HP001 - **VARIANT ANTIGEN PROFILING IN TRYPANOSOMA VIVAX - A HIGH-THROUG**HP0**UT APPROACH TO ANTIGENIC DIVERSITY IN NATURAL POPULATIONS**

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Trypanosoma vivax is a unicellular hemoparasite responsible for animal African trypanosomiasis in Africa and South America. African trypanosomes establish long-lasting infections partly due to antigenic variation. The parasite surface is coated with a variant surface glycoprotein (VSG) monolayer, each cell expressing a single VSG from a genomic repetoire of many hundreds. Sequential replacement of VSG allows the parasite to evade immunity and survive long term. Population genetics of VSG leading to an understanding of antigenic diversity is vital to explaining variation in disease outcome and to developing effective vaccines. However, due to their number and complexity, we have lacked the tools to analyse VSG diversity from high-througHP0ut data. We have developed variant antigen profiling, a novel bioinformatic approach to measure VSG abundance from deep sequencing data. We have produced 19 T.vivax genome sequences from natural infections and compared their 'variant antigen profiles' (VAP). We show that VSG diversity across T.vivax is directly correlated to geography and population structure, and indeed is remarkably homogenous, raising the possibility of a universal vaccine. However, we also identified a panel of location-specific VSG that can be used as diagnostic markers in epidemiological studies. Variant antigen profiling can also be applied to examine variation in VSG expression. We illustrate this with analyses of two published transcriptomes from South American and African T.vivax, which expose the dynamism and complexity of VSG expression profiles. Studying antigenic diversity on genomic and population scales is a great computational challenge. Our population genomics study is the first attempt to solve this issue for T.vivax. The VAP will provide unprecedented ability to discriminate among antigens. Ultimately, this will allow us to associate variant antigens with specific infection phenotypes leading to a better understanding of disease outcomes. **Supported by:**BBSRC **Keywords:**Trypanosoma vivax; variant surface glycoproteins; variant antigen profiling

HP002 - **HIGH MOBILITY GROUP B FROM TRYPANOSOMA CRUZI: A PUTATIVE INFLAMMATORY MEDIATOR IN ACUTE CHAGAS**

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High Mobility Group B (HMGB) proteins are conserved nuclear architectural factors involved in chromatin remodeling and important nuclear events. HMGBs also play key roles outside the cell acting as alarmins or Damage Associated Molecular Patterns (DAMPs). In response to a damage or danger signal these proteins act as immune mediators in the extracellular milieu. Moreover, DAMPs play a central role in the pathogenesis of many autoimmune, infectious and inflammatory chronic diseases.

We have previously identified a High mobility group B protein from Trypanosoma cruzi (TcHMGB) and showed that it has architectural properties interacting with DNA like HMGBs from other organisms. The aim of this study was to determine if the parasite protein can also act as an inflammatory mediator as a first attempt to study its putative role in the pathogenesis of Chagas disease. Using a recombinant TcHMGB protein, we observed that the parasite HMGB is able to induce an inflammatory response in vitro and in vivo, evidenced by the production of Nitric Oxide and induction of inflammatory cytokines like TNF-α, IL-1β and IFN-γ gene expression. Interestingly, TGF-β and IL-10, which are not inflammatory cytokines but do play key roles in Chagas disease, were induced by rTcHMGB. In order to play an inflammatory role during T. cruzi infection, TcHMGB is expected to be secreted or released by the parasite. Indeed, we showed that TcHMGB can be translocated to the cytoplasm and secreted out of the parasite, a process that seems to be stimulated by acetylation. Additionally, immunohistochemistry in mice hearts during acute experimental T. cruzi infection showed high production of TcHMGB by amastigotes that appears to be secreted and co-exists with inflammatory cells and pro-inflammatory cytokines.

These results suggest that TcHMGB can act as an exogenous immune mediator and can be envisioned as a pathogen associated molecular pattern (PAMP) partially overlapping in function with the host DAMPs **Supported by:**CONICET, ANPCYT, CONACYT **Keywords:**High mobility group b; danger associated molecular pattern; inflammation

HP003 - **THE TNF RECEPTOR 1 IN LEISHMANIA AMAZONENSIS INFECTION: PROMOTION AND REGULATION OF INFLAMMATION**

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The Th1/Th2 paradigm that explains most models of infection with Leishmania major does not apply to the experimental models of infection with L. amazonensis. In this latter model, mixed immunological responses rather than polarization are observed, in contrast with the Th1/Th2 paradigm of resistance and susceptibility. TNF is a pleiotropic cytokine that mediates inflammation, among other functions. TNF binds to two receptors, TNFR1 and TNFR2. Both receptors trigger inflammatory responses but only by binding to TNFR1 TNF mediates regulation or supression of inflammation, through induction of apoptosis via caspase 8/3. Hence, our work aimed at the identification of the role of TNFR1 the mouse model of infection with L. amazonensis. Our data reveal the importance of TNFR1 in the control of lesion development, but not on the control of parasite replication. This control was more efficient in the subcutaneous model of infection where, in addition to promoting lesion control, TNFR1 was importante in the maintainance of tissue homeostasis, probably due to IL-10 production. Furthermore, in the accute phase of the subcutaneous infection, TNFR1 mediated recruitment of mieloid cells and lymphocytes to the site of infection and, in the chronic phase, regulated cell recruitment. TNFR1-mediated apoptosis was inhibited during intradermic infection by L. amazonensis, and an alternative pathway was in play. However, our data indicate that apoptosis seems to be inhibited by the pro-inflammatory signaling through TNFR1, since apoptosis was lesser in wild-type mice. In conclusion, our data suggest the involvement of TNFR1 in resistance to L. amazonensis. This conclusion is supported by the fact that TNFR1 acts both in the promotion and in the regulation of inflammation. Regulation is crucial for preservation of the infected tissue, and seems to be related to continuous production of IL-10, observed mainly in the subcutaneous infection. **Supported by:**CAPES, FAPEMIG, CNPq, REDOXOMA **Keywords:**Leishmania amazonensis; tnf; tnfr1

HP004 - **TRYPANOSOMA CRUZI LONG READS BASED ASSEMBLY: REVEALING THE REAL EXTENT OF GENOME COMPLEXITY.**

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Although the genome of the Trypanosoma cruzi (CL Brener strain) is available since 2005, and those from some additional strains were later reported, very high-quality genomic sequences are not yet available due to the intrinsic genome complexity of this parasite. This limitation poses a number complications for diverse types of analyses that require high degree of precision. Third-generation sequencing technologies are particularly suitable to address the challenges associated with the high degree of repetition of T. cruzi`s genome since they allow obtaining sequencing reads of 15 kb in length and many larger than 30 kb. This opens the possibility to directly determine the full sequence of large clusters of repetitive sequences (without collapsing them), as well as determining the single copy sequences that surround both sides of these clusters. As a consequence assembly fragmentation is largely avoided. Furthermore this approach allows separated assembly of homologous chromosomes, namely haplotypes are retrieved as separate contigs/scaffolds instead of a unique mosaic sequence. We present the genome of the hybrid T. cruzi strain TCC, sequenced using PacBio SMRT technology. Its final assembly consists in a diploid genome of 86.7Mb, the 9% of which being composed by a well known satellite of 195pb. The remaining genomic regions are distributed in only 692 contigs with an N50 of 300Kb, the longer ones being larger than 1 MB and correspond to entire chromosomes. New tandem and disperse repetitive sequences were identified, including some located inside coding sequences. A total of 30947 genes were identified, 62% having an assigned function. The quality of this new T. cruzi genome allows one to separate the two parent halpotypes as well as to identify the centromeres, the real extension of repetitive elements and genes. **Keywords:**Comparative genomics; pacbio; assembly

HP005 - **IL-32γ PLAYS A PROTECTIVE ROLE IN EXPERIMENTAL VISCERAL LEISHMANIASIS**

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Visceral Leishmaniasis (VL) is a chronic infectious-parasitic disease caused by *Leishmania infantum chagasi* in the Americas. Brazil is among the countries responsible for 90% of the cases of VL in the world. The factors that determine the control or persistence of the parasite are not yet fully understood. IL-32 is an intracellular cytokine that has nine different isoforms, being IL-32γ the most active. IL-32 can induce other pro-inflammatory cytokines in innate immunity cells and polarize the acquired immune response to a Th1 and Th17 profile. Since rodents do not express any gene homologous to IL-32, despite responding to this cytokine, we used C57BL/6 mice carrying the human IL-32 gene (IL-32γTg) to assess the role of IL-32 in infection by *L. i. chagasi*. Wild-type (WT) and IL-32γTg mice were infected i.p. with 10^7 stationary phase-promastigote forms and euthanized after 30 days. Infection with *L. i. chagasi* induced expression and production of IL-32γ in the spleen and liver of the IL-32γTg mice. The IL-32γTg mice presented lower parasitism in both spleen and liver compared to WT mice. The protection was associated with a significant increase in the production of nitric oxide by spleen cells from the IL-32γTg mice, after ex vivo restimulation with *L. i. chagasi* antigen for 72 h. In addition, in the supernatants of *ex vivo L. i. chagasi* antigen re-stimulated spleen cells cultures from infected IL-32γTg mice produced higher levels of IFN-γ, IL-17A and TNF-α than WT mice . Similar results were found in the spleen and liver lysates of the animals. The IL-32γTg mice had a higher percentage of $CD4^+IFN\text{-}\gamma^+$ (Th1) and $CD4^+IL\text{-}17A^+$ (Th17) cells in the spleen compared to the WT mice. The data indicate that IL-32γ promotes a mixed Th1/Th17 profile immune response during experimental visceral leishmaniasis, which contributes to the control of infection with *L. infantum chagasi*. **Supported by:**CNPq, FAPEG, CAPES
Keywords: Visceral leishmaniasis; il-32; immune response

HP007 - ***TRYPANOSOMA RANGELI* RESISTANCE TO COMPLEMENT SYSTEM: EVIDENCES OF PROTEIN-PROTEIN INTERACTION AND C3 CONVERTASE INHIBITION**

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The complement system (CS) of mammals contains a number of proteins that interact in response to infections mainly via opsonization of pathogens and induction of inflammation. In order to establish the infection, it is crucial for pathogens to avoid the action of the CS and invade the host cells. While such mechanisms are well known for *Trypanosoma cruzi*, the etiologic agent of Chagas' disease, it is not clear for *Trypanosoma rangeli*. Despite infecting the same hosts and vectors in Central and South America, little is known about the *T. rangeli* survival strategies within mammals. Unlike *T. cruzi, T. rangeli* epimastigotes are resistant to CS, but the factors mediating such resistance are unknown. The aim of the present study is to characterize the resistance of different forms of *T. rangeli* to mammalian CS and to characterize proteins involved in this process. Exposure of *T. rangeli* (Choachí strain) to fresh human or Balb/C mice serum revealed that ~80% of *in vitro*-derived trypomastigotes and 95% of epimastigotes are resistant. Assessment of C3b deposition on *T. rangeli* membrane after exposure to fresh human serum was carried out by indirect immunofluorescence assays using epimastigotes and trypomastigotes and revealed negative. Pull-down assays using total protein extracts from *T. rangeli* and early CS proteins (C1q, C3b and Mannan-binding lectin) immobilized in magnetic streptavidin beads allowed the identification of 251 proteins by mass spectrometry (MS). Using a gel-free approach on MS analysis, 61 proteins related to the classical CS pathway, 68 to the alternative CS pathway and 61 to the lectin-based CS pathway were obtained. This work demonstrates the *T. rangeli* ability to subvert the alternative CS pathway by preventing C3b binding and C3 convertase formation on the parasite membrane. Therefore, we suggest that *T. rangeli* trypomastigotes, as observed for *T. cruzi*, may be resistant to lysis via alternative CS pathway. **Supported by:**CNPq, CAPES, FINEP and UFSC
Keywords: Innate immunity; c3b deposition; alternative cs pathway

HP008 - **TOWARDS IDENTIFICATION OF PHOSPHOLIPID RECOGNIZED BY MACROPHAGES RAW IN *LEISHMANIA (L.) AMAZONENSIS*.**

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Background: *Leishmania* uses several mechanisms to escape from the host immune response. As a strategy, the parasite is internalized by macrophage without the activation of mechanisms to kill parasite. The aim of this communication is to identify the phospholipid present in the external plasma membrane layer of *Leishmania (L.) amazonensis* that leads to its macrophage recognition and internalization that could help to understand the molecular mechanism of immune response evasion. Methods: We performed macrophage infection assays (4h and 24h) with promastigotes of *L. (L.) amazonensis* in a competition with liposomes composed of defined phospholipid classes. In addition, inward phospholipid transport by promastigotes was analyzed using fluorescent phospholipids. Results: Infection assays performed with PC liposomes resulted in a dose dependent decrease in the rate of infected macrophage and in the infectivity rate, in both 4h and 24h of infection, nearly to 10, 30 and 40% in 0.2 µM, 20 µM or 2 mM of PC, respectively. By contrast, the decrease was not observed in the presence of liposomes composed of PS or PC:PE (1:1). As a control, the viability assay showed that 0.2 µM and 20 µM of PC or PC:PE are not toxic to parasite or macrophage; exposition with 2 mM of PC or PC:PE liposomes reduced in 50% the viability of the parasite, but not of the macrophage. Internalization of PC by the parasite was higher when compared to the PS or PE uptake. Competition assay between the labeled and non-labeled phospholipid in stationary phase promastigotes showed that PS and PE reduced the PC uptake, PS and PC reduces the PS uptake, but PC and PS increased the PE uptake. Conclusion: The results suggest that PC is the preferential phospholipid recognized by the macrophage, possibly inhibiting the entry of the parasites. The number of amastigotes inside the infected macrophage was not altered, indicating that the parasites phagocytosed by macrophages replicates normally.
Supported by:FAPESP, CAPES, CNPq e Danish National Research Foundation (DNRF85).
Keywords: Phosphatidylserine (ps); phosphatidylcholine (pc); phosphatidylethanolamine (pe)

HP009 - **DEVELOPMENT OF A THREE-DIMENSIONAL MIGRATION ASSAY FOR STUDY OF TRYPANOSOMA CRUZI-MAMMALIAN CELL INTERACTION**

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Three-dimensional (3D) cultures are physiologically relevant because mimic the microarchitecture of tissues and provide an environment similar to the encountered in natural infections, bridging the gap between traditional monolayer cultures (2D) and animal models. In this work, we combined the 3D culture technology with host-pathogen interaction, by studying transmigration of *T. cruzi* trypomastigotes of two representative strains (CL Brener DTU VI; SylvioX10 DTU I) into 3D spheroids. Spheroids of HeLa cells expressing Actin-RFP were cultured with CFSE-labelled trypomastigotes and transmigration into spheroids was analyzed at 24 h post infection. Images obtained by 3D-reconstruction from confocal microscopy stacks showed that CL Brener was more migratory than SylvioX10, which was retained at the surface of spheroids (>40 µm vs <10 µm inside the spheroids, respectively). Also, CL Brener trypomastigotes were evenly distributed in all the spheroid surface and preferentially located in the paracellular space, while SylvioX10 parasites presented a "patch-like" distribution pattern. Spheroids cultured with CL Brener showed a ~four-fold higher percentage of infected cells than those infected with SylvioX10 (by qPCR and flow cytometry in disaggregated spheroids and in agreement with confocal microscopy). Moreover, transmigratory capacity seem to be an intrinsic and non-transferable feature of each strain, since the co-infection of spheroids simultaneously with CL Brener and SylvioX10, did not affect the characteristic pattern of each strain. Distinctive migration patterns into spheroids were also detected for other *T. cruzi* strains, and were independent of the DTU. Altogether, our results suggest that, in a 3D microenvironment, each strain presents a characteristic migration pattern and tissular distribution that can be associated to their *in vivo* behavior; certainly, the findings presented here cannot be studied with traditional 2D monolayer cultures. **Supported by:**CONICET, FONCyT, UNSAM
Keywords: Trypanosome cruzi; 3d-spheroid; migration assay

HP010 - **ALTERING THE FLAGELLAR POCKET SHAPE IN LEISHMANIA WITH FLAGELLUM ATTACHMENT ZONE MUTANTS CAUSES A LOSS OF DEVELOPMENT AND PROLIFERATION IN THE SAND FLY VECTOR AND PATHOGENICITY IN THE MAMMALIAN HOST**

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The flagellar pocket of Leishmania is often assumed, without direct evidence, to be an essential conduit through which the cell interacts with the environment and therefore critical for development and pathogenicity. In Leishmania promastigotes the flagellum is described as “free”, extending from the flagellar pocket at the cell anterior; yet these parasites have homologs of many flagellum attachment zone (FAZ) proteins, which in trypanosomes attach the flagellum laterally to the cell body. We have shown that Leishmania have a FAZ structure, which is intrinsically linked to the morphogenesis of the flagellar pocket. We have perturbed expression of certain FAZ proteins such that the flagellar pocket shape is altered yet these cells are able to proliferate in culture. We have used these mutants to address the assumption that flagellar pocket presence and architecture is an essential feature of parasite development and pathogenicity.
Here, we show that LmFAZ5 knockout mutant promastigotes display a disruption of FAZ organisation resulting in a loss of attachment between the flagellum and the cell body, with concomitant changes to the shape and size of the flagellar pocket and neck region. In LmFAZ5 knockout axenic amastigotes the loss of attachment in the neck region results in a dramatically different flagellar pocket and neck organisation and an increase in the length of flagellum outside the cell body. These data show that perturbation of the FAZ in Leishmania can produce alterations in flagellar pocket morphology yet both promastigotes and amastigotes are able to proliferate normally in culture. However, infection studies show that these changes have a large impact on the development and proliferation of Leishmania in sand flies and a dramatic reduction in the parasite burden in mice. We believe that this provides the first direct demonstration of the importance of flagellar pocket morphogenesis and function for kinetoplastid parasite development and pathogenicity. **Supported by:**Wellcome Trust **Keywords:**Flagellar pocket; pathogenicity; morphology

HP011 - **EXTRACELLULAR VESICLES RELEASED FROM B-1 CELLS INFECTED WITH *LEISHMANIA (LEISHMANIA) AMAZONENSIS* INCREASED INFLAMMATORY RESPONSE IN MURINE MACROPHAGES**

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Immune cells can release different type of extracellular vesicles (EVs) that are relevant vehicles of intercellular communication. EVs targeted various types of immune cells and are involved in immune regulation depending on the context. B-1 cells are a subtype of B lymphocytes with peculiar functions in immunity. These cells are able to produce regulatory cytokines (mainly IL-10), natural antibodies and to differentiate into phagocytic cells. The macrophages modulation was evaluated after their treatment with EVs from B-1 cells infected with *L. (L.) amazonensis*. Our results showed that B-1 cells spontaneously released EVs but we observed an increase in EVs releasing after 24 or 48 hours of in vitro infection, as demonstrated by nanoparticle tracking analysis (NTA) and Scanning Electron Microscopy. Medullar macrophages from BALB/c mice showed a significant increase in IL-6 and IL-10 after treatment with EVs from B-1 cells infected with the parasites by 24 hours. No differences were observed to TNF-α and iNOS. However, EVs obtained after 48 hours of B-1 cells infection with *L. (L.) amazonensis* led to a significant increase in TNF-α, IL-6, IL-10 and iNOS production, as compared to macrophages stimulated with EVs released by non-infected B-1 cells. Macrophages did not alter the phagocytic index (PI) after treatment with EVs from infected or non-infected B-1 cells. In vivo studies were performed to evaluate the influence of EVs from B-1 cells in the course of experimental cutaneous leishmaniasis. Our results demonstrated that B-1 cells can release EVs which are able to alter macrophages functions. This is the first evidence that EVs from B-1 cells can act as a new mechanism of communication between these cells and macrophages.
Supported by:FAPESP **Keywords:**Extracellular vesicles; b-1 cells; leishmania

HP012 - **ANTIGENICITY OF THE *TRYPANOSOMA CRUZI* MASP MULTIGENE FAMILY IN THE VERTEBRATE HOSTS**

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The protozoan parasite *Trypanosoma cruzi* is the causative agent of Chagas disease. Comparative genomics have shown that *T. cruzi* had the largest expansion of multigene families among the Tritryps. One of these families is the Mucin-Associated Surface Protein (MASP), a highly variable multigene family that encode surface proteins mainly expressed in trypomastigotes. Here we have shown that members of this family are among the *T. cruzi* proteins with the higher density of B-cell epitope predictions. Despite this, little is known about the vertebrate humoral response against MASPs. In order to better characterize the vertebrate immune response against this family, we separated the MASP predicted proteins into seven subgroups based on sequence similarity and selected a central representative member of each subgroup to be expressed as recombinant proteins. The antigenic profile of the seven MASP subgroups were initially accessed by ELISA using sera from mice (i) in the acute and chronical forms of Chagas disease (ii) infected with *T. cruzi* from different DTUs in the acute phase and (iii) infected with parasites provenient from successive passages in mice model. The results show that the MASP members are differently recognized during the acute phase of *T. cruzi* infection and between strains. Next, we evaluated the MASP reactivity to sera from patients with Chagas' disease reactivation, showing that the MASP antigenic profile also varies among patients. Finally, the seven recombinant MASP proteins were coupled to fluorescent microspheres in order to evaluate the recognition profile of several MASP members in a single assay. The MASP proteins coupled to the microspheres were incubated alone or pooled with the sera from mice infected with *T. cruzi* or uninfected mice. The results show differential reactivity against distinct MASP members during the acute phase of infection, suggesting that MASP antigens may play a role in immune evasion mechanisms.
Supported by:CNPq, FAPEMIG, CAPES **Keywords:**Masp; antigenicity; immune response

HP013 - **COMPARISON AMONG IMMUNOLOGICAL AND MOLECULAR TESTS APPLYING LESS INVASIVE SAMPLING TO DETECT *LEISHMANIA INFANTUM* INFECTION IN ASYMPTOMATIC ANIMALS FROM AN ENDEMIC AREA IN BRAZIL**

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Visceral leishmaniasis (VL) is a vector-borne zoonotic disease caused by *Leishmania infantum*. Domestic dogs are the principal reservoir hosts. In endemic areas dogs are the target of control measures because their presence in households increases the risk of humans developing VL. The objective of this study was to evaluate *L. infantum* infection in asymptomatic dogs from an endemic area, Governador Valadares-MG-Brazil. Sixty two asymptomatic dogs were analyzed. Blood sample was collected in an EDTA tube and popliteal lymph node aspirate in a vial containing RNA Later®. Plasma was used for DPP®CVL (Bio-Manguinhos) serology, while total blood and lymph node aspirate were utilized for qPCR using ssrRNA primers. The HP0RT gene was used as endogenous control. Results showed that 16/62 dogs were positive for DPP, 3/62 dogs were positive by qPCR from blood and 13/51 were positive by qPCR from lymph node aspirate. Eleven animals showed no amplification of HP0RT gene in lymph node samples. Two dogs were positive for all methods. Nine animals were positive by DPP and qPCR from lymph node. Seven animals were positive only by DPP. Four animals were positive only by qPCR from lymph node. One animal was positive only by qPCR from blood. The parasite burden varies between 0.14 to 1148.4 parasites/1000 dog cells in lymph node and 0.29 to 1 parasites/1000 dog cells in blood. A significant correlation was found between DPP and qPCR from lymph node ($p=0.001$). The lymph node aspirates are better samples for asymptomatic dogs than blood. Our results show that dogs living in endemic areas may become infected but remain seronegative while some seropositive dogs may present undetectable parasite DNA. Studies have shown that both symptomatic and asymptomatic dogs were infectious for sand flies with different intensity (>polysymptomatic dogs). Therefore, studies aimed at following infection must be performed to identify animals with high parasite burden that may be important in VL transmission. **Supported by:**NIH, CAPES, IOC-FIOCRUZ
Keywords: Canine visceral leishmaniasis; serology; molecular test

HP014 - **ENDOSOMAL TOLL-LIKE RECEPTORS SIGNALING FACILITATES MACROPHAGE RESISTANCE TO *LEISHMANIA MAJOR* INFECTION THROUGH INDUCTION OF AUTOPHAGY**

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Leishmania major is one of the causative species of Leishmaniasis, a neglected disease that affects and kills people in many countries worldwide every year. During human infection, the parasite infects mainly innate immune cells like macrophages and neutrophils, which have several mechanisms of detection and effective defense. Pattern recognition receptors, such as endosomal Toll-like receptors (TLRs) have been shown to be important for resistance to *L. major in vivo*, however the mechanisms are still unknown. Since it has been previously demonstrated that endosomal TLR signaling is important to protect host against *L. major* infection, we evaluated the engagement of endosomal TLRs triggering autophagy and if contributes to resistance to pathogen infection in macrophages. We demonstrated that infected bone marrow-derived macrophages (BMDMs) from C57BL6 mice undergo autophagy, a well known lisosome-dependent degradation pathway, and that was important to restrict parasite replication. Our results demonstrated that autophagy signalling required endosomal TLRs, once deficient macrophages for TLR3, -7, and -9 (Tlr3/7/9$^{-/-}$), UNC93B1, or MyD88 failed to undergo *L. major*-induced autophagy. When C57BL/6 and Tlr3/7/9$^{-/-}$ BMDMs infected with *L. major* were treated with rapamycin, an autophagy inductor drug, the parasite replication was reduced. Similar results were obtained in vivo, with reduced lesion size after rapamicyn treatment. All together, our results indicate that autophagy is triggered via endosomal TLRs during *L. major* infection and is important to parasite replication control. **Supported by:**FAPESP; CNPq; CAPES; CRID; FAEPA; INCTV **Keywords:**Autophagy; toll-like receptors; l. major

HP015 - **THE USE OF MESENCHYMAL STROMAL CELLS AS AN ALTERNATIVE TREATMENT FOR *LEISHMANIA AMAZONENSIS* INFECTION**

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Cell therapy using stem cells can be used to reestablish the homeostasis of the immune system using the immunological properties of these cells. Leishmaniasis is a disease caused by protozoa of the genus *Leishmania*. One of the characteristics of the disease is the unbalance of host immune responses to foster parasite survival. In our work we evaluated the use of bone marrow-derived mesenchymal stromal cells (MSC-BM) and adipose tissue-derived mesenchymal stromal cells (MSC-AT) as an alternative therapy for the murine cutaneous leishmaniasis in C57BL/6 mice. First we infected macrophages *in vitro* with *L. amazonensis* for 4 hours and placed them in co-culture for 48 hours with MSC-BM or MSC-AT. We observed that macrophages in co-culture with MSC-BM had a worsening of infection, unlike macrophages in co-culture with MSC-AT. In the *in vivo* infection model, animals were infected and received two doses of $1x10^5$ stromal cells into the jugular vein on the 15th and the 21st day after infection. Animals treated with MSC-AT had partial protection in lesion growth, whereas those treated with MSC-BM had no differences in their lesion, but neither group showed any difference in parasite load of the footpads compared to their controls. In an attempt to control the parasitic load, we combined treatment of MSC-AT with pentavalent antimoniate (PA) from 18th days post infection. The combined treatment group had a smaller lesion, lower parasitic load on the footpads and draining lymph node (LN), and lower number of total cells in LNs. By flow cytometry no significant percentage differences were found in the populations of T reg, T CD4$^+$ and T CD8$^+$. And despite some changes in the percentages of IFN-γ and TNF-α producing CD4$^+$ and CD8$^+$ T cells, there were no differences in the total populations of these cells. Our results indicate that mesenchymal stromal cells derived from adipose tissue can be an alternative form of treatment or an adjuvant to PA, in infection with *L. amazonensis*. **Supported by:**CNPq **Keywords:**Leishmania; leishmaniasis; stem cell

HP016 - **BMOOMP-αLPHA-I: A POTENTIAL METALOPROTEASE FOR TREATING ILEITIS INDUCED BY TOXOPLASMA GONDII.**

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Toxoplasma gondii oral infection mediates an exacerbated production of TNF leading to an intestinal inflammation which is quite similar to the response during Crohn's disease. TNF cytokine overexpression has a strong correlation with the intestinal damage during the infection with T. gondii and the neutralization of this cytokine is a potential therapeutic intervention. BmooMP-α-I is a snake venom metalloprotease isolated from Brothrops moojeni able of hidrolise TNF. Thus, we aimed to evaluate whether the deleterious effects of the T. gondii-induced ileitis could be reduce by the treatment with BmooMP-α-I. For this porpose, an oral infection with T. gondii were done in C57BL/6 mice for inducing ileitis. After 3 days p.i., animals were treated with the protein BmooMP-α-I or placebo as control during 3 days. Morbidity and mortality score were determined and we colleted the tissue samples on day 7 post-infection. Then, macroscopic and microscopic aspect of ileum as well as the cytokine profile production were evaluated. We could observe that the treatment with BmooP-α-I were able of augment the survival rates, ameliorate the clinical signs of disease of animals, such as weight loss. The macroscopic aspect of intestine were significantly reduced and the according to microscopic score the inflammation was reduced from very severe to severe. Furthermore, the cytokine production and expression of inflammatory mediators by mesenteric lymph node cells. Furthermore, occurred a diminished parasite burden in the ileum and a reduced ex vivo response by spleen and mesenteric lymph node cells were reduced. The findings of this study provide an evidence that the BmooMP-α-I treatment is able to reduce ileal inflammation, making possible to hypothetize that this type of approach could be also helpful for treating intestinal inflammation. **Supported by:**cnpq fapemig capes
Keywords: Ileitis; toxoplasma gondii; metaloprotease treatment

PV001 - **INTRACELLULAR HEME LEVELS MODULATES TCHTE EXPRESSION IN TRYPANOSOMA CRUZI**

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Trypanosoma cruzi lacks a heme biosynthesis pathway, then the heme quota should be covered by its incorporation from the hosts. Our group is interested in elucidating how *T. cruzi* takes heme from different hosts, how its transport is regulated and how it is distributed inside the cell. We clearly showed that *T. cruzi* is able to import heme during the replicative life-cycle stages and also identified a protein, TcHTE, that is involved in heme transport (Merli, 2016). TcHTE, member of the HRG family and located in the flagellar pocket, can modulate heme uptake in epimastigotes. When heme availability was altered during epimastigotes' growth, TcHTE protein and mRNA levels also changed. TcHTE protein was almost no detected when epimastigotes were grown in a medium with sufficient heme (5 µM or higher). However, when heme was depleted (less than 5 µM), TcHTE level increased and the accumulation of its mRNA was higher compared to values obtained in a heme sufficient medium. Besides, we studied heme uptake by growing epimastigotes in presence of hemin and heme fluorescent analogs (HAs) following its incorporation by fluorescent confocal microscopy and total fluorescence measurements. We found that not all tested HAs were imported by epimastigotes. Those HAs that were incorporated (ZnPP, ZnMP and GaPP), negatively affected epimastigotes' growth due to their toxicity, and also reduced TcHTE accumulation. On the other hand, SnMP, the only analog that was not incorporated by the parasite, did not affect epimastigotes' growth and surprisingly TcHTE's accumulation was not altered. Based on these facts, it is reasonable to postulate that *T. cruzi* presents a specific transport system responsible for heme uptake that can differentiate structurally similar compounds. TcHTE protein, is a relevant protein that promotes and/or regulate heme uptake, where its mRNA and protein levels respond to intracellular heme availability. **Supported by:**ANPCYT
Keywords: Trypanosoma cruzi; heme; transport

PV002 - **QUANTIFYING SUBCELLULAR STRUCTURE AND ORGANELLE DYNAMICS OF THE *EIMERIA TENELLA* SPOROZOITE**

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Eimeria tenella is a key agent of poultry coccidiosis, a disease that is characterised by the destruction of intestinal epithelial cells and has a significant impact on both animal welfare and economic security of poultry production worldwide. *Eimeria spp*. exhibit complex life cycles involving several morphologically distinct stages, many aspects of which remain poorly understood.
Using three-dimensional electron microscopy techniques (serial block face – scanning electron microscopy and transmission electron tomography) we were able to quantify organelle numbers and volumes for the initial invasive (sporozoite) stage of *E. tenella*. We have resolved and modelled the conoid (a specialised cytoskeletal structure important for cellular invasion) and have quantified the number, length, curvature and spatial positioning of the individual tubulin fibres. In addition, our tomogram data has revealed the presence of extensive vesicular networks associated with the secretory invasion organelles: micronemes and rhoptries.
Both confocal and widefield light microscopy were used to investigate the dynamics of a largely cryptic class of organelle: the refractile bodies; these were shown to reduce in number from two to one per sporozoite within the first few hours following the invasion of mammalian cells in vitro. Increasing our knowledge of the natural biology of *E. tenella* will help to identify novel ways to combat this pathogen. **Supported by:**Royal Veterinary College research fund and Oxford Brookes 150 anniversary research fund. **Keywords:**Eimeria; conoid; electron microscopy

PV003 - **PROTEIN KINASES THAT REGULATE LEISHMANIA DIFFERENTIATION**

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Leishmania encounters a number of challenges during its life cycle following environmental changes that trigger the transition from an amastigote that replicates inside a parasitophorous vacuole in the mammalian host, to the extracellular insect stages, a replicative procyclic and an infective metacyclic promastigote. The differentiation process is thought to be regulated in part by phosphorylation resulting in dramatic changes in protein expression followed by adaptation. Here we aim to identify and characterise protein kinases (PKs) involved in this process, and understand the mechanisms Leishmania use to overcome stress and regulate life cycle. We have applied the CRISPR-Cas9 genome editing system to L. mexicana in order to investigate the kinome, including 2 PKs previously characterized in Trypanosoma brucei as regulators of bloodstream to procyclic form differentiation, RDK1 and RDK2 (Repressor of Differentiation Kinase). To start we focused on PKs found in Leishmania but not Trypanosoma genus, hereby referred as Leishmania unique kinases (LUKs), as well as Leishmania Orphan kinases. We targeted in total 23 genes for knock out and in situ tagging and were able to localise those PKs in the nucleus, kinetoplast, flagellum, endocytic compartments endoplasmic reticulum, as well as the cytoplasm of promastigotes. Phenotyping of null mutants assessed the ability to differentiate to amastigotes, infect macrophages and generate footpad lesions in BALB/c. For example, RDK1 is non-essential for L. mexicana promastigotes, amastigote survival in macrophages and infectivity in vivo, but its absence impacts on amastigote proliferation and differentiation to promastigotes in vitro. High-througHP0ut phenotyping strategies allow us to generate PKs null mutants and in situ tagged cell lines, and investigate their involvement and interactions during Leishmania life cycle progression. We are now one step forward on understanding signalling pathways that regulate this process. **Supported by:**Wellcome **Keywords:**Leishmania; crispr-cas9; kinome

PV004 - **TRYPANOSOMATIDS USE MORE ORIGINS THAN THE MINIMUM REQUIRED TO COMPLETE DNA REPLICATION WITHIN S-PHASE PERIOD DUE TO REPLICATION-TRANSCRIPTION CONFLICTS**

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Trypanosomatids are a group of eukaryotic parasites that have peculiar characteristics, such as polycistronic transcription and tolerance to aneuploidies. This group includes human pathogens of great medical relevance, such as Trypanosoma spp. and Leishmania spp. Here, we used DAPI morphological patterns of T. brucei, L. major, and L. mexicana to estimate, for each of these parasites, the duration of the mitosis and cytokinesis. Also, using EdU to monitor DNA replication, we estimate precisely the duration of G1, S, and G2 phases. Then, we developed a formula to estimate the minimum number of replication origins required to duplicate each chromosome of these parasites. This formula is a function of the newly estimated S phase duration, chromosome size, and DNA replication rate. After the calculations, we compared the values found with the number of origins detected experimentally, concluding that trypanosomatids use more DNA replication origins than the theoretical minimum required. To investigate this feature, we developed computational models involving the frequency of origin firing and replication-transcription conflicts. These simulations provide us clues to speculate that replication-transcription conflicts activate dormant replication origins, contributing to increasing the number of total origins detected. This hypothesis was supported by the measurement of the γH2A and DNA/RNA hybrids levels by IIF in the absence of transcription. Moreover, this approach indicates that replication-transcript conflicts are responsible for the basal level of γH2A observed in some trypanosomatids. Analysis of the number of active origins (by DNA combing) after transcription inhibition are been carried out. Taken together, these results point to a non-stochastic usage of the total number of replication origins, putting the firing of dormant origins as a result of replication-transcription conflicts, which contributes to maintain the robustness of S-phase period. **Supported by:**FAPESP
Keywords: Dna replication origins; s-phase duration; replication-transcription conflicts

PV005 - **LUTZOMYIA LONGIPALPIS TGF-β HAS A ROLE IN LEISHMANIA INFANTUM CHAGASI SURVIVAL IN THE VECTOR**

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Leishmaniasis is transmitted to the vertebrate host mainly through the bite of sand flies. The intimate vector-parasite relationship leads to insect immune responses. In the malaria vector Anopheles the modulation of a TGF-β influences the production of NO and this modulation affects Plasmodium infection.
We found that an L. longipalpis Transforming Growth Factor–β from the activin-inhibin family (LlTGF-β) was overexpressed 72h post infection with L. i. chagasi, when parasites are starting to attach to the midgut epithelium. LlTGF-β seems to be modulated by the Toll pathway, since the silencing of cactus caused an increase in LlTGF-β expression. In the absence of infection, the silencing of LlTGF-β increased the expression of the immune effector molecules cecropin and defensin, but not INOS. We also investigated the effect of LlTGF-β silencing on immune responses and L. longipalpis infection with L. i. chagasi. With the depletion of LlTGF-β there is a drastic reduction of infection starting at 48h and also an increase of AMPs and INOS expression indicating that LlTGF-β probably is a negative regulator of immune responses and has a possible role in controlling L. i. chagasi infection inside the sand fly. We have also used the TGF-β receptor inhibitor SB-431542 in similar studies and found that depletion using gene silencing or receptor inhibition led to similar results, namely increased immune responses and decreased Leishmania infection.
Thereby we can assume that in L. longipalpis the Leishmania contact with the gut or the increase of NOS might be causing an activation of LlTGF-β and a negative regulation of immune effectors. **Supported by: Keywords:**Lutzomyia longipalpis; innate immunity; tgf-beta

PV006 - **ARGININE METHYLATION AS A NOVEL MODULATOR OF RNA-BINDING PROTEIN EXPRESSION AND FUNCTION IN *LEISHMANIA MAJOR***

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Arginine methylation is a post-translational modification present in multiple cellular processes and is catalyzed by protein arginine methyltransferases (PRMTs). *Leishmania* spp. genomes encode five different PRMT genes. PRMT7 is implicated in parasite virulence and it is the only enzyme known to exclusively catalyze monomethyl arginine (MMA). To investigate the impact of PRMT7 function on global monomethylation in *Leishmania* parasites, we performed comparative methyl-SILAC proteomics of wild-type versus Δ*prmt7* parasites. We find that 3% of the *Leishmania major* predicted proteome carry at least one MMA modification with a strong selection of trans-regulatory RNA-binding proteins (RBPs). Forty candidate target proteins were hypomethylated in the absence of the PRMT7 enzyme, isolating putative methylation sites in 17 RBPs. We confirmed the direct monomethylation of candidate targets RBP16 and Alba3 by PRMT7 *in vitro* and validated amino acids essential to this modification. To evaluate the impact of arginine methylation on protein function, we endogenously tagged candidate target RBPs to examine expression and RNA binding capacity in response to PRMT7 levels. Expression levels of three PRMT7 target RBPs reveal altered expression in Δ*prmt7* parasites, possibly implicating MMA in protein stability. In contrast, the RNA-binding capacity of Alba3 to *delta-amastin* and *p1/s1* nuclease mRNAs is reduced in Δ*prmt7* mutant parasites despite unchanged protein levels. Ongoing experiments will identify transcripts regulated by PRMT7 target proteins of potential relevance to parasite virulence. Our results suggest MMA modification by LmjPRMT7 epigenetically regulates *Leishmania* virulence by altering the function of trans-regulatory RBPs which control parasite gene expression. **Supported by:**MRC-FAPESP and CNPq
Keywords: Arginine methylation; prmt; rbp

PV007 - **PROTEIN KINASE C SIGNALING INTERFERES IN PROLIFERATION, DIFFERENTIATION AND METACYCLOGENESIS OF *LEISHMANIA AMAZONENSIS***

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Protein Kinase C (PKC) comprises a group of multifunctional proteins that catalyze phosphorylation in serine and threonine residues. The PKC was characterized in *Leishmania amazonensis* promastigote, related to tegumentar leishmaniasis. During the life cycle, the parasite in the invertebrate host differentiates from procyclical to metacyclic promastigotes in a process named metacyclogenesis, which is accompanied by morphological and metabolic changes regulated by protein kinases. Thus, the goal of this work is to investigate the involvement of *L. amazonensis* PKC in the promastigote proliferation, differentiation and in the metacyclogenesis. To investigate the differentiation, promastigotes were cultivated in Schneider medium, pH 5.5, with 20% of Fetal Bovine Serum (FBS), with or without different concentrations of RO32-0432 (PKC inhibitor) and PMA (PKC activator), and the differentiation to axenic amastigotes was monitored. The promastigotes proliferation was evaluated in Schneider medium, pH 7.2, 10% FBS with or without different concentrations of RO32-0432 and PMA, and parasites numbers counted in Neubauer chamber every 24 h for 5 days. Metacyclogenesis was evaluated in promastigotes cultivated as above for 5 days after metacyclic purification using ficoll gradient. Our results have shown that RO32-0432 inhibited both differentiation and proliferation in a dose dependent manner, and interferes in the metacyclogenesis, by decreasing the rate of metacyclic promastigotes *in vitro*. On the other hand, PMA had the opposite effect, because both proliferation and differentiation were stimulated. Taken together, these results demonstrated that activation and inhibition of PKC interfere directly in the processes of differentiation to amastigotes, in promastigotes proliferation and in metacyclogenesis *in vitro.* **Supported by:**FAPERJ, CNPq, PAPES, CAPES, IOC/Fiocruz
Keywords: Leishmania amazonensis; protein kinase c; tegumentar leishmaniasis

PV008 - **COMPARATIVE TRANSCRIPTOME ANALYSIS OF PROCYCLIC, METACYCLIC AND AMASTIGOTE STAGES IN *LEISHMANIA BRAZILIENSIS***

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Leishmania (Viannia) braziliensis is the causative agent of cutaneous and muco-cutaneous leishmaniasis in Brazil. In this study, modulation of gene expression between *L. braziliensis* developmental stages was analysed at the transcriptome level by RNA-seq. The transcriptomes of procyclic promastigotes, ficoll purified metacyclis and axenic amastigotes of *L. braziliensis* Lb 2903 strain were analysed and compared. The analyses revealed 6576 genes differentially expressed (DE) between procyclic promastigotes and amastigotes; 5689 DE genes between procyclic and metacyclic promastigotes and 4856 DE genes between metacyclic and amastigotes. A Gene Ontology analysis was also conducted. Based on the annotation of the genome the main functional categories of genes, differentially expressed throughout development, were defined. In addition, *L. braziliensis* specific genes were selected and evaluated concerning possible differential expression. Herein, *Leishmania spp.* putative ncRNAs at different life stages were computationally identified and characterized. Large-scale approaches combined with bioinformatic tools allowed the identification of 11,175 putative ncRNAs in *L. braziliensis*. Genes DE were identified and categorized according to their size, genome location and conservation among *Leishmania* species. Those genes were also submitted to ncRNA predictors (ncRNAs PORTRAIT, RNAcon, ptRNApred, snoscan and tRNAscan-SE). Thus, this work contributes to improve the understanding of gene expression regulation processes in *Leishmania*. In addition, this data offers important information about parasite genetic organization, gene expression regulation, along the development, and a large database of *L. braziliensis* transcriptome. **Supported by:**Fapesp
Keywords: Leishmania (viannia) braziliensis; transcriptome; differentially expressed genes

PV009 - **HEME A SYNTHESIS IS ESSENTIAL FOR THE SURVIVAL AND INFECTIVITY OF *TRYPANOSOMA CRUZI***

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Although *Trypanosoma cruzi* is an aerobic organism, it does not produce heme. Heme is acquired from the hosts, distributed and inserted into heme-proteins such as respiratory complexes in the parasite's mitochondrion. It has been proposed that *T. cruzi*'s energy metabolism relies on a branched respiratory chain with a cytochrome c oxidase (CcO) as the main terminal oxidase, which contains two molecules of heme A as cofactors. Two enzymes, heme O synthase (HOS) and heme A synthase (HAS), are involved in heme A synthesis. We present here our results on heme A synthesis and its relevance in T. cruzi.
We identified the HOS and HAS enzymes in *T. cruzi*, named TcCox10 and TcCox15, respectively. TcCox15 was differentially detected in *T. cruzi*'s stages by Western blot assays, being more abundant in the replicative forms, reflecting the necessity of more heme A synthesis and therefore more CcO activity at these stages. We designed and constructed TcCox15 mutants replacing conserved His residues by Ala. The overexpression of these non-functional mutants caused a reduction in heme A content with a concomitant drop in CcO activity in epimastigotes, confirming TcCox15 as HAS in T. cruzi. Also, the overexpression of these non-functional mutants negatively affects trypomastigotes' infection and intracellular amastigotes' replication. Interestingly, the over-expression of TcCox10 protein caused a negative effect on epimastigotes growth, suggesting that heme A synthesis should be regulated at the first step of this pathway.
Our results clearly showed that a drawback in heme A synthesis provoked a reduction on CcO activity and, in consequence, an impairment on *T. cruzi* survival, proliferation and infectivity. This evidence supports that *T. cruzi* depends on the respiratory chain activity along its life cycle, being CcO an essential terminal oxidase. This reinforces the idea of parasite energy metabolism as an attractive therapeutic target to control infection and transmission. **Supported by:** CONICET and ANPCyT (Argentina) **Keywords:**Heme-proteins; heme a; cytochrome c oxidase

PV010 - **TRYPANOSOMA CRUZI HIGH MOBILITY GROUP B PROTEIN (TCHMGB) CAN ALTER CHROMATIN STRUCTURE AND AFFECTS IMPORTANT PARASITE FUNCTIONS**

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HIGH MOBILITY GROUP Bs are abundant non-histone chromatin proteins. The DNA-HMGB interaction produces changes in chromatin structure thus affecting important nuclear processes like transcription, replication, recombination, DNA repair and chromosome segregation.
TcHMGB, the HMGB from Trypanosoma cruzi, has two "HMG box" DNA binding domains and a unique N-terminal sequence that bears a nuclear localization signal (NLS) and a "DEK-C terminal" domain. We previously demonstrated that TcHMGB is expressed in the nucleus in all the parasite life stages and has architectural properties on DNA structure like its mammalian orthologs. Given the TcHMGB "architectural" properties, the particular characteristics of transcription in trypanosomatids and evidence gathered about epigenetic mechanisms controlling gene expression, we decided to evaluate the role of TcHMGB in vivo.
With the aim of investigating TcHMGB functions, we constructed transgenic parasites capable of overexpressing the protein under tetracycline induction. We showed that TcHMGB can interact with chromatin DNA in vivo, altering where an overexpression of the protein altered the chromatin state in epimastigotes making it more sensible to micrococcal nuclease treatment. Overexpression of TcHMGB caused a dramatic decrease in epimastigotes growth and an arrest in G2/M phase. Also, other stages´ functions resulted impaired: we observed a decrease in trypomastigote in vitro infectivity on Vero cells, amastigotes proliferation and trypomastigotes release from infected cells in vitro in overexpressing parasites. These results suggest that trypanosomatid HMGB proteins can be considered pleiotropic players involved in trypanosomes key cellular processes, and they should also be considered as putative actors in Chagas disease pathogenesis. **Supported by:**CONICET, ANPCYT
Keywords: High mobility group b; chromatin; epigenetic control

PV011 - **FLAGELLUM LENGTH AND BASAL BODY ORIENTATION IS AFFECTED BY THE CELL CYCLE DEPENDENT PROTEIN CEP164C IN *TRYPANOSOMA BRUCEI***

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Trypanosoma brucei is an important model for studying the function of the flagellum as these single celled organisms assemble a new flagellum alongside the old during each cell cycle. The flagellum has a diverse set of roles in trypanosomes including propulsion and morphogenesis. In trypanosomes the flagellum exits the cell body through the flagellar pocket and nucleation occurs from the basal body, which consists of microtubules and acts as a template to build the flagellum. The distal section of the basal body contains the transitional fibres which anchor the basal body to the base of the flagellar pocket. In mammalian cells, centrosomal protein 164 (Cep164) is present in the transitional fibres, and is involved with docking of the basal bodies to the plasma membrane. We investigated the function of Cep164 in *T. brucei*, where three diverse orthologues of the mammalian Cep164 protein were identified. We endogenously tagged all three copies of Cep164 with mNeonGreen and showed that all three localised to the transitional fibres of the basal body. Interestingly, one orthologue (Cep164C) shows a cell cycle dependant localisation, and localises to the transitional fibres of the old basal body. Ablation of Cep164C led to an increased rate of duplicating cells demonstrating morphological changes, displacement of the basal body, kinetoplast and flagellar pocket. The basal bodies appeared to be displaced anterior to the nucleus, or to the far posterior in early dividing cells. Cep164C knockdown also led to asymmetrical divisions generating cells with either longer or shorter cell bodies, with longer or shorter flagella. It is hypothesized that Cep164C could be important for the anchoring of the basal body to the flagellar pocket in trypanosomes. We are now investigating this phenomenon at the ultrastructural level to determine whether this protein is essential for allowing the basal body to dock to the plasma membrane and whether this protein is required for ciliogenesis **Supported by:**Oxford Brookes 150 anniversary fund
Keywords: Trypanosoma brucei; basal bodies; cep164c

PV012 - **A NOVEL ABC THIOL TRANSPORTER IN *TRYPANOSOMA CRUZI* INVOLVED IN THE ACQUIRED RESISTANCE TO BENZNIDAZOLE**

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The protozoan *T. cruzi* is the etiologic agent of Chagas disease, whose chemotherapy is performed with benznidazole (Bz). *T. cruzi* strains can present resistance due to the presence of ABC transporters, involved in cellular detoxification. Our aim was to investigate ABCC activity in *T. cruzi* and to evaluate its role in the resistance to Bz. ABCC activity was evaluated via a fluorescent substrate efflux assay performed by flow cytometry. Briefly, 10^6 parasites of Y strain were incubated with CFDA in the absence or presence of the transporter modulators probenecid, indomethacin or MK571. All modulators inhibited the CFDA efflux, as seen by the increase of median fluorescence intensity and percentage of CFDA+ epimastigotes. Trypomastigotes were inhibited only with MK-571, specific to the ABCC subfamily. In epimastigotes, CFDA efflux was similarly inhibited by ATP depletion with sodium azide or iodoacetic acid, and by thiol depletion with N-ethylmaleimide. Also, the CMFDA (fluorescent thiol-conjugated compound) efflux was inhibited by indomethacin in epimastigotes. The CFDA efflux was inhibited in epimastigotes of CL Brener, Berenice and Colombian strains by transporter modulators. The Bz-responsive CL Brener and Berenice strains showed greater CFDA transport compared to Y and Colombian strains, more resistant to Bz. Y strain epimastigotes selected by a 4 month-exposure to Bz in vitro presented higher CFDA transport than parental parasites. For the first time, ABCC-mediated thiol transport was demonstrated in *T. cruzi*, being the activity higher in epimastigotes than trypomastigotes of Y strain. Despite being present in all strains, ABCC activity does not participate in natural resistance to Bz. On the other hand, activity increased after induced resistance to Bz, indicating its relevance to the resistant phenotype after prolonged chemotherapy. Thus, the study of its biologic role is important to reveal new therapeutic approaches against acquired resistance mechanisms.
Supported by:CNPq; FAPERJ; UFRJ **Keywords:**Thiol transport; abc transporters; multidrug resistance

PV013 - **EIF2α PHOSPHORYLATION IS AN IMPORTANT FACTOR FOR *TRYPANOSOMA CRUZI* HOMEOSTASIS MAINTENANCE AND STRESS RESPONSE**

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The eukaryotic initiation factor 2 (eIF2) is a key protein involved in the control of translation initiation. Upon stress conditions, its alpha subunit is phosphorylated by specific kinases that cause a general translation arrest except of proteins that act to reverse the stress situation. Several lines of evidence suggest that a similar response also occurs in protozoan parasites, including the Trypanosoma cruzi that undergoes several transformations during its life cycle, most likely induced by environmental changes. To better understand the importance of eIF2α and its phosphorylation in T. cruzi, we generated parasite lineage overexpressing the wild-type eIF2α, or eIF2α in which the possible phosphorylated residues (T169 and S43) were replaced individually or together by alanine to prevent phosphorylation. Replication assays demonstrated that epimastigotes overexpressing eIF2α with a mutation for alanine at T169 (T169A) showed an increase multiplication rate and delay the concentration in which they enter in stationary phase. An intriguing finding was this T169A mutated lineage produced high levels of GP-90, a glycoprotein expressed preferentially in metacyclic-trypomastigotes (MCT). Nevertheless, all lineages were able to differentiate to MCT, infected LLC-MK2 cells and generated tissue culture derived (TCT) parasites. After infection with TCTs, all lineages became into amastigotes but the T169A overexpressor multiplied 30% less than all control lineages. In comparison with other lineages, the T169A lineage was more susceptible to benznidazol, the drug used to treat Chagas' disease. Indeed, benznidazol treatment induced eIF2α phosphorylation in a time dependent manner. These results indicated that the phosphorylation at T169 is crucial to maintain homeostasis in T. cruzi and to properly respond against stress conditions. Therefore, inhibition of eIF2α phosphorylation can be consider an adjuvant treatment for Chagas' disease.
Supported by: Fapesp; Cnpq **Keywords:**Eif2α drug treatment

PV014 - **SI-2, A QUINONE DERIVATIVE SIRTUIN INHIBITOR INDUCES PROGRAMMED CELL-DEATH IN BLOODSTREAM AND PROCYCLIC FORMS OF *T. BRUCEI.***

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Trypanosoma brucei is the etiological agent of Human African Trypanosomiasis (HAT), an endemic Sub-Saharan disease, responsible for 10000 new cases per year. Current treatment options present several limitations in terms of: toxicity, administration and efficiency. Sirtuins, a group of deacetylases, are essential in a number of biological processes, with atypical activities and unique functions in parasitic protozoa, are potential drug targets. Our group designed and synthetized 46 new compounds based on known sirtuin inhibitors and tested this collection against *T. brucei* Lister 427 sirtuin activities in cell-free extracts and against bloodstream (BSF) and procyclic (PCF) forms of the parasite. The compound 2 (SI-2) exhibited a promising growth inhibition with dose-dependent profile in both, procyclic and bloodstream forms, with IC50 values of 0.75 and 0.8 µM, respectively. Remarkably, SI-2 completely abolished the sirtuin activities in PCF cell-free extracts at a concentration corresponding to the IC50 for PCF growth. When PCF were treated for 48 h with SI-2 at concentrations of IC50 and IC80 for PCF growth, the percents of cells exposing extracellular phosphatidylserine but not labeled with propidium iodide were 10.3% and 29.3%, respectively, indicating an apoptotic process in these sub-population. We also found that for both IC50 and IC80 treatments cells had an increased reactive oxygen species (ROS) production and intracellular calcium levels and induces mitochondrial depolarization. We conclude that SI-2 is a promising drug against *T. brucei* that triggers an apoptotic cell death mechanism. Three putative isoforms of sirtuin 2 were described for *T. brucei*. Our results show that SI-2 is able to target all them at sub-micromolar concentrations. Further studies are being conducted to better characterize this inhibition.
Supported by:FAPESP/ CAPES/ CNPQ
Keywords: Sirtuin inhibitor; t. brucei; cell death program

PV015 - **DIFFERENTIAL ANALYSIS OF MRNA POPULATIONS ASSOCIATED TO DISTINCT EIF4E TRANSLATION INITIATION FACTORS IN *TRYPANOSOMA BRUCEI***

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Gene expression in trypanosomatids is primarily regulated by post-transcriptional processes and the initiation of protein synthesis, mediated by the eukaryotic initiation factors (eIFs), is a likely point for this regulation. During translation initiation in eukaryotes, the binding of the cap binding protein, eIF4E, to the mRNAs is a key step for ribosome recruitment and a likely target for regulation. Six eIF4E homologues were described in trypanosomatids, but only two, EIF4E3 and EIF4E4, have been implicated in translation initiation. Neither the mRNAs to which they bind to have been characterized, however, nor how this binding would interfere with translation regulation. This work aimed to identify the mRNA populations associated with EIF4E3 and EIF4E4 and to evaluate if the depletion of these proteins would affect the binding dynamics to these mRNAs. This investigation was carried out in *Trypanosoma brucei* procyclic cells, using RNAi to drastically reduce the levels of EIF4E3 and EIF4E4, followed by CLIP-seq, using Illumina sequencing. EIF4E4 is associated with mRNAs encoding proteins with structural roles, especially ribosomal proteins, while EIF4E3 binds preferentially to mRNAs encoding catalytic proteins and those involved in the cell structure, such as cytoskeleton constituents. The reduction of EIF4E3 levels did not change the mRNA populations associated with EIF4E4, although the cells displayed an aberrant morphological phenotype, similar to what was observed for EIF4G4, the EIF4E3 partner. The depletion of EIF4E4, however, led to differences in the EIF4E3-bound mRNAs, with enrichment in those for ribosome constituents. This is compatible with previous RNAi phenotype data for procyclic cells where depletion of EIF4E3, but not EIF4E4, leads to cell death. A complementary profile is then observed with different mRNA populations for each protein, but nonetheless a more critical role for EIF4E3 in the procyclic life stage. **Supported by:**FACEPE/FIOCRUZ
Keywords: Trypanosomatids; translation initiation; eif4e

PV016 - **FUNCTIONAL CHARACTERIZATION OF AP-1 IN TRYPANOSOMA CRUZI**

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The adaptor protein complex 1 (AP-1), which assists in mounting clathrin-coated vesicles from the trans-Golgi network (TGN) and endosomes, is composed of two large subunits (γ and β1) and two smaller subunits (σ1 and μ1). There is scarce information involving this adaptor complex in trypanosomatids. Studies showed that deletion of μ1 and σ1 subunits affects *Leishmania mexicana* infectivity. Furthermore, the silencing of the gene encoding the μ1 subunit of *Trypanosoma brucei* showed that this protein is essential for the parasite survival. *Trypanosoma cruzi* is the causative agent of Chagas disease and the function of AP-1 has not been addressed in this parasite. The goal of this work is to perform the localization and functional characterization of AP-1 in this trypanosomatid. For that, we raised a monoclonal antibody against *T. cruzi* γ adaptin which identified this AP-1 subunit in the Golgi complex (GC) in different *T. cruzi* life cycle forms. We also have generated a *T. cruzi* null mutant for γ adaptin (γKO), using targeted gene replacement by homologous recombination. Our results showed that the ability of the replicative epimastigote forms of the γKO to proliferate and undergo differentiation to the infective metacyclic trypomastigote forms is decreased when compared to the wild type (WT) parasite. In addition, we observed that, although the metacyclic form of the γKO is able infect mammalian cells and differentiate to amastigote, the number of infected cells as well as the number of amastigotes per cell was drastically reduced. We also showed that γ null mutant impaired maturation and transport of cruzipain (the major cysteine protease of *T. cruzi*) to reservosomes in epimastigote forms. In this sense, ours results show that AP-1 machinery mediates the transport of cruzipain to reservosomes in epimastigote form of *T. cruzi* and that AP-1 function is important for parasite proliferation, differenciation and infectivity towards host cells. **Supported by:**CAPES/ FIOCRUZ
Keywords: Ap-1; trypanosoma cruzi; cruzipain

TB001 - **A MODEL FOR VACCINE TESTING AGAINST TRYPANOSOMA CRUZI INFECTION THAT EXPLOITS HIGHLY SENSITIVE BIOLUMINESCENSE IMAGING**

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Chagas disease, caused by the protozoan parasite Trypanosoma cruzi, affects 6-8 million people. Those who develop chronic stage pathology often suffer severe, life-threatening cardiac and/or gastrointestinal symptoms. The only drugs available to treat the disease can exhibit severe side effects and treatment failures are frequently reported. Pre-clinical testing of vaccine candidates has been limited by the complex nature of the disease and difficulties in detecting the extremely low parasite burden during chronic infections. Here, we describe a new murine model for vaccine testing, based on highly sensitive bioluminescence imaging, which circumvents these problems and provides new insights into conferred immunity. In preliminary experiments, mice were infected with bioluminescent T. cruzi, cured by benznidazole treatment, and then subjected to re-infection. The mice were found to be highly resistant to re-infection with the same strain, whereas cross-strain immunity, although effective at reducing the parasite burden, was insufficient to confer sterile protection. We next assessed if the level of protection was influenced by the route of inoculation or by the length of the primary infections. Mice that had overcome the peak of the parasite burden before drug cure were highly resistant to re-infection. Whereas mice that were drug cured prior to the peak of the parasite burden were less resistant. We have also used this model to investigate the protective correlates of immunity. The data demonstrate that this predictive model can have a central role in studying the efficacy of vaccines, correlates of immunity and the relative merits of prophylactic and therapeutic vaccines as strategies for combatting this complex parasitic infection. **Supported by:**Medical Research Council
Keywords: Immunity; vaccine; bioluminescence

TB002 - **APPROACHING A NEW THERAPY FOR CHAGAS DISEASE: INHIBITORS OF THE *TRYPANOSOMA CRUZI* PROLINE RACEMASE IN FOCUS**

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Chagas disease, caused by the parasitic protozoan *Trypanosoma cruzi*, is a neglected disease once restricted to the Latin America that has become a global concern with human migrations. This infection affects about 8 million people worldwide, with a mortality rate of close to 10 thousand people/year. Only one drug is recommended and its efficiency in chronic phases is questionable, allied to occurrence of undesirable side effects and drug resistance. Consequently, due to the lack of a vaccine and the urgent need to develop new safe and efficient therapies to treat this disease, we focused our efforts on the characterization of the proline racemase of the parasite (*Tc*PRAC) and how its inhibition could interfere with parasite viability. TcPRAC is essential for *T. cruzi*, plays a role in the parasite development and acts a virulence factor by its B-cell mitogenic properties and, therefore, TcPRAC proved to be an encouraging drug target. Structural Bioinformatics analysis of the conformational transition pathways of *Tc*PRAC from its active to its ligand bound 3D-structure, allowed us to generate hundreds of compounds that could virtually inhibit the enzyme. After in vitro screenings to identify which of these compounds could indeed inhibit *Tc*PRAC activity and kill the parasite in all its life cycle stages, we generated ester prodrugs to start in vivo tests, aiming to increase drug diffusion across cellular membranes. Using our rapid and sensitive non-invasive method to follow the infection of bioluminescent and fluorescent *T. cruzi* parasites in mice, we observed a reduction in parasite load of mice treated with these esters. The effect was dose-dependent and the esters acted synergistically with benznidazol, favoring a reduction in the doses of both drugs, which is essential to avoid toxicity and side effects. Studies of treatment frequency and dosing intervals are in progress to treat acute and chronic mouse infections and envisage a new chemotherapy for Chagas disease. **Supported by:**Agence Nationale de la Recherche (ANR; grant number 14-CE16-0001) - Institut Pasteur **Keywords:**Drug discovery; drug screening; preclinical testing

TB003 - **OVEREXPRESSION OF HMGCOA REDUTASE INFLUENCES THE SUSCEPTIBILITY TO AZOLES AND ALTERS THE STEROL HOMEOSTASIS OF LEISHMANIA AMAZONENSIS**

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Trypanosomatids have their own machinery for sterol synthesis, producing ergosterol as final product. The enzyme 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCoA reductase), an enzyme catalyzes the synthesis of mevalonic acid from HMG-CoA, is a rate-limiting enzyme in sterol synthesis for mammalian cell and protozoan. This project aims to understand the role of HMGCoA reductase in sterol homeostasis of Leishmania amazonensis. The first step was to generate parasites overexpressing HMGCoA redutase (LaHMG). The gene was cloned in the pGEMT vector using E. coli DH5α competent bacteria, subcloned in PSP72αNEOα vector and transfected in L. amazonensis. Gene sequencing and qPCR were performed to confirm the gene overexpression. In susceptibility assays, the LaHMG parasites showed an increased resistance to ketoconazole, in a dose-dependent fashion, with an IC50 of 40,64uM, which is 4 times the control value. To investigate the resistance to azoles, we evaluated the total content of sterols by Bodipy staining and the uptake of exogenous sterols, using 7-dehydrocholesterol. After cholesterol starvation, LaHMG parasites showed lower uptake of 7-dehydrocholesterol and reduced lipid staining by Bodipy. TLC analysis of LaHMG parasites showed 51% increase in ergosterol content. When cultured under cholesterol deprivation by 72h, LaHMG increased the production of sterol with ergostane skeleton by 45.8% and decreased the cholesterol content. Taken together, these results show that the overexpression of HMGCoA reductase influences the sterol homeostasis and promotes a difference in the susceptibility to ketoconazole, without the need to increase the capture of cholesterol from the medium. **Supported by:**CNPq, CAPES **Keywords:**Hmgcoa redutase; leishmania amazonensis; sterol biosynthesis

TB004 - **FLUORESCENT-BASED CLICK CHEMISTRY DEMONSTRATES INCORPORATION OF THYMIDINE DERIVATIVES INTO INTRACELLULAR *TRYPANOSOMA CRUZI* DNA**

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The nitro-heterocyclic drugs used to treat infection with *Trypanosoma cruzi* have questionable efficacy and associated toxicity. New drugs are therefore needed, however high attrition rates in drug discovery remains an issue. An increased understanding of the mode of action of new compounds effective against T. cruzi would support new discoveries. We have developed a high-content assay to assess compound activity against intracellular *T. cruzi* which can detect as few as 5 parasites per host cell. In addition to this sensitivity, it would be beneficial to determine the effects of compounds on parasite replication and survival. To understand the replicative capability of *T. cruzi* following compound treatment, we have utilised fluorescent, image-based methods to assess incorporation of pyrimidine analogues into *T. cruzi* DNA. Incorporation is detected using click chemistry, by linking modified nucleosides to a fluorescently labelled azide. This is the first time the incorporation of synthetic nucleoside analogues have been used to assess replication in intracellular kinetoplastids. A collection of new thymidine analogues were incubated with intracellular *T. cruzi* parasites to determine incorporation into parasite DNA. Fluorescent labelling of the most effectively incorporated thymidine analogue/s using click chemistry was used to determine the effect of a collection of compounds containing known drugs, failed drugs, and mammalian cell cycle inhibitors with activity against *T. cruzi* on parasite replication. An effect on parasite replication was assessed following 2 hours which may suggest a mode of action against the parasite related to cell division. A longer incubation of 48 hours was used to evaluate potential resistance or slow effectiveness of compounds, not favourable for drug discovery. Early prioritisation of effective compound classes by providing more information about compound action is imperative to the success of future treatments against *T. cruzi*. **Keywords:**Thymidine analogues; click chemistry; drug discovery

TB005 - **HIGH CONTENT SCREENING OF PHARMACOLOGICALLY ACTIVE COMPOUNDS AGAINST *TRYPANOSOMA CRUZI*: IMPACT OF DIFFERENT HOST CELL LINEAGES FOR HIT SELECTION**

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High content screening technology enables the triage of a large quantity of molecules within a relatively short period in a robust fashion. As *Trypanosoma cruzi* is promiscuous with regards to mammalian host cells invasion, virtually any nucleated mammalian cell line can be used for *in vitro* triage. However, cellular dynamics and host-parasite interactions may play an important role when assessing the potential effect of compounds. To verify this hypothesis, we have screened a pharmacologically active molecule library of 1,280 compounds, at 10 µM, using four distinct cell lines infected with *T. cruzi* Y strain: U2OS, THP0-1, VERO and L6 cell lines. Depending on the host cell used, different readouts were observed following infection for 120 h: the infected cells ratio observed for L6 cells was about 2.5-fold higher than that detected for VERO cells; likewise, the mean of amastigotes per infected cell was nearly 10-fold higher in L6 when compared to VERO; values of infection ratio (76%) and average amastigotes per infected cell (13) detected in THP0-1 were also relatively high, while intermediate levels were obtained for U2OS (52% and 5). Primary screening results yielded 82 compounds whose activity were superior to 50% inhibition of infection and with toxicity to host cell lower than 50%. Infected U2OS cell lines were the most sensitive hit-generation system, as 27 exclusive compounds were identified when using this cell line as host cell; conversely, THP0-1 and L6 infected cell lineages had the lowest hit rates, generating six and five exclusive hit compounds, respectively. Dose-response assays of selected hits were performed against three T. cruzi strains (Y, CL Brener and Sylvio) infecting U2OS cells. Compounds CB1954, FPL64176, AEG3482 and entecavir presented EC50 values lower than 10 µM and CC50 higher than 100 µM for at least two *T. cruzi* strains. These compounds could be repurposed or serve as starting points for the development of novel anti-chagasic drugs. **Supported by:**CNPq/DNDi
Keywords: Trypanosoma cruzi; high content screening; host cell

TB006 - **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). 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:BBSRC **Keywords:**Diagnostic; trypanosoma; recombinant

TB007 - **IMMUNE-STRUCTURAL ASSOCIATION BETWEEN EPITOPES AND PREDICTED DISORDERED REGIONS**

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Despite considerable scientific efforts, development of neglected diseases vaccines remains a great challenge. This scientific scenario had led to a great expansion of neglected diseases scientific data in the last years. Regarding the seeking for new antigens, biological data integration originated from multiple analytical approaches could be a great strategy in the search for novel vaccine candidates. In this context, the presence of protein disordered regions near immunogenic epitopes could be a valuable information for optimize the transformation of biological knowledge into diagnostics and therapeutics for medicine. The main question addressed by this work is: Is there a statistic correlation between the proximity of immunogenic regions and disordered regions that could be used to better predict new targets for vaccine development? In order to address our hypothesis we initially downloaded all experimentally validated epitopes information about *Trypanosoma cruzi* (ID:353153), *Leishmania spp.* (ID:5658) and *Trypanosoma brucei* (ID:5691) from IEDB, including assays for T cell, B cell and MHC ligand. Disorder regions were predicted using the approach described by Ruy et al, 2014 and the biological data integration was performed using a MySQL database. According to contingency analysis, statistically significant (p-value less than 0.05) and a higher than expected frequency of B cell epitopes in regions predicted as disordered were observed in *Leishmania infantum*, considering as host *Cannis lupus* and also using data from *T. cruzi* considering *Homo sapiens* as host.
In addition, this immune-structural association between predicted disordered regions and experimentally tested epitopes was verified in six *L. braziliensis* proteins and five *L. infantum* proteins selected via reverse vaccinology. These epitopes are being currently tested for Leishmania vaccine development by our research group. **Supported by:** FAPEMIG, CNPq, CAPES **Keywords:**Protein disorder; epitope; prediction

TB008 - **IMMUNOINFORMATICS AND DATA INTEGRATION: *IN SILICO* STUDY OF FEATURES ASSOCIATED TO EXPERIMENTALLY TESTED TARGETS FOR *LEISHMANIA* VACCINE DEVELOPMENT**

FORTES DE BRITO, R.C.[*1]; GUIMARÃES, F.G.[2]; VELLOSO, J.P.L.[2]; CORRÊA-OLIVEIRA, R.[2]; RUIZ, J.C.[2]; REIS, A.B.[1]; RESENDE, D.M.[3]
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Leishmaniasis is a wide-spectrum disease caused by parasites from *Leishmania* genus. There is no human vaccine available and it is considered by many studies as a potential effective tool for disease control. To discover novel antigens, computational programs have been used in reverse vaccinology strategies. In this work, we developed a validation antigen approach that integrates prediction of B and T cell epitopes, analysis of Protein–Protein Interaction (PPI) networks and metabolic pathways. We selected twenty candidate proteins from *Leishmania* tested in murine model, with experimental outcome published in the literature. The predictions for CD4+ and CD8+ T cell epitopes were correlated with protection in experimental outcomes. We also mapped immunogenic proteins on PPI networks in order to find Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways associated with them. Our results suggest that non-protective antigens have lowest frequency of predicted T CD4+ and T CD8+ epitopes, compared with protective ones. T CD4+ and T CD8+ cells are more related to leishmaniasis protection in experimental outcomes than B cell predicted epitopes. Considering KEGG analysis, the proteins considered protective are connected to nodes with few pathways, including those associated with ribosome biosynthesis and purine metabolism.
Supported by:FAPEMIG, CAPES, CNPq, UFOP, Fiocruz **Keywords:**Immunoinformatics; epitope prediction; leishmaniasis

TB009 - **TRYPANOSOMA CRUZI PROLINE RACEMASE (TCPRAC): A TARGET FOR DEVELOPMENT OF MOLECULAR, SEROLOGICAL AND IMAGING TOOLS FOR CHAGAS DISEASE DIAGNOSIS AND TREATMENT MONITORING**

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Nowadays, Chagas disease transmission by blood transfusion has become a public health problem in Northern countries due to people migration. TcPRAC is an essential parasite enzyme, not present in humans and in other pathogenic Trypanosomatids, conferring a particular interest in terms of parasite specificity for diagnosis and criteria of cure. We have previously shown that TcPRAC gene and expression are closely related to the parasite viability. This has a particular importance for the chronic phases of Chagas disease where the parasite is almost exclusively intracellular and hardly detected, imposing serious difficulties for the diagnosis and for assessing healing after treatment.
To improve the sensitivity and specificity of diagnostic tests, we have developed different successful approaches, with the TcPRAC as the main target, by using different specific mouse monoclonal and VHH lama antibodies raised against TcPRAC. Details will be discussed.
Experiments using parasites constitutively expressing bioluminescence (Redshift Luciferase gene) and fluorescence (GFP or E2-Crimson genes) are used to follow the infection in real-time. To reveal the localization of live parasites in deep tissues during the experimental chronic phases, we are developing a prototype imaging test by using appropriate anti-TcPRAC antibodies labeled with far infra-red fluorescent molecules with using recent imaging techniques. The detection and the co-localization of the intracellular TcPRAC is possible in vitro using live parasite culture. Preliminary tests show that those antibodies can also reveal intracellular TcPRAC in amastigotes inside Vero cells. Preliminary in vivo data using subcutaneous infected mice confirm the co-localization of the parasite and the racemase signals in the infection site. These experiments may pave the way for the development of potential non-invasive tests in humans to confirm diagnosis in late phases of Chagas disease and to monitor cure after treatment. **Keywords:**Tcprac; imaging; diagnosis

TB010 - **IDENTIFICATION AND EVALUATION OF GTP BINDING PROTEIN OF THE *LEISHMANIA INFANTUM* FOR THE SERODIAGNOSIS OF VISCERAL LEISHMANIASIS OF SYMPTOMATIC AND ASYMPTOMATIC DOGS**

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Leishmaniasis is a complex group of diseases caused by intracellular protozoan of the *Leishmania* genus that infect macrophages of a variety of mammals. The visceral form of the disease is fatal, if not treated. Despite the continuous development of immunodiagnostic tests, intrinsic limitations of each technique result in an uncertain diagnosis. A high sensitivity test is desired for screening and early detection of cases and high specificity is determinant for prognosis and treatment. Our group has been developing immunogenic studies to identify new antigens with potential use to improve the diagnosis of canine visceral leishmaniasis (CVL). Thus, the present study aimed to identify and evaluate a new antigen of *L. infantum* for use in the diagnosis of CVL in symptomatic and asymptomatic dogs. The GTP binding protein (Myxo*Li*) was selected for presenting similarity with proteins associated to host defense processes, from the ImmunomeBase database. The gene encoding the Myxo*Li* protein was cloned into *Escherichia coli* for heterologous expression. The antigenic properties of the protein were investigated and linear B cell epitopes were identified and used to evaluate the potential use as antigen in the CVL serodiagnosis. The Myxo*Li* antigen was tested in ELISA using sera from symptomatic (n=26), asymptomatic (n=31) dogs, non-infected dogs (negative) (n=37), in addition to sera from dogs experimentally infected with *Trypanosoma cruzi* (n=53) and naturally infected with *Babesia* sp. (n=9) to evaluate possible cross reactivity. Myxo*Li* presented a sensitivity of 91% and specificity of 93%, with the area under the ROC curve of 0.9739, indicating a test with excellent quality for diagnosis of CVL. The positive and negative predictive values obtained in the test were 90% and 94%, respectively, with an accuracy of 93%. These results suggest that the use of genomic approaches may support the perspectives for identification of new targets to improve the accuracy of CVL diagnosis.
Supported by:CAPES,FAPEMIG,CNPq. **Keywords:**Leishmaniasis; immunodiagnosis; recombinant antigens

TB011 - **LEISHMANICIDAL ACTIVITY OF WITHANOLIDES FROM SPECIE OF GENUS *AURELIANA***

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Leishmaniasis is a widely distributed infectious and neglected disease with a high cost treatment, and many cases of resistance have been reported, making treatment difficult. Literature provides some evidences that withanolides, a group of steroidal substances characterized for a moiety ergostane with 28 carbon atoms, possess potent trypanosomicidal and antileishmanial activity. Found in many genera of the family Solanaceae, the mode of action of these substances have not been elucidated yet. In this study, we evaluated the leishmanicidal activity of three withanolides (VIT1, VIT2 and VIT3), which were isolated from dichlorometanic (Di) fraction from leaves of Aureliana sp. Promastigotes of Leishmania amazonensis were cultured in the presence of several concentrations of the compounds up to 100 µM for 72 h and the cells viability was measured by resazurin assay. All molecules tested inhibited the parasite growth, in a dose-dependent manner, with 3.90 µg/mL IC50 (VIT1), 4.56 µg/mL IC50 (VIT2), 7.58 µg/mL IC50 (VIT3) and 1.85 µg/mL IC50 (Di). Macrophages were infected with *L. amazonensis* to evaluate antiamastigote activity. VIT1 showed to be effective in decreasing the infection of the macrophages, with IC50 less than 0.1 µg/mL. The molecules were incubated with murine macrophages for 72 h in order to determine the toxicity, and the effect on macrophage viability was quantified by resazurin. VIT1 showed selectivity index of 187.55, with LD50 13.41 µg/mL. These results show that withanolides from dichlorometanic fraction have leishmanicidal activity and additional studies are needed to extend these findings.
Supported by:CAPES, CNPq, IOC/FIOCRUZ **Keywords:**Withanolides; leishmaniasis; chemotherapy

TB012 - **THE EFFECT OF THE α-PHENYL-TERT-BUTYL-NITRONE (PBN) DERIVATIVE, LQB 303, IN THE MITOCHONDRIAL PHYSIOLOGY OF *TRYPANOSOMA CRUZI* AMASTIGOTE FORMS AND NON-INFECTED MAMMALIAN CELLS**

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Chagas disease is a neglected tropical disease caused by the *Trypanosoma cruzi* that affects 6 to 7 million people worldwide. Despite of that chemotherapy relies on benznidazole (BZ), a drug with low efficiency in chronic phase of the disease. Thus, more effective and less toxic alternatives are mandatory. Data in the literature showed that α-phenyl-tert-butyl-nitrone (PBN) decreased mitochondrial reactive oxygen species levels and preserved the respiratory system efficiency and energy status, but failed to decrease parasite persistence in chronically infected mice hearts. Therefore, we evaluated the effect of a PBN derivative, LQB 303 upon *T. cruzi* clinically relevant forms: bloodstream trypomastigotes (BT) and amastigotes. For that, we incubated BT and infected murine macrophages with increasing concentrations of LQB 303 for 24 and 48 hours, respectively. We observed an increase in BT lysis after 24h and a great decrease in intracellular amastigote proliferation after 48h treatment. Also, LQB 303 treatment did not alter non-infected macrophages viability. Since PBN improved infected cardiomyocytes bioenergetics, we investigated the LQB 303 effect over amastigotes and non-infected cells mitochondrial physiology. For that, we used high-resolution respirometry to determine the mitochondrial metabolism in axenic amastigotes incubated with LQB 303 for 24h. We observed that comparing to untreated parasites, LQB 303 decreased amastigotes ROUTINE respiration in 70%, also impairing LEAK and ETS oxygen consumption, but increased residual respiration in three-fold. Conversely, LQB 303 treatment did not alter cell oxygen consumption of non-infected VERO, suggesting the drug specificity to the parasite mitochondrion. In conclusion, our results suggest that LQB 303 specifically targets *T. cruzi* mitochondrion, inducing functional alterations that leads to an impairment in oxygen consumption, that ultimately, may contribute to decrease amastigotes proliferation. **Supported by:**FAPERJ, CNPq and INCT-EM **Keywords:**Trypanosoma cruzi; chemoterapy; mitochondrial physiology

TB013 - **COMPARATIVE TRANSCRIPTOME ANALYSIS OF ANTIMONY RESISTANT AND SUSCEPTIBLE *LEISHMANIA INFANTUM* LINES**

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Leishmaniasis are a complex of diseases caused by different species of the protozoan parasite *Leishmania* that represent a major public health problem in developing countries. One major challenge for leishmaniasis treatment is the emergence of parasites resistant to pentavalent antimony. In this context, in order to identify differentially expressed genes associated with drug resistance mechanism, we performed a comparative transcriptomic analysis of antimony trivalent (SbIII) resistant (LiSbR) and susceptible *Leishmania (L.) infantum* (LiWTS) lines (MHOM/BR/74/PP75) using high-througHP0ut RNA sequencing (RNA-Seq). For this analysis, we used Illumina HiSeq 2000 sequencing platform. Samples were evaluated for quality with Prinseq program. Adapters and low quality sequences were removed using Trimmomatic. TopHat2 was used on mapping process of the reads in the genome and DESeq2 for differential expression analyses. In order to identify differentially expressed genes the adjusted p-value less than 0.05 and fold-change greater than two was set as threshold to define the significance. When comparing the data from drug treated SbIII resistant and susceptible lines, the analytical pipeline allowed the identification of 1067 differentially expressed genes. According to the Pfam database these genes were classified into 16 functional classes, implicated in RNA/DNA processing, phosphorylation, cytoskeleton proteins, transporters, metabolism of protein, carbohydrate and fatty acids and hypothetical proteins. Our study provides a view of the global transcriptome of SbIII resistant and susceptible *L. infantum* lines allowing the identification of several biochemical pathways that are potentially associated with antimony resistance. **Supported by:**FAPEMIG, CNPq, CAPES, Institut Pasteur/FIOCRUZ funding; PRONEX/FAPEMIG and PROEP/CNPq/FIOCRUZ **Keywords:**Transcriptome; leishmania infantum; drug resistance

TB014 - ***IN SILICO* PREDICTION OF DRUGS AND NUTRACEUTICALS SUPPLEMENTARY TO ANTIMONIAL CHEMOTHERAPY FROM *LEISHMANIA AMAZONENSIS***

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Leishmaniasis are a disease complex related to parasites of the genus Leishmania and neglected by public authorities. Different responses to treatment leads to development of new therapeutic approaches. Usually, Leishmania changes its gene expression pattern in response to chemotherapy; but these genes can be modulated by nutraceutical and drugs, improving the traditional chemotherapy and cure efficiency. In this work, drugs and nutraceuticals that potentially modulate the response of L. amazonensis to antimonials were identified. Genes and transcripts associated to drug resistance were identified by DNA microarray methodology and the proteins were compared to public drug-protein interaction databases (https://www.drugbank.ca). Identification of homolog target proteins for drugs was performed by local alignment using the BLAST (Basic Local Alignment Sequence Tool) algorithm and functional annotation. The compounds identified were classified in drugs and nutraceutical and then sorted by biochemical properties. The full dataset was compiled in a specific database for this research. The results showed that 2788 genes are differentially expressed in antimony trivalent-resistant L. amazonensis line compared to wild-type parental line. These, 1093 are homologous to protein targets for drugs (417 amplified/upregulated and 676 deleted/ downregulated). Altogether were identified 634 compounds (608 drugs and 26 nutraceuticals). Curiously, 70% of the drugs belongs to “new product” or “experimental” groups. The nutraceuticals founded were: vitamins (15%), amino acids (27%), mineral salts (8%) and others (50%). The in silico prediction of modulating biocompounds to parasitic response drives to low experimental costs and boosts the drawing of therapeutical schemes for new drugs treatments.
Supported by:CNPq; FAPEMIG; FIOCRUZ **Keywords:**Nutraceutical; drugs; leishmania amazonensis

TB015 - **N’N-DIARYLUREA I-17 AFFECT TRYPANOSOMATIDS DUE TO ACTIVATION OF EIF2α SPECIFIC KINASE**

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Derivatives of urea containing two aromatic rings (N’N-diarylurea) were able to arrest cancer cell replication by activating heme-regulated inhibitor (HRI) kinase and inhibiting protein synthesis via phosphorylation of eukaryotic translation initiation factor (eIF2). Here we evaluate the effects of N’N-diarylureas in *Trypanosoma cruzi* and *Trypanosoma brucei*. An initial screening against *T. cruzi* epimastigotes and mammalian LLC-MK2 cells using a set of 25 N’N-diarylurea derivatives identified compound I-17 effective against epimastigote and amastigotes at concentration of 3 µM and 9-fold less effective in mammalian cells. Also, I-17 was able to affects *T. brucei* bloodstream and procyclics forms replication at 5 µM and 3 µM, respectively. Indeed, epimastigotes treated with I-17 have an aberrant morphology and an impairment of cell cycle progression resulting in 20% increased G1 phase. To test if I-17 was affecting eIF2, we treated epimastigotes with 10 µM of I-17 for 4 hours and using immunoblotting we observe a phosphorylation of the alpha subunit (eIF2α) when compared to non-treated parasites. In addition, a robust decreasing in translation is detected by polyssomal profile, suggesting that I-17 is a protein synthesis inhibitor. To determine if I-17 was acting directing at trypanosomatids homologues eIF2-kinases, we knockdown the expression of those kinases by RNAi in *T. brucei* and tested I-17 effect in parasite replication. The eIF2-kinase 3 (TB0K3) knockdown parasites were more resistant to I-17 compared to wild-type parasites. These results suggest that N’N-diarylurea I-17 is a kinase-activator with trypanocidal effects against *T. cruzi* and *T. brucei*.
Supported by:FAPESP, CNPq
Keywords: Eif2α -kinase 3; drug discovery

TB016 - **EVALUATION OF THE LEISHMANICIDAL ACTIVITY OF NEW QUINONES DERIVATIVES AND ITS ACTION ON THE RESPIRATORY CHAIN OF LEISHMANIA INFANTUM**

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Quinones are present in virtually all living beings, performing essential functions. Experimental quinones can interfere with these systems and induce oxidative stress or inhibit mitochondrial respiratory complexes. In this study, we evaluated different synthetic naphthoquinones in relation to their ability to selectively induce mitochondrial alterations in *L. infantum*. The naphthoquinones (LQBs) showed good leishmanicidal activity against promastigotes and axenic amastigotes. However, throughout the series, only the LQBs 32 and 182 were active against intracellular amastigotes, with IC_{50} of 3 and 3.6 µM. LQB-182 also induced increase in ROS production and we choose it to continue the study. In addition, we evaluated the respiratory activity of intact parasites in oxygraph. Parasites incubated with LQB-182 in presence of antimycin A or KCN, showed significantly higher oxygen consumption in relation to the parasites treated with inhibitors only, due to the redox cycle suffered by naphthoquinone. LQB-182 also inhibited significantly the respiratory complexes II-III and IV of *L. infantum* promastigotes and the antioxidant enzyme trypanothione reductase. To study the selectivity of this substance, we extracted murine liver mitochondria and evaluated the respiratory complexes. Our results suggest that LQB-182 may be inhibiting the respiratory complex III, in higher concentrations than the IC_{50} in Leishmania. LQB-182 also induced alteration in mitochondrial membrane potential ($\Delta\Psi m$) of parasites. As well as, the association of LQB-182 with miltefosine, the only oral treatment for leishmaniasis, was effective. Moreover, LQB-182 showed a favorable profile regarding the adherence of the chemical characteristics to Lipinski's "Rule of Five" and in silico ADMET properties. Thus, our results indicate that LQB-182 has selective antileishmanial activity by a mechanism involving inhibition of parasite respiration and oxidative stress, becoming a good candidate for in vivo studies. **Supported by:**FAPERJ
Keywords: Leishmania infantum; respiratory chain ; quinones derivatives