## HP1 - **UNRAVELLING THE ACTIVATION MECHANISMS OF THE RECEPTOR-TYPE ADENYLATE CYCLASE FROM TRYPANOSOMA BRUCEI, A KEY MODULATOR OF THE HOST INNATE IMMUNE RESPONSE OF THE HOST.**

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Trypanosomal adenylate cyclases (ACs), unlike those of mammals, possess a catalytic domain directly fused to a transmembrane receptor. Little is known about how ACs are activated. Until recently no clear function was attributed for most of these enzymes, which in African trypanosomes, such as T. brucei, constitute a large multigene family encoding distinct isoforms expressed at the surface of the parasite. In the bloodstream stage of this parasite, it was demonstrated that activation of these enzymes during trypanosome lysis by myeloid cells triggered inhibition of TNF-α synthesis in host cells promoting host colonization. The presence of two conserved Venus Flytrap (VFT) domains in the AC N-terminal extracellular domain suggests that their activity could also be triggered through specific ligands. Because most VFT domains usually form dimers, we have assessed the function of these domains in modulation of AC activity by using a dominant negative (DN) strategy. Experiments with constitutive overexpression of ESAG4, an AC specific for the bloodstream form of T. brucei, lacking both VFTs domains significantly decreased both AC activity and parasite's ability to control the innate immune defense of the host. In addition, overexpression of several conditional ESAG4 deletion mutants affecting either the catalytic domain or the N-terminal domain displayed a transitory growth phenotype in monomorphic cell lines associated with a reduction of AC activity by around 40-50%. In mice, ESAG4 pleomorphic mutants lacking one of the two VFT domains or both of them affected the infection pattern, similarly to the mutant devoid of catalytic domain, with a parasitaemia significantly lower than control parasitaemia and host survival time longer. This phenotype was reversed in TNF-α–/– mice suggesting that the deletion mutants affecting the VFT domains display a DN phenotype and thereby are important for the regulation of AC activity. **Supported by:**WBI
**Keywords:** Trypanosoma brucei; adenylate cyclase; innate immune response

## HP2 - **THE FLAGELLUM CONSTRUCTION IS AN EARLY EVENT IN THE LIFE CYCLE OF *TRYPANOSOMA BRUCEI* REVEALED BY FIB-SEM**

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*Trypanosoma brucei* possesses a complex life cycle alternating between tsetse flies and mammals. When parasites infect the fly, they differentiate to adapt to life in various tissues, which is accompanied by changes in flagellum length (from 3 to 40 µm), especially when parasites migrate to the proventriculus. The flagellum is a cylindrical organelle made of 9+2 structure present during the entire cell and life cycle. It is assembled by intraflagellar transport (IFT) a bi-directional movement of protein complexes along microtubules doublets driven by kinesin and dynein. In the proventriculus the organelles duplication takes place in the epimastigote morphotype (Sharma *et al.*, 2008). We took advantage of the Focused Ion Bean Scanning Electron Microscopy approach to study in 3D flagellum construction of *T. brucei* in the proventriculus by giving the two main information needed resolution and orientation. The duplication process is first observed by the basal body maturation followed by the construction of the new flagellum by IFT. Initially, the short new flagellum and the mature one share the same flagellar pocket. This was observed in long trypomastigotes with an elongated nucleus. The flagellum elongates while the distance between the nucleus and the kinetoplast is reduced until the organelles occupy the equivalent longitudinal position in the cell. During the elongation of the new flagellum, it is physically connected to the mature one through an electron-dense plate-like structure similar to the flagella connector. The duplication of the flagellum in long trypomastigotes is supported by the duplication of the transition zone marker FTZC observed by immunofluorescence assays and by the presence of a second IFT pool at the base of flagellum using the anti-IFT22 antibody. This led to a novel model for flagellum duplication that takes place much earlier in *T. brucei* life cycle than originally thought and it is simultaneous to the differentiation process in the cell. **Supported by:**Fondation Recherche Medical - FRM and ISERM **Keywords:** Trypanosoma brucei; flagellum construction; proventriculus

HP3 - **SAND FLY SALIVARY PROTEINS DRIVE NEUTROPHIL RECRUITMENT AND EXACERBATE LEISHMANIA INFECTION**

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Neutrophils are the first cells recruited to the site of an infected sand fly bite and interact with Leishmania parasites. It has been shown that neutrophil recruitment increases the ability of Leishmania to establish an infection. Here we investigate the effect of sand fly salivary proteins on neutrophil recruitment. All experimental procedures were approved by the National Institutes of Health Animal Care and Use Committee. We purified bone marrow neutrophils from C57BL/6 mice or from peripheral blood of healthy human donors. Chemotaxis driven by sand fly saliva of vectors of visceral (Lutzomyia longipalpis) or cutaneous (Phlebotomus duboscqi) disease was measured by modified Boyden chamber assay and by imaging with the EZTAXIScan assay. Neutrophils migrated towards saliva glands of the vectors P. duboscqi and L. longipalpis, in a dose-dependent manner. To unearth the identity of the chemotactic factor, we have generated plasmids coding for the 20 most abundant salivary proteins from P. duboscqi and injected them in the ears of C57BL/6 mice. In vivo neutrophil recruitment was analyzed by flow cytometry. We have identified 3 different salivary protein-coding plasmids with neutrophil recruitment activity. We next produced these 3 recombinant proteins in the HEK293 cell line system and we observed that a family of 40kDa salivary proteins (known as yellow proteins) acts as chemoattractant for neutrophils both in our in vitro and in vivo assays. Strikingly, intravital microscopy confirmed that recombinant yellow proteins recruit neutrophils in a time dependent manner. Finally, we furtherevaluated whether yellow proteins would affect Leishmania infection outcome. Coinjection of Leishmania major with P. duboscqi yellow proteins resulted in higher numbers of infected neutrophils at the site of injection leading to an exacerbation of the infection. In this study, we are showing a new insect chemoattractant, ascribing a new role to the sand fly salivary yellow proteins. **Supported by:**Ciência sem Fronteiras/CNPq - NIH **Keywords:** Neutrophils; chemotaxis; leishmania

HP4 - **DUAL ROLES FOR IFN-γ AND INFAMMATORY MONOCYTES DURING EARLY *LEISHMANIA AMAZONENSIS* INFECTION**

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Protection against intracellular parasites relies on the canonical Th-1 immune cytokine IFN-γ, which mediates the recruitment and activation of Ly6ChiCX3CR1+CCR2+ inflammatory monocytes that have a fundamental role in priming adaptive immunity and reactive oxygen-mediated pathogen elimination. Paradoxically, IFN-γ mediates only partial protection in the late phase of infection with the intracellular parasite Leishmania amazonensis and no protection during early infection. Why this is remains unclear. In order to investigate the role of IFN-γ during early L.a infection we infected C57BL/6 Wt and IFN-γ-/- mice with in the ear dermis and followed disease for 6 wks. We characterized the inflammatory cell infiltrate, innate cell activation status, and genic expression of cytokines during infection. Despite a highly polarized Th-2 immune response and reduced activation of phagocytic cells versus Wt mice, IFN-γ-/- mice had equivalent lesion sizes and parasite numbers during the acute rapid phase of parasite expansion. However, Th2 immunity in IFN-γ-/- mice was associated with a deficit in inflammatory monocyte recruitment and infection. In addition, these inflammatory monocytes in Wt mice were iNOS low and harbored viable parasites while monocyte derived cells were iNOS+ and harbored a smaller proportion of parasites. Employing IFN-γR1-/- bone marrow (BM) and mixed-BM chimera mice to discriminate between monocyte recruiting versus parasite killing roles of IFN-γ we found IFN-γR1-/- BM chimera mice had equivalent monocyte recruitment versus Wt-chimera mice but negligible iNOS production and enhanced parasite loads. Therefore, IFN-γ mediated expansion of the permissive host cell reservoir via inflammatory monocyte recruitment counteracts IFN-γ-receptor dependent iNOS-production and parasite killing by monocyte derived cells. This dual role for IFN-γ explains how an intracellular pathogen can thrive in a setting of Th1 immunity. **Supported by:**Beverley Phillips Rising Star Program; CIHR; CNPq, FAPEMIG, CAPES **Keywords:** Monocytes; leishmania amazonensis; ifn-gamma

HP5 - **ROLE OF MIRNAS IN THE REGULATION OF POLYAMINES/NO PRODUCTION PATHWAYS IN THP-1 DERIVED MACROPHAGES INFECTED WITH *LEISHMANIA AMAZONENSIS***

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In the mammalian host, *Leishmania* infects phagocytic cells of the immune system. Inside the ...ge, the amastigote can subvert the hostile environment of the phagolysosome modulating ho... ...n. L-arginine is a common substrate for both enzymes nitric oxide synthase 2 (NOS2) fo... ...tion and arginase 1 (ARG1) to produce ornithine and subsequently polyamines ... C). Polyamines are essential for parasite proliferation and survival, in detri... ...h. In this study, we investigated the role of macrophage miRNAs i... ...t immune response in human THP-1 derived macrophages ... ) or the arginase knockout (*La-arg*⁻) strain.
The hsa-miR-372-3p, hsa-miR-373-3p, hsa... ...egulated in human infections, such as formerly observed ... *infantum*. These miRNAs share the same seed sequence... ...get-mRNA, as mmu-miR-294-3p, previously described in t... ...he cationic amino acid transporter 2 (CAT2) is a predicte... ...NA hsa-miR-340-5p is downregulated during *La*-WT infecti... ...es ARG1 and ODC as putative targets.
The qu... ...of macrophage *Arg1* in *La*-WT infection model, but not in *L*... ...ed at 4 and 24 hours post infection only of *La*-WT but NO pr... ...n models due to the higher availability of L-arginine in the infection of *La*-... ...nibitors we observed modulation on L-arginine metabolism and infectivity outc... ...is study helps to comprehend the molecular mechanisms involved in the estab... ...*mazonensis* infection in human macrophages. **Supported by:**FAPESP and CNPq
**Keyw...** ...rna; leishmania; arginine metabolism

HP6 - **FUNCTIONAL, STRUCTURAL AND EVOLUTIONARY CHARACTERIZATION OF PROSTAGLANDIN F2α SYNTHASES FROM *TRYPANOSOMA CRUZI***

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The discovery that trypanosomatids are capable of synthesizing prostaglandins raised questions about the role of these molecules during parasitic infections. Multiple studies indicate that prostaglandins could be related to the infection processes and pathogenesis in trypanosomatids. This work aimed to unveil the role of the prostaglandin $F_2\alpha$ synthases, *Tc*OYE and *Tc*AKR, in the establishment of *Trypanosoma cruzi* infection. The vast majority of characterized $PGF_2\alpha$ synthases belong to the aldo-keto reductase (AKR) protein family. However, in *T. cruzi* the $PGF_2\alpha$ synthesis is catalyzed by *Tc*OYE, which belongs to the Old Yellow Enzyme family. This enzyme does not present homologs in mammals and other trypanosomatids. Accordingly, we performed an evolutionary analysis which led us to propose a prokaryotic evolutionary for *Tc*OYE. Then, we used *in vitro* and *in vivo* experiments to show that *T. cruzi* prostaglandin $F_2\alpha$ synthases play an important role in modulating the infection process. In this sense, *Tc*OYE overexpressing parasites were less able to complete the infective cycle in cell culture infections and increased cardiac tissue parasitic load in infected mice. We also demonstrate that parasites overexpressing *Tc*OYE and *Tc*AKR increased $PGF_2\alpha$ synthesis from arachidonic acid. Despite both proteins are able to catalyzed the $PGF_2\alpha$ synthesis *in vivo*, they present different subcellular localization, indicating the may have different biological implications. Finally, different susceptibility to Benznidazole and Nifurtimox in *Tc*OYE and *Tc*AKR overexpressing parasites showed its participation in the metabolism of the currently anti-chagasic drugs, which added to its observed ability to confer resistance to hydrogen peroxide, highlights the relevance of these enzymes in multiple events including host–parasite interaction.
**Supported by: Keywords:** Prostaglandin f2α synthases; old yellow enzyme

HP7 - **NEUTROPHIL ELASTASE PROMOTES INFECTION BY *LEISHMANIA DONOVANI* VIA INTERFERON β**

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Visceral leishmaniasis is a debilitating illness that causes liver and spleen inflammation and tissue destruction. The molecular mechanisms that determine *L. donovani* or *L. infantum* tissue tropism and disease progression remain elusive. In the causative agent of cutaneous leishmaniasis, *L. major*, the virulence factor named inhibitor of serine peptidases (ISP2) prevents triggering of TLR4 and TLR2 during parasite phagocytosis due to inactivation of neutrophil elastase (NE) in macrophages, guaranteeing parasite survival. ISP2 was associated with prevention of TNFα and IFNβ production by infected macrophages and with reduction of iNOS expressing monocytes in lesions in mice. Here, we report poor intracellular growth of *L. donovani* in macrophages upon inhibition of NE activity, or in macrophages from *ela*$^{-/-}$, *tlr*4$^{-/-}$ or *tlr*2$^{-/-}$ mice. NE and TLR4 co-localized with the parasite in the parasitophorous vacuole. Parasite load in the liver and spleen of *ela*$^{-/-}$ mice were reduced and accompanied by increased nitric oxide and decreased TGFβ production. We did not find detectable expression of ISP2 in *L. donovani* and a transgenic *L. donovani* line constitutively expressing the *L. major* ISP2 gene, displayed poor intracellular growth *in vitro* and decreased parasite burden in mice. We found that macrophages infected with either *L. donovani* or *L. donovani*:ISP2 display marked differences in the kinetics of accumulation of IRF3 and IRF7 transcription factors in the nucleus. qPCR analyses of infected macrophages revealed upregulation of IFNβ in response to *L. donovani* but not to *L. donovani*:ISP2 or in the ela$^{-/-}$ macrophages. Addition of exogenous IFNβ fully restored the intracellular growth of *L. donovani* in ela-/- macrophages or of transgenic *L. donovani*:ISP2- parasites to wild type levels. We propose that *L. donovani* utilizes the host NE-TLR molecular machinery to generate IFNβ and promote parasite growth in the early phases of infection and that this pathway is blocked by ISP2. **Supported by:**CNPq, FAPERJ, NEWTON **Keywords:** L. donovani; infection; interferon

HP8 - **M1-M2 MIX IN *LEISHMANIA AMAZONENSIS*-MACROPHAGE INTERACTION: A TRANSCRIPTOMIC VIEW**

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*Leishmania* infection in murine models varies according to parasite species and host genetic background. In this work, we analyzed the impact of *L. amazonensis* infection on BALB/c and C57BL/6 macrophages through transcriptomic profiling, pointing some markers that define the different susceptibility between these two mouse strains. We identified a total of 12,641 transcripts and the profile of differentially expressed genes (DEG) revealed only 22 DEG in BALB/c infected vs. BALB/c uninfected macrophages. This low level of gene modulation could explain the discrete immune response activation and susceptibility in this host. In contrast, in C57BL/6 infected vs. C57BL/6 uninfected macrophages we identified 497 DEG. The high level of gene modulation could explain the strong immune response activation and moderate resistance in this host. Further, in BALB/c infected vs. C57BL/6 infected macrophages we identified 752 DEG, emphasizing that different host genetic backgrounds define the fate of infection. The identification of essential molecular signatures from both host and parasite is the basis for the development of new approaches for the diagnosis, treatment and control of leishmaniases. Based on these data, we identified particular markers of each host involved in response to *L. amazonensis* infection as part of recognition and signaling cascades, cytokine and chemokine production/interaction, as well as other immunomodulatory molecules involved in the polyamines pathway, nitric oxide production and oxidative stress. Transcriptomic profiles revealed that both BALB/c and C57BL/6 macrophages present a mix of M1 and M2 immune responses against *L. amazonensis* infection, with higher amplitude in the modulation of DEGs in C57BL/6 infected macrophages. **Supported by:**Fapesp, CNPq, CAPES, SiU **Keywords:** Rna-seq; transcriptome; immune response

HP9 - **DEUBIQUITINATING CYSTEINE PEPTIDASES (DUBS) OF *LEISHMANIA* ARE ESSENTIAL FOR LIFE CYCLE PROGRESSION**

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*Leishmania* has a functional ubiquitin conjugation system that includes 20 Deubiquitinating enzymes (DUBs) orthologues of the C12 and C19 cysteine peptidase families. However, the identity, function and essentiality of DUBs remains to be revealed. A CRISPR-Cas9 gene editing approach identified 4 out of 20 DUBs to be essential for promastigote viability. The 16 null mutants produced contain a unique barcode allowing for screening using a BarSeq (barcode sequencing) approach which relies on next generation sequencing of amplicons from pooled mutants, and proved to be a powerful method for detection of loss of fitness during differentiation and infection. We detected 5 DUBs involved in metacyclogenesis and 10 DUBs required for infection in macrophages and mice. Our results were validated by individual assessment of the mutants on in vitro differentiation combined with a fluorescent ubiquitin-based probe used for activity protein profiling and revealed the presence of many active DUBs in different life cycle stages. Among the DUBs we could not generate null mutants, DUB1 and DUB2 were further validated using DiCRE inducible gene deletion for novel insights into their function in the host. Active recombinant DUB1 and DUB2 expressed and purified using baculovirus expression system were used with dimeric ubiquitin (Di-Ub) representing eight different Ub linkage to define DUB poly-Ub specificity. Analysis on DUB1 preferred chain types identified Lysine 6 (K6), K48 and K63 and suggest involvement in DNA damage and endocytosis, as corroborated by a glucose transporter recycling assay. Thus, we combined a genetic method with a Barseq screening that allow for assessment of function for multiple genes and can be scalable to big families of proteins for medium-throughput analysis. Combined with chemical strategies we identified essential *Leishmania* DUBs, determined their function during the life cycle and infection, and further validated DUBs as potential drug targets. **Supported by:**WELLCOME AND MRC **Keywords:** Crispr-cas9; dubs; differentiation

HP10 - **MOLECULAR IDENTIFICATION OF *LEISHMANIA* SP. FIELD ISOLATES FROM DOGS WITH VISCERAL LEISHMANIASIS REVEALS COINFECTION WITH PARASITES FROM *VIANNIA* AND *LEISHMANIA* SUBGENUS**

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Leishmaniasis is a disease complex caused by protozoans from the *Leishmania* genus and endemic in Brazil. The manifestation of the disease depends on the infecting species and host immune response. *Leishmania* (*L.*) *infantum* is associated to the visceral form of Leishmaniasis in dogs and humans, and some recent studies also point a possible role of *L.* (*L.*) *amazonensis* in this clinical manifestation. Coinfections involving different *Leishmania* species are however less described in the literature. Thus, the tracking of seropositive dogs with clinical manifestation of visceral leishmaniasis followed by the isolation and characterization of *Leishmania* isolates circulating in endemic areas represents an important approach for a better understanding of the biological behavior of these parasites and possible associations that may exist between the clinical status of the disease and the different *Leishmania* populations involved in the infection. In this study, dogs from São Joaquim de Bicas and Belo Horizonte, endemic regions in Minas Gerais state, are being constantly clinically evaluated and their blood samples submitted to serological assays such as immunochromatographic (SNAP *Leishmania* – IDEXX, Maine, USA) and ELISA, which employs KDDR as primary antigen to detect *Leishmania* infection. Positive dogs are being submitted to a bone marrow aspiration and parasite isolation followed by PCR targeting a conserved region of *Leishmania* kDNA minicircle to differentiate *Leishmania* and *Viannia* subgenus. So far, five *Leishmania* isolates were analyzed by PCR: one representative of the *Viannia* subgenus (70bp), another one from *Leishmania* subgenus (135bp), while three dogs were found coinfected with parasites from both *Viannia* and *Leishmania* subgenus. These results were confirmed by sequencing the PCR products. Further analyzes are being performed in order to better comprehend coinfection patterns in canine population in these endemic areas. **Supported by:**CNPq, CAPES, FAPEMIG **Keywords:** Visceral leishmaniasis; dogs; leishmania sp. coinfection

## HP11 - **RAPID ACCUMULATION OF SURAMIN IN BLOODSTREAM FORM TRYPANOSOMES LEADS TO DIFFERENTIATION RELATED METABOLIC SWITCHING**

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Suramin, a frontline drug against African trypanosomes was introduced over a century ago, yet remains in the clinic for treatment of some early stage disease. Recent genetic and biochemical studies suggest a prominent role of endocytosis for suramin uptake, a receptor function for the invariant surface protein 75 (ISG75) and transport into the cytosol via the MFSF lysosomal channel. However, the mechanism by which suramin exerts its toxic effect once within the cell has remained unclear, but with genetic and other evidence suggesting a broad impact on metabolism, together with many examples of activity against disparate targets. Suramin is taken up rapidly, accumulates to high intracellular concentrations proportional to ISG75 expression levels and rapidly perturbs the mitochondrial membrane potential. This is followed by decreased cellular ATP, accumulation of intracellular pyruvate and significant upregulation of enzymes of the Kreb's cycle, proline dehydrogenase, glutamate dehydrogenase, pyruvate dehydrogenase and mitochondrial metabolite transporters, suggesting a dramatic switch in mitochondrial metabolism. Additionally, several proteins involved in differentiation to stumpy forms, including PAD1, PAD2 and PIP39, are induced. Highly significantly, >90% of upregulated proteins are more abundant in procyclic forms compared to bloodstream stage parasites. Taken together these data suggest that suramin induces partial differentiation, including stumpy stage signalling pathway and mitochondrion activation and that suramin acts to propel parasites into quiescent stumpy-like forms, which cease replication and are cleared from the infected host.

## HP12 - **THE MICROBIOTA DETERMINES THE MACROPHAGE IMMUNE RESPONSE PATTERN AFTER TRYPANOSOMA CRUZI INFECTION IN VITRO**

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The microbiota plays an important role in mediating the immune and tolerogenic response in some cases. The intestinal microbiota may benefit the host in a variety of ways, including metabolic functions, systemic immune response and intestinal homeostasis. T. cruzi promotes systemic infection, impair the intestinal homeostasis and function. Moreover, this parasite infects and multiply in macrophages and this cell of innate immune response plays an important role to primarily control the infection. So our goal was evaluating the crosstalk between microbiota and macrophages during the infection with T. cruzi in vitro. For this, we used bone marrow macrophages of germ free (GF) mice, macrophages of mice with depleted microbiota by antibiotic (Abx) and group control without antibiotic treatment. The macrophages of all groups were infected to proceed the assays. We evaluate reactive oxygen species (ROS), nitric oxide (NO), arginase I (ArgI), infection index and parasite number in the supernatant. We observed that the Abx macrophages presented low infection index compare to control group after 2, 24, 48 and 72 hours of infection. Moreover, GF macrophages had less phagocytic capacity. When we quantify the number of parasites in the supernatant, we showed the Abx and GF macrophages presented low parasite number during 7 days of quantification compared to control. In addition, Abx macrophages after IFN-γ/LPS stimulus had reduced production of NO without infection and after T. cruzi infection compared to control group. And the same trend we can observe with ROS assay. About ArgI, the macrophages were stimulated with IL-4 and we observed that Abx macrophages presented high levels without infection and after T. cruzi infection. Then, we conclude that the microbiota plays an important role in the macrophages polarization profile and determine how the macrophages acts after a challenge with T. cruzi. Additionally, microbiota influences the phagocytic capacity of macrophages. **Supported by:**CAPES, FAPEMIG, CNPQ
**Keywords:** Trypanosoma cruzi; macrophages; microbiota

HP13 - **RADIAL GLIA CELLS INFECTION BY *TOXOPLASMA GONDII* IMPAIRS NEUROGENESIS AND DISRUPTS BRAIN MICROVASCULAR ENDOTHELIAL CELLS INTEGRITY**

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Congenital toxoplasmosis is a parasitic disease that occurs due vertical transmission of the protozoan *Toxoplasma gondii* during pregnancy. The parasite crosses the placental barrier and reaches the developing brain, infecting progenitor, glial, neuronal and vascular cell types. Although the role of Radial glia (RG) neural stem cells in the development of the brain vasculature have been recently investigated, the impact of *T. gondii* infection in these events is not known. Here we studied the role of *T. gondii* infection on RG cells function and its interaction with endothelial cells. Isolated RG cells were infected with *T. gondii* tachyzoites for 24 h. Cells were fixed and conditioned medium (CM) was collected for treatments and cytokine analyses. Cells were immunostained for specific neural markers and proliferation; CM was used on mouse brain microvascular endothelial cell line (bEnd.3). Alternatively, CM was used for TGF-β ELISA and cytokine profiling with cytokine bead array (CBA). bEnd.3 cells were plated on coverslips for ZO-1 immunostaining or transwell inserts for transendothelial electrical resistance (TEER) measurements. After 24 h of treatment with CM or infection with T. gondii cells were analyzed. We observed reduced RG cell proliferation and neurogenesis without affect gliogenesis. CM from RG control cultures increased ZO-1 and □-catenin on endothelial bEnd.3 cell membranes, which was completely impaired by CM-Inf or by direct infection, accompanied by reduced TEER. CBA and ELISA assays revealed increased levels of the pro-inflammatory cytokine IL-6 and reduced levels of anti-inflammatory TGF-β1 in CM-Inf. Recombinant TGF-β1 restored ZO-1 immunoreactivity in CM-Inf-treated bEnd.3 cells. Our results suggest that infection of RG cells by *T. gondii* modulates the secretion of cytokines that might contribute to endothelial loss of barrier properties, thus contributing to impairment of neurovascular interactions. **Supported by:**CNPq, Fiocruz **Keywords:** Congenital toxoplasmosis; neurogenesis; neurovascular interactions

HP14 - **ROLE OF TOLL-LIKE RECEPTORS AND MICRORNA-LET-7E TO MACROPHAGE INFLAMMATORY RESPONSE DURING *LEISHMANIA AMAZONENSIS* INFECTION**

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Toll-like receptors (TLRs) trigger the innate inflammatory response by the recognition of molecular patterns from pathogens or cell damages. Its signaling pathway can be regulated by microRNAs (miRNAs) at a post-transcriptional control level. To evaluate the role of those mechanisms in the *Leishmania amazonensis* infection, we compared TLR signaling as well as miRNA profile expression of macrophages (MØ) obtained from wild type (WT) or TLR2, TLR4 or MyD88 (myeloid differentiation primary response 88) knockout mice. The absence of these genes increased infectivity of L. amazonensis. The TLR pathway also influences in the L-arginine metabolism leading to nitric oxide (NO) production to kill parasite by increasing Nitric Oxide Synthase 2 mRNA expression in the WT- MØ, but not in TLR2, TLR4 and MyD88 knockouts. Also, in MyD88$^{-/-}$-MØ presented an increase in mRNA amounts of Cationic Amino Acid Transporters. TLR2$^{-/-}$-MØ presented an increase in Arginase 1 transcripts, enzyme involved in polyamines production and parasite survival. Moreover, *L. amazonensis* infection of WT-MØ modulated 32% of 84 analyzed miRNAs, being 50% up regulated; while MyD88$^{-/-}$, TLR2$^{-/-}$, and TLR4$^{-/-}$ showed distinct modulation of miRNAs. Remarkably, the miRNA let-7e expression was increased in WT-MØ infected but reduced in the knockout mice strains. The let-7e functional inhibition impacted in the TLR pathway gene expression, involving recognition and adaptors molecules, such as TLR9, MD-2 (myeloid differentiation protein 2), TIRAP (TIR domain-containing adaptor protein) and TRAF6 (TNF receptor associated factor), NF-kB (nuclear factor kappa-light-chain-enhancer of activated B cells) signaling and immunomodulators, such as COX2 (Cyclooxygenase), GM-CSF (granulocyte/macrophage colony stimulating factor) and NOS2, promoting a reduction in infectivity. In conclusion, TLR and miRNA impact in the macrophage response to *L. amazonensis* infection. **Supported by:**FAPESP and CNPq **Keywords:** Inflammation; innate immune response; c57bl/6 mice

HP15 - **SWIM LIKE YOUR LIFECYCLE DEPENDS ON IT: INVESTIGATING CHEMOTAXIS AND NAVIGATION IN LEISHMANIA PARASITES.**

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Background: *Leishmania* motility is essential for lifecycle progression. An oscillating flagellum pulls the promastigote through changing environments and the parasite transforms to survive. During the host transition from sandfly to mammals, the egestion from the sandfly is highly enriched in metacyclic promastigotes due to the viscous promastigote secretory gel (PSG) creating a 'sieve'. Exactly how the metacyclics are capable of swimming through PSG remains obscure, but is fundamentally linked to infectivity and transmission. It is therefore the focus of our investigation. We use a novel imaging technology, digital inline holographic microscopy (DIHM), to track promastigote swimming in three dimensions (3D). Objectives:
1. To quantitatively define and compare swimming parameters of sandfly- vs. human-infective promastigote stages in 3D.
2. To quantify changes in parasite swimming behavior in response to different environmental gradients and examine for evidence of taxis.
3. To quantify the effect of Promastigote Secretory Gel on promastigote parasite motility.
Results: Tracking the swimming behavior of >10,000 cells, we find differences between the promastigote stages: procyclics swim with a 'corkscrew' motion and metacyclics swim with a striking run-and tumble motif similar to chemotactic bacteria (E. coli). Both lifecycle stages were placed in controlled chemical gradients, and their swimming behavior examined. While procyclic swimming does not change, metacyclics undergo a chemokinetic shift, visibly slowing their swimming speed. The presence of a visco-elastic fluid slows both lifecycle stages' swimming speeds. As in the sandfly, the metacyclics swim more easily in a complex fluid, demonstrate higher levels of motility and swim faster in PSG than procyclics.
Conclusions: Metacyclic cells are able to swim through PSG and swim differently in the presence of cells they infect. 3D swimming patterns may provide a quantitative test of transmission fitness.
**Supported by:**Wellcome **Keywords:** Leishmania motility; sandfly transmission; macrophage chemotaxis

HP16 - **EXTRACELLULAR VESICLES (EVS) FROM DIFFERENT *LEISHMANIA* SPECIES HAS IMMUNOMODULATORY ROLE IN MURINE MACROPHAGES**

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*1.FUNDAÇÃO OSWALDO CRUZ - IRR, MG, Brazil; 2.FUNDAÇÃO OSWALDO CRUZ- IGM, BA, Brazil; 3.INSTITUT ARMAND-FRAPPIER, Canada; 4.UNIVERSIDADE FEDERAL DE SÃO PAULO, SP, Brazil.*

Extracellular vesicles are secreted by most eukaryotic cell types, including the parasites. *Leishmania* can release vesicles and play a crucial role in host-pathogen interactions. These EVs contain the surface metallopeptidase gp63 and others molecules that possess signaling-inducing activities. *Leishmania* cell surfaces are dominated by lipophosphoglycan (LPG). The LPG display inter-species polymorphisms and has been shown to interfere with host immune response. In this study, we evaluated the in vitro immunomodulatory properties of EVs from three New World *Leishmania* species: *Leishmania braziliensis*, *Leishmania amazonensis* and *Leishmania infantum*, causative agents of cutaneous and visceral leishmaniasis. Peritoneal macrophages recovered from mice (Balb/C, C57BL/6, TLR2-/- and TLR4 -/-), were primed with INF-γ (0,5 µg/ml) prior to incubation with EVs ($1x10^{10}$ particles). LPS (100 ng/mL) was used as positive control. Following incubation (48h), NO and cytokines were measured in the supernatants by Griess reaction and CBA-multiplex flow cytometry. Our preliminary results suggest a species-specific stimulation pattern. EVs from *L. braziliensis* and *L. infantum* (WT and LPG-KO) did not induced the production of nitrite, whereas *L. amazonensis* EVs were able to induce its production via TLR4. In another set of experiments, Balb/C and C57BL/6 macrophages were pre-incubated with EVs, prior to stimulation with LPS (100 ng/mL). Here, pre-stimulation with EVs abrogated nitrite production by EVs from *L. amazonensis*. However, it induced a higher nitrite production by *L. braziliensis* EVs. In order to investigate if LPG was present in the EVs, EVs were purified and probed with CA7AE and anti-gp63 (positive control). LPG was detected in the EVs. In conclusion, EVs from New World species of *Leishmania* differentially stimulate murine macrophages and *Leishmania infantum* EVs also express LPG in their surface and together with gp63 can also be considered an EV marker. **Supported by:**CNPq, FAPEMIG **Keywords:** Extracellular vesicles; leishmania; new world

PV1 - **INVESTIGATION OF ARGININE METHYLATION IN *LEISHMANIA (V.) BRAZILIENSIS* GROWTH AND INFECTION**

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Leishmania (V.) braziliensis is the predominant cause of cutaneous and mucocutaneous leishmaniasis in Brazil. In trypanosomatids, control of gene expression occurs mainly at the post-transcriptional level, and RNA binding proteins (RBPs) are key players in the determination of transcript fate. Among other post-translational modifiers, RBPs are targets of Arginine Methyltransferases (PRMTs), which transfer a methyl group to arginine residues of proteins affecting their RNA-binding capacities. Herein we present the preliminary characterization of the five predicted arginine methyltransferases of L. (V.) braziliensis. The comparative immunoblotting analysis of the arginine methylation profile of L. (V.) braziliensis between procyclic promastigotes, metacyclics and amastigotes reveal a diverse profile of arginine methylation in the different stages, as well as modulation of expression of the five putative PRMTs. Overexpression (OE) and knockout (KO) of these genes led to changes in parasite protein methylation profile. Based on the KO and OE phenotypic analyses, PRMTs are not essential genes for L. (V.) braziliensis promastigote survival, at least independently, and investigation of candidate targets is in progress. Although no differences were observed in axenic promastigote growth for any of the OE transfectants when compared to wild-type cells, macrophage infection by PRMT5 OE parasites is significantly lower than by other cell lines. According to preliminary data, PRMT5 is the only PRMT which is expressed both in nucleus and cytoplasm in L. (V.) braziliensis, which may suggest divergent functions. Overall, the data indicate that these PRMTs promote methylation of a diverse group of proteins in different stages of the parasite life cycle and the relevance of the post-translational modification for the nucleic-acid binding activities, gene expression regulation, and virulence will be investigated. **Supported by:**FAPESP, CAPES, CNPq **Keywords:** Leishmania; arginine methylation; crispr/cas9

PV2 - **ISOLATION AND CHARACTERIZATION OF FOUR NOVEL SOUTHAMERICAN STRAINS REVEALS UNDERAPRECIATED GENETIC DIVERSTIY, WITH PHENOTYPICAL IMPLICATIONS, AMONG NEOSPORA CANINUM ISOLATES**

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*Neospora caninum* causes neosporosis, one of the leading causes of bovine abortion. Uruguay is a developing economy in South America producing milk to feed twenty million people annually: seven times its population. Naturally, dairy production is of massive importance to the country's economy. Recent studies have shown that all dairy farms in Uruguay have at least one seropositive cow for *N. caninum,* and that the vast majority of abortions are caused by this agent. Despite this, very little is known about the basic biology of N. caninum in the country. To delve into the local situation, and contextualize it within the international standing, we set out to characterize the prevalent strains of N. caninum in Uruguay. We isolated four strains from three distinct regions, which by multi-locus microsatellite typing represented three unique genetic lineages, distinct from those reported previously. This genetic diversity correlated with phenotypical differences on vertical transmission, virulence and abortigenic potential, in a mouse model. Importantly, antigen recognition by infected cow sera, normally relied upon in diagnostic tests, is strain-specific. Microsatellite typing of *N. caninum* DNA from spontaneously aborted bovine fetuses from dairy farms correlated more often with one of the isolates, suggesting that different strains may have different abortigenic potential in the field. Nonetheless, our study also revealed that additional strains are present in aborted fetuses, suggesting that greater diversity exists. Our results suggest that the strain´s genetic background likely impacts differently the outcome of a bovine pregnancy, rising questions on the importance of strain-specific diagnosis. Finally, whole genome sequencing using PacBio technology revealed an imprecise assembly of the reference N. caninum Liverpool genome, prompting us to challenge the paradigm of genome synteny between *N. caninum* and its apicomplexan relative *Toxoplasma gondii.* **Supported by:**AGENCIA NACIONAL DE INNOVACION E INVESTIGACION - ANII **Keywords:** Neospora; toxoplasma; apicomplexa

PV3 - **CHARACTERIZATION OF ANGIOGENESIS INDUCED BY *T. CRUZI* AND *L. BRAZILIENSIS* IN HAMSTER USING INTRAVITAL MICROSCOPY AND COMPUTER BASED TECHNOLOGY.**

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Introduction: Angiogenesis is a physiological and also a pathological process of great complexity, that is difficult to measure objectively and automatically. The hamster cheek pouch (HCP) prepared for intravital microscopy (IVM) has been used to characterize inflammation induced microvascular changes and was chosen to investigate microvascular characteristics of HCPs parasitized by green protein labeled T. cruzi Dm28c trypomastigotes (TCT-GFPs), epimastigotes-GFP and L. brasiliensis MHOM/BR/00/BA788 promastigotes (Lb) and compare such HCPs with non-infected HCPs using an image processing method, Bulant et al. (2017). Methods and Results: Images of HCPs captured at IVM were subjected to computer based measurements of TCT-GFP and Lb induced angiogenesis with an image segmentation approach that discriminates between fluorescence emitted by FITC-dextran inside the vasculature and the extravascular space. The method computes several independent indices that can measure morphological characteristics of a normal and pathological microvasculature such as fluorescence of FITC-dextran, vascular area, total vascular length (TVL), mean length of vascular segments (MSL), number of vascular segments, tortuosity, and non-vascular fluorescence. Measurements in TCT-GFP infected hamsters (n=36) showed that all indices were significantly different ($p < 0.05$) from the same indices measured in non-infected hamsters (n = 33) on day 7 d.p.i. A negative linear correlation was observed between the indices TVL and MSL suggesting that increased angiogenesis resulted in shorter vascular segments. Similar results were observed in Lb infected HCPs (n = 6). Conclusion: The significant differences between seven indices of angiogenesis in T. cruzi infected and non-infected HCPs and in L.brasiliensis infected HCPs showed that our method could be useful to investigate mechanisms and pathophysiological relevance of angiogenesis induced in HCPs parasitized by intracellular protozoa. **Supported by:**CNPq, CAPES, FAPERJ **Keywords:** Angiogenesis; image processing ; trypanosoma cruzi

PV4 - **SPOT THE DIFFERENCE: USING PROTEOMICS TO INVESTIGATE THE EFFECT OF THE BCAAS ON METACYCLOGENESIS IN *TRYPANOSOMA CRUZI***

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*Trypanosoma cruzi*'s epimastigotes differentiate into infective non-proliferative metacyclic trypomastigotes in a process called metacyclogenesis. It happens in the final portion the insect vector's gut and is crucial to the progress of the parasite life cycle. Factors such as nutritional stress and low pH trigger metacyclogenesis. In this context, the supply of amino acids have divergent effects on this process: whereas His, Pro and Gln are able to support it, the branched–chain amino acids (BCAAs) Ile, Leu and Val diminish it. To investigate the mechanism by which BCAAs impact on metacyclogenesis, we differentiated epimastigotes in triatomine artificial urine (TAU) in the presence of Pro or Pro combined with one of the BCAAs. Metacyclogenesis in TAU-Pro yielded, 24.6% of metacyclics whereas differentiation in TAU-Pro-Leu, TAU-Pro-Ile and TAU-Pro-Val yielded 10.6%, 15.7% and 16.7% of metacyclics, respectively. Comparative proteomics analysis identified 1457 proteins as present in all the analysed groups. When compared to the metacyclics originated in TAU-Pro, 40 proteins are differentially regulated in cells differentiated in TAU-Pro-Leu, whereas 131 are differentially regulated in TAU-Pro-Ile and 179 are differentially regulated in TAU-Pro-Val. Interestingly, only 3.3% of the total differentially regulated proteins are shared by metacyclics differentiated in the presence of BCAAs. However, 21.1% of the total differentially regulated proteins are common between TAU-Pro-Ile and TAU-Pro-Val metacyclics. Proteins related to gene expression are differentially regulated in all conditions. Noteworthy, proteins involved in the metabolism of amino acids, metabolism of lipids as well as in autophagy are upregulated in at least two of the BCAAs metacyclics. Together, the data points that the impact of BCAAs on metacyclogenesis goes beyond the use of these amino acids as carbon sources, and they may also play a major role as negative modulators of the differentiation process. **Supported by:**FAPESP **Keywords:** Amino acids; metabolism; proteomics

PV5 - **ON THE IMPORTANCE OF VALIDATING *DIENTAMOEBA FRAGILIS* REAL-TIME PCR ASSAYS**

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*Dientamoeba fragilis* is a gastrointestinal trichomonad parasite whose pathogenicity is yet to be determined. The difficulty involved in microscopically diagnosing *D. fragilis* in faeces led to the development of RT-PCR methodologies for the detection of *D. fragilis* in stool samples, two of which are widely used across the globe. Prevalence studies in Europe show much higher levels of infection where an in house real time assay is the predominant assay for the detection of Dientamoeba fragilis, compared to regions that use the EasyScreen assay for detection of gastrointestinal pathogens. This study compared the commercially available *Dientamoeba fragilis* assay (Genetic Signatures EasyScreen assay) against the widely used in house real time PCR method. 250 faecal samples were screened using the in house real time assay on four real time PCR platforms producing a number of discrepant results. The presence or absence of *Dientamoeba fragilis* DNA in discrepant samples was shown using PCR amplicon next generation sequencing. Eukaryotic 18S diversity profiling was conducted on discrepant samples to identify the presence or absence of additional protozoan species in samples that may be responsible for cross reactivity seen in these samples. The results revealed multiple false positive results when using the in house real time assay across multiple real time platforms. This study has highlighted several problems regarding the sensitivity and specificity of the in house real time assay for the detection of *Dientamoeba fragilis* and emphasises the need for standardisation of detection assays across all nations researching D. fragilis. **Keywords:** Dientamoeba; real-time pcr; pcr validation

PV6 - **EXPLOITING THE CRISPR/CAS9 REPERTOIRE FOR PRECISE GENE EDITING IN *TRYPANOSOMA CRUZI***

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In higher eukaryotes during stress conditions kinases are activated to phosphorylate the eIF2α in the S51 causing translational arrest. To understand the importance of eIF2α and its phosphorylation in *T. cruzi*, a lineage of epimastigotes overexpressing the wild-type eIF2α was generated in parallel with a lineage overexpressing an alanine replacing the T169 phosphorylation site, which correspond to the S51 of higher eukaryotes. The resulting cells showed abnormal growth and lack the regulation of stage specific expression of metacyclic proteins (GP-90), confirming that eIF2α phosphorylation is important for the parasite. To assess if the observed effects weren't an artefact due to the overexpressing approach, we introduced the T169A mutation in the endogenous eIF2α gene using the CRISPR/CAS9 system in combination with a restriction site for the enzyme PauI. Parasites of the strains DM28c and Y expressing the Cas9 protein were transfected with guides RNA produced *in vitro* and a ssDNA containing the desired mutations. The correct insertion of the mutations in more than 90% of the parasites was confirmed and clonal selection was made for both lineages. We were not able to obtain a clonal population using limiting dilution for the Y strain but even after 4 months of culturing, the parasites conserved the mutation in more than 93% of the parasites.On the other hand, several clones were obtained for the DM28c strain, although surprisingly none of 20 clones analyzed maintained the mutation, indicating that either during the cloning process the modified parasites did not grow efficiently or that the parasites were able to repair the mutations introduced. These parasites did not express the GP-90 protein, but the amount of non-mutated DNA increased significantly during the process of differentiation from epimastigotes to infective metacyclic parasites. These results confirm the importance of the adequate balance of eIF2α expression levels and its phosphorylation for *T. cruzi*. **Supported by:**FAPESP **Keywords:** Translation; eif2; phosphorylation

PV7 - **DUPLICATION AND DELETION OF MOTIFS HAVE SHAPED THE EVOLUTION OF *TRYPANOSOMA CRUZI* TRANS-SIALIDASE, MUCIN AND MASP MULTIGENE FAMILIES, CORRELATING WITH THEIR EXPRESSION LEVELS**

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Copy number variation, the gain or loss of genomic material is extensively documented in the evolution of trypanosomatid parasites. Among the Tritryps, *T. cruzi* owns the largest expansion of multigene families encoding surface proteins that are enrolled in host-parasite interactions. The repetitive content of these families hinders the assignment of next generation sequencing (NGS) reads to a specific gene, as they can align with the same reliability to several loci. However, these genes present motifs (short DNA sequences) that are shared among distinct members, which can be explored to estimate variability among the parasite DTUs. To this end, we developed a methodology to evaluate the copy number variation of motifs derived from repetitive gene families based on unassembled NGS reads. Our methodology was used to estimate the copy number variation of motifs in MASP, TcMUC and trans-sialidase multigene families in the genome of 36 *T. cruzi* strains, including members of the 6 DTUs. The variation in motifs composition display high phylogenetic resolution, suggesting that these families have undergone large copy number variations along the parasite evolution. A comparison of the copy number of genomic and transcriptomic motifs in TcI, TcII and TcVI revealed a positive Pearson correlation, suggesting that motif duplication influences gene expression. However, a small number of motifs with high expression levels and low genomic copy number was observed, suggesting a positive selection for the expression of these motifs, especially for the MASP family. These highly expressed motifs are localized in MASP genes with high density of B-cell predicted epitopes. We have selected and synthesized by SPOT-synthesis the peptide sequence corresponding to the 300 motifs with higher B-cell epitope prediction scores in the three families. We are currently screening this membrane with the sera from mice infected with different *T. cruzi* DTUs as well as with sera from chagasic patients. **Supported by:**CNPq, FAPEMIG, CAPES **Keywords:** Multigene families; copy number variation; trypanosoma cruzi

PV8 - **A POSSIBLE ROLE OF MSH6 IN THE OXIDATIVE STRESS RESPONSE IN TRYPANOSOMATIDS**

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DNA repair mechanisms are crucial for maintenance of the genome in all organisms, including parasites where successful infection is dependent both on genomic stability and sequence variation. MSH2 and MSH6 are early acting, central component of the Mismatch Repair (MMR) pathway, responsible for the recognition and correction of base mismatches that occur during DNA replication and recombination. Previous work from our group showed that MSH2 plays an important, but poorly understood, role in responding to oxidative damage in both African and American trypanosomes. To investigate the involvement of other MMR components in the oxidative stress response, null mutants of MSH6 were generated in *Trypanosoma brucei* procyclic forms and single allele knockout in *Trypanosoma cruzi* epimastigote forms. Here we show that differently from MSH2 knockout in *T. cruzi*, knockout of one MSH6 allele resulted in increased susceptibility to $H_2O_2$ exposure, but not to impaired MMR as no difference in tolerance to alkylation by MNNG is observed. In *T. brucei* MSH6 double knockout, but not single knockout showed increased resistance to MNNG. No difference in the oxidative stress response is observed for MSH6 mutants in *T. brucei*. Taken together our results suggest that both proteins of the MMR complex act in response to oxidative stress in a new role that operates differently in *T. brucei* and *T. cruzi*. **Supported by:**CNPq, Capes **Keywords:** Trypanosoma cruzi; trypanosoma brucei; dna repair

PV9 - **LAMIN PROTEIN NUP-1 IN TRYPANOSOMA BRUCEI ASSEMBLY SUGGESTS A 'HUB AND SPOKE' MODEL**

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The nuclear lamina supports multiple roles in the nucleus, including structural integrity, chro atin organisation and developmental gene expression, for which correct assembly is es tly it has emerged that there are multiple lamina systems across eukaryot or independent descent, these systems have remarkably analo al importance. In the trypanosomatids several elem two, NUP-1 and NUP-2, demonstrated are large proteins, indicati deletion mutant na. We find that h ption of this process also a and nuclear envelope. Proteomics a ing proteins, including a kinase, a peptidyl-p ally involved in the regulation of NUP-1 assembly. These da domains of NUP-1 have important roles in the assembly of the try and suggest a 'hub and spoke' architecture. **Supported by: Keywords:** Try soma brucei; nuclear envelope; lamina



PV10 - **A NEW WAY TO PLAY AN OLD GAME: LESSONS FROM REPLICATION PROTEIN A IN TRYPANOSOMATIDS.**

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*Trypanosoma cruzi* and *Trypanosoma brucei* are parasitic protozoa responsible for causing Chagas disease and sleeping sickness that result in a high number of deaths annually. These parasites possess a complex life cycle that alternates between replicative and non-replicative lifeforms. Despite the characterization of a great number of molecular pathways, there are still many gaps in the understanding of the processes that coordinate the DNA metabolism of these organisms. Replication protein A (RPA), the major eukaryotic single-stranded binding protein, is a well-known heterotrimeric complex formed by three subunits RPA-1, RPA2, and RPA-3 that participates in various vital functions during replication, repair, and checkpoint signaling. RPA from trypanosomatids presents significant structural peculiarities compared to other eukaryotes such as the lacking of DBDF-domain that interacts with proteins majorly involved in DNA damage response (DDR) pathways and amino acids substitutions in conserved regions, raising questions regarding the conservation of canonical functions described in mammals and yeast. In this work, we show that RPA from trypanosomatids can interact with single-stranded DNA and is indeed important for replication and DNA damage response pathways. Moreover, we could find new features concerning trypanosomatids RPA such as the discovery of (i) non-described post-translation modifications (ii) a new RPA-like protein that seems to be exclusive of trypanosomatids interacting with RPA complex and (iii) a nucleus-cytoplasm shuttle that is lifecycle dependant. **Supported by:**FAPESP **Keywords:** Replication protein a; dna replication; ssdna binding protein

PV11 - **USING CONDITIONAL GENOME ENGINEERING WITH DICRE AND CRISPR/CAS9 TO UNDERSTAND THE ROLE OF HOMOLOGOUS RECOMBINATION IN DNA REPLICATION IN *LEISHMANIA*.**

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*Leishmania* present remarkable genome plasticity, which is manifested as gene amplification and chromosome ploidy changes and hinders both genetic manipulation and the development of anti-leishmanial therapies. Homologous recombination (HR) factors, including Rad51 and its paralogue Rad51_4, act in gene amplification in *Leishmania*. However, the depth and breadth of HR roles in *Leishmania* genome maintenance remains unexplored. In other eukaryotes, HR is canonically implicated in DNA double-strand break repair and in the replication stress response. Due to the potentially unusual nature of DNA replication initiation in *Leishmania*, in which a single locus-specific origin is activated per chromosome, we set out to investigate the role of HR in the replication process of this parasite. We used CRISPR/Cas9 to rapidly engineer cell lines in which HR genes were endogenously tagged and also flanked by *LoxP* sites (Flox) to allow rapamycin-inducible knockout (KO) with DiCre. KO induced loss of Rad51 (*Rad51-HA*$^{Flox}$), each Rad51 paralogue (*Rad51_3-HA*$^{Flox}$, *Rad51_4-HA*$^{Flox}$, *Rad51_6-HA*$^{Flox}$ ) and the helicase PIF6 (*PIF6*$^{Flox}$) resulted in accumulation of DNA damage, as measured by γH2A, demonstrating all these factors act in the preservation of *Leishmania* genome integrity. Contrary to previous reports, loss of none of the HR genes was essential. Interestingly, we observed that while induction of Rad51 KO led to increased DNA synthesis, decreased DNA synthesis was seen upon the absence of each Rad51 paralogue or PIF6. Also, cell cycle progression analysis revealed that KO induction of Rad51 or its paralogue Rad51_6 led to faster G2/M to G1 transition, while Rad51_3 KO led to the opposite effect. Altogether, these data suggest that these HR factors have distinct and/or antagonistic functions during DNA synthesis and G2/M transition in *Leishmania*. Currently, we are characterizing single strand DNA accumulation and genome-wide mutation patterns upon KO induction of each of these HR factors. **Supported by:**Marie Skłodowska-Curie Actions / European Commission **Keywords:** Dna replication; homologous recombination; dna damage repair

PV12 - **DISSECTING THE ATR PATHWAY OF LEISHMANIA MAJOR: CHARACTERIZATION OF AN ATR KINASE-DEFICIENT CELL LINE.**

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Species of the protozoan Leishmania are deep-rooted eukaryotes with an unusually plastic genome characterized by gene and chromosome copy number variation (CNV), mosaic aneuploidy and chromosome rearrangements, which are frequent hallmarks of genome instability. The ATR kinase is the central player of a signaling pathway (ATR pathway) that regulates the eukaryotic response to replication stress and a few types of DNA damage, and orchestrates cell cycle arrest, replication fork stabilization and DNA repair recruitment, which altogether are determinants of genome stability and maintenance. Considering Leishmania's genome plasticity we sought to investigate the conservation and functional relevance of ATR kinase in these protozoa. We used CRISPR/Cas9 genome editing to target the kinase domain of L. major putative ATR gene (LmjF.32.1460) to generate an ATR-deficient cell line. Selected clones were heterozygous as confirmed by Southern blotting and PCR analysis. Despite the similar growth phenotype, the ATR-deficient cell response to replication stress was remarkably different when compared to wild type cells. The mutant cell line was exposed to different concentrations of Hydroxyurea (HU) and the levels of histone H2A phosphorylation, a DNA damage marker in trypanosomatids (□H2A), were analyzed by western blotting. ATR-deficient cells have reduced levels of □H2A when compared to WT cells. This difference is exacerbated when cells are exposed to either mild or acute HU-mediated replication stress. These findings suggest that ATR is a functional kinase that participates in DNA damage signaling in Leishmania. Current work is focused on the characterization of ATR deficiency and its consequence to cell homeostasis. **Supported by:**FAPESP **Keywords:** Atr pathway; dna damage response; atr deficiency

PV13 - **TB8870, A CYTOPLASMIC PROTEIN IS CRITICAL FOR PFR ASSEMBLY IN *TRYPANOSOMA CRUZI***

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The paraflagellar rod (PFR) is an extra-axonemal structure present in kinetoplastids that runs alongside the flagellum linked to the axoneme and is important for the motility of these parasites. The PFR has a complex, trilaminar lattice organisation, which will require a regulated and ordered assembly process. In a search for potential PFR assembly factors in Trypanosoma brucei, we identified the gene Tb927.10.8870 that encodes a coiled-coil protein which we called Tb8870. We generated Tb8870 null mutants using a CRISPR-Cas9 approach. Immunofluorescence and transmission electron microscopy analysis of the null mutant show that the PFR is disorganised, much reduced, and the trilaminar organisation is completely absent; however, the connections to the axoneme are still present. The disruption to PFR assembly caused a reduced growth rate with errors in cytokinesis, yet despite the reduced, unstructured PFR there is only a minor effect on cell motility. Surprisingly, Tb8870 is a cytoplasmic protein and together this suggests that important steps in PFR assembly occur in the cytoplasm before entry into flagellum as is found for axonemal components such as the outer dynein arms. **Supported by:**CAPES, MRC-CONFAP **Keywords:** Paraflagellar rod; flagellum; crispr-cas9

PV14 - **INORGANIC POLYPHOSPHATE INTERACTS WITH NUCLEOLAR AND GLYCOSOMAL PROTEINS IN TRYPANOSOMATIDS**

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Inorganic polyphosphate (polyP) is a polymer of three to hundreds of phosphate units bound by high-energy phosphoanhydride bonds and present from bacteria to humans. Most polyP in trypanosomatids is concentrated in acidocalcisomes, acidic calcium stores that possess a number of pumps, exchangers, and channels, and are important for their survival. In this work, using polyP as bait we identified > 25 putative protein targets in cell lysates of both Trypanosoma cruzi and T. brucei. Gene ontology analysis of the binding partners found a significant over-representation of nucleolar and glycosomal proteins. Using the polyphosphate-binding domain (PPBD) of Escherichia coli exopolyphosphatase we localized long chain polyP to the nucleoli and glycosomes of trypanosomes. A competitive assay based on the pre-incubation of PPBD with exogenous polyP and subsequent immunofluorescence assay of procyclic forms of T. brucei showed polyP concentration-dependent and chain length-dependent decrease in the fluorescence signal. Targeting of yeast exopolyphosphatase to the glycosomes of procyclic forms resulted in polyphosphate hydrolysis, alteration in their glycolytic flux and increase in their susceptibility to oxidative stress. **Supported by:**CNPq; U.S. National Institutes of Health (NIH) **Keywords:** Polyphosphate; glycosomes; nucleolus

PV15 - **EDITING DELTA- AND DELTA-AMA40/50 AMASTINS FROM *TRYPANOSOMA CRUZI* BY CRISPR-CAS9 SYSTEM**

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The amastins are membrane proteins differentially expressed during *T. cruzi* life cycle, and it has been shown in *T. cruzi* and *Leishmania* that they can be associated to invasion, parasite survival, or multiplication in their hosts. *In silico* analysis allowed amastin classification in families in Trypanosomatids, being that we identified α, β and δ-amastins in T. cruzi. Therefore, we would like to improve the functional characterization of amastins. The δ-amastin gene is a multi copy gene family highly expressed in amastigotes and trypomastigotes, not only δ-amastins but also upregulated in amastigote forms. In this system, a sgRNA to target each of δ-amastins was designed using EuPaGDT. After that, G epimastigotes were co-transfected with *Staphylococcus aureus* Cas9 and *in vitro* transcribed sgRNA targeting δ-amastin and donor sequence containing stop codons. One week after transfection, the δ-amastin knockout was confirmed by PCR. The flow cytometry analysis showed specific δ-amastin::GFP expression (90% with donor and 74% without donor) in δ-amastin::GFP epimastigotes. Southern analysis also showed that besides the ectopic copy, the endogenous copy was edited. To test the possible effects of this editing in different strains, parasites derived from Y strain and DM28c clones were edited in a similar fashion and the gene editing was checked by RFLP-PCR. The phenotypic analysis of these edited parasites is in progress as well as the editing of the divergent δ-amastin, Ama40/50. **Supported by:**CAPES, CNPq, FUNDAÇÃO ARAUCÁRIA **Keywords:** Amastin; crispr-cas9; donor

PV16 - **DISRUPTION OF GENES ENCODING ACTIVE TRANS-SIALIDASES IN TRYPANOSOMA CRUZI USING CRISPR/CAS9 TECHNOLOGY**

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The trans-sialidases (TSs) are proteins from Trypanosoma cruzi responsible for transfering sialic acid from host glycoconjugates to acceptors at the surface of the parasite. Sialylation of trypomastigote surface is described as an important process in parasite invasion of host cells and in prevent killing of parasites by lytic antibodies. TSs are part of a multigenic family with more than 1400 members, however only few members have catalytic activity, based on the presence of a conserved tyrosine at position 342 crucial for TSs activity. In addition, some active TSs have a repetitive amino acid sequence named SAPA in their C terminal portion, which has been show to improve enzyme pharmacokinetics. Due to TAC to CAC substitution, inactive TSs posses an histidine at position 342. Aiming at knocking out only active TS genes using the CRISPR/Cas9 technology we designed two single guide RNAs (sgRNAs) that target TSs genes encoding active enzymes. As a donor template to be used for homologous recombination repair of the double strand break generated by Cas9, we used a single stranded oligonucleotide containing three stop codons, the M13 universal sequence and an EcoRV restriction site, flanked by 25 nucleotides corresponding to conserved sequences encoding active TSs. After transfection of epimastigotes constitutively expressing Cas9 nuclease with both sgRNAs and donor, PCR analyses of genomic DNA purified from transfected cloned cell lines followed by EcoRV digestion indicated the insertion of the donor sequence in the right position disrupting TSs genes. Quantitative PCR as well as enzymatic assays are currently been done to estimate the proportion of TSs genes that have been disrupted and the level of TSs activity that remained in the mutant cell lines compared to WT trypomastigotes. Preliminary in vitro infection assays using parasites with disrupted TSs suggests that after lysis of cells by tryposmatigotes those became amastigotes faster than WT parasites. **Supported by:**FAPEMIG, INCTV **Keywords:** Trypanosoma cruzi; trans-sialidases; crispr/cas9

TB1 - **DRUG TARGET DECONVOLUTION STUDIES IN *LEISHMANIA DONOVANI***

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Visceral leishmaniasis (VL) is caused by protozoan parasites from the *Leishmania* genus and is potentially fatal if left untreated. Despite its significant health impact, there are limited anti-leishmanial drugs available, with the lack of validated drug targets a serious impediment to the development of effective treatments for these diseases. Whole-cell phenotypic high-throughput screening of a set of 1.8 million compounds against *L. donovani* was performed by GlaxoSmithKline and resulted in the generation of a Leish-box containing approximately 200 active compounds. Since there is no information regarding the molecular targets and/or the mode of action (MoA) of these phenotypically-active compounds, comprehensive studies to determine their molecular targets are required. With this in mind, we have selected a small cohort of promising Leish-box compounds in order to carry out drug target deconvolution studies. Principally, we have exploited two genomic approaches to determine the molecular targets of these compounds: generation of resistant lines followed by whole genome sequencing (WGS) and screening of a *Leishmania* cosmid-based over-expression library. Resistant lines are selected by growing parasites under increasing drug pressure until a resistant population emerges. Genetic mutations that may be associated with the drug resistance phenotype will be identified by WGS. In the second approach, the *L. donovani* cosmid library is subjected to sub-lethal concentrations of each compound and the cosmid DNA harboured by the resistant parasite population will be analysed by Next Generation Sequencing. Both approaches provide valuable and complementary information to determine the targets and MoA of phenotypically active compounds. Progress in determining the molecular target(s) of these promising anti-leishmanial compounds will be presented. **Supported by:**Medical Research Council; GlaxoSmithKline **Keywords:** Leishmania; targets; genomic approaches

TB2 - **THE ROLE OF THE OVEREXPRESSION OF ABCG1, ABCG4 AND HMGCOA REDUCTASE ENZYME IN STEROLS HOMEOSTASIS OF LEISHMANIA SPP.**

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Trypanosomatids have their own machinery for sterol synthesis. However, a high percentage of cholesterol is found in Leishmania spp. The transport of cholesterol and other sterols in their free form is not clear. In mammalian cells, ABCG1/G4 are related with translocation of cholesterol and phospholipids. The genome of Leishmania spp. has 42 genes of ABC family, but their functions are unknown. HMGCoA reductase is a rate-limiting enzyme in sterol synthesis. To study the role of HMGCoA reductase, ABCG1 and ABCG4, leishmania strains overexpressing these proteins were generate. The gene was cloned in the pGEMT vector using E. coli DH5α competent bacteria, subcloned in PSP72αNEOα vector and transfected in Leishmania spp. Gene sequencing and qPCR were performed to confirm the gene overexpression. The ABCG1-high and HMGCoA-high showed resistance to ketoconazole (IC50 23,7 and 6,1 μM, respectively; and the control 4,6□M).We also evaluated the uptake of exogenous sterols, using 7-dehydrocholesterol as a probe. After cholesterol starvation, ABCG4-high and HMG-high parasites showed lower uptake, but ABCG1-high get more exogenous sterol than the control. TLC analysis of HMG-high showed an increase of sterol ergostane skeleton, showing 51% increase in ergosterol content. While ABCG1-high had a dose-dependent increase in cholesterol content in relation to the mock. After that, we evaluated the uptake of NBD-cholesterol by FACS. The strain HMG-high and ABCG4 get the cholesterol increasing up until 4 h, and ABCG1 get less cholesterol in relation with the control. Taken together, these results show that the overexpression of these proteins influences the sterol homeostasis. **Supported by:**CNPq **Keywords:** Cholesterol; leishmania spp; sterol biosynthesis

TB3 - **SCREENING OF THE PATHOGEN BOX LIBRARY FOR DISCOVERY OF BROAD-SPECTRUM ANTI-TRYPANOSOMATIDIC AGENTS**

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High content screening (HCS) has become the mainstream technology for drugs discovery for Chagas disease and Leishmaniasis. However, most assays are still performed using a single Trypanosoma cruzi strain or Leishmania species, although drug activity can drastically vary among different strains, species and assays. Thus, secondary assays, such as a panel of diverse parasites strains/species should be incorporated into the screening cascade to better understand the compound spectrum of activity and to perform a proper selection/prioritization of candidate compounds. Pathogen Box, a library containing 400 molecules with known antiparasitic activity, was screened parallelly against T. cruzi strains (Y, CL Brener and Sylvio X1/10) and Leishmania species (L. amazonensis, L. braziliensis, L. donovani and L. infantum), using HCS. Compounds with activity ≥ 80% were selected as “hits” from each strain/species assay. The T. cruzi screening yielded more hits than the Leishmania screening, corroborating previous screening campaigns results. Together, 72 hit compounds were identified from both parasite screenings, of which 21 hits were identified only in Leishmania screens, 41 hits were exclusive to T. cruzi screens and 10 hits were shared among T. cruzi and Leishmania strains/species. Another seven broad-spectrum compounds presented activity greater than 50% against distinct T. cruzi strains and Leishmania species. Four compounds were comparably active (activity > 65%) against all T. cruzi strains and Leishmania species, indicating possibly a common molecular/metabolic target. Of this set, one compound, MMV011903, has not been previously reported for these parasites. The parallel screening against distinct T. cruzi strains and Leishmania species panel provided novel broad-spectrum starting points for trypanosomatidic drug discovery. Next experiments will focus on determination of EC50, CC50 and selective index parameters, and combination assays with reference drugs. **Supported by:**DNDi - Drugs for neglected diseases initiative/MMV - Medicines for Malaria Venture **Keywords:** High content screening; t. cruzi strains; leishmania species

TB4 - **NEW MOLECULAR MARKERS FOR THE GENOTYPING OF SPECIES OF THE *LEISHMANIA* GENUS BY MULTIPLEX PCR**

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Leishmaniasis encompasses a group of diverse clinical diseases caused by protozoan parasites of the *Leishmania* genus. Multilocus Enzyme Electrophoresis (MLEE) is one of the most used methods in the last years for the genotyping of *Leishmania* strains. Despite this scenario, the identification of the species still represents a challenge, hampering the molecular epidemiology studies, appropriate treatment of the patients and the efforts to control. The multiplex PCR is an attractive alternative since it is a fast technique, which presents potentially high sensitivity and specificity at low cost. Thus, the objective of this work was to generate a panel of primers optimized for multiplex PCR for the identification of different species of the *Leishmania* genus in a single PCR reaction. The species-specific primers were designed based on genomic data, using the online tool TipMT, developed by our group. Potential non-specific annealing of the primers with genomes of other trypanosomatids, and human, dog and sandfly hosts was evaluated using the Primer-Blast tool. It was possible to obtain species-specific primers for *L. amazonensis*, *L. braziliensis*, *L. donovani*, *L. infantum*, *L. mexicana* and for the *L. guyanensis* complex. The primers have on average a detection sensitivity of up to 0.01 ng of parasite gDNA, and were optimized for use at the same annealing temperature in the PCR. In addition, these primers were specific for their target gDNA when tested against gDNA from 13 species of *Leishmania,* as well as 6 other trypanosomatids and *Babesia sp*. Multiplex PCR using a pool of all primers and individual gDNA was able to identify specifically each one of the six *Leishmania* species tested. The primers were also individually tested in a pool of gDNA from the six species, being able to detect only the target species. Thus, this study may contribute to the development of a more efficient, fast and reliable technique for the genotyping of parasites of the *Leishmania* genus.
**Supported by:**FAPEMIG, CAPES and CNPq **Keywords:** Leishmania genotyping; molecular markers; multiplex pcr

TB5 - **OVEREXPRESSION OF STEROL 24-C- METHYLTRANSFERASE (ERG6) IN *LEISHMANIA SPP*. AND LEISHMANICIDAL ACTIVITY OF AZAPTEROCARPANS**

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Leishmaniasis is a serious public health problem and is considered an extremely neglected disease with a high cost treatment, and many cases of resistance have been reported, making treatment difficult. Aiming the rational development of new drugs, the choice of a selective target in the parasite is essential. Sterols play crucial roles in the organization and functionality of eukaryotic membranes. While mammals produce cholesterol, trypanosomatids produce sterols with ergostane skeleton, such as ergosterol. The divergent step between them is catalyzed by the enzyme sterol C-24 methyltransferase (ERG6). In this study, aiming to investigate the mode of action of new and/or previously known drug candidates and to validate ERG6 as a pharmacological target, promastigotes of *Leishmania amazonensis* and *L. infantum* were transformed using the pSP72αNEOα expression vector containing the ERG6 coding sequence cloned from both strains. ERG6 overexpression was confirmed by quantitative PCR (qPCR). We evaluated the activity of azopterocarpan derivatives as possible inhibitors of ERG6. Promastigotes of *L. infantum* overexpressing the enzyme (Li*erg6*$^{high}$) were cultured in the presence of several concentrations of the compounds up to 100 µM for 72 h. Four molecules inhibited the parasite growth (Li*erg6*$^{high}$), with 20.9 µM IC50 (LQB336), 45.5 µM IC50 (LQB339), 51.7 µM IC50 (LQB341) and 33.1 µM IC50 (LQB343). Promastigotes of *L. infantum* that received the expression vector but not the insert were used as control (Li + pSP72αNEOα), with 17.5 µM IC50 (LQB336), 35.6 µM IC50 (LQB339), 24.8 µM IC50 (LQB341) and 25.0 µM IC50 (LQB343). The IC50 value for LQB341 against Li*erg6*$^{high}$ was twice higher than Li + psp72αNEOα. These results suggest that the azopterocarpans LQB339, LQB341, LQB343 might have specific activity against ERG6 and additional studies are needed to evaluate the effect of this compounds on parasite sterol biosynthesis. **Supported by:**CAPES, CNPq, FAPERJ, IOC/FIOCRUZ **Keywords:** Sterol c-24 methyltransferase; leishmaniasis; chemotherapy

TB6 - ***IN VITRO* HUMAN VISCERAL LEISHMANIASIS MODEL BASED ON THE ORGANS-ON-A-CHIP TECHNOLOGY**

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Current preclinical drug screening for leishmaniasis based on *in vitro* cell culture and animal models presents many limitations, such as low accuracy in predicting drug responses in humans. Multi-organ microphysiological systems that recreate organ-organ interactions *in vitro* hold great potential to simulate drug pharmacokinetics and pharmacodynamics, in order to better identify drug candidates for clinical trials. We developed a tissue engineered microphysiological system with five organ compartments representing “intestine”, “liver”, “spleen”, “bone marrow”, and “kidney” to create an *in vitro* human visceral leishmaniasis model based on a physiologically-based pharmacokinetic model. Organ chamber volumes and flow rates were scaled down proportionally to maintain physiological organ size ratios and in vivo fluid resident time in the corresponding organs. Medium recirculation among organ chambers was achieved using gravity-driven flow and rocking motion. The flow rates were measured and shown to be comparable to the desired values. Organ chambers were filled out with human cell lines representative of each organ (Caco-2 cells for intestine, HepG2 C3A for liver, HL60 cells for bone marrow, HL60-derived macrophages for spleen/ liver, and HK2 cells for kidney) and the microfluidic system supported the survival of all organ cells for 7 days (viability above 85 %). The infection of HL-60 derived macrophages present in the microdevice was achieved using a green fluorescent protein expressing line of *Leishmania infantum*. Further testing of cells/organs behavior and function are under way. This device will provide a relevant replica of a human visceral leishmaniasis model where drug efficacy, metabolism and interactions can be screened simultaneously. **Supported by:**FAPESP, CNPq **Keywords:** Leishmaniasis; organs-on-a-chip; drug discovery

TB7 - **SELECTION OF VACCINE CANDIDATES FOR LEISHMANIASIS USING THE PHAGE DISPLAY TECHNIQUE**

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Leishmaniasis comprises a group of diseases caused by protozoa of the *Leishmania* genus, affecting approximately 350 million people. Although leishmaniasis control through vaccination is promising, no effective vaccine is currently available. It has previously been shown that immunization with a *L. donovani* strain from which centrin has been deleted (LdCen-/-) confers protection against several species of *Leishmania* spp. Based on gene recombination, the Phage Display technique consists in the fusion of peptides to the surface of bacteriophage capsid, producing wide repertoire libraries which are used to identify epitopes recognized by specific antibodies. Thus, this work proposes the use of Phage Display to identify peptides that are able to confer protection against leishmaniasis. Three immunizations were performed on BALB/c mice using LdCen-/- strain. The serum collected was used to evaluate the increase in antibody production through ELISA assay. Anti-*Leishmania* and basal IgG were extracted by addition of ammonium sulfate and purified using Protein A-Sepharose 4B column. For selection of IgG-specific peptides, three biopannings were performed using 12, 15 and 17 aminoacids peptide libraries. The clones were screened from the third biopanning and their reactivity to IgG was assessed by ELISA assay. The most reactive clones were sent for sequencing and the obtained peptides were analyzed by BLASTn against *Leishmania* spp. genomes. Initial results revealed gradual and significant increase in IgG production after immunizations. Ninety five potential candidates were selected through Phage Display, all presenting significant reactivity to *Leishmania*-specific IgG. After sequencing, two 15 aminoacids peptides were obtained and exhibited a match with non-gene chromosome regions, with 85% identity and 57% coverage, indicating the conformational nature of the peptides. The protection conferred by them will be evaluated in mice experimentally infected with *Leishmania* spp. **Supported by:**CNPq **Keywords:** Leishmaniasis; vaccine; phage display

TB8 - **IMMUNOLOGICAL ANALYSIS INDUCED BY CHIMERIC PROTEIN AS A VACCINE AGAINST TRYPANOSOMATIDS**

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American Tegumentary Leishmaniasis (ATL), Visceral Leishmaniasis (VL) and Chagas disease are among the major parasitic diseases in tropical countries with more than 8 million infected people worldwide. These parasites may cause serious damages to health, leading to debilitation and even death of affected patients. Leishmaniasis are generally transmitted by the sandfly Lutzomyia longipalpis and the main species that cause these diseases in Latin America are L. (V.) braziliensis, L. (L.) mexicana, L. (V.) panamensis, L. donovani and L. infantum, the last two being responsible for LV. Chagas disease is caused by the protozoan Trypanosoma cruzi and transmitted by triatomine bugs of the genera Triatoma, Rhodnius and Panstrongylus. In order to control the trypanosomatid infections worldwide and impair the continuous expansion of the number of infected people and the morbidity associated with such infections, the development of vaccines might comprise an important additional tool for parasite load and infection. In the current study, we aimed to develop a polyvalent multicomponent synthetic vaccine against all the trypanosomatid infections and further evaluate the specific immune response induced by this vaccine by using the indirect Enzyme-Linked Immunosorbent Assay (ELISA) method. Serum samples (n = 6) were collected before, between and after immunization with 3 doses of the synthetic vaccine (administered every 15 days). Our results demonstrated the specific antibody production elicited by vaccination of the synthetic vaccine, indicating the potential immunogenicity associated with the vaccination process. **Supported by:**CNPq, FAPEMIG **Keywords:** Vaccine; immunology; trypanosomatids

TB9 - **FUNCTIONAL CHARACTERIZATION OF A COMPOUND CAPABLE OF INDUCING *T. CRUZI* CELL CYCLE ARREST AT G1 PHASE WITHOUT CAUSING LOSS OF PARASITE VIABILITY**

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Little is known about what pathways or proteins are responsible for *Trypanosoma cruzi* intracellular development in mammalian cell. We have identified a small molecule capable of interfering with *T. cruzi* intracellular development, causing inhibition of parasite replication without affecting parasite or host cell viability. The functional characterization of the compound activity showed that: (i) the phenotype of intracellular development arrest was not restricted to a host cell type; (ii) the compound was able to inhibit the replication of different T. cruzi strains (Y, Sylvio X10/1 and CL Brener strain); (iii) the phenotype of arrest was reversible upon compound removal, once the parasite from that point grows normally concluding its intracellular cycle by differentiation to trypomastigotes; (iv) the compound did not show cytotoxicity for distinct host cells (U2OS, LLC-MK2, NRK-52E and BHK-51) for up to 200 µM for 48 h, being highly selective for the parasite; (v) the molecule also is able to induce arrest in the epimastigote form of *T. cruzi* without loss of viability up to 96 h, but displayed a dose-dependent cidal activity against bloodstream forms of *Trypanosoma brucei* and promastigote forms of *Leishmania donovani*; and (vi) the compound interfered with *T. cruzi cell cycle*, arresting most of the cell population at G0/G1. Altogether, these results suggest that this compound acts via a regulator of cell cycle that can specifically cause arrest in *T. cruzi* and not in other trypanosomatids or mammalian cells. Future experiments will focus on target deconvolution to uncover the parasite proteins related to the phenotype studied. **Keywords:** Trypanosoma cruzi; phenotypic compound; cell arrest

TB10 - **FROM WASTE TO "FUEL FOR LIFE": ALANINE AS A "FLEX" METABOLITE IN TRYPANOSOMA CRUZI**

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In *Trypanosoma cruzi*, the etiological agent of Chagas's disease, amino acids participate of several critical processes in the parasite biology, such as osmoregulation, cell differentiation and host cell invasion. Some of them provide reducing power for mitochondrial ATP synthesis. Alanine is known to be a secreted main end product of the *T. cruzi* energy metabolism in a medium containing glucose and amino acids. Interestingly, *T. cruzi* possess putatives Alanine racemase (AR). To our knowledge, the only reported racemase activities in trypanosomatids until now are a proline racemase activity in *T. cruzi* and AR activity in Leishmania amazonensis respectively. These data suggest the relevance of the D-amino acids metabolism in this parasite. In this work we showed that *T. cruzi* encodes a functional enzyme. We cloned and expressed its recombinant version (rTcAR) which allowed us to characterize and evidence its activity through parasites life cycle with at least 4 fold higher values in epimastigote compared to the other stages. Km and Vmax were determined for the reaction in both directions (21.5 ± 6.04 mM and 65 ± 9.4 µmol/min/mg (D to L) and 26.1 ± 7.9 mM and 53 ± 6.3 µmol/min/mg (L to D)). Additionally, we showed that both, D-and L- isomers of Ala can be taken up by epimastigotes through a low specificity non-stereoselective active transport system. Both isoforms presents similar uptake kinetic parameters (Vmax of 1.86 ± 0.3 cells and 1.14 ± 0.75 nmol•min−1 per 2 × 107 and a Km of 1.81 0.6 ± and 1.46 ± 1.4 mM for L-Ala and D-Ala respectively) Our results also indicated that the incorporated L-Ala as well D-Ala can be completely oxidized to CO2, supplying electrons to OXPHOS. Our data indicate that the D-Ala oxidation mainly occurs through the TcAR. To conclude, the metabolism of DL-Ala and the related AR activity, underlines the parasites outstanding parasites metabolic flexibility as well the relevance of the D-amino acids metabolism in these organisms. **Supported by:**CNPq, FAPESP **Keywords:** Alanine; bioenergetics; t. cruzi

## TB11 - **PHENOTYPIC AND GENETIC CHARACTERIZATION OF TRYPANOSOMA CRUZI RESISTANT TO RAVUCONAZOLE**

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Studies on the molecular mechanisms underlying drug resistance to different drug classes in Trypanosoma cruzi are lacking. In order to study triazole resistance in Trypanosoma cruzi, a clone of the Y strain, H10, was cultured under increasing concentrations of ravuconazole, until in vitro stability was achieved at 500 nM of this drug. The resistant clone exhibited a reduced infectivity and a reduced number of intracellular amastigotes, when compared to the parent wild type clone. The resistant clone also presented a slowed epimastigogenesis process and increased epimastigote population doubling time. In drug activity assays, ravuconazole was at least 1,000-fold less potent in the resistant clone, and the resistant phenotype was maintained even after several weeks off drug pressure and also after cycling the parasite through a complete life cycle in vitro. The ravuconazole-resistant clone also displayed cross-resistance to other azolic and non-azolic CYP51 inhibitors, but not to the nitroheterocyclic antichagasic benznidazole and nifurtimox, which do not target TcCYP51. Interestingly, both the parent/wild type and the resistant clones present partial resistance to benznidazole at comparable levels under the studied conditions. A novel amino acid residue change, T297M, was found in the TcCYP51 gene in the resistant but not in the sensitive clones. The structural effects of the T297M, and of the previously described P355S residue changes, were modelled to understand their impact on interaction with CYP51 inhibitors. **Supported by:**CNPq, DNDi **Keywords:** Trypanosoma cruzi; azole drug resistance; tccyp51 mutation

## TB12 - **DRUG REPUSPOING FOR LEISHMANIASIS TREATMENT: IDENTIFICATION OF NEW MOLECULAR TARGETS**

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Enzymes of cytochrome P450 complex (CYP450) are well known for their role in drug metabolism and other metabolic pathways. Several authors have suggested inhibition of CYP51 (sterol C14-demethylase) from Leishmania spp. as a possible target for chemotherapy of leishmaniasis. Many in silico tools can provide insight into potential candidates for drug repositioning by constructing predictive models. Here, we aim to perform a comprehensive identification and characterization of CYP450 enzymes encoded in the genome of Leishmania infantum, to reveal new putative targets for the repositioning of CYP450 inhibitors for leishmaniasis. The set of human CYP450 enzymes deposited and annotated in the SwissProt database was compared with the set of proteins of Leishmania infantum predicted and annotated in the online database TrytripDB. The comparisons were made through a search for local similarity with the use of the blastp program. Four statistically significant hits (E-value ≤ 0.001) were found, representing putative homologues to distinct sequences of human CYP450. All sequences have characteristic motifs found in the CYP450 family. Functional annotation analyses demonstrated characteristic sites of the CYP450 family and biological activities related to oxidation-reduction process. Secondary structure analyses showed a structural conservation between the sequences, with co-location of the structures α-helix and β-sheet. Transcriptomic and proteomic analyses showed that all sequences have mRNA annotation, but only 2 proteins were found to be potentially expressed in L. infantum. About antileishmanial activity, 8 CYP450 inhibitors medicines were selected for antipromastigote assays and 2 of them, an anti hypertriglycemic and an antihistamine, obtained promising IC50/72h values against L. infantum promastigotes: 2.4μM and 2.2μM. Antiamastigote and cytotoxicity assays will be performed to confirm their potential as antileishmanial drugs. **Supported by:**FAPERJ/CNPq **Keywords:** Cyp450; leishmania sp.; therapeutic target

TB13 - **NITRO-HETEROARYL NITRONES AS NITROREDUCTASES-BIOACTIVATED LEISHMANICIDAL COMPOUNDS**

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Trypanosomatids nitroreductases (NTRs) are a bacterial like enzymes that catalyses the reduction of nitro group enabling nitro-compounds to be effective and generate more potent intermediates in parasite interior. So far, *Leishmania* sp. had two different NTRs characterized and the study of its role point to nitro-compounds reassessment as selective and safer prodrugs for leishmaniasis. Hence, in this present study, we evaluated the leishmanicidal activity of nitro-heteroaryl nitrones bioactivated by NTR1 and NTR2 of *Leishmania* sp. and their structure activity relationship. Promastigotes of *Leishmania* sp. were cultured in the presence of thirteen nitro-heteroaryl nitrones derivatives up to 100 µM for 72 hours. Eight compounds were able to inhibit *Leishmania* sp. proliferation. Thereafter, we evaluated macrophage toxicity and anti-amastigote activity of *L. amazonensis* and *L. infantum*. Both derivatives LQB303 and LQB484 showed selectivity index above 30-fold for *L. amazonensis* and over 100-fold for *L. infantum*. In addition, *L. amazonensis* ROS levels were measured using $H_2$DCFDA dye for six derivatives up to a 4h kinetic and a significant increase in ROS production was observed for LQB303AL. Overexpressing NTR1 and NTR2 *L. donovani* cell lines were also cultured with eight most active nitro-heteroaryl nitrones derivatives for 72h, indicating that they were able of undergoing nitro reductions preferentially for NTR1, highlighting the LQB303 and LQB484, respectively, 58-fold and 32-fold more potent on *L. donovani* NTR1+. Preliminary ADMET in silico analysis, indicates that they adhere to Lipinski's rule of five, may have high intestinal absorption, low distribution volume and are unlikely to be metabolized by the main CYPP450 oxidases, but may inhibit CYP1A2 (expect LQB303). Besides that, they are not likely to be hepatotoxic however could be mutagenic in Ames test. Together these results indicates promising drug candidates to treat of visceral and cutaneous leishmaniasis. **Supported by:**FIOCRUZ, CNPQ, WCAIR **Keywords:** Leishmania; nitro-heteroaryl nitrones; nitroreductase

TB14 - **STRUCTURE-ACTIVITY RELATIONSHIP OF THE THIOUREIA DERIVATIVES AGAINST *LEISHMANIA AMAZONENSIS.***

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Leishmaniasis treatment faces the challenge of old and new concerns about toxicity, therapeutic regimens, parasite resistance and few therapeutic options. Thus, the identification of antileishmanial drug candidates is essential to fill the drug discovery pipeline for leishmaniasis. In the search for new drugs, we propose a structure-activity relationship study of new synthetic N,N'-substituted ureas and thioureas (LabTIFs) against L. amazonensis. Compounds were evaluated in antipromastigote, antiamastigote and toxicity assays. First, our group evaluated 56 compounds and four N,N'-substituted thioureas (LabTIFs 21, 38, 85 and 87) had the best activity against promastigotes. The best hit of this series was LabTIF38, with IC50 5µM and high selectivity. In the optimization of this hit, a new series was designed incorporating one of the nitrogen atoms of the N,N'-thiourea into a piperazine ring, giving 4-substituted-N-substituted-piperazine-1-carbothioamide derivatives, synthesized in one step in yield between 60-90%. Three LabTIFs of this new series (162, 166 and 168) were more active inhibiting promastigotes proliferation, with IC50<10 µM. The analysis of SAR in promastigotes evidenced that a spacer is important for the activity, once that in 4-benzhydryl-N-X-piperazine-1-carbothioamides, when X is N-phenyl or N-benzyl or N-phenethyl, showed IC50 30.7, 8.0 and 6.9µM, respectively. In addition to this substitution, the replacement of 4-benzhydryl by 4-butyl (LabTIF-162) potentiates the leishmanicidal action, with IC50 2.9µM. Preliminary toxicity and anti-amastigote assays indicate LabTIF162 as the best hit, with IC50 in amastigote less than 1.25µM and high selectivity. In conclusion, this study points to 4-butyl-N-phenethylpiperazine-1-carbothioamide as a hit for preclinical studies and prototype for optimization to find a lead compound for the treatment of leishmaniasis. **Supported by:**FIOCRUZ **Keywords:** Thioureia; structure-activity relationship; leishmania amazonensis

TB15 - **REPURPOSING DRUG CANDIDATE FOR THE TREATMENT OF LEISHMANIASIS INTERFERES ON STEROL BIOSYNTHESIS PATHWAY AND POTENTIATES THE EFFICACY OF GLUCANTIME ON EXPERIMENTAL MODEL OF VISCERAL LEISHMANIASIS**

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The drug arsenal against leishmaniasis is small, and each has a disadvantage in terms of toxicity, efficacy, price or treatment regimen. In the search for new drugs, we performed a drug screening on *L. amazonensis* promastigotes and intracellular amastigotes of fifty available drugs belonging to several classes according to their pharmacophoric group, and spironolactone, a potassium-sparing diuretic, proved to be the most promising drug candidate. In the search of the mechanism of action of spironolactone, we analyzed the sterol biosynthesis of the parasite, due to the steroidal structure of this drug. Promastigotes treated with spironolactone were incubated for 72 h for extraction of neutral lipids for GC/MS analysis. As result, we noted accumulation of sterols methylated at positions 4 and 14, inferring a possible inhibition of the enzyme that demethylates at these positions to produce ergosterol. After demonstrating the *in vitro* antileishmanial activity and a possible mechanism of action, we evaluated the efficacy on murine experimental model with *L. amazonensis* or *L. infantum*. The treatment controlled the cutaneous lesion and reduced the parasite burden of *L. amazonensis* significantly, as effective as meglumine antimoniate. The treatment of VL was effective in reducing the parasite load on the main affected organs (spleen and liver); however, it was less effective than the antimonial. Thus, we evaluated the efficacy of the association of spironolactone and glucantime in VL model. The association promoted a better control of the parasite load in the spleen compared with the antimonial alone (98 % x 90 % of reduction). In liver, we noted a reduction of 97% in the group treated with glucantime while the association reduced 100%. These results evidenced a possibility of repositioning spironolactone in association with glucantime for the treatment of leishmaniasis in Brazil. **Supported by:**CNPq FAPERJ CAPES **Keywords:** Leishmania; sterols; repurposing

TB16 - **UNVEILING THE PATHWAYS RELATED TO INTRINSIC MILTEFOSINE TOLERANCE IN *LEISHMANIA (VIANNIA) BRAZILIENSIS* CLINICAL ISOLATES: TAKE IT IN AND DIE.**

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We have previously described differences in the susceptibility in vitro of Brazilian clinical isolates of *L. braziliensis* to miltefosine. Even though these parasites were never exposed to the drug, the half maximal inhibitory concentration varied by a factor of 6 and 15 for promastigotes and amastigotes, respectively. Aiming to elucidate the reason for this differential susceptibility, we evaluated the uptake of labelled-phosphocholine and labelled-miltefosine in promastigotes of the most and least susceptible isolates by flow cytometry and of labelled-miltefosine by confocal microscopy as well. An inverse correlation of uptake and miltefosine susceptibility was observed. We also determined the nucleotide sequence of the two copies of the genes encoding the miltefosine transporter (*MT*) and the *Ros3* genes, both of which have been previously associated with miltefosine resistance in *Leishmania*. Even though polymorphisms in *MT* gene were already described as the main reason for resistance in miltefosine selected resistant parasites, in these clinical isolates no correlation between polymorphisms in this gene and drug susceptibility was found. RNA sequencing experiments were then employed to widen the search for miltefosine tolerance mechanisms in these isolates. We found 36 differentially expressed genes when the two less tolerant isolates were compared to the most sensitive one and the reference strain M2903. These results reinforced the hypothesis of intrinsic molecular differences among these clinical isolates of *L. braziliensis*. We are now working in the functional validation of differentially expressed genes in these clinical isolates. **Supported by:**FAPESP, CNPq **Keywords:** Leishmania braziliensis; miltefosine; resistance