

HP001 - IN VIVO IMAGING APPROACHES REVEAL THAT *LEISHMANIA* VIRULENCE FACTOR, ISP2, ATTENUATES HOST INNATE RESPONSES ASSOCIATED WITH PARASITE KILLING

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L. major has 3 genes encoding peptide inhibitors of serine peptidases (ISPs), which are orthologues of a bacterial protease inhibitor, ecotin. Trypanosomatids are the only known eukaryotes to possess these ecotin-like inhibitors. *L. major* mutants deficient in ISP2 and ISP3 (Δ isp2/3) are internalised and killed more efficiently by macrophages *in vitro* due to the unregulated activity of the host serine peptidase neutrophil elastase (NE). Serine peptidases regulate host immunity through the proteolytic cleavage of cytokines, chemokines, and cell surface receptors including Toll-like receptors. Here, we investigated the role of ISP2 in immune modulation at the dermal site of *Leishmania* infection in the C57BL/6 mouse model. Bioluminescent cell lines of *L. major* WT, Δ isp2/3, and a mutant re-expressing ISP2/3 (Δ isp2/3:ISP2/3) were employed to track parasite dissemination using the whole animal *in vivo* imaging system (IVIS). This revealed the importance of ISP in the establishment and persistence of *Leishmania* infection at the site of inoculation. Longitudinal flow cytometric investigations revealed larger recruitment of monocytes, monocyte-derived macrophages and dendritic cells (moDCs) after 2 wk of Δ isp2/3 infection compared to WT infection. In the absence of ISP2/3, the co-stimulatory molecule CD80 was upregulated on moDCs, IFN- γ was higher, and monocytes and macrophages exhibited an increase in iNOS expression. In addition, quantitative IVIS imaging of myeloperoxidase activity through the administration of luminol enabled us to investigate phagocyte activation in real-time at the site of infection. Taken together, these findings indicate that the immune response is more primed towards parasite killing in Δ isp2/3 infection compared to WT, which suggests that *L. major* ISP modulates these responses to facilitate survival *in vivo*. Furthermore, transgenic mice deficient in NE (*Ela*^{-/-}) were used to assess the role of this serine peptidase during *Leishmania* infection *in vivo*.

Supported by:MRC

Keywords:In vivo imaging; innate response; virulence

HP002 - ASSOCIATION BETWEEN TGF- β 1 POLYMORPHISMS AND SUSCEPTIBILITY TO CHAGAS DISEASE IN BRAZILIAN PATIENTS

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Studies developed by our group have shown the involvement of TGF- β in Chagas heart disease. It was observed that patients with cardiac damage show elevated plasmatic levels of TGF- β compared to asymptomatic patients and those patients presented a worse clinical outcome after 10 years of follow up. The polymorphism at codon 10 in the TGF- β 1 gene has been described to influence the susceptibility to *T. cruzi* infection in patients from Colombia and Peru. The present study assessed polymorphisms of the TGF- β 1 gene in patients with chronic Chagas disease, correlating the presence of polymorphisms on TGF- β 1 gene with serum levels of this cytokine. A cohort of 181 individuals was invited to participate in the study. We investigated five SNPs (-800G>A, -509C>T +10T>C +25G>C and +263C>T) by sequencing of PCR products. In addition, serum levels of TGF- β 1 were measured by ELISA. We observed a significant difference in the distribution of the -509C/T and +10 T/C between the controls and patients groups, suggesting a genetic influence of these TGF- β 1 variants on *T. cruzi* infection susceptibility. The genotype C/T and T/T at position -509 of the TGF- β 1 gene were significantly increased in patients ($p<0.01$ and $p<0.05$), while the reference genotype C/C were increased in the healthy individuals. Patients homozygous for this allele have an increased risk of Chagas disease (OR= 3.2; 95% CI= 1.06-9.5, $P<0.05$). Similar results were observed for the genotype C/T and C/C at codon +10: patients homozygous for this allele also have an increased risk of Chagas disease (OR=3.2; 95% CI= 1.0-9.4, $P=0.04$). Thus, -509C/T and +10T/C TGF- β 1 polymorphisms were significantly associated with Chagas disease susceptibility in Brazilian patients. Furthermore, TT genotype at position -509 was associated with higher serum levels of TGF- β 1. Our data suggest that genetic polymorphisms at position -509 and codon 10 of the TGF- β 1 gene may be a risk factor to Chagas disease development in Brazilian patients.

Supported by:DECIT □ FAPERJ □ IOC/FIOCRUZ □ CAPES □ INSERM **Keywords:**Chagas disease; polymorphism; tgf- β

HP003 - IMPLICATIONS OF *LEISHMANIA BRAZILIENSIS* PROSTAGLANDIN F2-ALPHA SYNTHASE EXPRESSION LEVELS IN HOST-PARASITE INTERACTION

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Prostaglandin (PG) production is not restricted to mammals; recent studies have shown parasites able to synthesize PG from metabolites of arachidonic acid (AA). Prostaglandin F2a synthase (PGF2S) of *Leishmania* shares 34% identity and 51.4% similarity with the mammalian homolog (AKR1C3) and similar AKR1C3 protein domains. Noteworthy, PGF2S was detected in the secretome of *L. braziliensis* and in the exosome of *L. donovani* and according to the TDR Targets Database the *L. major* PGF2S homolog is one of the most antigenic proteins of *L. major*. In this work, we have shown that *Leishmania braziliensis* promastigotes express Prostaglandin F2a Synthase (LbrPGF2S) during all phases of axenic growth and synthesizes Prostaglandin F2a in presence of arachidonic acid in the culture. In addition, we showed the possible localization of LbrPGF2S near the flagellar pocket in promastigotes and amastigotes. Additionally, we have generated *L. braziliensis* overexpressing LbrPGF2S ectopically and integrated in the ribosomal locus in a fusion with mCherry (mCh-PGF2S). These transfectants displayed a higher percentage of infected macrophages and number of amastigotes per cell when compared to control transfectants. Furthermore, time-lapse video of in vitro infection using mCh-PGF2S overexpressor revealed the secretion of LbrPGF2S into the host cell cytoplasm. The effect of LbrPGF2S expression levels on BALB/c mice infection is under analysis. We showed that LbrPGF2S is constitutively expressed, that its overexpression led to an increment of virulence in vitro and that the protein LbrPGF2S is secreted into the cytoplasm of the host cell. **Supported by:**FAPESP CNPq

Keywords:Leishmania braziliensis; pgf2s; prostaglandin f2a

HP004 - FULL PROTECTION OF α -GALACTOSYLTRANSFERASE-KNOCKOUT MICE VACCINATED BY Q β - α GAL PARTICLE ASSOCIATED WITH ADASP-2 IN EXPERIMENTAL TRYPANOSOMA CRUZI INFECTION.

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Background: Chagas disease (CD), caused by the protozoan parasite *Trypanosoma cruzi*, is an endemic and deadly infectious disease in Latin America. About 7.7 million people are infected and thousands will die each year due to medical complications, particularly cardiomyopathy. The trisaccharide Gal α (1,3)Gal β (1,4)GlcNAc α is an immunodominant antigen highly expressed on the *T. cruzi* parasite surface, mainly in glycosylphosphatidylinositol-anchored mucins of the trypomastigotes infective form of *T. cruzi*. Results obtained in our lab showed the presence of α -Gal epitope in Y (60%), Colombiana (32%), CL-Brener (58%) and Arequipa strains (72%). Type 5 recombinant adenoviruses (rAd) carrying sequences of amastigote surface protein-2 (rAdASP2) have been successfully used for immunotherapy in experimental Chagas disease. Also, the use of an animal model that mimics human with improved immune response against CD may facilitate the search for more effective treatment of the disease. Design Methods: In this work we employed a stable particle, derived from the bacteriophage Q-beta capsid as a model system, coupled to 540 molecules of the α -Gal carbohydrate, named Q β - α Gal in association with AdASP-2 for immunotherapy in experimental CD using α -Galactosyltransferase knockout (α GalT-KO) mice infected with 10e6 trypomastigotes of Y strain. Results: Vaccinated mice were full protected, presenting higher anti- α -Gal antibody titers with lower parasitemia and 100% survival rate. In addition, vaccinated α 1,3-GalT-KO mice showed much higher pro-inflammatory (IL-12, IFN- γ) and lower anti-inflammatory (IL-10, IL-4) cytokine levels, measured in the heart homogenate. Taken together, our results demonstrate that the lytic, protective anti- α -Gal antibodies and pro-inflammatory immune response produced by vaccinated α 1,3-GalT-KO mice can contribute to the effective parasite control in the acute phase of experimental CD.

Supported by:CAPES, FAPEMIG

Keywords:Q β β

HP005 - CCR4-DEPENDENT HOMING OF CD4⁺CD25⁺ REGULATORY T CELLS FAVORS THE *L. AMAZONENSIS* PERSISTENCE

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The expansion and persistence of parasites are favored by engaging of regulatory T cells (Treg). These cells rapidly accumulate within chronic dermal sites of *Leishmania* infection where they suppress anti-pathogen CD4⁺ T cell responses, favoring the parasite persistence and dermal pathology. The migratory properties of lymphocyte to inflamed lymphoid and nonlymphoid tissues are tightly regulated by expression of distinct sets of chemokine receptors, which provide directional cues for recruitment and homing of T cells into sites of inflammation. The chemokine receptor CCR4 is selectively expressed on CD4⁺CD25⁺ Treg cells, which present a strong chemotatic response to CCR4 ligands. Here, we postulated that CCR4 might direct Treg cell homing to the sites of *Leishmania* infection contributing to parasite maintenance. Thus, we explored the *in vivo* role of CCR4 in the homing of regulatory T cells to the dermal sites as well as its functional significance during the infection induced by protozoan parasites *L. amazonensis* and *L. braziliensis*. We found out that CCR4 deficiency induced an increased resistance to *L. amazonensis* infection. It was associated with an increased effector T cells expansion and IFN- γ production in the draining lymph node and *L. amazonensis*-infection sites. Furthermore, we saw that CD4⁺CD25⁺ Tregs cells failed to migrate to infected-dermal sites and draining lymph node in the absence of CCR4. Such response was specie-specific since that CCR4-dependent effect in the resistance to infection, lymphocyte activation and Tregs recruitment was not observed during *L. braziliensis* infection. Interestingly, the resistance to *L. amazonensis* infection was related to an inefficient migration of *Ccr4*^{-/-} Treg cells to infected dermal sites compared with WT Treg cells. Taken together, our results show that CCR4-dependent homing of CD4⁺CD25⁺ Treg cells to *L. amazonensis*-infected dermal sites promoting the persistence of parasite and the establishment of infection. **Supported by:**FAPESP, CAPES, CNPq **Keywords:**Regulatory t cells; chemokines ; leishmania

HP006 - TRYPANOSOMA CRUZI: IN VIVO IMAGING OF SINGLE CELLS INSIDE INFECTED MICE TISSUES

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Live imaging is a powerful tool to examine how parasites interact with host cells and tissues, as well as their vectors. Imaging *Trypanosoma cruzi* live parasites in its different evolutive forms is a routine procedure and detailed information has been acquired using modern approaches, such as multidimensional confocal microscopy. Over the last years, *intravital* microscopy emerged as powerful technique to study host-pathogen interactions in live tissues and animals, but this method is limited by tissue penetration depths of up to few hundred microns and blurring due to the overlaying connective tissue. To circumvent these limitations, *ex vivo* slices of tissues and organs may be used. We developed a simple method for *in vivo* observation of live single cell *T. cruzi* parasites inside mammalian host tissues: slicing organs with a sandwich of single edge blades to gain access to the interior, then image *ex vivo* slices. BALB/c or C57BL/6 mice infected with DsRed-CL or GFP-G trypomastigotes had their organs removed and sectioned at acute phase of infection. *Ex vivo* organ sections were observed under confocal microscopy. For the first time, this procedure enabled imaging of live individual amastigotes, intermediate forms and motile trypomastigotes within infected tissues of mammalian hosts. **Supported by:**FAPESP, CAPES & CNPq **Keywords:**Trypanosoma cruzi; ex vivo; live imaging

HP007 - RELATIONSHIP BETWEEN INHIBITORY RECEPTORS EXPRESSION ON CD8+ T CELLS AND POLYFUNCTIONAL RESPONSE AGAINST *TRYPANOSOMA CRUZI* ANTIGENS IN CHRONIC CHAGASIC PATIENTS

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Some models of chronic infections caused by infectious agents have been associated with a deficient functional T cell response, a high frequency of terminally differentiated T cells, presence of monofunctional antigen-specific T cells and an increased inhibitory receptors co-expression. As in other chronic infections, during *Trypanosoma cruzi* infection, the progressive loss of certain functional activities may cause the inability to control the parasite replication. To test this hypothesis, it was evaluated the cell effector function of CD8+ T cells as well as the frequency of CD8+ T cells expressing inhibitory receptors in chronic chagasic patients with different degrees of disease severity. It was found that patients at the advanced stage of disease severity had a higher frequency of monofunctional CD8+ T cells while patients with less severe disease had a higher frequency of polyfunctional CD8+ T cells. In parallel, it was observed that symptomatic patients had higher frequency of CD8+ T cells producing granzyme B and perforin compared with the asymptomatic patients. On the other hand, a high frequency of CD8+ T cells expressing or co-expressing inhibitory receptors was found in symptomatic patients compared with asymptomatic patients and healthy donors. All together, these findings suggest that during Chagas disease, CD8+ T cells undergo a process of gradual loss of function characterized by an impaired cytokine production and increased frequency of CD8+ T cells expressing inhibitory receptors.

Keywords:Chagas disease; polyfunctional cd8 t cells; inhibitory receptors

HP008 - PATIENTS WITH ACTIVE VISCERAL LEISHMANIASIS EXHIBIT AN IMBALANCE BETWEEN SYSTEMIC LEVELS OF PROSTANOIDS AND CYTOKINES

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Visceral leishmaniasis (VL) remains a major public health problem worldwide. Cytokine balance is thought to play a critical role in the development of this disease. Hereby, we perform an exploratory study addressing whether simultaneous assessment of circulating levels of prostaglandins (PGE₂ and PGF_{2α}) and cytokines previously associated VL immunopathogenesis (IL-6, IL-8, IL-12p70, TNF, IL-1β, IL-10 and TGFβ) could highlight a distinct biosignature of this disease in treatment naïve patients compared to uninfected endemic controls. Moreover, we prospectively evaluate changes in the expression of these markers at 15 and 30 days after initiation of treatment with leishmanicidal drugs. We found that plasma levels of PGF_{2α}, IL-12p70, TNF, IL-1β and IL-10 were significantly increased in VL patients whereas concentrations of TGFβ were higher in endemic controls. Hierarchical clustering and interactome analysis of the plasma concentrations of these biomarkers revealed unique relationships with platelets and neutrophil levels. Importantly, the inflammatory profile depicted here in VL patients was gradually reversed after initiation of effective anti-microbial therapy. These findings demonstrate that an inflammatory imbalance involving cytokines and prostanoids hallmarks active VL disease and argue that manipulation of these pathways could be employed in a potential host-directed therapy to reduce the clinical burden of VL. **Supported by:**CNPQ / iii-INCT

Keywords:Visceral leishmaniasis; plasma biosignature; immunopathology

HP009 - THE ROLE OF MACROPHAGE MIGRATION INHIBITORY FACTOR IN THE INFECTION INDUCED BY NEOSPORA CANINUM

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Neospora caninum is a protozoan of the phylum Apicomplexa, distributed worldwide, and relevant due to the induction of abortions in bovine leading to considerable economic loss. MIF is a cytokine known to be involved in response against intracellular protozoa, which is produced by several immune cells and is found intracellularly preformed, turning this cytokine an important factor in acute inflammatory response, exhibiting a central role in innate immunity. Considering the lack of information about the role of MIF in this specific model, the present study aimed to evaluate the relevance of this cytokine during an infection by *N. caninum*. Therefore, we analysed the cytokine production and phenotype induced by itself, using wild type (WT) and genetically deficient in MIF (MIF^{-/-}) C57BL/6 mice, infected with 5x10⁶ tachyzoites of Nc-Liv. Then, to determine the interaction between MIF secreted by parasite or host and chemokines receptors, we proceed an in silico analysis. Firstly, it was possible to observe in the infection, an acute production of MIF in cells and peritoneal cavity fluids, spleen and lungs as well as a chronic production in the serum. Additionally, it was demonstrated that MIF^{-/-} mice possesses decreased parasitism during acute and chronic phases of infection. Moreover, it was observed an elevated production of TNF-α and NO and increased pulmonary inflammation in those animals and a slight increase in survival and recovery of body weight in comparison to WT mice. Furthermore, TNF-α is involved in the secretion of MIF, while adapter molecules MyD88 and TRIF are not required. In silico analysis suggests that excreted MIF, by the host or parasite, interacts with the chemokine receptor CXCR4. Moreover, an interaction between CD74 and MIF is unlike to happen, indicating that MIF is primordially endocytosed. In sum, MIF presents a relevant modulatory role during the establishment of infection by *N. caninum*. **Supported by:**FAPEMIG CNPQ CAPES **Keywords:**Neospora caninum; mif; immune evasion

HP010 - CEREBRAL VASCULATURE AND COGNITIVE IMPAIRMENT IN ACUTE TRYPAOSOMA CRUZI INFECTION

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Chagas disease (CD), caused by *Trypanosoma cruzi* (*T. cruzi*), remains a leading cause of illness and death in endemic areas of Latin America. While research on CD has focused on the heart and gastrointestinal tract, involvement of the brain, although appreciated since its discovery, has received less attention. Damage to the cerebral vasculature is an important feature tied to neurological impairments in CD. We postulated that the vasoactive peptide, ET-1 contributes to cerebral endothelial activation and the subsequent cognitive impairment in neuro-CD. To induce experiment CD (ECD), C57BL/6 mice were infected with trypomastigotes of the Tulahuen strain of *T. cruzi*. Cognitive function, degree of illness, and levels of inflammatory markers were assessed in the brains of the mice. Acute ECD caused an increase in parasitemia, low blood glucose levels, decreased weight and body temperature changes and an increase in level of illness as determined by rapid murine coma and behavior score. Object recognition and placement tests revealed significant visual and spatial memory impairments in infected mice. These cognitive deficits correlated to elevated levels of pro-inflammatory cytokine IL-1β and cell adhesion molecules e selectin and ICAM-1. Angiopoietin (Ang)-1, an angiogenic growth factor, inhibits these molecules on endothelial cells, while Ang-2 promotes their expression. qRT-PCR performed on whole brain homogenates revealed elevated levels of ET-1 mRNA in the brains of infected mice correlated with decreased Ang-1, resulting in an increased ratio of Ang-2:Ang-1. An angiopoietin system disruption illustrates cerebral endothelial dysfunction. Conditional ET-1 knockout mice (Tie-2-Cre: ET-1flox/flox) infected with *T. cruzi* showed improved memory function and survival rate than infected wild type mice. Further examination of the mechanistic basis of these observations may provide insight into adjunctive therapy to prevent neurological complications associated with CD. **Supported by:**NIH Training Grant in Geographic Medicine and Emerging Infections - T32AI070117 (BDF) **Keywords:**Endothelin; chagas disease; angiopoietin

HP011 - MITOCHONDRIAL DYSFUNCTION AND REACTIVE OXYGEN SPECIES IN NEONATAL PRIMARY CARDIOMYOCYTES: RELATION TO *T. CRUZI* INTRACELLULAR DEVELOPMENT

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A differential tissue tropism of *Trypanosoma cruzi* clonal populations has been observed in BALB/c mice infected with Col1.7G2 and JG parasites (*T. cruzi* I and II, respectively), where JG predominated in the heart tissue. The same phenotype was observed during infection of primary cultures of BALB/c mice embryonic cardiomyocytes or heart explants with these two parasite populations. These data strongly suggest that the differential distribution of *T. cruzi* strains during infection is a result of the direct interaction between parasite and infected host cell, with low contribution of the immune system, and most likely dependable on host cell response to infection. To elucidate the mechanisms involved in this process, we decided to investigate the production of reactive oxygen species (ROS) along distinct hours of infection of isolated neonatal BALB/c primary cardiomyocytes (CM) with JG and Col1.7G2. For this we incubated these cultures with CM-H2DCFDA, a probe that fluoresces in the presence of ROS. We showed that JG strain is able to trigger higher levels of ROS, 48 hours post infection, in comparison to Col1.7G2. Additionally we showed that this higher ROS production is likely due to an increase in mitochondrial dysfunction observed in JG infected CM cultures, which is not found in CM cultures infected with Col1.7G2. To investigate whether this two parasite populations may be differentially resistant to ROS, we evaluated the antioxidant enzymes produced by COL1.7G2 and JG using both trypomastigote and amastigote forms. Although no differences were observed in the production of these enzymes by trypomastigotes, Col1.7G2 amastigotes did show higher levels of Superoxide Dismutase A (SODA) and B (SODB), responsible for detoxification of superoxide, when compared with JG. We believe this higher ROS production, as shown previously in the literature, is powering JG higher intracellular multiplication in CM and contributing to the observed tissue tropism in BALB/c mice. **Supported by:** CAPES, CNPq, FAPEMIG

Keywords: *Trypanosoma cruzi*; cardiomiócitos primários; espécies reativas de oxigênio

HP012 - TRANSCRIPTOMIC PROFILE OF HUMAN CELLS STIMULATED WITH SALIVA FROM LU. INTERMEDIA OR FROM LU. LONGIPALPIS, VECTORS OF LEISHMANIASIS IN BRAZIL.

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The saliva of sand flies, vectors of leishmania parasite, has a variety of pharmacological agents, such as anticoagulants, vasodilators as well as immunomodulatory and anti-inflammatory molecules. In this work, we aimed at understanding the innate response to salivary antigens, given the different scenarios observed following immunization with *Lu. longipalpis* saliva (protection against infection) or with *Lu. intermedia* saliva (exacerbation of leishmaniasis). Whole blood obtained from healthy volunteers was stimulated with salivary gland sonicate from *Lu. intermedia* or *Lu. longipalpis* for 2 and 6 hours. Total RNA was extracted and used in large-scale sequencing (RNAseq) followed by bioinformatics analysis. Both *Lu. intermedia* and *Lu. longipalpis* saliva modulate the expression of a number of genes and functional enrichment analysis showed that six biological processes are commonly upregulated following stimulation with saliva of the two sand flies: taxis, chemotaxis, locomotory behavior, positive regulation of immune system process, regulation of cytokine production and regulation of cell activation. Among the genes that characterize these six biological processes, are genes encoding chemokines, cytokines and surface molecules. We also found a set of genes upregulated exclusively in cells stimulated with *Lu. intermedia* saliva compared to cells stimulated *Lu. longipalpis* saliva. These differences may provide clues for the development of leishmaniasis in individuals continuously exposed to *Lu. intermedia* saliva. **Supported by:** FAPESB and Programa Brasil-Suíça de Cooperação Científica e Tecnológica. **Keywords:** Sand fly; saliva; transcriptome

HP013 - *TRYPANOSOMA CRUZI* ISOLATES FROM DIFFERENT CHAGAS DISEASE CLINICAL FORMS HAVE A DISTINCT PROFILE REGARDING RESISTANCE TO OXIDATIVE STRESS AND REACTIVE OXYGEN SPECIES PRODUCTION

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The variability of Chagas disease clinical manifestations has been ascribed to the differences in the host response and to the heterogeneity of *Trypanosoma cruzi*. The acute phase of the disease when left untreated, progresses to the chronic indeterminate, cardiac, digestive or cardiodigestive clinical forms. The purpose of this work was to establish a possible correlation between the clinical form of the disease and parasite resistance to H₂O₂-treatment and reactive oxygen species (ROS) production. In this sense, *T. cruzi* epimastigotes derived from trypomastigotes isolated from the blood of four Chagas disease patients: cardiac (MAMA), digestive (AP), indeterminate (MJFL) and cardiodigestive (SAO) were obtained. Regarding the IC₅₀ for H₂O₂, AP was more sensitive to H₂O₂-treatment (IC₅₀= 156.5 ± 0.7 µM) than the others where no significant difference among them was observed (170.0 ± 0.0 µM, 187.5 ± 9.2 µM and 175.3 ± 1.8µM, for MAMA, SAO and MJFL, respectively). Regarding the superoxide production, through the Mitosox technique it was possible to establish that no significant difference was observed among the isolates (≈ 2 oxMitosox production/min.10⁸ cells), with the exception of MJFL where no production could be detected. H₂O₂ release was determined using succinate as the mitochondrial respiratory chain (MRC) substrate and as observed with other *T. cruzi* strains with a higher resistance to oxidative stress, no H₂O₂ release could be detected under basal conditions or even in the presence of antimycin A, an inhibitor of the MRC. Western blotting analysis of mitochondrial and cytosolic trypanothione peroxidases was also performed. Together, these results suggest differences in the resistance/production of ROS of the *T. cruzi* isolates from patients with different clinical forms of Chagas disease that could influence the virulence of the parasite. **Supported by:**FAPESP, CNPq and Capes **Keywords:**Chagas disease; clinical forms; reactive oxygen species

HP014 - THE ROLE OF INTEGRATED ENDOPLASMIC RETICULUM STRESS RESPONSE IN THE PATHOGENESIS OF *LEISHMANIA AMAZONENSIS*

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The Integrated Endoplasmic Reticulum Stress Response (IERSR) restores cellular homeostasis and ensures cellular survival under ER stress. This mechanism is mediated by activating transcription factor 6 (ATF6), inositol requiring kinase 1 (IRE1), and PKR-like endoplasmic reticulum kinase (PERK). Activation of IRE1α activates the X box binding protein 1 (XBP1). XBP1 is a transcriptional factor with critical role in cellular homeostasis and regulate the expression of cytokines like IFN-γ. Activation of PERK induces activation of transcriptional factor 4 (ATF4) and expression of genes related with cellular homeostasis and resistance to oxidative stress. We previously demonstrated that *L. amazonensis* infection was able to increase IFN-γ expression which favors infection. In this work we showed that macrophages treated with ER stress inducers increased *L. amazonensis* infection and that *L. amazonensis* activates both PERK/ATF4 and IRE-1/XBP1 splicing arms of the IERSR. In ChIP assays we observed that *L. amazonensis* was able to increase occupancy of enhancer and promoter for ifn gene by XBP1. In macrophages knocked down for XBP-1 expression we observed a significantly reduction in parasite growth. These could be explained by the impaired translocation of phosphoIRF3 to the nucleus and a decrease in IFN-γ expression in these cells. We also knocked down the expression of PERK and ATF4 and observed a significantly reduction in parasite growth. In further studies we demonstrated that knocking down PERK and ATF4 expression reduces the translocation of the NRF2 transcriptional factor and the expression of antioxidant genes such heme oxygenase (HO1) that protect parasites from oxidative stress in infected cells. The hypothesis underlying this work is that *L. amazonensis* induces IERSR to protect them from intracellular defense mechanism, particularly oxidative stress, and increase expression of important cytokines that favors *L. amazonensis* infection. **Supported by:**CNPq **Keywords:**Er stress; leishmania amazonensis; xbp1/perk/atf4

HP015 - ROLE OF CXCR3 CHEMOKINE RECEPTOR FOR T-CELL MEDIATED PROTECTIVE IMMUNITY ELICITED BY HETEROLOGOUS PRIME-BOOST VACCINATION AGAINST A SYSTEMIC PARASITIC INFECTION.

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Chemokine receptors are molecules responsible for leukocyte migration to non-lymphoid peripheral tissues where they exert their effector functions. The CXCR3 receptor is expressed on activated T cells during Th1 type response induced by intracellular pathogens. We have shown previously that heterologous prime-boost vaccine induces specific CD8⁺ T cells which play a key role in the protective immune response against *Trypanosoma cruzi* infection. Also, after phenotype analysis we showed that these cells expressed the receptor either after vaccination or infection. The aim of this work was analyze the role of CXCR3 receptor after immunization and infection with *T. cruzi*. For that purpose, A/Sn mice were immunized with heterologous prime-boost vaccine and 15 days after last dose were challenged with 150 tripomastigotes forms of *T. cruzi*. One group was treated with 250 µg of anti-CXCR3 monoclonal antibody every other day beginning on the same day of infection and the other treated with IgG isotype control. The blood parasitemia and survival were monitored. Mice treated with the monoclonal antibody αCXCR3 have increased blood parasitemia compared to the control group and all died after the 36 days of infection. Furthermore, we analyze the effector function of specific CD8⁺ T cells by *in vivo* cytotoxicity, ELISPOT and intracellular staining analyses. The treatment did not affect the frequency of specific CD8⁺ T cells in spleen, and these cells after *in vitro* stimulation degranulate and secreted IFN-γ and TNF-α at similar levels as compared to the immunized and infected group treated with IgG isotype control. Interestingly, the specific CD8⁺ T *in vivo* cytotoxicity was decreased in the immunized and αCXCR3 treated group compared to the control group. We concluded that CXCR3 receptor play a critical role in the protective immune response generated by the heterologous prime-boost vaccination possibly by blocking CD8⁺ T cell cytotoxicity mechanism after challenge. **Supported by:**FAPESP

Keywords:Chemokines; cd8+ t cells; trypanosoma cruzi

HP016 - ROLE OF PD-1/PD-L1 IN THE INDUCTION OF REGULATORY T CELLS DURING LEISHMANIA AMAZONENSIS INFECTION

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Leishmania amazonensis is the etiological agent of diffuse cutaneous leishmaniasis in South America. In murine models of this infection, dysregulated expansion of effector T cells is related to pathogenesis, while the induction of regulatory T cells (Treg) promotes lesion resolution. The most important co-stimulator/receptor pairs for Treg induction are PD1/PDL1, ICOS/ICOSL, OX40/OX40L and GITR/GITR. In this study, we examined the roles of these molecules in *L. amazonensis*-infected C57BL/6 mice. We found that infected foot tissues had a 10- and 5-fold increase in PD1 and PDL1 expression levels, respectively, with minimal changes for other receptor/ligand pairs. In skin-draining lymph nodes of infected mice, there were an increase in the percentage of CD11c+PDL1+ dendritic cells (DC) and PD1+CD4+ T cells. To evaluate PDL1 expression on DC, we performed *in vitro* infection with promastigotes and amastigotes. *L. amazonensis* infection resulted in an increased PDL1, but decreased PD-L2, expression on DC surface. This induction-triggered PDL1 expression was dependent of mTor, partially dependent on STAT3 and PI3K with minor participation of MAPK and MYD88, but not on JKI, JKII, JKIII, ERK and STAT5. Infected DCs were more competent in inducing CD25+FoxP3+ Treg *in vitro* than the control cells, and this Treg-promoting effect was dependent on PDL1 expression but not on TGF-beta production. Together, these data suggest a role for PD1/PDL1 in the regulation of local immune responses during *L. amazonensis* infection. This study provides new insights on immune regulation of cutaneous leishmaniasis. **Supported by:**CNPQ

Keywords:Leishmania amazonensis; pdl1; célula dendrítica

HP017 - CHARACTERIZATION OF THE INFECTION OF MOUSE NEUROSPHERES BY TOXOPLASMA GONDII AS AN IN VITRO MODEL OF CONGENITAL TOXOPLASMOSIS.

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Toxoplasma gondii is an obligate, intracellular parasite belonging to the phylum Apicomplexa and up to a third of the world's population is believed to be infected. *T. gondii* is commonly transmitted to any warm-blooded vertebrate through oocysts expelled by its definitive host (felines) or bradyzoite-containing tissue cysts residing in undercooked meat. The tachyzoite stage of the parasite's life cycle is characterized by rapid proliferation inside the host cell and can cause acute disease associated with tissue destruction. In immune competent hosts, tachyzoites differentiate into bradyzoites, which are believed to be largely quiescent for the remainder of the host's life. *T. gondii* acute infection during pregnancy cause congenital toxoplasmosis resulting in severe damage to the Central Nervous System, including blindness and hydrocephaly. To assess the impact of infection on neuro/gliogenesis and the fate of intracellular parasites, we infected murine neural progenitor cells (NPCs) with tachyzoite forms of the avirulent ME49 strain of *T. gondii*. NPCs are maintained undifferentiated as neurospheres and can spontaneously differentiate into neurons, astrocytes and oligodendrocytes upon removal of the mitogens present in the culture media. Cultures were analyzed for parasite load and specific genes expression at different times using qRT-PCR and Confocal and Electron Microscopy. Our preliminary analysis showed that infected NPCs have altered levels of connexin43 and pannexin1, both described as playing important roles on neurogenesis. In addition, we verified the presence of bradyzoites after 96 hours of infection, indicative of spontaneous stage-conversion – without any additional treatment such as INF- γ as described elsewhere. This model has proven to be a valuable tool to investigate in vitro the mechanisms involved in the pathogenesis of congenital toxoplasmosis and elucidate how this infection alters the correct development of the CNS. **Supported by:** CNPq, Faperj, Fundação Oswaldo Cruz

Keywords: Congenital toxoplasmosis; developmental biology; stage-conversion

HP018 - ABSENCE OF INDIGENOUS MICROBIOTA IMPAIRS MICROBICIDAL ACTIVITY OF MACROPHAGES

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Macrophages have diverse functions during pathologic processes that depend on their activation by external stimuli. They can be classically activated by LPS and IFN- γ . If stimulated with IL-4, they are alternatively activated. In addition, the production of IL-10 by these cells can characterize a regulatory macrophage, and this phenotype is achieved by stimulation with PGE2+LPS. The microbiota is important for *Leishmania major* killing in vivo, since germ-free mice (GF) fail to control parasite replication in comparison with conventional mice (CV). Our aim in this study was to investigate if the microbiome could be determinant for the phenotype of macrophage activation during *L. major* infection and contribute to CV resistance. We compared the activation of bone marrow derived macrophages stimulated in vitro by LPS+IFN- γ , IL-4 or PGE2+ LPS and infected with *L. major* amastigotes or promastigotes. We observed higher production of classically activated macrophage markers (CXCL9, IL-12, TNF, NO, ROS) by CV when stimulated by LPS and IFN- γ and infected with the parasite. On the other hand, there was higher expression of arginase I, FIZZ1 and YM1, markers of alternatively activated macrophages in GF. In addition, macrophages from GF showed higher expression of IL-10 after regulatory stimulation. We infected mice with 106 metacyclic forms of *L. major* in the footpad. GF showed bigger lesions and higher parasite load in the footpad from the fifth week compared to CV. Arginase activity was higher in the GF footpads after 6 weeks post infection. Taken together our data suggest that the absence of microbiota enhances, in vitro, the alternative activation and regulatory profile of macrophages indicated by higher expression of arginase I, FIZZ1, YM1 and IL-10. Thus, the differences we found for macrophage activation in vitro could explain the fact that CV are able to control the infection and GF are not. **Supported by:** CNPq, CAPES, FAPEMIG, REDOXOMA, REDE DE PESQUISA EM DOENÇAS INFECCIOSAS HUMANAS E ANIMAIS-MG

Keywords: Microbiota; macrophages; microbicidal activity

HP019 - LACK OF SAPA DOMAIN OF TRANS-SIALIDASES AS A FACTOR THAT CONTRIBUTE TO THE ATTENUATED PHENOTYPE OF THE CLONE CL-14 OF *TRYPANOSOMA CRUZI*

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CL-14 is a highly attenuated clone of *T. cruzi* that was isolated from the CL strain, as well as its parental clone CL Brener. In contrast to CL Brener, CL-14 is a non-pathogenic *T. cruzi*, which is not able to induce parasitemia and tissue parasitism. This clone was originally described as a vaccinal strain, since it leads to protection against a challenge with virulent strains. Recent studies from our group used the attenuated clone as a vaccine delivery vector expressing the cancer antigen NY-ESO-1 to be employed as an antitumor vaccine. Considering the promising results obtained with the vaccine vector, we decided to investigate the molecular basis of the low pathogenicity of the CL-14 clone in order to confirm its biosafety for vaccination protocols. Aiming at achieving this goal, the CL-14 genome was fully sequenced, as well as its transcriptome. Comparative analysis of multigenic families encoding surface proteins known to be involved with host-parasite interactions, revealed that CL-14 lacks the SAPA domain present in a subset of trans-sialidase genes of CL Brener. The lack of SAPA repeats in TS from CL-14 was confirmed by southern blot and immunodetection assays. Since this protein is known to play a key role in the invasion process and, being one of the main immunodominant *T. cruzi* antigen, leading to immunomodulation and parasite escape, we sought to test the effect of reconstituting the expression of TS SAPA in CL-14. The expression of TS-SAPA in CL-14 resulted in increased release of trypomastigotes in vitro infection and increased virulence in vivo. Together our data provide new insights about the importance of trans-sialidase containing SAPA as a virulence factor for *T. cruzi*. Financial Support: INCTV, CNPq, CAPES, Fapemig
Supported by:INCTV, CNPq, CAPES, Fapemig **Keywords:**Trypanosoma cruzi; tras-sialidase;virulence

HP020 - CIRCULATING NEUTROPHILS PRESENT A HEIGHTENED INFLAMMATORY STATE DURING ACTIVE CUTANEOUS LEISHMANIASIS

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Different species of the protozoa of the genus *Leishmania* can promote several clinical manifestations associated with an exacerbated response in skin. Although macrophages are the main harbor cells for these parasites, neutrophils (PMN) are the first inflammatory cells to migrate to site of infection. PMN participation in murine infection by *Leishmania* has been documented to promote parasite clearance or contribute to disease aggravation depending on the susceptible or resistant status of the host. Based on the hypothesis that PMN contribute to a systemic inflammatory state in humans with symptomatic *L. braziliensis* infection, we evaluated the phenotype of neutrophils from patients with cutaneous leishmaniasis (CL). PMN were obtained from peripheral blood from CL patients during the course of *L. braziliensis* infection. The phenotype of these cells were assessed by CD62L and CD66b expression by flow cytometry and the production of oxidative burst and some chemokines were evaluated by flow cytometry and sandwich ELISA technique, respectively. CL patient neutrophils produced more reactive oxygen species (ROS) and higher levels of CXCL8 and CXCL9 than neutrophils from control healthy subjects (HS). Despite this, CL patient and HS neutrophils were equally infected with *L. braziliensis*. There was no difference between the degree of activation of PMN from CL versus healthy subjects, assessed by CD66b and CD62L expression. Interestingly, these studies revealed that both parasite-infected and bystander PMN became activated during incubation with *L. braziliensis*. After treatment of disease, the enhanced ROS and chemokine production in neutrophils from CL patients reverted to baseline. These findings suggest that PMN from CL patients do not have necessarily greater ability to kill *L. braziliensis* but have more pro-inflammatory profile than healthy subjects cells and the altered phenotype in circulating neutrophils is predominantly related to the environment observed during CL. **Supported by:**National Institute of Health (NIH) grant AI30639-20 **Keywords:**Human cutaneous leishmaniasis; neutrophils; leishmania braziliensis

PV001 - LUTZOMYIA UMBRATILIS POPULATION CAPTURED IN THE SOUTH OF THE NEGRO RIVER IS REFRACTORY TO INTERACTION WITH LEISHMANIA GUYANENSIS.

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Lutzomyia umbratilis is the vector *Leishmania guyanensis* in Northern South America. This vector has been found naturally infected with this species only east of the Negro River and north of the Amazonas River. However, populations of this sand fly species are also present in areas south of those rivers, natural geographical barriers. An interesting aspect is that they were never found infect and/or transmitting *L. guyanensis* in those areas. Genetic differences among *L. umbratilis* populations suggest that they may be in the process of speciation. However, no studies on the parasite host interactions are available. Here, the interaction of *L. guyanensis* (MHOM/BR/75/M4147) with *L. umbratilis* captured in two sites UFAM and Manacapuru (North and South of the Negro River), respectively, was evaluated. Procyclic and metacyclic attachment was quantitated in vitro after interaction with the midguts of field collected *L. umbratilis*. Midguts (11 per group) were incubated for 20 min with procyclic and metacyclic promastigotes (2×10^7 cells/mL) and the number of attached parasites counted. No attachment of parasites was observed in the midguts from all the insects of Manacapuru. On the other hand, a higher binding of procyclic parasites was observed in the midguts from UFAM and this attachment was more pronounced than that observed for metacyclics ($P < 0.001$). Those data suggest that the population of *L. umbratilis* south of the Negro River is refractory to interaction with *L. guyanensis*. Those findings also confirmed the previous epidemiological information on *L. guyanensis* transmission in different regions of the Amazon. **Supported by:**CNPq and FAPEMIG

Keywords:Lutzomyia umbratilis; leishmania guyanensis; interaction

PV002 - USING THE ORFEOME PROJECT AS A TOOL FOR FUNCTIONAL CHARACTERIZATION OF TRYPANOSOMA CRUZI CANDIDATES PROTEINS

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Trypanosoma cruzi is very divergent when compared to model organisms, due to its early separation from the eukaryotic lineage. This results in a great lack of knowledge regarding the functional properties of its proteins, mostly classified as of unknown or generic function, based on weak sequence/domain similarity identification by bioinformatics analyzes. Aiming to increase our knowledge of protein function, we have produced a library containing the great majority of *T. cruzi* coding regions (CDSs) in a suitable vector for posterior functional characterization (*T. cruzi* ORFeome). These clones are being used in several projects, as protein expression in bacteria and antibody production (immunolocalization, quantification and precipitation), protein expression in *T. cruzi* with specific tags (distinct fluorescent proteins, immunolocalization and immunoprecipitation tags) and in a large scale Y2H protein-protein interactome project. Here, we present the initial results using the ORFeome for characterizing physiological modifications of *T. cruzi* after heterologous protein over-expression. As our initial dataset, we selected RNA-binding proteins (RBPs) containing RRM (n=60), zf-CCCH (n=42), PUF (n=11) and Alba (n=4) domains fused to a *T. cruzi* specific vector, containing the 3xFLAG epitope in the N-terminus and UTR regions that were selected as potentially increasing the mRNA and protein levels during its life cycle. After transfection, the recombinant cultures were evaluated for over-expression impact in cell growth and morphology, stress response, metacyclogenesis and cell infection. The cultures were also assessed for mRNA steady state alterations in epimastigotes. These results serve primarily to select potential candidates for further functional characterization. Nevertheless, they can also be taken together as a generic, but parasite specific, functional characterization very useful for omics analyzes as a broad biological process gene ontology classification. **Supported by:**CNPq, Fundação Araucária, Fiocruz

Keywords:Trypanosoma cruzi; omics analyzes; protein function characterization

PV003 - ANALYSIS OF *LEISHMANIA MAJOR* OVEREXPRESSING THE EXONUCLEASE-1 HOMOLOGUE

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Leishmaniasis are caused by protozoan parasites of the genus *Leishmania* spp. They are heteroxenic organisms that can adapt to a great variety of hosts and their gene expression is controlled mainly by post-transcriptional mechanisms. These organisms can modulate the expression of specific genes by gene amplification, which depends upon mechanisms of DNA repair. In trypanosomatids, double strand breaks may happen after exposure to exogenous agents or during physiological processes, and they can be resolved by homologous recombination. The enzyme Exonuclease-1 participates in many cellular processes and it is one of the main proteins involved in homologous recombination. The objective of this study was to evaluate the phenotype of *Leishmania major* overexpressing the *LmjEXO1* - *Lmj*-[pXG1 EXO1] - gene product using bioinformatics tools, growth curves after exposure to DNA damaging agents, analysis of the molecular karyotype and determination of *LmjEXO1* transcript level. Predicted nuclear localization signals were found for the protein *LmjEXO1*, besides possible interactions with other DNA repair proteins, which suggests its participation in nuclear processes. Growth curve analysis demonstrated that the *L. major* lineage overexpressing the gene *LmjEXO1* has clear difficulty of growth after treatment with zeocin and hydroxyurea, and that this doesn't seem to be related to an accumulation of chromosomal damage. Moreover, no changes in the level of expression of the transcript *LmjEXO1* were found for the controls and overexpressor lineage. Finally, the results suggest that sensibility of the *Lmj*-[pXG1 EXO1] lineage to DNA damaging agents could be associated with effects caused by the exacerbated activity of the enzyme Exonuclease-1 in *Leishmania major*. **Supported by:**FAPEMIG
Keywords:Leishmania major; homologous recombination; exonuclease-1

PV004 - BIOENERGETIC APPROACH TO HYPOXIC CHALLENGE IN TRYPANOSOMA CRUZI EPIMASTIGOTES

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Trypanosoma cruzi inhabit different environments that challenge it to divert its metabolism. In most cells, oxygen levels variation induce a shift towards aerobic to anaerobic metabolism. Unlike mammal cells, *T. cruzi* has a single mitochondrion, making it an important target for Chagas disease chemotherapy. Since little is known about the influence of oxygen fluctuation upon *T. cruzi* forms, we analyzed hypoxia effect on mitochondrial *T. cruzi* epimastigotes metabolism. Thereby, hypoxic condition was induced by N₂ atmosphere saturation and after 5 days, normoxia or hypoxia parasites were quantified by counting. Hypoxic condition induced a three-fold increase in proliferation. We also evaluated DCFDA fluorescence in hypoxia or normoxia epimastigotes and hypoxic condition increased ROS production. Thus, to compare O₂ consumption between normoxia or hypoxia parasites we performed high resolution respirometry using a substrate-uncoupler-inhibitor-tritration protocol in digitonin-permeabilized cells. Normoxia cells showed an increased in O₂ consumption when succinate and ADP were added. In contrast, hypoxia did not present any induction, that suggests hypoxic condition might impairing mitochondrial ATP production. Besides hypoxia epimastigotes routine O₂ consumption was three times higher than normoxia cells. Interestingly, antimycin A totally abolished normoxia parasites O₂ consumption but did not eliminate of hypoxia parasites consumption, probably due to ROS production or lipid peroxidation that might consuming O₂ outside mitochondria during reoxygenation process. We also analyzed complex II-III activity and hypoxia cells presented a 5 times lower complex activity than normoxia, which corroborates that hypoxic condition decreases mitochondrial contribution for epimastigotes bioenergetics. Taken together, our results suggest that hypoxia induces a shift on epimastigotes bioenergetic metabolism that allows parasite to survive and proliferate under hypoxic conditions. **Supported by:**CNPq, INCT e Faperj

Keywords:Trypanosoma cruzi; hypoxia; mitochondria

PV005 - LUTZOMYIA LONGIPALPIS LL5 EMBRYONIC CELL LINE AS A MODEL FOR INSECT INNATE IMMUNITY STUDIES

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Lutzomyia longipalpis is the main vector of visceral leishmaniasis. In addition to *Leishmania* parasites these insects are exposed to viruses, bacteria and fungi. Studies on insect immunity are necessary to understand how these insects survive and control infections. There are three major pathways related to innate immunity in insects: Toll, IMD and JAK/STAT, which regulates the expression of antimicrobial peptides (AMPs). Previous work on *Drosophila* has shown that the Toll pathway is activated upon Gram positive bacteria and fungi while the IMD pathway is activated upon Gram negative bacteria and fungi. Our objective is to study the expression of genes related to immune response upon microbial challenge. We used *L. longipalpis* embryonic cells LL5 challenged with the Gram negative *Escherichia coli* and *Serratia marcescens*, the Gram positive bacteria *Staphylococcus aureus*, the yeast *Saccharomyces cerevisiae* and *Leishmania infantum chagasi*. By qPCR we assessed the expression of Cactus (negative regulator) and Dorsal (transcription factor) of the Toll pathway; Caspar (negative regulator) and Relish (transcription factor) of the IMD pathway; and three AMPs Attacin, Cecropin, and Defensin. We observed increased expression of Relish in response to both Gram+ and Gram- bacteria at 6 hours after challenge. Furthermore, *Serratia marcescens* challenge showed an increased expression of Dorsal, 24 hours after infection. There was also increased expression of Cecropin and Defensin in all bacterial challenges. When LL5 cells were challenged by yeast, we observed an increased expression of Relish and Defensin at 6 hours and 24 hours post challenge. On the other hand *Leishmania* infection triggered the expression of the negative regulators Caspar and Cactus 24 hours after challenge. Our results indicate that LL5 cells are capable of developing immune response to bacteria and yeast challenges. Curiously, *L. i. chagasi* may be able to reduce the immune response of the insect cells. **Supported by:**FAPERJ, CNPq, IOC-Fiocruz **Keywords:**Leishmania infantum chagasi; lutzomyia longipalpis; immunity

PV006 - INORGANIC PHOSPHATE TRANSPORTER IN TOXOPLASMA GONDII

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Inorganic phosphate (P_i) is a central compound in the metabolism of all organisms, including parasites. The first step for P_i utilization begins with P_i transport across the plasma membrane. This work is the first report regarding the mechanism of P_i acquisition by *Toxoplasma gondii*. The intracellular concentration of free P_i in *T. gondii* is 0.5 mM, compared to 0.1 mM for host fibroblasts at confluence, showing a capacity for P_i accumulation in *T. gondii* cytosol. Due to the negative charge on P_i , this accumulation may be mediated by a protein transporter. The annotated *T. gondii* genome has a single putative plasma membrane P_i transporter, *TgPIT* that bears a close resemblance to Na^+ -dependent transporters of the PiT family from fungi and animals. *T. gondii* expresses a *TgPIT* with 92 kDa in the membrane fraction, recognized by an antibody raised against recombinant *TgPIT*. By immunofluorescence analysis, it is possible to visualize *TgPIT* in non-permeabilized extracellular parasites, using an antibody against *TgPIT*, demonstrating the plasma membrane localization of this protein. These data are confirmed by the immunolocalization within cells expressing *TgPIT*-mCherry. The mechanism of P_i uptake in *T. gondii* was evaluated by biochemical methods using extracellular parasites. P_i uptake was 8-fold higher in the presence of Na^+ compared to the presence of choline chloride, suggesting a requirement for transmembrane coupling between P_i and Na^+ fluxes for P_i internalization into the parasite. P_i uptake inhibition by the Na^+ ionophore monensin or the Na^+ - K^+ -ATPase inhibitor ouabain confirms that a steadily sustained Na^+ gradient is required for the secondary active Na^+ : P_i uptake. The P_i uptake is specific for P_i as substrate, as demonstrated by competition assay, where the monovalent ionic form of P_i is preferred. In summary, we provide strong preliminary evidence that *T. gondii* concentrates P_i provided by the environment and has a specific transport system for P_i acquisition. **Supported by:**CNPq Ciencia sem Fronteiras **Keywords:**Toxoplasma gondii; inorganic phosphate transporter; pit family

PV007 - ELUCIDATING THE ROLE OF MITOCHONDRIAL CALCIUM UNIORTER (MCU) IN TRYPANOSOMA CRUZI BY A CRISPR/CAS9 APPROACH

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Calcium ion (Ca²⁺) is an important second messenger for a variety of cell functions through the complex life cycle of trypanosomatids, including host cell invasion. The mitochondria of these organisms possess a uniporter (mitochondrial calcium uniporter or MCU) that, in *Trypanosoma brucei*, is essential for survival. The recent development of the CRISPR/Cas9 system for gene disruption in *Trypanosoma cruzi* is facilitating the functional analysis of proteins in this parasite. In order to determine the physiological role of the mitochondrial Ca²⁺ uptake in *T. cruzi* we obtained mutant cell lines where the gene encoding MCU (TcMCU) has been either knocked out or overexpressed. We performed the ablation of TcMCU (TcMCU-KO) by co-transfecting the CRISPR/Cas9 plasmid (TcMCU-sgRNA/Cas9/pTREX-n) with a DNA template containing the blasticidin-S deaminase resistance marker flanked by homology arms corresponding to the 5'-UTR and 3'-UTR of the target gene to induce DNA double-strand break repair by homologous recombination. The TcMCU-KO cell line displayed a complete absence of mitochondrial Ca²⁺ uptake without affecting the membrane potential of digitonin-permeabilized *T. cruzi* epimastigotes. Additionally, the overexpression of the TcMCU using the pTREX vector caused a significant increase in the ability of mitochondria to accumulate Ca²⁺ and generates an increase in reactive oxygen species production. Our results indicate that mitochondrial Ca²⁺ uptake is solely performed by MCU in *T. cruzi*. Unexpectedly, MCU is not essential for the growth of *T. cruzi* epimastigotes in vitro. However, TcMCU-KO epimastigotes take longer in reaching the stationary phase; and in low-glucose LIT medium they show a long-lived phenotype. Evaluation of other phenotypic features, including autophagy and apoptosis pathways is currently in progress. **Supported by:**FAPESP N° 2013/50624-0, 2011/50400-0 and 2014/13148-9

Keywords:Crispr/cas9 ; mitochondrial calcium uniporter; trypanosoma cruzi

PV008 - INVESTIGATION OF THE BIOLOGICAL ROLE OF LEISHMANIA INFANTUM DUAL-SPECIFICITY TYROSINE-REGULATED KINASE 1 (LINDYRK1) AND EVIDENCE FOR ITS PARTICIPATION IN PARASITE SPECIFIC PATHWAYS

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Protein kinases are attractive drug targets for developing new anti-leishmanial drugs. DYRKs comprise a family of kinases with pleiotropic functions, known to autophosphorylate activation loop Tyr and phosphorylate Ser/Thr in their substrates. In *Leishmania* the *L. infantum* homologue of DYRK1 (LinJ.15.01800) displays differences in the ATP binding pocket from the host homologue and a *Leishmania*-specific domain, thus fulfilling an important criterion for drug target prediction. For the above reasons we selected DYRK1 for genetic validation and employed a facilitated null mutant analysis to sequentially knocking out the two endogenous kinase alleles in transgenic parasites that ectopically express DYRK1 from the negative selectable plasmid pXNG that also carries a GFP reporter. pXNG-DYRK1 persistence after 15 passages under negative selection was used as a readout of essentiality. Our results showed that episome loss was tolerated, suggesting that DYRK1 is not essential for parasite viability. However knockouts (KOs) rounded up and increased hypodiploid DNA content in stationary phase. KOs were also more sensitive to different stresses (SbIII, heat-shock 26 to 37° C) and displayed reduced intracellular survival. These phenotypes were linked to cell-cycle dysregulation both in DYRK1 KOs and over-expressing parasites, in agreement with an inhibitory role for DYRK1 in G1/S transition. Moreover parasites expressing GFP-DYRK1 displayed increased median fluorescence in stationary phase, reinforcing the notion that DYRK1 activity is required in this phase. GFP-DYRK1 displayed a parasite-specific localization, showed proximity to the kinetoplast, and distinct localization to endosomal markers (ConA-TexasRed & FM4-64). Overall our results suggest that DYRK1 is required in sustaining stationary phase and modulates stress-induced response, explaining its significance in parasite survival and infectivity, and thus defining this protein kinase a potential drug target. **Keywords:**Leishmania; kinase; dyrk1

**PV009 - CODON USAGE IS THE MAJOR DETERMINANT OF MRNA LEVELS IN
TRYPANOSOMA BRUCEI**

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In trypanosomes, most or all protein coding genes are constitutively transcribed and there is little evidence for selective regulation of expression by RNA polymerase II. Thus, regulation of mRNA levels is largely post-transcriptional. In this work we developed a novel codon usage metric called the 'gene expression codon adaptation index' (geCAI) that is predictive of both relative protein abundance and relative mRNA abundance with a coefficient of determination of 0.84 and 0.55 respectively. The predictive capacity of this novel scoring metric was tested using GFP reporter gene expression. 22 synonymous GFP mRNAs were expressed in procyclic cell lines. Protein expression and mRNA levels were modifiable over a ~40-fold range. The range and expression levels were similar to the measured mRNAs per haploid gene copy for the transcriptome and thus geCAI score is sufficient to account for 50% of the variation observed in mRNA copy number. GFP mRNAs with low geCAI scores decayed more rapidly than those with high scores suggesting translational efficiency is the mechanism that produces the differences in mRNA steady state levels. The translational efficiency model was tested by selectively blocking translation of individual GFP mRNAs by insertion of a hairpin in the 5'UTR, this equalized steady state levels to that of high geCAI score GFP mRNAs. In contrast, inclusion of a short upstream open reading frame in the 5'UTR greatly decreased translation of GFP and decreased steady state mRNA levels to that of a very low geCAI score GFP mRNA. Thus, geCAI is a good predictor of mRNA levels and efficiency of translation is a major determinant of mRNA levels. **Supported by:**Ciencias sem Fronteiras, Wellcome Trust, Medical Research council **Keywords:**Gene expression; protein translation; mrna

**PV010 - ALTERNATIVE MECHANISMS OF GENE EXPRESSION MODULATION IN T. CRUZI
MUCINS**

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The protozoan parasite *Trypanosoma cruzi* is the etiological agent of Chagas disease, a major public health issue in Latin America. Due to its predominant clonal proliferation, this species is composed by multiple strains or "discrete typing units", displaying considerable genetic diversity. TcSMUG is a family of Thr-rich *T. cruzi* mucins genes, which are expressed in the insect-dwelling stages and display key roles in the parasite biology. Interestingly, some of TcSMUG products present substantial differences in their expression levels across strains. Although it has been established that control of gene expression in *T. cruzi* is essentially concerted by posttranscriptional mechanisms, we here explore a possible role of tRNA editing mediated by ADATs (adenosine deaminases acting on tRNA, ADATs) in this phenomenon. Briefly, we demonstrate that inter-strain variation in the expression of TcSMUG products correlate with significant differences in their codon usage and with changes in the levels of adenosine-to-inosine (A-to-I) editing on the anticodon loop of the tRNA^{Thr}AGU. Furthermore, over-expression of individual ADAT enzymes led to an increase in the levels of tRNA^{Thr}AGU A-to-I editing, and significant changes in expression of TcSMUG products, but not on their encoding mRNAs. Together these findings suggest an additional and specific mechanism of gene expression regulation that is transcript-specific and relies on the efficient interaction of codons and anticodons, thus on tRNA availability. **Supported by:**CONICET

Keywords:Adat; trna; regulation

PV011 - L. AMAZONENSIS METABOLOMICS ASSOCIATED WITH LOSS OF VIRULENCE AND IN VIVO CELL DIFFERENTIATION

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Leishmaniasis are widespread neglected endemic diseases with an incidence 1.6 million new cases and 40 thousand deaths per year. Some Leishmania strains or isolates may lose virulence and infectivity after successive *in vivo* passages. Also, parasites may return to its virulent form after *in vivo* differentiation into amastigotes-like, and then to promastigotes, or after being recovered from infection in mammalian host. However, the mechanisms and metabolic pathways involved in these processes are not well known. This work aimed to perform a metabolomic fingerprint approach to scan the alterations in the metabolic pathways related to these processes. A multiplatform assay based on gas chromatography, liquid chromatography and capillary electrophoresis, coupled to a mass spectrometry was used. Features were tested in multivariate and univariate statistical analysis, and those that presented at least two statistical significance indexes were selected for analysis using chemoinformatics' softwares to putative identification of metabolites. The identified metabolites were validated by fragmentation profile or by comparative standards analyses, and then mapped on metabolic pathways using bioinformatics softwares. Loss of virulence profile included 83 metabolites identified (34 confirmed), associated to 11 metabolic pathways. The cell differentiation assay has shown 215 metabolites (67 confirmed), associated to 15 metabolic pathways. Two metabolic pathways stand out among those identified on the loss of virulence essays: ABC transporters and the pathway of biosynthesis of fatty acids. *In vivo* and *in vivo* assays are currently underway as to validate the involvement of the aforementioned pathways. Considering the data obtained thus far, it can be concluded that metabolomics has great value as an alternative tool for the analysis of Leishmania physiological processes and mechanisms. **Supported by:** CAPES, FAPEMIG, MS-DECIT **Keywords:** Metabolomics; leishmania; virulence

PV012 - PROTEOMIC ANALYSIS OF EXTRACELLULAR VESICLES RELEASED FROM TRYPANOSOMA CRUZI DURING METACYCLOGENESIS

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Extracellular vesicles (EVs) are important vehicles to cell-cell communication and may influence physiologic and behavior aspects of cellular targets. *Trypanosoma cruzi*, the etiologic agent of Chagas disease, release EVs during its life cycle. The metacyclogenesis is a process that occurs in the invertebrate host, where the parasite under nutritional stress converts from a noninfective to an infective form. It is presented herewith the morphological and proteomic characterization of EVs released from *T. cruzi* in the course of the *in vitro* metacyclogenesis process, namely epimastigotes (EPI), epimastigotes under nutritional stress (ST), epimastigotes adhered on the substrate 24h after nutritional stress (ADHE) and metacyclic tripomastigotes (MET) forms. To obtain the EVs samples, *T. cruzi* (Dm28c strain) was cultivated as described by Contreras *et al.*, 1985. To discard EVs contaminants, all the cultures were washed in PBS (EPI) or TAU (ST, ADHE and MET) 3 times and then incubated in RPMI 1640 (EPI), TAU (ST) or TAU3AAG (ADHE and MET). The supernatant free from parasites was filtered through 0.45µm membrane and extracellular vesicles isolation was performed by ultracentrifugation at 120,000xg during 2 hours. The pellets were resuspended in 200µl of PBS. Morphological characterization of EVs was performed using NanoSight LM10 and transmission electron microscopy, showing that the size of the samples ranged between 32 and 200nm. Protein levels were assessed by the Qubit fluorometric quantification. Three EVs sample preparations, independently obtained from EPI, ST, ADHE and MET were digested with trypsin and analyzed by nano-chromatography (Easy-nLC 1000) coupled online to an LTQ Orbitrap XL. The protein identification was done using PatternLab platform. We highlight the presence of several trans-sialidases, glycoproteins and heat-shock proteins that are important for the invasion pathway. **Supported by:** CNPq, PRONEX, Fundação Araucária, Fiocruz-CAPES **Keywords:** Extracellular vesicles; proteomic analysis; trypanosoma cruzi

PV013 - ASSESSING THE GUT MICROBIAL DIVERSITY OF FIELD Aedes Aegypti MOSQUITOES

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A microbiome is a set of microorganisms present in a determined environment and in association with other organisms it could aid in physiological process, as nutrition, development and reproduction.. The *Aedes aegypti* gut microbiota has been implicated as a potent modulator of its vector competence dengue virus (DENV), representing a promising resource to the development of innovative strategies to control the dissemination of dengue. Although there is evidence that some bacteria can influence DENV replication in the mosquito midgut either directly or through the activation of the mosquito antiviral defenses, it is still unknown how the diversity of the gut microbiota may influence the mosquito's susceptibility to DENV. With the intention to explore these relationships, in this study we assessed the midgut microbiome of field *Ae. aegypti* collected from different breeding sites in São Paulo State, Brazil. Larvae were collected with breeding site water, and brought to the insectary, were develop into adult. On the second day post emersion their midgut were dissected and the DNA isolated. Libraries of V4 hypervariable region amplicons amplified from the bacterial 16S rDNA gene were generated, sequenced in the MiSeq Illumina platform. Sequencing data was processed and analyzed with the CLC bio workbench software. Our results revealed a high variability among the microbiomes of *Ae. aegypti* collected in the field with no obvious association between bacterial diversity and a given type of breeding site (e.g. disposed tires, plant vases and garbage). The more abundant genera found, such as *Acinetobacter*, *Pseudomonas*, *Tepidimonas* and *Stenotrophomonas*, appeared in similar proportions in all samples, even though mosquitoes have been obtained from different cities and breeding sites (Botucatu, Avaré and Itanhaém/SP). Our next steps are directed to analysing the inheritance of such bacteria throughout successive generations of those mosquitoes maintained in the laboratory. **Supported by:**CNPQ

Keywords:Microbiome; aedes; sequencing

PV014 - CHARACTERIZATION OF TELOMERIC REPEAT-CONTAINING RNA (TERRA) EXPRESSION IN LEISHMANIA SPP.

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Telomeres are nucleoprotein structures that protect chromosome ends from fusion and degradation. Although generally considered transcriptionally silenced, it was previously shown that different types of RNA polymerases transcribe telomeres in Kinetoplastida protozoa. However, at that time it was not clearly defined which telomeric strand was being transcribed, since a double stranded telomeric probe was used in the assays. More recently, it was demonstrated that telomeres from different eukaryotes including mammals and yeast are transcribed into telomeric repeat-containing RNA (TERRA). TERRA is a long non-coding RNA (lncRNA) transcribed from subtelomeric regions towards the 3'ends of telomeric repeats, whereby the C-rich telomeric strand is used as template. Several lines of evidence indicate that TERRA regulates telomere length, telomerase activity and heterochromatin deposition and although its biogenesis is well defined the functions associated to TERRA are still very controversial. In this work, we tested the existence of TERRA in *Leishmania* using independent RNAs-Seq libraries constructed from the three parasite life stages (promastigotes, metacyclic and amastigotes) and identified putative TERRA transcripts originating from several chromosome ends (right and left arms). We then focused on chromosome ends that share structural similarities with human subtelomeres and analyzed their transcription profiles by northern blot and RT-PCR. Both approaches confirmed the existence of TERRA transcripts comprising subtelomeric sequences followed by tracts of G-rich telomeric repeats of variable length. The existence of TERRA in *Leishmania* further indicates that telomere transcription is highly conserved among eukaryotes and opens new avenues to study TERRA-associated functions in a parasite. **Keywords:**Terra; telomeres; leishmania

PV015 - OVEREXPRESSION OF GLUCOKINASE IN *TRYPANOSOMA CRUZI* EPIMASTIGOTES IS TOXIC

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Trypanosoma cruzi energy metabolism is based on the use of glucose as a carbon and energy source. Trypanosomatids have compartmentalized most of the glycolytic pathway inside glycosomes. The first step of glycolysis is the conversion of glucose to glucose 6-phosphate (Glc6P), coupled to the hydrolysis of ATP. In *T. cruzi* this step is catalyzed by two enzymes, hexokinase (HK) and glucokinase (GlcK), which kinetic characteristics suggest distinct biological functions. We evaluated the effects of GlcK overexpression in epimastigotes metabolism by using the inducible expression system pTcINDEX. To this aim, a cell line, pLEW13-pTcINDEXGlcK was obtained. GlcK overexpression was confirmed by RT-PCR and Western blot after tetracycline (Tet) induction. Recombinant cells were cultured in presence and absence of Tet and samples were taken each 24 hours. We evaluated growth rate, enzyme localization, GlcK expression levels by western blot and enzymatic activity and glucose and amino acids consumption. We found that GlcK overexpression caused a decrease in parasites growth rate (68.9%) and a change in cell morphology. Glucose consumption was dramatically reduced (90%) while aminoacids consumption increased (14.4%). This phenotype reverted slowly when tetracycline was removed from the medium. GlcK specific activity increased by 5 times; in agreement with the expression levels determined by western blot. Immunofluorescence analysis showed that overexpressed GlcK was properly targeted to glycosomes. These results suggest that GlcK participates in the distribution of Glc6P inside the glycosome, controlling the flux between glycolysis and pentose phosphate pathway (PPP). An increase in the specific activity of GlcK entails a deviation of more β -D-Glc6P toward PPP route, due to the selectivity of glucose-6-phosphate dehydrogenase for this substrate. Consequently, an unbalance ATP/ADP is generated leading to an intraglycosomal accumulation of Glc6P and parasite's growth arrest. **Supported by:**CDCHT-ULA N° C-1829-13-03-B, Misión Ciencia N° 2007001425, CNPq

Keywords:Trypanosoma cruzi; glucokinasa; energy metabolism

PV016 - TRYPANOSOMA RANGELI INFECTION MODULATES ANTIMICROBIAL PEPTIDES MRNA LEVELS IN RHODNIUS PROLIXUS MIDGUT AND FAT BODY

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Trypanosoma rangeli is a protozoan that infects a variety of mammalian hosts, including humans. Its main insect vector is *Rhodnius prolixus* that has a strictly blood-sucking habit in all life stages, ingesting large amounts of blood from vertebrate hosts from which it can acquire a number of microorganisms, as *T. rangeli*. One of the most important antimicrobial defences of insects is the challenge-induced synthesis of several antimicrobial peptides by fat body and epithelial cells. The *R. prolixus* vector competence depends on the *T. rangeli* strain and the molecular interactions, as well as the insect's immune responses in the gut and haemocoel. Regarding the life cycle of *T. rangeli* in the insect, the present work focuses on the modulation of the antimicrobial peptides gene expression in the fat body and the midgut of *R. prolixus* infected with *T. rangeli* Macias strain. The parasite population density was analysed in different *R. prolixus* midgut compartments in short and long-term experiments. The modulation of *R. prolixus* immune responses was studied by analysis of the antimicrobial activity in vitro against different bacteria using turbidimetric tests; the abundance of mRNAs encoding antimicrobial peptides defensin (DefA, DefB, DefC), prolixicin (Prol) and lysozymes (LysA, LysB) by RT-PCR and analysis of the phenoloxidase (PO) activity. Our results showed that *T. rangeli* successfully colonized *R. prolixus* midgut altering the insect immune responses as follows: enhanced midgut antibacterial activities against *Serratia marcescens* and *Staphylococcus aureus*; down-regulated LysB and Prol mRNA levels; altered DefB, DefC and LysA depending on the infection (short and long-term), midgut region and days after infection/feeding; inhibited phenoloxidase activity. Our results suggest that *T. rangeli* Macias strain modulates *R. prolixus* immune responses in order to ensure parasite population survival in the insect midgut. **Supported by:**PAPES FIOCRUZ; FAPERJ **Keywords:**Rhodnius prolixus; trypanosoma rangeli; antimicrobial peptides

PV017 - THE INTRASPECIFIC POLYMORPHISMS IN THE LIPOPHOSPHOGLYCAN OF LEISHMANIA AMAZONENSIS DOES NOT INFLUENCE IN THEIR INTERACTION WITH THE SAND FLY VECTOR LUTZOMYIA MIGONEI

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Leishmania amazonensis is the causative agent of diffuse cutaneous leishmaniasis in Latin America. Lipophosphoglycan (LPG) is the major surface molecule found on *Leishmania* promastigotes and plays a key role during the interaction with the sand fly. Besides the biochemical and morphological changes in the parasite from procyclic to infective metacyclic promastigotes, intra and interspecies polymorphisms in the LPGs may occur. The LPG of *L. amazonensis* (PH8 and Josefa strains) from Brazil exhibit polymorphisms in its structure. The former has 1-2 beta-glucoses and the latter 1-3 beta-galactoses in their side-chains, respectively. Our previous results indicated that LPGs from both strains could induce different NO and cytokine production in murine macrophages via TLR2 and TLR4. However, the role of LPGs from this species in the interaction with the vector *Lutzomyia migonei* is unknown. Here, we investigated whether variations in the sugar residues in the LPGs of those two strains could account for differences during in vivo and in vitro binding in *Lutzomyia migonei*. Sand flies were kept under laboratory conditions and were artificially fed on chicken skin (2×10^7 /mL). The midguts were dissected and the infections were determined on days 2 and 4 using a Newbauer chamber. After day 4, both strains were able to sustain infection in the vector. This suggested that LPG structural polymorphisms were not crucial for development of those species in this sand fly. To test if the parasite attachment could be intermediate by LPG molecules, a competitive binding experiment was developed only in Josefa strain, where midguts were previously incubated with phosphoglycans (PGs) (10 ug/mL) derived from procyclic and metacyclic forms. PGs derived from procyclic parasites were able to bind to the insect midgut and inhibit *L. amazonensis* attachment in vitro. These data also suggest the existence of an LPG-ligand for this strain in the sand fly midgut. **Supported by:** CNPq and FAPEMIG
Keywords: *Leishmania amazonensis*; *Lutzomyia migonei*; interaction

PV018 - METACYCLOGENESIS OF TRYPANOSOMA CRUZI INCLUDES STARVATION-INDUCED TRANSIENT ACCUMULATION OF MITOCHONDRIAL GENE TRANSCRIPTS

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Trypanosoma cruzi parasites causing Chagas' disease are passed between mammals by an insect vector. Within the insect, *T. cruzi* epimastigote stage cells replicate and progress through the increasingly nutrient-restricted digestive tract, differentiating to infectious, non-replicative metacyclic trypomastigotes. To better understand *T. cruzi* gene expression during insect developmental changes, we analyzed mitochondrial RNA abundances. We generated axenic cultures containing replicating epimastigotes, or cultures containing pre-adapted epimastigotes and metacyclic trypomastigotes. We observed increases in the 9S, 12S, CO1, and mature (edited) CO2, CO3, CYb, and A6 mitochondrial RNAs in cultures induced to differentiate. For only the edited CYb did this increase appear to predominate in metacyclic trypomastigotes. For many of these RNAs, abundance increases are linked to starvation and are strongest in culture fractions with a high population of pre-adapted epimastigotes. We show that loss of both glucose and amino acids results in rapid increases in RNA abundances that are quickly reduced after feeding. Furthermore, the individual RNAs exhibit distinct temporal abundance patterns, suggestive of multiple mechanisms regulating transcript abundance. Finally, increases in mitochondrial respiratory complex subunit mRNA abundances were not matched by nuclear-encoded subunit mRNAs. Epimastigotes are maintained long term between insect feedings and can continuously differentiate to infectious cells or resume replication upon insect feeding. We speculate that epimastigotes gain fitness in starvation conditions between feedings by increasing some mitochondrial transcript abundances to drive changes in metabolism.
Keywords: Mitochondria; metacyclogenesis; starvation

PV019 - DISSECTING SOCIAL MOTILITY IN AFRICAN TRYPANOSOMES BY A RNA-SEQ TRANSCRIPTOME ANALYSIS

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Microbes are mostly thought of as individualized cells but they live in groups and are able to communicate with each other and undergo social behavior. Cell-cell communication and social behavior are widespread in microbes, such as fungi or bacteria. Social behavior was recently discovered in protozoan parasites. However, its contribution to disease pathogenesis and transmission are poorly understood. African trypanosomes (*Trypanosoma brucei*) are flagellated protozoan parasites that cause sleeping sickness in humans and related diseases in livestock in Sub-Saharan Africa. *T. brucei* parasites engage in social behavior or "social motility" when cultivated on semisolid agarose surfaces. This behavior is characterized by trypanosomes assembling into multicellular communities that engage in polarized migrations across the agarose surface and cooperate to divert their movements in response to external stimuli. In bacteria, transition from planktonic conditions to a multicellular lifestyle on a surface is associated with a series of adaptations, which is reflected in changes in gene transcription. Similarly, developmental transformation from single cell to multicellular life-style in *T. brucei* is also expected to be regulated by changes in gene expression. To identify the mechanisms underlying social motility we applied a RNA-Seq transcriptome comparison of trypanosomes cultivated in suspension culture versus surface conditions. The results indicate that a set of genes is differentially regulated between the two culture conditions, suggesting that these genes might contribute to social motility. Current effort is focused on dissecting the contribution of identified genes to social motility on the molecular level using RNA interference (RNAi) knockdown approaches. The study of social behavior in microbes is expected to open new paradigms for considering host-parasite interaction and cell-cell communication, giving way to new strategies for therapeutic intervention. **Keywords:** African trypanosomes; social motility; rna sequencing

PV020 - THE ROLE OF RBP9 AND RBP10 IN THE MODULATION OF GENE EXPRESSION IN TRYPANOSOMA CRUZI

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Trypanosoma cruzi is the protozoan causative of Chagas disease and its life cycle involves two hosts and three stages of development: epimastigotes, trypomastigotes and amastigotes. The regulation of gene expression in trypanosomes differs from other eukaryotes for not having transcriptional regulation for most of their genes. The association between mRNAs and specific proteins form mRNPs complexes, which have great significance in post-transcriptional regulation. The RRM domain (RNA Recognition Motif) is present in RBP9 and RBP10 and is one of the most abundant protein domains in RNA-binding proteins. Proteins containing this domain participate in most post-transcriptional processes of regulation, such as processing, nuclear export, transport and localization of nascent mRNAs. Few RNA-binding proteins have been described so far in *T. cruzi*, so we decided to investigate the role of RBP9 and RBP10 in regulating gene expression in the parasite. Based on a phylogenetic analysis, both proteins were shown to be exclusive of trypanosomatids with orthologs in *T. brucei* and *Leishmania* sp., what qualifies them as suitable targets for drugs. RBP10 is expressed throughout the parasite's life cycle while RBP9 is regulated, not being expressed in metacyclic trypomastigotes. Analysis of ribosome footprint profile showed that RBP9 mRNA is stalled on polysomes and not being translated in this stage, confirming the expression profile and indicating that RBP9 is regulated at the mRNA-polysome level. Both RBP9 and RBP10 showed a cytoplasmatic localization along the metacyclogenesis. After sodium arsenite treatment (oxidative stress), RBP9 was detected by immunofluorescence in cytoplasmatic granules, resembling stress granules (SGs), indicating a possible role in stress response. RBP10 is associated to polysomes and the protein sedimentation profile in sucrose gradient after puromycin treatment changed to the lighter fractions, implying its role in translation. **Keywords:** *Trypanosoma cruzi*; rbp; rrm motif

TB001 - PHOTODYNAMIC THERAPY USING THE PHOTSENSITIZER ALUMINUM-CHLORIDE PHTHALOCYANINE ENTRAPPED IN PLGA NANOCAPSULES (NC-ALPC) IMPROVES CUTANEOUS LEISHMANIASIS TREATMENT BY SUBCUTANEOUS ROUTE

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Conventional therapeutics against Cutaneous Localized Leishmaniasis (LCL) are based on long-term drug administration by invasive routes, submitting patients to severe toxic effects. Localized treatments by topical and subcutaneous routes represent a great advance by its local therapeutic effect on the target tissue and to minimize systemic side effects. In this aspect, the aim of this work was to study the feasibility of local LCL treatment using the topical and subcutaneous route and photodynamic therapy (TFD). For this purpose, we used biodegradable nanocapsules of poly-(L-lactide-co-glycolide) (PLGA) entrapping the photosensitizer chloroaluminum phthalocyanine (AlPc), herein named Nc-AlPc, followed by local LED light application. Characterization of these nanocapsules showed an average size of 230 nm, zeta potential of + 13 mV and a suitable polydispersity index (P.D.I.) of 0,28. In vitro studies showed that encapsulation of AlPc increased their activity against *L. amazonensis* promastigotes and amastigotes. Furthermore, Nc-AlPc but not AlPc, was able to activate ROS production by infected and uninfected macrophages, without increasing cytotoxicity. In the mouse model of *L. amazonensis* infection of the ear, the efficacy of Nc-AlPc and free AlPc was first tested by intralesional route to maximize the TFD. Nc-AlPc significantly reduced the parasite burden by subcutaneous route. Subsequently, topical Nc-AlPc was evaluated in a gel formulation. Nc-AlPc did not reduce parasite burden by topical route compared to untreated mice. Possibly, the rigid PLGA nanocapsules did not permeate through the thickened LCL skin. In conclusion, this study indicates the new formulation Nc-AlPc as a potential CL treatment by intralesional, but not by topical route. **Supported by:**CNPq

Keywords:Photodynamic therapy; leishmania amazonensis; plga nanocapsules

TB002 - A NEW AROMATIC DIAMIDINE PRODUCES GDNA DAMAGE, DYSKINETOPLASTY AND ARRESTS *TRYPANOSOMA CRUZI* CELL CYCLE.

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Chagas disease, caused by the intracellular protozoan *Trypanosoma cruzi*, is a parasitic illness endemic in Americas affecting approximately 10 million people, representing an important public health problem. The existing chemotherapy, based on benznidazole and nifurtimox, is not considered to be ideal due to substantial toxicity and well-known poor activity on the chronic phase of the infection. Within this context, three new diamidines, MB17, MB19 and MB38, were evaluated against *T. cruzi*. All three compounds showed a dose-dependent inhibition of epimastigote growth. As MB17 exhibited the more promising IC₅₀ (0.5 µM), we focused our investigation on its mechanisms of action. Cells treated with MB17 did not show a significant increase of annexin and/or propidium iodide labeling when compared to untreated parasite, ruling out an apoptosis-like death. We could determine that MB17 targets the parasites DNA, preferentially kDNA affecting the cell cycle. In addition, MB17 caused DNA damages, with higher effect on kinetoplastid which can leads to dyskinetoplasty. Finally, non-toxic concentrations of MB17 were assayed on infected cells. The infection index was decreased by 60% and the trypomastigote release by 50% at 0.1 µM when compared to control. In synthesis, MB17 was effective against epimastigotes replication, induces parasite cell cycle arrest, produces DNA damages, especially on kDNA and decreases both infection index and trypomastigote bursting. Taking together, the results indicate that MB17 is promising compound against *T. cruzi*. **Supported by:**CNPQ,FAPESP,The Ministry of Science, Education and Sports of the Republic of Croatia

Keywords:Trypanosoma cruzi; dna/rna binder; cell cycle

TB003 - GOLDLEISH: NOVEL GOLD(I) PHOSPHINE COMPLEXES ARE HIGHLY ACTIVE AGAINST ANTIMONY-SENSITIVE AND RESISTANT *LEISHMANIA* PARASITES
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Current chemotherapy arsenal for leishmaniasis is restricted, toxic, has limited administration, efficacy and emergence of drug resistance has been reported. Auranofin, a gold(I) phosphine complex has primarily been used to treat rheumatoid arthritis. More recently, it has shown anticancer and antileishmanial activities. Inspired on drug repurposing strategy based on auranofin as prototype, we synthesized 24 novel gold(I) complexes and performed in vitro screening against amastigote forms of *Leishmania infantum* and promastigotes of *L. infantum*, *L. braziliensis* and *L. guyanensis*, etiological agents of visceral, cutaneous and mucocutaneous leishmaniasis, respectively. Antileishmanial activity was determined by luciferase activity and growth measurement in amastigotes and promastigotes, respectively. Gold(I)-based complexes were obtained using triethylphosphine [Au(PET3)Cl] and triphenylphosphine gold chloride [Au(PPh3)Cl] as precursors and 3-benzyl-1,3-thiazolidine-2-thione; 5-phenyl-1,3,4-oxadiazole-2-thione or adamantane-derived azoles as ligands. We found two Au(PET3)Cl-oxadiazole derivatives highly active against intracellular amastigotes of *L. infantum* presenting EC50 of 0.54 μ M (0.45-0.63) and 0.97 μ M (0.82-1.14), 6 times more selective to the parasites. Gold(I) phosphine complexes presented antipromastigote activity with EC50 values ranging from 0.95 to 10.6 μ M, and toxicity against THP-1 cells varying from 3.08 up to 14.4 μ M. We further investigate the antileishmanial activity of most active Au(PET3)Cl-derivatives against antimony-resistant *L. guyanensis* mutant (SbR). Complexes 4, 3, 17 and 18 were active against both sensitive and SbR line with EC50 of 1 μ M, while complexes 20 and 21 were cross resistant with RI of 2.34 and 1.5, respectively. These findings highlight the gold complexes as alternative metallodrug to be considered as topical and/or oral formulations for treatment of cutaneous and visceral leishmaniasis. Financial support: CNPq, FAPEMIG **Supported by:** CNPq; CAPES; FAPEMIG **Keywords:** Leishmania; gold; antimony resistance

TB004 - 2'-HYDROXYFLAVANONE EFFECTS IN VITRO AND IN VIVO AGAINST *LEISHMANIA AMAZONENSIS*

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Leishmaniasis is a disease that deserves attention due to the wide variety of clinical manifestations and its high annual incidence. Pure compounds obtained from plants have significant antiprotozoal activity. 2'-hydroxyflavanone is a flavanone, currently known to inhibit metastasis, vascularization and induce apoptosis in many types of cancer cells. This study evaluated the effect of 2'-hydroxyflavanone on both forms of *L. amazonensis* *in vitro*, *in silico* pharmacokinetic analysis and its effect *in vivo*. Promastigotes were treated with different concentrations of 2'-hydroxyflavanone for 9 hours and aliquots were removed from culture medium and counting every hour. 2'-hydroxyflavanone demonstrated a dose dependent inhibition profile from 3 hours of incubation with an IC₅₀ of 11 μ M, reaching 80% of inhibition at the highest concentration (96 μ M). To evaluate antiamastigote activity, peritoneal macrophages were infected with *L. amazonensis* and incubated with 2'-hydroxyflavanone (3-12 μ M) for 72 hours. 2'-hydroxyflavanone demonstrated a decrease on infected index in a dose-dependent manner with an IC₅₀ of 3.4 μ M. All the tested concentrations were not toxic to macrophages, with an IC₅₀ of 72 μ M and a selectivity index of 21.3. *In silico* analysis qualified 2'-hydroxyflavanone as a good candidate to oral treatment *in vivo* and fulfilled the Lipinski rule of five. Furthermore, oral treatment with 2'-hydroxyflavanone (50mg/kg/day) in BALB/c mice infected with *L. amazonensis* was able to control the lesion size and reduce the parasitic load. Toxicological analysis showed no change in biochemical and hematological parameters. The selective *in vitro* activity of 2'-hydroxyflavanone, together with excellent theoretical predictions of oral availability, clear decreases in parasite load and lesion size, and no observed compromises to the overall health of the infected mice encourage us to support further studies of 2'-hydroxyflavanone as a candidate for Leishmaniasis chemotherapy. **Supported by:** CNPq; CAPES; FAPERJ; IOC/FIOCRUZ **Keywords:** Leishmaniasis; 2'-hydroxyflavanone; chemotherapy

TB005 - ENCAPSULATION IN PLGA-PVP MICROPARTICLES INCREASES THE LOCAL EFFICACY OF CHALCONE CH8 AGAINST CUTANEOUS LEISHMANIASIS

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Conventional leishmaniasis treatment is given by systemic routes, producing severe generalized toxicity which is particularly unacceptable in the case of localized cutaneous leishmaniasis (CL). We have previously shown that subcutaneous implants made of biodegradable microparticles promote sustained local drug release allowing an effective 3-dose treatment with a novel antileishmanial chalcone (CH8) in mice infected with *Leishmania amazonensis*. Here, we proposed to optimize CH8 formulation by increasing its drug loading. For that, PLGA (poly lactide-co-glycolide acid) microparticles with varying polymeric matrixes were prepared by spray drying technique, as contrasting with the previous solvent evaporation technique that maximally incorporated only 10% of CH8 (10%CH8-PLGA). Two formulations (18%CH8-PLGA and 18%CH8-PLGA-PVP) were produced with increased drug loading (18% CH8), round smooth surface, 8 µm mean diameter, and packed with CH8 crystals in the inner core as seen by RAMAN microscopy. These were tested for efficacy against CL in *L. amazonensis* GFP-infected BALB/c mice. After 7 days of infection in the ear, the animals were given a single s.c. injection in the infected ear pinna with a single dose of 10%CH8-PLGA, 18%CH8-PLGA or 18%CH8-PLGA-PVP at a dose of 30 µg of CH8. Controls received the same dose of free CH8, empty PLGA, or 10 µL of PBS alone. On days 30 or 60 of infection, animals were sacrificed, the ears removed, grinded and assayed by fluorometry for instant determination of parasite loads. We found that all CH8 microparticle implants were effective in controlling parasite growth. 18%CH8-PLGA-PVP was the most effective, reducing parasite loads by 98% as compared with 79% of free CH8. These findings show that the slow-release implant was successfully optimized by using spray-drying and addition of polyvinylpyrrolidone PVP onto the polymeric matrix allowing an effective single-dose treatment of CL.

Supported by: CNPq and CAPES. Technical **Supported by:** Rapsodee Centre and the French Gala technological platform **Keywords:** Cutaneous leishmaniasis; chemotherapy; microparticles

TB006 - GPI FUSED RECOMBINANT PROTEINS: USE IN NANOTECHNOLOGY FOR CELL TARGETING, DRUG DELIVERY OR VACCINATION

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Liposomes are widely used as drug delivery systems or immunological particles for vaccines. They are versatile to be guided against specific cell/or tissues and can carry large amounts of drugs or antigens inside of it. On the other hand, there are still significant problems to be solved such as low efficiency of conjugation of the proteins responsible to guide these particles to specific cells or tissues or enable antigens to be recognized by B cells in antibody inducing vaccines. Here, we produce a recombinant system to create any desired protein with a lipophilic domain (GPI) that can be easily attached to liposomes. As a proof of principle, we used GFP_GPI for technical analyses, PfRH5_GPI as a malaria vaccine and ProtA_GPI to guide particles using antibodies attached to the ProtA moiety. For this, CHO cells were transfected with three pcDNA3-based plasmids for the production of secreted GFP_GPI, PfRH5_GPI and ProtA_GPI. Recombinant proteins were recovered from CHO cells and loaded on liposomes composed of DPPC:cholesterol (4:1). Mice were immunized with liposomes containing PfRH5_GPI on the outside. GFP_GPI was used to measure technical aspects such as efficiency of coupling and loading. ProtA_GPI on liposomes was used to guide particles to specific tissues. Here we used an antiCD4 antibody to guide particles to CD4 exposing cells. Using GFP_GPI, we showed that liposomes can be efficiently loaded and then stably maintain their load. Immunization of mice with the PfRH5_GPI liposomes resulted in antisera containing titers up to 104 in ELISA endpoint assays. Moreover, the IgG fraction in these sera blocked *P. falciparum* in vitro reinvasion at 26-30% when using 0.5mg/ml IgG. Finally, using ProtA as adaptor for CD4 specific antibodies we could guide particles specifically to CD4+ cells and much less to other cells such as CD8+ cells. Taken together, we successfully created a recombinant system for use as vaccine or guided drug/antigen delivery to specific cells.

Supported by: FAPESP, CAPES **Keywords:** Vaccine; nanotechnology; pfrh5

TB007 - ALCOHOL DEHYDROGENASE AND L-TREONINE DEHYDROGENASE ARE INVOLVED IN THE MECHANISM OF ACTION AND RESISTANCE TO BENZNIDAZOL IN TRYPANOSOMA CRUZI

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The understanding of the mechanisms of action and resistance to drugs used to treat *Trypanosoma cruzi* have focused on strategies to detect specific changes in transcripts or proteins between sensitive and resistant parasites. However, it is not enough to find differential expression of some genes to involve them in these mechanisms. The aim of our work was to confirm the role in the mechanisms of action and resistance to Benznidazol (Bz) of alcohol dehydrogenase (ADH) and L-threonine dehydrogenase (TDH) genes that were found differentially expressed between sensitive and resistant clones. This was achieved overexpressing these genes in *T. cruzi* (*T. cruzi*-ADH, *T. cruzi*-TDH) and quantifying the parasites survival by analyzing the cell cycle when they were exposed to Bz, glyoxal and hydrogen peroxide. Additionally, the analyses of expression profiles of other genes were evaluated when ADH and TDH were overexpressed. The results indicated a cytostatic effect in the parasites exposed to Bz, arresting the parasites in G2/M. Interestingly, the overexpression of ADH and TDH genes generated resistance to all of the products tested and allowed to the parasites continue its cell cycle normally. Additionally, we found that *T. cruzi*-TDH transfected parasites also overexpressed ADH gene. Our results suggest that both genes are involved in the mechanism of action and resistance to Bz. TDH could be involved in the mechanism indirectly, regulating the expression of ADH, and this latter could be detoxifying toxic products such as glyoxal, as was reported in yeast. Finally, our results indicated that functional genomics studies are necessary to know the mechanism of action of drugs. **Supported by:**Colciencias, U de A **Keywords:***Trypanosoma cruzi*; cytometry; functional genomics

TB008 - CROTOXIN DERIVED FROM CROTALUS DURISSUS TERRIFICUS STIMULATES M1 ACTIVATION PROFILE DURING LEISHMANIA AMAZONENSIS INFECTION.

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American Tegumentary Leishmaniasis (ATL) is caused by different species of the *Leishmania*. This protozoan has mechanisms to subvert the microbicidal activity of macrophages. Current indicated drugs to leishmaniasis treatment, such as pentavalent antimonials and Amphotericin B, present high cost and toxicity. Given the limited efficacy of current therapies, development of alternative treatment is essential. Thereby, animal venoms are known to exhibit a variety of pharmacological activities, including antiparasitic effect. Crotoxin (CTX) is the main component of *Crotalus durissus terrificus* venom and has several biological effects range from anti-inflammatory to anti-tumoral activities. Nevertheless, there is no report on the activity of CTX during macrophage - *Leishmania* interaction. The aim of this study was evaluate the stimulatory effect of CTX on infected host cells, analyzing its inflammatory profile during *Leishmania* interaction and treatment. Macrophages treated with 2.4 µg/mL of CTX demonstrated an increase of 2.85-fold capacity to phagocyte promastigotes after 24h when compared to untreated cells showed higher phagocytic capacity when challenged with *Leishmania* after CTX treatment. *L. amazonensis* is able to inhibit macrophage NO production. Despite this, CTX reverted this action after treatment with 2.4 and 4.8 µg/mL for 24h, increasing NO production (1.12-fold and 1.35-fold, respectively). Flow cytometry analysis demonstrated increased secretion of IL-6 and TNF-α in macrophages treated with 2.4 µg/mL (5.5- fold, 6.9-fold, respectively) and 4.8 µg/mL (6.4 - fold, 7.1-fold, respectively) compared with infected macrophage without treatment. In conclusion, CTX is able to revert *L. amazonensis* inhibitory action upon macrophage immune response, and is capable to polarize macrophage-M1 profile, which leads to a better prognostic for cutaneous leishmaniasis treatment. **Supported by:**Fapespa; UFPA; CAPES; Instituto Evandro Chagas; Instituto Butantan. **Keywords:**Crotoxin; leishmaniasis; snake venom

TB009 - THE SURFACE LANDSCAPE OF AFRICAN TRYPANOSOMES

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Surface membrane organization and composition is key to parasite growth, immune evasion and transmission, with surface-exposed proteins that represent promising therapeutic targets. For extracellular African trypanosomes, the surface is partitioned such that all endo- and exocytosis is directed through a specific membrane region, the flagellar pocket, in which it is thought the majority of invariant surface proteins reside. However, very few of these proteins have been identified, severely limiting functional studies, and hampering the development of potential treatments. Here we used an integrated biochemical, proteomic and bioinformatic strategy to identify surface components of the human parasite *Trypanosoma brucei*. This 'surfeome' contains previously known flagellar pocket proteins as well as multiple novel components, and is significantly enriched in proteins that are essential for parasite survival. Molecules with receptor-like properties are almost exclusively parasite-specific, whereas transporter-like proteins are conserved in model organisms. Validation shows that the majority of surfeome constituents are bona fide surface-associated proteins, and as expected, the majority present at the flagellar pocket. Moreover, our work represents the largest systematic analysis of trypanosome surface molecules to date and provides evidence that the cell surface is compartmentalized into three distinct domains with free diffusion of molecules in each, but selective, asymmetric traffic between. This work provides a paradigm for the compartmentalization of a cell surface and a resource for its analysis.

Supported by: Medical Research Council & Royal Society, UK

Keywords: Flagellar pocket; cell surface; esag

TB010 - IDENTIFICATION OF NEW DRUGS AGAINST CHAGAS DISEASE THROUGH A CHEMOGENOMICS DRUG REPOSITIONING STRATEGY

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Introduction: Chagas, a Neglected Tropical Disease (NTD), is caused by *Trypanosoma cruzi*, a flagellate protozoan parasite, accounting for approximately 12.000 deaths in South America alone, where the disease is endemic. Currently available treatments for Chagas diseases are scarce and toxic. Objectives: We used the paradigm of "Drug Repositioning" in order to identify treatments already available for clinical use in humans for other conditions, but that may hold potential efficacy against *T. cruzi*. Methods: We first used the TriTrypDB database to carry out a comparative genomics strategy to generate a list of therapeutic targets present in *T. cruzi*, but absent in humans, in order to increase the inhibitory selectivity and decrease the probability of toxicity to humans. Subsequently, primary sequences of proteins selected as potential therapeutic targets were used to interrogate publicly available databases, which provide synoptic data on drugs and their targets, namely DrugBank and Therapeutic Target DataBase (TTD). Results: We identified a total of 8277 potential targets exclusively present in *T. cruzi*, but absent in humans. Of these, 4761 and 983 were classified as hypothetical proteins and pseudogenes, respectively. Of the remaining 2533 proteins, 43 were found to have homologous targets potentially amenable to inhibition by a total of 37 drugs, distributed among 14 pharmacological action categories, according to DrugBank data. Regarding TTD, 32 proteins were shown to have homologous targets, which are predicted to be inhibited by a total of 59 drugs distributed among 32 categories. Conclusions: We used comparative and drug repositioning to identify several clinically approved drugs that may be efficacious against *T. cruzi*. We are now applying further bioinformatics filters in order to select the best predicted drug candidates, whose efficacy will be tested experimentally both in vitro and in vivo.

Keywords: Chagas; comparative genomics; drug repositioning

TB011 - DRUG TARGETS AND INHIBITORS OF INOSITOL PHOSPHATE METABOLISM IN KINETOPLASTID PARASITES

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Kinetoplastid drug development has been hindered by the limited knowledge of proteins or processes essential for parasite survival. We took a genetic and chemical approach using *Trypanosoma brucei* as a surrogate system to identify drug targets and inhibitors for *T. cruzi* and *Leishmania*. Knockdown of genes encoding *T. brucei* inositol phosphate and phosphatidylinositol metabolism enzymes identified genes essential for survival and mouse infection. PIP5K, PIP5Pase, IPMK and CDS were all essential for infection, whereas IMPase null cells had reduced mice infectivity. Exclusive expression of *T. cruzi* or *L. major* genes in *T. brucei* conditional nulls showed that TcIPMK, LmIPMK, and TcCDS all rescued *T. brucei* growth. However, TcPIP5K failed to complement *T. brucei* PIP5K conditional nulls, likely due to the PIP5K-specific role in VSG transcriptional control in *T. brucei*. Thus, one enzyme at each step of the pathway is essential for *T. brucei* infection and some steps are functionally conserved in related kinetoplastids. We performed affinity purification and mass spectrometry analysis of proteins that interact with the pathway metabolites IP3, IP4, PI(4,5)P2 and PI(3,4,5)P3 and identified a network of proteins enriched for cell signaling, RNA processing, protein synthesis, proteasome, traffic, and ion/solute transporters. These data imply that genetic lethality is likely due to perturbation of metabolic flux and of processes regulated by these metabolites. We screened a GlaxoSmithKline library against *T. brucei* to identify inhibitors of the pathway and found fifty inhibitors with EC50s ranging from 0.01 to 1µM. Alteration of enzyme levels by gene overexpression or elimination identified 14 inhibitors targeting various steps of the pathway. The efficacy of these inhibitors against *T. cruzi* is currently being studied. Overall, we identified new kinetoplastid drug targets and inhibitors for their chemical validation in *T. cruzi* and *Leishmania*. **Supported by:** NIAID, AHA, FAPESP **Keywords:** Trypanosoma; inhibitor; inositol metabolism

TB012 - DEVELOPMENT OF A PROTOTYPE KIT FOR MOLECULAR DIAGNOSIS AND PARASITE LOAD QUANTIFICATION OF PATIENTS WITH CHAGAS DISEASE

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Chagas disease (CD) has a prevalence of 7 million infected individuals in endemic areas, where 30-40% of patients will develop cardiomyopathy or other symptoms. Currently, serology is the gold standard for the diagnosis of CD. However, conventional and Real Time PCR are used as complementary tools for diagnosis in cases of congenital transmission, discordant serological results and for evaluating etiological treatment. In this work, we standardized a multiplex Real Time PCR TaqMan assay targeting *T. cruzi* nuclear satellite DNA and an exogenous internal amplification control (IAC). Reagents produced by IBMP/FIOCRUZ were assayed in order to generate a proof of concept for developing a prototype kit to be used for the molecular diagnosis of CD, capable to quantify *T. cruzi* load from blood samples. The standardization consisted in defining the best concentration of some components from the FIOCRUZ master mix (MgCl₂, dNTPs, ROX), as well as primers, probes and PCR cycling. Following this methodology, the quantification presented linearity between 104 to 0.5 parasite equivalents/mL with a Limit of Detection (LOD) of 0.40 Par. Eq./mL, which was not statistically different of the observed using commercial reagents. Using blood samples from 50 patients with chronic CD and 20 patients with negative serology, we found 100% sensitivity and 100% specificity in the reaction containing the FIOCRUZ reagents. Nevertheless, in order to improve the LOD, we are now developing a triplex assay by adding a set of primers and probe directed to *T. cruzi* kDNA in the reaction. Preliminary tests combining the fluorophores FAM, VIC and NED were very promising, showing amplification for the three targets, without Ct alterations, in comparison to the duplex reaction. However, the assay still needs to be further standardized. Taking together, our results indicate the quality of the present assay, even in duplex or triplex format, to be tested as a prototype kit for the molecular diagnosis of CD. **Supported by:** CNPq / FAPERJ **Keywords:** Chagas disease; molecular diagnosis; real time pcr

TB013 - A HIGH CONTENT ANALYSIS FOCUSING ON A COMPOUND ABLE OF INTERFERING WITH INTRACELLULAR DEVELOPMENT OF *TRYPANOSOMA CRUZI*

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Chagas disease has been studied for several years, nevertheless *Trypanosoma cruzi* biology remains still poorly understood in comparison to other trypanosomatids. Moreover, proteins involved in *T. cruzi* differentiation remain poorly characterized. A high-content screening against *T. cruzi* using a targeted library of 4000 anti-kinase/phosphatase compounds was performed and compounds capable of interfering with the parasite intracellular development were identified. More specifically, a determined compound may cause early intracellular cycle arrest at the amastigote stage. Therefore, the aim of this work is to make a functional characterization of such compound, thus generating chemical probe that will aid the discovery of molecular players behind the differentiation processes in *T. cruzi* and possibly generate new drug candidate for antichagasic chemotherapy. Regarding the results, a high content analysis (HCA) was performed for determination of EC₅₀, CC₅₀ and selectivity index (SI) of the compound considering different periods of incubation in the phenotypic assay. A HCA using the compound with non-infected cell was also performed in order to investigate whether there would be differences in terms of cytotoxicity between non-infected and infected cell. The compound displayed satisfactory EC₅₀ (1.39µM) and CC₅₀ (> 200µM), as well as satisfactory SI (>143). Moreover, it was not cytotoxic for both non-infected and infected cell. The specific induced phenotype of such compound has been confirmed and the next step will be to investigate possible targets, which are involved in this process. To achieve this goal, the technique Differential Scanning Fluorimetry will be performed using the current library of kinases from our laboratory. Once identified the compound's target, it will be possible to elucidate *T. cruzi* biology and the cellular mechanisms related to intracellular development of the parasite.

Supported by:CNPq

Keywords:Chagas disease; intracellular development; phenotypic hit

TB014 - TESTING PROTEIN RECOGNIZED BY RECOVERED VISCERAL LEISHMANIASES PATIENTS IN EXPERIMENTAL MODEL OF CUTANEOUS LEISHMANIASES.

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Leishmaniasis is a parasite disease caused by different species of genus *Leishmania*. Due to the lack of an effective treatment or vaccine, the search of novel antigens to confer protection is important. In this study, four proteins were identified by their reactivity with the sera from asymptomatic and recovered visceral leishmaniasis (VL) Brazilian patients' with cellular positive responses against the parasite (DTH). The objective was to analyze their protective potential in different models of cutaneous experimental leishmaniasis. For this purpose, coding regions of the enolase, S-adenosylmethionine synthetase (MAT2), alpha-tubulin and 83 kDa heat shock protein (HSP83) were cloned in prokaryotic and eukaryotic expression vectors. Different formulations of the four component vaccine were tested in a *Leishmania* major BALB/c mice experimental model (5 × 10⁴ stationary-phase promastigotes in footpads): DNA vaccine, prime-boost and recombinant protein vaccine with or without a cellular inducing adjuvant. The prophylactic properties of the first formulation were also tested against challenge of 10⁷ stationary-phase promastigotes of *Leishmania braziliensis* in the ear. In both models a very weak protection was observed; reduction of lesions but not a decrease in parasite burdens. The immune correlate of the lack of protection was the induction of a limited cellular response and the production of high titers of antibodies against vaccine constituents, being the enolase and the HSP83 the most antigenic proteins of the mixed vaccines. Testing formulations based on individual antigens will be performed trying to improve protection against *Leishmania* infection.

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Keywords:Leishmania; recombinant; vaccine

TB015 - BACTERIAL CELLULOSE BIO-CURATIVES CONTAINING DETC FOR THE TOPICAL TREATMENT OF CUTANEOUS LEISHMANIASIS CAUSED BY *LEISHMANIA BRAZILIENSIS*

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Leishmaniasis remains a worldwide public health problem. The limited therapeutic options, drug toxicity and reports of resistance reinforce the need for the development of new treatment options. Herein, we tested a topical formulation of bacterial cellulose (BC) membranes containing Diethyldithiocarbamate (DETC), a superoxide dismutase 1 inhibitor. Our hypothesis is that SOD inhibition increases intracellular superoxide levels, enhancing parasite killing. Scanning Electron Microscope analysis of BC-DETC membranes showed a homogenous distribution of DETC inside the BC membranes. In vitro release studies using cellulose acetate membranes showed that DETC is released from BC membranes at a constant rate, indicating the feasibility of topical application. Exposure of *Leishmania*-infected murine macrophages to BC-DETC resulted in a dose-dependent killing of intracellular parasites, without pronounced toxic effects to host cells. Parasite killing was associated with decreased SOD1 activity paralleled by the increased production of superoxide and pro-inflammatory mediators. Topical application of BC-DETC to dermal lesions significantly decreased ear thickness and parasite load at the infection site. Additionally, expression of IFN- γ and TNF- α was down modulated in situ, as well as in recall responses employing draining lymph node cells. BC-DETC also decreased parasite load following exposure to human macrophages infected with *L. braziliensis*, an effect reversed in the presence of anti-oxidants. These results highlight the feasibility of using BC-DETC as a topical formulation for chemotherapy of cutaneous leishmaniasis caused by *L. braziliensis*. **Supported by:**PAPES-FIOCRUZ; CNPq; CAPES **Keywords:**Cutaneous leishmaniasis ; dithiocarb; bacterial cellulose

TB016 - EFFECT OF CALPAIN INHIBITOR MDL28170 ON GROWTH, MACROPHAGE INTERACTION AND PEPTIDASES EXPRESSION OF *LEISHMANIA BRAZILIENSIS*

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Leishmania spp. are parasites of considerable medical importance. The drugs used in leishmaniasis treatment have adverse effects, high toxicity, high cost and face the emergence of resistant strains. Considering the critical roles played by peptidases in the parasite life cycle, proteolytic inhibitors are interesting compounds for alternative chemotherapy. This study is focused on calpains, which comprise a family of neutral cysteine peptidases, which are strictly dependent on calcium. Through multiple alignment of several trypanosomatids calpains, a consensus region was selected for the synthesis of a peptide for rabbits immunization. The reactivity of the antibody (anti-TriTryp-calpain) against *Leishmania braziliensis* was evaluated by Western blotting and flow cytometry. In Western blotting assays, we found that the anti-TriTryp-calpain antibody was able to recognize a 50 kDa protein. Also, by flow cytometry, molecules homologous to calpains have been identified in abundance within cells. In inhibition assays employing MDL28170, a potent and specific calpain inhibitor, it was possible to observe a dose-dependent reduction in the proliferation rate of either recently isolated promastigotes or culture-adapted strain (more than 20 passages *in vitro* in culture medium). The treatment of parasites with the inhibitor before the interaction with peritoneal macrophages led to a dose-dependent decrease in the association index and an increase in the percentage of host cells with attached parasites. Finally, MDL28170 enhanced the expression of Gp63 molecules, while Cpb and calpains were not affected. Further studies to better characterize calpains in *L. braziliensis* are in progress, aiming to add new possibilities for the exploitation of calpain inhibitors as an alternative treatment of leishmaniasis. **Supported by:**MCT/CNPq, FAPERJ, CAPES and FIOCRUZ **Keywords:**Leishmania braziliensis ; calpain; mdl28170

TB017 - EVALUATION OF CASPASES AND TREM IN SKIN BYOPSIES OF LOCALIZED LESIONS FROM PATIENTS WITH TEGUMENTARY LEISHMANIASIS IN ENDEMIC AREA IN THE STATE OF BAHIA, BRAZIL

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Leishmaniasis remains as one of the most seriously neglected tropical diseases. The pathology is caused by an intracellular parasite transmitted to mammals through the bite of *Leishmania*-infected sandflies. The host immune response has a critical role not only in protection from human leishmaniasis but also in promoting disease severity. Many studies have examined the systemic immune response in *Leishmania braziliensis* infected patients, and show that cells from patients release pro-inflammatory molecules in response to leishmania antigen. Such inflammatory response is mediated through recognition of pathogen associated molecular patterns (PAMPs) by PRRs, among them, the Triggering Receptors Expressed on Myeloid Cells (TREM), recently identified. Our group characterized as that 59 proteins in the lesions from patients with localized lesions (LC), and the bioinformatic analyses revealed that the cytotoxicity mediated by T cells and apoptosis are the main canonical pathway identified in the lesions from patients. Our hypothesis in the present study is that caspases are associated with tissue necrosis observed in the patients with LC, and that could exacerbate inflammation mediated by recognition of DAMPs, recognized by TREM. To test this hypothesis, we collected biopsies from skin lesions of patients with LC from endemic area of Bahia. It was evaluated gene expression (through qPCR) for caspases-1, -3, -8 and -9 and TREM1 and TREM2, as well for, the molecular adaptor of TREM, DAP12. Skin biopsy from health individuals were evaluated as controls. All the caspases with exception of caspase-8 were upregulated in patients with LC compared to health donors, with a >2 fold change increased of gene expression. TREM1 and its molecular adaptor DAP12 were upregulated as well in patients with LC. Further experiments will be made to correlate the pathological severity of lesions with increased expression of caspases and TREM1 in patients with LC. **Supported by:**FAPESB e CNPq **Keywords:**Leishmania braziliensis; skin; inflammation

TB018 - THE COST EFFECTIVENESS OF METHODS TO EVALUATE DRUG ACTIVITY AGAINST PLASMODIUM FALCIPARUM IN VITRO

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Malaria is an important parasitic disease, its control depends on specific treatment of patients acutely infected. New treatments are required and the evaluation of their in vitro activity, in comparison with that of commercially available antimalarial drugs, is performed through tests based on diverse methodologies. The test chosen depends on the availability of fresh *P. falciparum* blood forms maintained in continuous cultures medium (RPMI medium supplemented with human serum or albumax) (Trager & Jensen, 1976), and on young trophozoites synchronized in sorbitol. These parasites are exposed (72h) to test and control compounds in the traditional test (TT) (Rieckman et. al., 1978). The drug concentration inhibiting 50% of parasite growth (IC₅₀) is evaluated after determining parasitemia in Giemsa-stained blood smears by optical microscopy. The contact parasites-compounds may be reduced to 48h using the modified TT or the semi-automatic tests like SYBR (a fluorescent DNA intercalator) (Smilkstein M et. al., 2004); or parasite incorporation of tritiated hypoxanthine (HYPH³) (Desjardins et. al., 1979); or immunoenzymatic assays with specific monoclonal antibodies (anti-HRP II, anti-LDH). In the present work, the best cost-effective test was determined comparing the modified TT, SYBR and HYPH³ in parallel. The antimalarial drugs (chloroquine, sulfadoxine, pyronaridine and amodiaquine) and other new drugs were evaluated. The results show that the modified TT was of lowest cost, but demanded longer time for evaluation of parasitemia, and qualified labor able to differentiate dead from live parasites. The semi-automatic tests allowed quick readings with no subjectivity, however, require specific reagents, mostly imported; high-cost equipment; plus long term storage of the test plates (HYPH³ residues). The IC₅₀ values of the compounds were similar in the different methods, their choice depend on the availability of financial support, time and specialized technical help. **Supported by:**CNPq (fellowships to AUK, TPS), MS, FIOCRUZ and FAPEMIG (PIBIC fellowship to PAAR). **Keywords:**P. falciparum; test; antimalarial

**TB019 - PHYSALIS ANGULATA, A LEISHMANICIDAL AGENT, INDUCES M1
MACROPHAGES POLARIZATION**

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Leishmania (Leishmania) amazonensis is a protozoa that cause cutaneous leishmaniasis american, diseases that have high incidence in developing countries such as Latin America. The treatment used for leishmaniasis are limited due to the high cost and high toxicity. Nowadays, some natural products with immunomodulatory properties are considered an important alternative source of a new leishmanicidal agent, which includes the Physalis angulata, one plant widely used in popular medicine and in the literature is well-known leishmanicidal activity. however, it is unknown if the immunomodulatory activity of aqueous extract from roots of Physalis angulata (AEPa) contribute to promote the Leishmania death. Thus this study aims to determine immunomodulatory action the AEPa on macrophages. Bone marrow cells (BMCs) were obtained from femurs, maintained in cultures and treated with AEPa at a concentration of 100 µg/mL by 96 hours. AEPa inducing the autophagy and Akt activation during the differentiation of monocytes into macrophages. Morphological analysis showed that AEPa induces a high number of cytoplasmic projections, increase of cytoplasm and cytoskeleton alterations, characteristics observed in differentiated cells and with intense metabolism. Nitro blue tetrazolium (NBT) cytochemistry showed that only cells with small cytoplasm volume presented an increased production of superoxide anions in the group of cells treated with AEPa. In addition, cytokines detection revealed that AEPa increased TNF production, but not IL-6, IL-2 and IL-4, inducing the macrophages polarization to M1 phenotype. AEPa-treated M1 macrophage causes Leishmania death. BMCs treated with AEPa by 96 hours before interaction and 24 hours after internalization of the parasite showed a reduction of 23.8%. These results show that AEPa promotes differentiation and maturation of BMCs in M1 macrophages, inflammatory cells responsible for promoting the death of the protozoa L. (L.) amazonensis. **Supported by:**CNPq (Brazil), CAPES (Brazil), INBEB (Brazil), FAPERJ and FAPESPA. **Keywords:**Antileishmanial activity; leishmania death; physalis angulata