tools (BT). The main goal of this work is to evaluate the in vitro capacity of selected peptides to induce proliferation and IFN-γ-producing T cells in Leishmania naturally infected dogs. In order to do this, naturally infected dogs were selected through leishmanin skin test, clinical signs and leukogram to screen the synthetic peptides. Therefore, peripheral blood mononuclear cells (PBMC) from dogs were stimulated by three peptides per mix using combinatorial analysis (CA). At that point, we evaluated in vitro capacity of CD4+ and CD8+ T cells to proliferate and produce IFN-γ. Then, we developed a score matrix to evaluate the individual response of five dogs, based on index of stimulated culture/control culture. Regarding the PBMC stimulation with mixes, 13 mixes met the criteria and were selected. Next, these mixes were combined resulting in 13 peptides selected. Afterwards, they were individually evaluated with the same parameters of the mixes to confirm the CA, thus 10 out of 13 peptides were capable of inducing T cell proliferation and IFN-γ production in dogs PBMC. In conclusion, peptides selected using BT are capable to induce proliferation and produce IFN-γ in T cells. As perspectives, the 10 peptides will be tested in vivo in dogs by Delay Type-Hypersensitivity (DTH) and the best ones will be combined in multi-peptide vaccines to the preliminary immunogenicity tests in mouse models. **Supported by:** FAPEMIG, CAPES, CNPq, UFOP, Fiocruz **Keywords:**Rational vaccinology; cvl vaccine; multi-peptide vaccine

TB005 - **NANOSTRUCTURED FORMULATIONS WITH LYCHNOPHOLIDE CURE TRYPANOSOMA CRUZI STRAIN RESISTANT TO BENZNIDAZOLE IN MICE TREATED BY ORAL ROUTE.**

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Chagas disease (CD) remains neglected and the only drug available for its treatment in BRA is the benznidazole (BZ), which causes side effects and low therapeutic efficacy. We already demonstrated that lychnopholide (LYC) showed anti-T. cruzi efficacy in the acute phase (AP) and in the chronic phase (CP) in vivo by EV route. Nanocapsules were used as strategy for to load LYC due its lipophilic nature in order to control its release and reduce toxicity. Thus, the objective of this study was to verify the effectiveness of free LYC and LYC-NC by oral route during the AP and CP infections in mice infected with VL-10 strain of T. cruzi treated for 20 consecutive days. Swiss female 20g were i.p inoculated with 10,000 blood trypomastigotes (AP) and 500 trypomastigotes (CP). The mice were divided: Free LYC 12, LYC-NC (8 and 12), BZ 100 mg/kg/day and controls. The treatment effectiveness was assessed by hemoculture (HC), PCR and ELISA. Animals infected and treated with LYC-NC 12mg/kg/day showed 75% (AP) and 88% (CP) of cure. Animals treated with LYC-NC 8 showed 38% of cure (AP) and 43% (CP), what revealed the phenomenon doseXeffect; whereas mice treated with BZ were not cured. Free LYC reduced the parasitemia when compared to controls, but none mice were cured. The qPCR results were negative and consistent with the results obtained with HC and PCR, but contrary to ELISA that remained positive only in 3/55, which usually became negative only later after treatment. Although qPCR technique had efficiency > 97.5%, these results strongly indicate absence of T. cruzi in the heart tissue in 100% of the treated animals infected withVL-10 strain at both phases of infection, suggestive of parasitological cure. NC lead to increase of LYC therapeutic effectiveness by oral route in animals infected with T. cruzi strain totally resistant to BZ. These findings represent a great perspective for treatment of CD which still constitutes one serious neglected parasitic disease in the world. **Supported by:**FAPEMIG, CNPq, UFOP **Keywords:**Chagas disease; chemotherapy; lychnopholide

TB006 - **EFFICACY OF BENZNIDAZOLE NANOPARTICLES AGAINST T. CRUZI NICARAGUA IN EXPERIMENTAL MURINE INFECTION.**

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Recently, we have shown that Benznidazole nanoparticles (BNZ-nps) are capable of reducing the infection of T. cruzi in-vitro cells. In addition, it was found that BNZ-nps are biocompatible in term of toxicity and hemolytic effects. In-vivo studies showed the survival (100%) of T. cruzi Nicaragua (TcN) infected acute mice during BNZ-nps treatment, in contrast with the non-treated infected mice (15% survival) (Scalise, Arrúa et al., 2016). BNZ-nps having a mean particle size of 63.3 nm, a size distribution of 3.35, and a zeta potential of −18.30 were prepared using poloxamer 188 as a stabilizer. In this work, we have evaluated the antiparasitic activity of BNZ-nps on TcN infected mice in the acute and chronic phases of the infection. Also, it was analyzed the ROS production by cells treated with this novel BZN formulation. The results obtained in this research exhibit a 100% survival of infected and treated mice with 25 or 50 mg/kg/day doses of BNZ-nps. The animals were monitored until the chronic stage, at 3 months pi. All T. cruzi-specific antibodies of serum treated mice decreased up to 50% and 100%, after the administration of 25 and 50 mg/kg/day, respectively. No parasite DNA could be detected by PCR in 60% of treated mice. The myocardium of untreated infected mice showed extensive and multiple inflammatory foci of mononuclear cell infiltrates and some necrotic areas and extensive fibrotic foci. In contrast, all BNZ-nps treated mice had lower mononuclear cell infiltrates and fewer structural changes. Equally fibrotic foci were reduced in all the animals treated. VERO cells show no significant differences in the production of reactive oxygen species between treatments with R-BNZ and BNZ-nps 25, this being significant production from cells with medium. These findings led to the conclusion that the BNZ-nps treatment is a convenient approach, at different doses and treatment regimens, to treat T. cruzi infection in the murine experimental model. **Keywords:**Trypanosoma cruzi; nanoparticles; benznidazole

TB007 - **IDENTIFYING AND EXPLOITING DEUBIQUITINATING CYSTEINE PEPTIDASES (DUBS) OF TRYPANOSOMES AND *LEISHMANIA***

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Deubiquitinases (DUB)s are peptidases that cleave the post-translational modifier ubiquitin from proteins or ubiquitin-conjugates. DUBs play crucial roles in many biological processes such as protein quality control and membrane trafficking. Trypanosomatids have a ubiquitin system and the genome of Trypanosoma brucei and Leishmania show the presence of 20 syntenic DUB orthologues, however, the identity and function of DUBs remain to be elucidated. A chemical proteomics approach using a fluorescent ubiquitin-based probe was used for activity-based protein profiling, revealing the presence of many active DUBs in T. brucei and L.mexicana. A number of stage-specific DUBs have been identified, including some in Leishmania that have amastigote-specific activity. RNAi of DUB1 in T. brucei indicates the gene is essential, whilst preliminary DiCRe inducible gene knockout of L.mexicana DUB1 suggesting that it is essential also. Furthermore, active recombinant DUB1 protein has been expressed and purified using a baculovirus expression system and an HTS-compatible fluorescence polarisation assay developed based on the proteolysis of tetramethylrhodamine-labelled Lys(Ub)Gly. Our approach combines chemical and genetic screening to identify essential trypanosomatid DUBs as a starting point for drug discovery activities and to investigate their cellular function.
Supported by:MRC
Keywords: Leishmania; drug target; peptidase

TB008 - **EXPRESSION OF *TRYPANOSOMA* ANTIGENS IN *LEISHMANIA TARENTOLAE*. POTENTIAL FOR USE IN RAPID SERODIAGNOSTIC TESTS (RDTS).**

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The development of rapid serodiagnostic tests for diseases caused by kinetoplastids relies on the affordable production of parasite-specific recombinant antigens. Exposure to Trypanosome and Leishmania parasites generally stimulates the production of parasite specific antibodies in the infected person. In the absence of symptoms the presence of antibodies can indicate early stages of disease. These antibodies are often detecting surface glycoproteins which can be produced by recombinant systems. However recombinant systems such as *E.coli* and Yeast do not carry out post-translational processing in the same way as kinetidoplastids and therefore may lack some epitopes. Here, we describe the production of recombinant antigens from *Trypanosoma brucei gambiense* (causative agent of sleeping sickness, HAT) in the related species *Leishmania tarentolae*, and compare their diagnostic sensitivity and specificity to native antigens currently used in diagnostic kits. 10 mg/L of recombinant protein was purified and subsequently tested against a WHO panel of sera from sleeping sickness patients and controls (without sleeping sickness but living in HAT endemic countries). The evaluation on sera from 172 T.b. gambiense human African trypanosomiasis (HAT) patients and from 119 controls showed very high diagnostic potential of the two recombinant VSG, and rISG65 fragments. The areas under the curve for recombinant VSG LiTat 1.3 and 1.5 were 0.97 and 0.98 compared to 0.98 and 0.99 for the native forms (statistically not different). The *L. tarentolae* expression system enables simple, cheap and efficient production of recombinant protein for a new test being developed for HAT. Production of other kinetoplatid proteins for use in diagnostic, vaccine and drug discovery research are being examined. Rooney, Barrie, et al. PLOS Negl Trop Dis 9.12 (2015): e0004271.
Supported by: Biotechnology and Biological Sciences Research Council UK (BBSRC)
Keywords: Diagnostics; antigens; kinetidoplastid

TB009 - **GOLDLEISH: ANTILEISHMANIAL ACTIVITY OF GOLD(I) PHOSPHINE COMPLEXES AGAINST SB-SENSITIVE AND RESISTANT *LEISHMANIA* IS DUE TO EARLY ROS PRODUCTION AND TR INHIBITION**

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The drugs currently used to treat leishmaniasis have limitations concerning cost, efficacy and safety, making the search for new therapeutic approaches urgent. We have previously reported the antileishmanial activity of 24 novel gold(I) complexes synthesized based on Auranofin, a gold(I) phosphine complex used to treat rheumatoid arthritis that has anticancer and antileishmanial activities. We then selected the 4 most active complexes on *L. infantum* amastigotes (IC_{50} from 0.5-1.5 µM) for further studies. Their antileishmanial activity was evaluated on *L. b*raziliensis amastigotes and antimony-sensitive and resistant (SbR) *L. guyanensis*, *L. amazonensis* and *L. chagasi* promastigotes. Their ability to generate reactive oxygen species (ROS) was assessed using H_2DCFDA as oxidative stress indicator. Their trypanothione reductase (TR) inhibition activity was determined by DTNB coupled enzymatic assay. We found that the complexes were also active in *L. b*raziliensis amastigotes with IC_{50} values ranging from 2.3 to 5.5 µM. There was no cross-resistance to antimony found in any of the studied strains; actually, SbR lines were hyper sensitive to some of the complexes than their wild type counterparts. 3-benzyl-1,3-thiazolidine-2-thione-based complexes were 2 to 5 times more active than their gold precursors on *L. chagasi* SbR, highlighting the importance of ligands for the differential antileishmanial activity. Triethylphosphine-derived complexes enhanced ROS production, at least twice, after short time exposure (2 h). All complexes and their precursors were highly active in TR inhibition with IC_{50} ranging from 1 to 7.8 µM. These results indicate that gold(I) complexes cause oxidative stress either by direct ROS production or by impairing TR activity and thus accumulating ROS. In this regard, gold(I) complexes are promising antileishmanial agents, with relevance for the treatment of leishmaniasis caused by antimony-resistant parasites. *In vivo* efficacy assays are being performed. **Supported by:**CNPq; CAPES; FAPEMIG; ISID **Keywords:**Leishmania; gold complexes; antimony resistance

TB010 - **GIANT SYNTHETIC VESICLES (GUVS): A MODEL TO EVALUATE THE TRYPANOCIDAL EFFECTS OF BENZOFUROXAN ACTIVE DERIVATIVES**

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Chagas disease is endemic in the Americas. Currently, the most widely used drug in BRA is benznidazole, whose action is limited according to the stage of the disease, *Trypanosoma cruzi* strain and severe side effects. Recently, studies have shown that derivatives benzofuroxan were more cytotoxic than benznidazole for epimastigotes and amastigotes of *T. cruzi* and not mutagenic in mice, and are produced in large quantities, which encourages further understanding of its mechanism of action. Similarly, polyene interactions with lipid membranes has been extensively studied employing small unilamellar vesicles (SUVs) and / or large (LUVs) using different fluorescent techniques. This study goals to evaluate mechanisms of drug interactions by examining the individual behavior of giant vesicles (GUVs), either ergosterol (as the membranes of trypanosomes) or cholesterol lipid membranes (as mammalian cells), with interesting observations on the effects induced by the activity of two derivatives benzofuroxan (BZFS and BZFTS) in different concentrations; these analyzes were carried out with phase contrast microscopy and micromanipulation system. The images were continuously acquired with a chilled blackand-white CCD camera and recorded on a hard disk drive. The images selected for the qualitative determination were stored in an external drive. All the images were processed and analyzed using in-house-produced software. Preliminary results have shown promise in evaluating the mechanism of action of the structures depending on the membrane lipid content. The results will lead interesting information for understanding the mechanism of action of the compounds across the membranes of trypanosomes compared to those of mammalian hosts. **Supported by:**FAPESP, CAPES, CNPq
Keywords: Benzofuroxan derivatives; giant phospholipid vesicles; ergosterol

TB011 - ***IN VITRO* PHENOTYPIC SCREENING AND ELECTRON MICROSCOPY APPROACHES OF NOVEL AROMATIC AMIDINES ON TRYPANOSOMA CRUZI**

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For the last five decades, Nifurtimox and Benznidazole (Bz) are the only drugs available for Chagas disease therapy, both exhibiting serious limitations. There is an urgent need to develop alternatives for the treatment of this neglected pathology. Therefore, our aim was to evaluate the biological effect of six novel amidines against Trypanosoma cruzi *in vitro* also exploring their primary cellular insults induced on the drug-treated. Regarding the activity against intracellular parasites (from different strains and DTUs as Tulahuen and Y strains), the aromatic molecules presented considerable trypanocidal effect, exhibiting EC_{50} values in a range of 0.3-10.5 µM. Considering the toxicity profile towards mammalian host cells, unfortunately the most active agents were also the most toxic (LC_{50} ranging between 6-15 µM). Still, 28SMB032 presented LC_{50}>100 µM and high selectivity (≥ 60) against both Y and Tulahuen strains. The phenotypic screening on bloodstream trypomastigotes (Y strain) showed that 5 out of 6 compounds were more active than BZ (EC_{50} 3 µM) after 24 h of drug exposition and 2 of them carried this profile with only 2 hours of incubation. Transmission Electron Microscopy (TEM) concerning these two fast killers and 28SMB 032 revealed interesting features like flagellar pocket dilatation, presence of myelin figures, large number of intracellular vesicles, distortions of the Golgi apparatus and altered profile of endoplasmic reticulum that may be related to autophagy cell death. Concerning our promisor compound 28SMB 032, morphological alterations included body shortening and twisting were observed by Scanning Electron Microscopy (SEM), although the majority exhibited a similar profile to the untreated ones. No morphological modification was noticed to the fast killers. Due to the high SI against both parasite forms, 28SMB 032 was moved to *in vivo* analysis of mouse acute T. cruzi infection that is underway. **Supported by:**Fiocruz, Capes, CNPq and FAPERJ.

Keywords: Chagas disease; experimental chemotherapy ; aromatic amidines

TB012 - **EFFICACY OF 14E THERAPY, A FUROXAN DERIVATIVE, IN VISCERAL LEISHMANIASIS USING HAMSTER MODEL OF INFECTION WITH LEISHMANIA INFANTUM AND THE POTENTIAL OF MULTIPLE TARGETS INVOLVEMENT**

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Furoxans have been described for their antileishmanial properties as well as NO-donors [1,2,3,4]. Herein we describe the *in vitro* and *in vivo* antileishmanial effect of 14e. This molecule exhibited good antileishmanial activity against *L. infantum* amastigotes (EC_{50}=3.14 µM). Although its potency was 4 times lower than amphotericin B (ampB) (EC_{50}=0,71 µM), its Selectivity Index indicated that 14e (SI=66.35) was 2 times more selective to the parasite than ampB (SI=32.54). Moreover, 14e was able to increase NO production in culture supernatants from macrophages infected with *L. infantum*. These data compelled us to explore the efficacy of this compound in an *in vivo* visceral leishmaniasis model. Golden hamsters were infected with *L. infantum* and after 45 days, the animals were intraperitoneally injected with 14e or ampB for 15 days. After this period, the parasite burden of liver and spleen was evaluated by limiting dilution. 14e reduced the parasitemia both in the liver (54.4%) and spleen (49.9%); interesting, the dose of 14e (3.0 mg/kg/day) was almost 7 times lower than ampB (20.0 mg/Kg/day) and we did not observe hepatic/renal toxicities followed by biochemical determination. Histopathological analysis was carried out and it was observed a reduction in the number of moderate and intense inflammatory infiltrate in periportal tracts in animals treated with 14e compared to non treated animals. Some mechanistic studies described in the literature have pointed out that the moyety N-acyl hydrazone is responsible for cysteine protease (CP) inhibition[3,5]. 14e, which also presents this chemical group, was able to inhibit the protease CPB2.8 of *L. mexicana* (IC_{50} of 1.04 µM). However, CP does not seem to be the exclusive target of 14e, since we were not able to develop a resistant strain which is suggestive that we are facing a multiple target phenotype. Indeed, preliminar results indicates that this compound could be interfering in calcium homeostasis in this parasite. **Supported by:**CNPq

Keywords: Furoxan; visceral leishmaniasis; multitarget

TB013 - ***IN SILICO* INVESTIGATION OF HIV PEPTIDASE INHIBITORS ON *TRYPANOSOMA CRUZI***

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The activity of aspartic peptidases in etiological agent of Chagas disease *Trypanosoma cruzi* were never deeply analyzed being the participation of aspartic peptidases in basic cellular functions demonstrated in some *in vitro* assays .The role of these peptidases in virulence was postulated, highlighting these enzymes as an interesting target for the development of new trypanocidal compounds. The aspartic peptidase named *DNA-damage inducible protein* (*Ddi-like*) was reported in *T. cruzi* genome, contains a conserved domain homologous to the retroviral aspartic peptidase, which in *Leishmania major* is the target of the aspartic peptidase inhibitors designed for the viral peptidase (PIs-HIV). Through homology modeling, a 3D structural organization of the aspartic peptidase domain of *Ddi-like* was validated and used in molecular docking studies with PIs-HIV. The 3D structure of the parasite *Ddi-like* domain forms a homodimer, which is characteristic of retroviral aspartic peptidase. Each dimer has the essential amino acid sequence for the active site Asp-Thr/Ser-Gly. The data obtained with molecular docking pointed out a marked relationship between the experimental inhibition and the electrostatic interaction energy for all IPs-HIV evaluated. Differences in binding affinity were observed, indicating the enzyme selectivity to form more stable enzyme-inhibitor complexes. The effect of IPs-HIV *in vitro* and *in silico* stimulates further studies about the interaction of the parasite aspartic peptidases with these inhibitors. Furthermore, these results showed that *T. cruzi* aspartic protease as a promising target for design of selective inhibitors and drugs repurpose. **Supported by:** CNPq, CAPES, FAPERJ and Fiocruz
Keywords: Aspartic peptidases ; molecular docking; pis-hiv

TB014 - ***TC*PRAC : A TARGET FOR DEVELOPMENT OF MOLECULAR, SEROLOGICAL AND IMAGING TOOLS FOR DIAGNOSIS OF CHAGAS DISEASE**

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Nowadays, Chagas disease transmission by blood transfusion has become a public health problem in North countries (USA, Europe). The *Trypanosoma cruzi* proline racemase (*Tc*PRAC) is an essential parasite enzyme, not present in humans and in others pathogenic trypanosomatids for humans, whose genes are expressed in all parasite genotypes in all stages. We are convinced in *Tc*PRAC is a marker of the viability of the parasite, especially during the chronic phase where the parasite is almost exclusively intracellular in host tissues. To improve the sensitivity and specificity of existing diagnostic tests, we are developing differents approaches using TcPRAC as main target. A qPCR strategy using primers based on TcPRAC genes confirmed the infection of acutely infected mice by *T. cruzi* with 100% sensitivity and 100% specificity. Different antibodies against *Tc*PRAC (rabbit polyclonal, mouse monoclonal and nanobodies) were tested by IMF and/or by ELISA to study infection in both acute and chronic disease. We are developping in vivo experiments using infected mice by parasites constitutively expressing bioluminescence (Luciferase gene) and fluorescence (GFP or E2-Crimson genes). Selected antibodies labeled with fluorescent protein (IR Dye) and recent imaging techniques (IVIS, 3D imaging) could allow the following of the infection in real-time and the parasites localization in tissues during the chronic phase of Chagas disease. These approaches may lead the development of potential non-invasive tests in humans using TEP-scan or RMI technology to confirm diagnosis in chronic phase and monitoring after treatment.
Keywords: Tcprac; diagnosis; monitoring

TB015 - **SEX DIFFERENCES ON THE BENZNIDAZOLE PHARMACOKINETICS IN HEALTHY VOLUNTEERS**

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Despite its toxicity and low efficacy in the chronic phase, benznidazole is the drug of choice in Chagas disease. Scarce information about pharmacokinetics of benznidazole has been published. Sex-related differences have been observed in clinical practice (increased likelihood of adverse event). The aim of our study was to evaluate the effect of sex differences on the pharmacokinetics of benznidazole. Eight healthy volunteers (n = 4 men and 4 women) received a single oral dose of 100 mg benznidazole (Abarax®, Elea, ARG) on an empty stomach. Serial blood samples (6 mL) were collected for 72 h following benznidazole administration. Benznidazole plasma concentrations were measured using UPLC–MS/MS. The results, reported as median, were compared using the Mann-Whitney test ($p \leq 0.05$). After benznidazole administration, median peak plasma concentration of benznidazole was lower in men than in women (1.6 *vs* 2.9 µg/mL), and median Vd/F was smaller in women than those in men (88.6 *vs* 125.9 L). These results suggest that sex-related differences in pharmacokinetics of benznidazole might be due to variations in the gastric pH, rate of gastric emptying, gut transit time, intestinal expression of transport proteins (i.e. P-glycoprotein) and body composition. However, further studies using a larger sample size will be required to understand implications among the dose adjustment, toxic profile of benznidazole and to discriminate between the mechanisms underlying these sex differences. **Supported by:**CAPES
Keywords: Benznidazole; pharmacokinetics; chagas disease

TB016 - **MOLECULAR MARKERS FOR MILTEFOSINE RESISTANCE IDENTIFIED IN *LEISHMANIA CHAGASI* ISOLATES FROM VISCERAL LEISHMANIASIS PATIENTS WITH DIFFERENT MILTEFOSINE TREATMENT OUTCOME**

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Visceral leishmaniasis (VL) treatment relies on a few chemotherapeutic drugs including pentavalent antimony, amphotericin B and miltefosine. Miltefosine has been highly effective against VL in India. However, the clinical trial conduced in BRA showed that about 50% of VL patient relapsed after the miltefosine treatment (2.5 mg/Kg during 28 or 42 days). Miltefosine resistance mechanisms are being elucidated in laboratory *Leishmania* spp. strains but are less clear in clinical isolates. In this study, we used comparative genomics to identify molecular differences between *L. chagasi* (= *L. infantum*) isolates from VL patients in BRA with different miltefosine treatment outcomes. Whole genome sequencing was carried out with isolates from cured (n=14) and relapsed (n=12) patients. 93 orthologs groups exhibited a significant difference ($p<0.01$) in gene dosage between the two groups, including a deletion of a locus on chromosome 31 (containing four genes; Miltefosine Sensitivity Locus, MSL) which was strongly associated ($p<0.001$; reciprocal relative risk=9.4) with treatment failure. It was inferred that this deletion process occurs by homologous recombination using repetitive sequence flanking the MSL and apparently is not induced by miltefosine pressure. Nevertheless, re-expression of individual MSL genes in a miltefosine resistant promastigote line did not restore the in vitro miltefosine susceptibility phenotype. Moreover, MSL screening in 107 more *L. chagasi* isolates from different regions of BRA (ES, MG, BA, MA, and PI) showed that ES and MG have a very low number of isolates that are likely to respond to miltefosine treatment (3.7% of isolates with MSL), whereas in MA and PI 67.7% of isolates have the MSL. These data indicate that miltefosine-resistance mechanisms in *Leishmania* clinical isolates are beginning to be elucidated and suggest that miltefosine could successfully be used for treatment of VL in BRA after stratification for the presence of the MSL. **Supported by:**CNPq, CAPES, FAPES and Wellcome Trust
Keywords: Leishmania chagasi; miltefosine-resistance ; whole genome sequence