HP1 - **THE HOST CELL SECRETORY PATHWAY MEDIATES THE EXPORT OF *LEISHMANIA* VIRULENCE FACTORS OUT OF THE PARASITOPHOROUS VACUOLE**

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To colonize phagocytes, *Leishmania* subverts microbicidal processes through components of its surface coat that include lipophosphoglycan and the GP63 metalloprotease. How these virulence glycoconjugates are shed, exit the parasitophorous vacuole (PV), and traffic within host cells is poorly understood. Here, we show that lipophosphoglycan and GP63 are released from the parasite surface following phagocytosis and redistribute to the endoplasmic reticulum (ER) of macrophages. Pharmacological disruption of the trafficking between the ER and the Golgi hindered the exit of these molecules from the PV and dampened the cleavage of host proteins by GP63. Silencing by RNA interference of the soluble N-ethylmaleimide-sensitive-factor attachment protein receptors Sec22b and syntaxin-5, which regulate ER-Golgi trafficking, identified these host proteins as components of the machinery that mediates the spreading of Leishmania effectors within host cells. Our findings unveil a mechanism whereby a vacuolar pathogen takes advantage of the host cell's secretory pathway to promote egress of virulence factors beyond the PV. **Supported by:**CIHR
Keywords: Leishmania; parasitophorous vacuole; trafficking

HP2 - **CYCLOPHILIN 19 A NEW PLAYER IN OXIDATIVE STRESS DURING *TRYPANOSOMA CRUZI* INFECTION.**

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Cyclophilins are ubiquitous and evolutionarily conserved enzymes, known for its peptide-prolyl-cis-trans-isomerase activity. Several types of cyclophilins are expressed from prokaryotes to eukaryotes. Mammalian cyclophilin A (CyPA) is an intracellular enzyme that under stress situations has an increased expression, which also causes its secretion into the extracellular environment. The CyPA plays an important role during inflammatory processes such as cell migration, proliferation, T-cells activation and helping others processes leading to an increase of reactive oxygen species (ROS) by the interaction with the p47Phox, a subunit that regulates the NOX2 activity. Trypanosoma cruzi, the protozoan parasite that causes Chagas disease, encodes the cyclophilin 19 (TcCyP19), which is very similar to CyPA. Here we found that TcCyP19 is expressed and distributed in the parasite cytosol of all lifecycle stages. Tissue culture trypomastigotes secrete several cytosolic proteins, including TcCyP19 in the soluble form. By using TcCyP19 tagged with the HA epitope, we observed that intracellular amastigotes also release TcCyP19 in the host cell cytosol. We produced a lineage of myoblast L6 cells expressing the TcCyP19 and when measured the basal ROS levels, we found an increased amount of H2O2 production. When these cells were infected, the intracellular amastigotes showed an augmented replication profile as the same occurs in the infection with overexpressing TcCyP19 parasites in wild type L6 cells. Microscopy analyzes in mammal cells shows that TcCyP19 is found in similar areas when compared with CyPA and suggests to colocalize with p47Phox as well. Taking together, these results, we are addressing the role of TcCyP19 released by mammalian stages of T. cruzi that could have a similar role as described to CyPA and affect the pathogenesis of Chagas disease by increasing the ROS levels during the infection leading to a faster replication of the parasite in the host cell. **Supported by:**FAPESP / CAPES / CNPq
Keywords: Ciclophilin; reative oxigen species; replication

HP3 - **EVALUATION OF THE ATTENUATED AND VIRULENT *LEISHMANIA AMAZONENSIS* INFECTION AND THE CHARACTERIZATION OF ITS EXTRACELLULAR VESICLES**

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Leishmaniasis is a group of neglected diseases, endemic in tropical and subtropical areas including Brazil. Studies have shown that some species of *Leishmania* spp. are able to release antigens, virulence factors, lipids, RNA and DNA by extracellular vesicles (EVs). These EVs play an important role in the parasite-host relationship, such as: facilitating infection in experimental models, promoting immunomodulation, and allowing adaptation of the parasite to the host environment.It is known that changes in the cargo of the EVs may have an impact on the immune response and disease progression, thus EVs derived from parasites with different virulence profile (virulent and attenuated parasites) may show relevant differences in the immune response activation.This work aims to establish virulent and attenuated *Leishmania amazonensis* (M2269), to characterize their EVs and to employ purified EVs in immunization protocols. Virulent *L.amazonensis* promastigotes were obtained by consecutive and successive recovering from lesions in infected animalsand attenuated parasites were derived after long live period in culture (100 passages in culture). To evaluate the parasite attenuation, BALB/c mice were infected in the footpad with $1x10^6$ virulent or attenuated *L. amazonensis* promastigotes, the lesion size was monitored and after 7 weeks the parasite load was evaluated.A significant decrease in the footpadwere observed in animals infected with attenuated *L. amazonensis*, as compared to virulent parasites. EVs from virulent and attenuated parasites were obtained and characterized.Previous experiments using these EVs showed that animals immunized with EVs from attenuated *L.amazonensis* emulsified in Alumen adjuvant had a significant decrease in parasite load, as compared to non-immunized animals.Studying EVs released by *L. amazonensis* with different virulence profiles may help to better understand the role of these EVs in modulating the immune response and the parasite-host interaction. **Supported by:**CAPES, FAPESP, CNpq **Keywords:** Extracellular vesicles; leishmania ; immunization

HP4 - **FUNCTIONS OF THE BBSOME PROTEIN COMPLEX IN LEISHMANIA MEXICANA**

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The BBSome is a protein complex which is associated with molecular trafficking to/from primary cilia and flagella in other eukaryotes. Previous work (Price et al 2013) showed that deletion of one of the subunits, BBS1, from Leishmania major severely reduces parasite virulence in mice. We hypothesise that the Leishmania BBSome is involved in the transportation of macromolecules to the parasite cell surface. We are in the process of testing this hypothesis by analysis of transgenic parasite cell lines with disrupted BBSome function. We have targeted BBS9, a core protein subunit of the BBSome. Our data shows that knocking out the BBS9 gene in L. mexicana causes a significant decrease in cell size, flagellum length and motility in promastigotes. The ability of stationary phase promastigotes to infect THP-1 macrophages is also significantly reduced in BBS9-/- mutant lines compared to the parental line. The next steps in this work are to analyse the effect these changes have on the distribution of macromolecules on the cell surface. We are using biotinylation and streptavidin pull down of cell surface proteins, which will be analysed by mass spectrometry for differences in protein levels. **Supported by:**Medical Research Council **Keywords:** Leishmaniasis; bbsome; protein trafficking

HP5 - **ASSESSING THE REPLICATION KINETICS OF INTRACELLULAR *TRYPANOSOMA CRUZI* DURING ACUTE AND CHRONIC MURINE INFECTIONS**

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Objective: To use a bioluminescent/fluorescent T. cruzi reporter strain to monitor the replication kinetics and tissue distribution of parasites at a single-cell level during murine infections.
Results: Using the dual reporter T. cruzi strain (CL Brener), in combination with confocal microscopy, it is possible to visualise individual amastigotes present within the tissues of infected C3H mice. In parallel, the replication status of each parasite can be established by the incorporation of EdU during S-phase. Mice were injected twice with EdU over a 6 hour time period. 18 hours later we found a marked decrease in the percentage of EdU+ amastigotes between the acute (70%) and chronic (20%) stages of infection. Although there is a major reduction in the number of infected host cells during the chronic stage, this is associated with a significant increase in the number of parasites per infected cell. In the acute stage, differentiation and egress typically occurs when the number of parasites reach around 50 per cell. In contrast, during the chronic stage, nests ranging from 100-1000 parasites can be detected. In C3H mice infected with CL Brener, smooth muscle cells in the GI tract are a major site of parasite persistence, with infections of the skin and skeletal muscle higher than in other mouse-parasite strain combinations.
Conclusions: In the acute stage, parasites appear to replicate rapidly at multiple sites within the mouse, with egress occurring after a relatively small number of replication cycles. During the chronic stage, parasites are restricted to a smaller number of tissue sites, are less likely to be in S-phase, with large “mega-nests” being a feature of the infection. The implications of these findings for drug efficacy and parasite persistence will be discussed. **Supported by:**Medical Research Counsel UK
Keywords: Trypanosoma cruzi; replication; persistence

HP6 - **LEISHMANIA RNA VIRUS WORSENS INFECTION BY LIMITING NLRP3 INFLAMMASOME ACTIVATION THROUGH A TLR3/TYPE I IFN/AUTOPHAGY PATHWAY**

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Leishmania RNA virus (LRV) was described as an important virulence factor associated with the severity of mucocutaneous Leishmaniasis, a severe form of cutaneous disease caused by some species, including L. guyanensis and L. braziliensis. Activation of TLR3 by the virus was reported to be key for LRV-mediated disease exacerbation, but downstream mechanisms remain largely unexplored. Here, we combine human and mouse data to demonstrate that LRV triggers TLR3 and TRIF to induce type I IFN production, which induces autophagy. This process results in autophagy-mediated degradation of NLRP3 and ASC, thereby limiting NLRP3 inflammasome activation in macrophages. NLRP3 is important to control Leishmania replication in macrophages and in vivo and the signaling pathway triggered by LRV results in increased parasite survival and disease progression. In support to this data, we found that lesions in patients infected with LRV+ Leishmania are associated with reduced inflammasome activation and the development of the severe mucocutaneous form of the disease. Our findings mechanistically reveal an evasion mechanism triggered by LRV to worsen Leishmaniasis, identifying autophagy as an important regulator of inflammasome activation by Leishmania. This work also unravels a previously unappreciated role for TLR3 in negatively regulating inflammasome assembly and activation, advancing our knowledge in TLR3-driven regulatory mechanisms and host-pathogen interactions influencing the outcome of the disease. These findings may also account for the definition of better treatments for the severe and debilitating mucocutaneous form of Leishmaniasis. **Supported by:**FAPESP **Keywords:** Leishmania rna virus; innate immunity; inflammasome

HP7 - **TRYPANOSOMA CRUZI TRYPOMASTIGOTES TRANSMIGRATE THROUGH 3D-CULTURES BY A PARACELLULAR ROUTE IN A PROTEASE-DEPENDENT MANNER**

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The mechanisms associated to dissemination and tissue colonization in T. cruzi infection are largely speculative. Transmigration is the capacity of traverse biological barriers enabling pathogens to spread deeper into tissues. The role of transmigration in the biology of T. cruzi is still unknown. This is probably because the extremely simplicity of monolayer cultures to reproduce a tissue-mimicking environment, as well as, the extremely complexity of animal models. Three-D cultures could be an excellent alternative because they reproduce the microarchitecture of tissues, providing an environment similar to the encountered during natural infections. In this work, we used 3D-spheroids of Hela-RFP cells to analyze the transmigration ability of T. cruzi trypomastigotes (tryp) of CL Brener (highly virulent) and SylvioX10 (low virulent) strains. In previous studies we determined that CL Brener tryp transmigrate and penetrate deeply inside spheroids, while most SylvioX10 parasites remain on the superficial layers. CL Brener tryp were usually found in between cell-cell contact sites, suggesting that they are using a paracellular migration pathway. Accordingly, disruption of cell-cell contacts (tight and adherent junctions) increased tryp transmigration and invasion. On the other hand, protease inhibitors strongly blocked the transmigration process of CL Brener tryp. Finally, comparative surface proteomes of tryp from CL Brener and SylvioX10 revealed more than 200 proteins exclusively expressed on the membrane of migrant parasites. Among them, we highlight extracellular matrix (ECM) binding proteins (gp85, CRT), proteases (gp63) and flagellar motility-related proteins (FM7, RSP11). Alltogether the results indicate that transmigration of T. cruzi trypomastigotes is a multistep process, which possibly involve (1) the disruption of cell-cell junctions; (2) the degradation of ECM; (3) the interaction with components of ECM and (4) the motility of parasites. **Supported by:**PICT-2016-0108; PICT-2017-2644, ANPyCT, Argentina **Keywords:** 3d-cultures; transmigration; trypanosoma cruzi

HP8 - ***TOXOPLASMA GONDII*** **INDUCES AUTOPHAGY AND SYNCITIALIZATION IN TROPHOBLASTS**

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Congenital toxoplasmosis is acquired during primary infection of pregnant women with *Toxoplasma gondii* by the ingestion of contaminated undercooked meat or water and vegetables. Available therapies for congenital toxoplasmosis present teratogenic effects and may also lead to abortion. A newly proposed therapy to prevent vertical transmission of pathogens is based on the modulation of host cell autophagy, a physiological pathway used for degradation of abnormal cellular protein aggregates and damaged organelles. In Zika model, autophagy blockade reduced virus burden in trophoblasts, suggesting that autophagy might favor pathogens during the infection. Here, we investigate the autophagic contribution to *T. gondii* invasion and establishment in trophoblasts. BeWo trophoblast cells were infected with *T. gondii* tachyzoites for up to 24h at different parasite:cell ratios (1:1 and 3:1). The infection index (% infected cells × intracellular parasites/total number of cells) was evaluated after Giemsa staining. We found that infection is dependent on time and parasite:cell ratio. BeWo cultures infected with RH or ME49 strains were also labeled with the autophagic marker monodansylcadaverine (MDC). Both strains induced MDC staining increase in a time-dependent manner. Although ME49 infection led to a MDC fluorescence decrease at late time points, RH infected cells showed a time-dependent increase. The morphometric analysis of syncytium area demonstrated an increase in syncytialization triggered by *T. gondi*i infection. Ultrastructural analysis revealed several multilamellar bodies as well as endoplasmic reticulum profiles surrounding organelles such as mitochondrion in infected BeWo cells, suggestive of autophagy exacerbation. Our further experiments involve autophagy modulation with pharmacological inducers and inhibitors during BeWo infection. **Supported by:**Fiocruz, CAPES, FAPERJ and CNPq **Keywords:** Congenital toxoplasmosis; bewo trophoblast cell line; infection

HP9 - **AMIODARONE CONTROLS THE *TRYPANOSOMA CRUZI* INFECTION IN PREGNANT WISTAR RATS**

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Trypanosoma cruzi is the causative agent of the Chagas disease, with high prevalence and burden in Latin America. The parasite is usually transmitted through feces of infected Triatomine bugs or contaminated food or water. However, congenital transmission is also important and is related to the spread of Chagas disease in non-endemic areas. The treatment of the Chagas disease is restricted to two drugs (Benznidazole and Nifurtimox), usually toxic for the pregnant infected woman. This study aims to evaluate the potential of the amiodarone for the control of Chagas disease in pregnant Wistar rats. Amiodarone is an anti-arrhythmic drug; with potential to alleviate the arrhythmia caused by Chagas disease. The animals were infected with *T. cruzi* (Y strain) and after 3 days, allowed to mate. The pregnant animals (first day of gestation) were treated for 18 days with 30 mg/Kg/day of amiodarone. At the 18th day of treatment, the animals were euthanized and hearts, fetuses and placentas collected, as well as the peritoneal exudate and spleens. The *T. cruzi* parasite burden was evaluated in hearts, fetuses and placentas by real-time PCR and normalized using primers for rats (GAPDH primers). Macrophages were used for nitrite quantification whereas the CD45RA molecule from splenocytes was also detected by flow cytometry. Amiodarone decreased the parasite burden in 84%, 78% and 62% for hearts, placentas and fetuses, respectively. In parallel, nitrite levels and the CD45RA were maintained compared to the infected group. The decrease of parasite burden, allied to the immune response maintenance, indicated that amiodarone has an anti-*T. cruzi* pattern in pregnant rats. Our results demonstrate that amiodarone is a potential candidate to control *T. cruzi*, especially for pregnant models. **Supported by:**CNPq **Keywords:** Chagas disease; amiodarone; immune response

HP10 - **EFFECTS OF MELATONIN SUPPLEMENTATION IN PREGNANT WISTAR RATS INFECTED WITH *TRYPANOSOMA CRUZI***

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Chagas disease is a tropical illness caused by the protozoan *Trypanosoma cruzi*. The disease afflicts millions in Latin America, causing cardiac and digestive injuries in the chronic phase of the disease. Chagas disease treatment remains partially ineffective, mostly for pregnant, due to the high toxicity of the current drugs. Therefore, novel candidates for disease control are necessary. Melatonin is a hormone related to the circadian rhythm regulation, besides the antioxidant and immune-regulatory properties. Moreover, the hormone has demonstrated interesting results for the immune response against *T. cruzi* in male Wistar rats. Thus, this study aimed to evaluate the immunomodulatory effects of melatonin in pregnant Wistar rats infected with the Y strain of *T. cruzi*. Animals (six/group) were infected with the Y strain of *T. cruzi* and after 3 days allowed to mate. After the pregnancy confirmation, the animals were treated with 5 mg/Kg/day of melatonin for 18 days. At the final period of pregnancy (18th day) the animals were euthanized and the hearts, placentas and fetuses collected. Spleens and peritoneal exudate were also collected. The *T. cruzi* parasite burden quantification was performed in hearts, placentas and fetuses using Real-Time PCR, normalized with rat GAPDH primers. Macrophages and spleen cells were used for nitrite oxide (NO) quantification and CD45RA detection by Griess method and flow cytometry, respectively. Melatonin treatment decreased the parasite burden in hearts (56%), whereas in placentas and fetuses no significant alteration was observed in relation to the infected and non-treated groups. Moreover, melatonin decreased the NO levels and maintained the CD45RA cells, indicating a regulated response against *T. cruzi*. Therefore, melatonin has a limited effect in placentas and fetuses, despite the positive effects in heart and immune response. **Supported by:**CNPq **Keywords:** Chagas disease; melatonin; immune response

HP11 - **MULTIMODAL LIVE IMAGING MODELS FOR INTEGRATED ANALYSES OF *TRYPANOSOMA CRUZI* INFECTIONS AND DIGESTIVE CHAGAS DISEASE PATHOGENESIS.**

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Objectives: Gastrointestinal (GI) symptoms are found in a substantial subset of chronic Chagas disease (CD) patients, but the mechanisms and pathogenesis of this form of the disease are poorly understood. The objective of this study is to establish robust animal models of digestive CD that will allow the pathological changes, including functional impairment of GI transit, to be analysed in the context of T. cruzi infection dynamics.
Results: We established BALB/c and C3H mice as mild and severe models, respectively, of GI transit dysfunction. Using the carmine red dye tracer method, we determined total gut transit time in concert with real-time infection imaging using bioluminescent parasites. C3H mice infected with the TcI-JR strain showed a significant delay in total GI transit time at both acute and late chronic stages. We then used this model to identify the specific regions of the gut responsible for the delayed transit. Fluorescent tracers were administered at different times before sacrifice of the mice to focus on gastric emptying and intestinal transit using ex vivo imaging. There were no significant alterations in gastric emptying, but a significant proximal shift in the geometric centre of fluorescence in the intestine was observed in the infected mice compared to naïve controls. We found that the delay in gut motility localised to the ileum and the colon, which were also sites of T. cruzi infection, as revealed by co-imaging of bioluminescence.
Conclusion: The C3H:TcI-JR mouse:parasite combination provides an excellent model of digestive CD, with symptoms closely resembling key clinical manifestations in humans. The experimental tractability of this model and the multimodal imaging technique represent an innovative platform to study digestive CD pathogenesis and facilitate drug discovery.
Supported by:MRC, UK **Keywords:** Trypanosoma cruzi ; imaging; chagas disease

HP12 - **ADDRESSING THE VSG EXPRESSION HIERARCHY IN *TRYPANOSOMA BRUCEI* USING SINGLE CELL RNA SEQUENCING.**

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African trypanosomes persist extracellularly through a remarkable capacity to exchange their variant surface glycoprotein (VSG) coat through a stochastic process termed antigenic variation. *Trypanosoma brucei* has a repetoire of ~2500 *VSG* genes, most of which are organised in long subtelomeric arrays on the megabase chromosomes. Expression of a VSG relies on the positioning of the *VSG* gene within one of ~15 expression sites (ESs) by transcriptional or recombinatorial mechanisms.
VSG expression is hierarchical, as demonstrated by the ordered expression of certain subsets of VSGs across chronic infection. Thus far, hierarchy studies have only been performed at the population level and do not account for potential cell to cell heterogeneity. To study the choices made in VSG switching at the single cell level, we have developed a highly sensitive single cell RNA sequencing method that can capture > 34% of genes.
It has been shown that a DNA double-strand break (DSB) within the active ES can trigger a VSG switch. Taking advantage of this and the development of CRISPR/Cas9 tools in *T. brucei*, we have used CRISPR/Cas9 to introduce sequence-specific DSBs into the active VSG gene. Upon induction of Cas9 expression we observe rapid formation of DSBs and highly reproducible kinetics of active VSG loss, with a population negative for the starting VSG emerging after 96 hours. Using our single cell RNA-seq approach, we expect to observe the progression of VSG expression and the mechanisms of switch events in a large number of single cells at regular timepoints during the course of a DSB-induced VSG switch.
Our results will allow us to track the choices made by *T. brucei* following the induction of a VSG switch and form the basis of work which will decipher how and why the T. brucei VSG expression hierarchy occurs.
Supported by:DFG, ERC **Keywords:** Trypanosoma; vsg; scrna-seq

HP13 - **CHANGES ON THE TRIATOMA INFESTANS MICROBIOTA INFLUENCES TRYPANOSOMA CRUZI DEVELOPMENT AND ALTERS THE PARASITE INFECTIVITY TO THE MURINE MODEL**

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The influence of triatomine's gut microbiota (GM) on Trypanosoma cruzi (Tc) development and the parasite infectivity to mammalian host has not been fully understood. Former studies using experimental infection of triatomines with Tc epimastigotes reported changes in the GM composition, on the parasitemia and immune response of experimentally infected mice. Since triatomine infection in the wild occurs via ingestion of Tc trypomastigotes, in this work we have comparatively evaluated the development of a Colombian Tc strain in T. infestans (Ti) with normal and disturbed GM as well as the infectivity of the metacyclic trypomastigotes to mice. Initially, the GM from laboratory-raised Ti was characterized by sequencing of the 16S rRNA gene from samples of the anterior (A), middle (M) and posterior (P) gut portions. Disturbance of the GM was induced by adding Gentamicin and Vancomycin to ex-vivo blood meals of Ti prior infection with Tc blood trypomastigotes. Triatomines feces were examined every 15 days for the presence of trypomastigotes and had their A, M and P gut portions removed for sequencing. As expected, the abundance of the GM composition was reduced all gut portions of the experimental group, differing overtime during Tc infection. Tc development in GM-disturbed Ti occurred faster than the control group, revealing an earlier appearance and a higher number of metacyclic trypomastigotes. Experimental intradermal infection in mice using metacyclics from both Ti groups revealed lower parasitemia and mortality rates of mice infected with feces from GM-disturbed Ti. Preliminary histological evaluation of mice infected with Tc from both groups indicates no differences on the inflammatory infiltrate. Our results seem to indicate that disturbance of the Ti GM positively influences the development and metacyclogenesis rates of Tc and reduces the acute pathological effects to mammals. **Supported by:**CAPES, CNPq, FINEP
Keywords: T. infestans; gut microbiota; t. cruzi

HP14 - **DIFFERENTIAL BINDING AFFINITY OF TRYPANOSOMA CRUZI P21 PROTEIN TO SYNTHETIC LIGAND PEPTIDE AND CXCR4 RECEPTOR SUGGESTS A NOVEL INHIBITORY MECHANISM OF P21 BIOLOGICAL ACTIVITIES**

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Upon entering the vertebrate host, *Trypanosoma cruzi* secrets proteins that play a role in intracellular invasion, immune response evasion and infection establishment. Among these proteins, P21 is involved in many biological activities. Previous studies showed that P21 promotes parasite entry into host cells, increases phagocytosis activating the PI3K signaling pathway through interaction to the CXCR4 receptor. Also, P21 induced actin cytoskeleton polymerization in different cell lines and exhibited chemotactic activity. Besides, P21 inhibits blood vessel formation, interfering with endothelial cell growth. In this study, we aimed to search for P21 protein-binding peptides to inhibit the P21 biological activities. We also aimed to use structural models to predict interactions of these ligand peptides with P21 and the receptor CXCR4. We selected a P21 high-affinity peptide denominated P1 by phage display. The inhibitory role of P1 synthetic peptide was evaluated by analyzing the actin polymerization, phagocytosis, angiogenesis and chemotaxis assays using cells treated with recombinant P21 (rP21) or P1. Invasion and replication assays were also performed using macrophages and parasites pretreated with rP21 or P1 or both. Our results demonstrated that P1 inhibited the effects promoted by treatment with rP21, such as actin polymerization, phagocytosis and antiangiogenic activity. Interestingly, P1 did not block the effects of rP21 on parasite replication or macrophage chemotaxis. Furthermore, we analyzed the interaction between P1, P21, and CXCR4 using predicting protein-ligand interactions. We demonstrated that P1 amino acid residues co-interact with CXCR4 and P21. Taken together our data demonstrated that P1 has P21 inhibitory activity through P1 interference in the interaction between P21 and CXCR4. Thus, P1 may be a new tool in the search for new therapeutic targets for Chagas disease. **Supported by:**CNPq, CAPES, FAPESP
Keywords: P21 protein; synthetic peptide; therapeutic target

HP15 - **EXTRACELLULAR TRAPS PRODUCTION IN RESPONSE TO *TOXOPLASMA GONDII* BY NEUTROPHILS FROM DOMESTIC CATS, THE DEFINITIVE HOST OF THE PARASITE**

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Toxoplasma gondii is an obligate intracellular parasite responsible for toxoplasmosis, an infectious disease that affects more than 30% of the human world population, causing fatal infections in immunocompromised individuals and neonates. The cell cycle of T. gondii is complex, and involves intermediary hosts (birds and mammals), where the asexual reproduction occurs in a process called endodyogeny, and definitive hosts (felines, including domestic cats), where, besides endodyogeny, sexual reproduction takes place in enterocytes. The reason by which sexual reproduction occurs only in felines is poorly understood, but it could involve yet unidentified differences in the immune response against the parasite. Therefore, the aims of this study were to investigate whether neutrophils from cats are able to produce extracellular traps (NETs) in response to T. gondii and the mechanisms involved in NETs production. Neutrophils were isolated by density gradient from peripheral blood of healthy domestic cats and cultivated with T. gondii tachyzoites from RH or ME49 strains, or with phorbol myristate acetate (PMA) for 15, 60 or 180 minutes. dsDNA on the supernatants was quantified using the PicoGreen assay. Histone H1 and myeloperoxidase (MPO) in released NETs was detected after staining neutrophils with anti-H1 and anti-MPO and secondary fluorescent antibodies and examining under a fluorescence microscope. DNA was stained with dihydrochloride (DAPI). We found that the production of NETs increased over time in a strain-dependent manner. MPO and H1 staining was detected in DNA closely associated to parasites, entrapping and possibly killing it. By scanning electron microscopy, we observed fibers of chromatin clearly trapping the parasites. We show that T. gondii efficiently trigger the release of NETs with its classical components by feline neutrophils. The biological relevance of such phenomenon and the mechanisms otherwise implied are subject of our current investigations. **Supported by:**Fiocruz, FAPERJ, CAPES and CNPq **Keywords:** Toxoplasma gondii; neutrophil extracellular traps; domestic cat

HP16 - **THE IMMUNE CHECKPOINT LIGAND CD200 UP-REGULATION IS MEDIATED BY TLR9 SIGNALING IN *LEISHMANIA* INFECTION**

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To survive and proliferate inside macrophages, amastigotes of *Leishmania amazonensis* induce the immune checkpoint ligand CD200, which inhibits the leishmanicidal mechanism mediated by iNOS-Nitric oxide. However, the signaling pathway associated with CD200 up-regulation is so far not well known. Understanding the underlying mechanism mediated by activation of this pathway has the potential to promote new insights for the development of new parasite-control strategies. Because the first molecules recognizing pathogens are the well-known Toll-like receptors (TLRs), we hypothesized that *L. amazonensis* activates TLR-mediated signaling to induce CD200 and increase parasite virulence in the host cell. To test this hypothesis, we investigated the expression of CD200 during Leishmania infection in murine macrophages from WT and different deficient TLRs mice. Here, we show that phagocytosis-mediated internalization of *L. amazonensis* amastigotes following activation of endosomal TLR9/MyD88/TRIF signaling is critical for inducing CD200 in infected macrophages. We also demonstrate that *Leishmania* microvesicles containing DNA fragments activate TLR9-dependent CD200 expression, which inhibits the iNOS/NO pathway. These results highlight new insights for this mechanism of subversion of the innate immune response by *Leishmania* parasites. **Supported by:**CAPES; CNPq; FAPESP **Keywords:** Cd200; leishmania; tlr9

HP17 - ***TOXOPLASMA GONDII*** **REDUCES NEUROGENESIS IN NEURO2A CELLS AND REDUCES PROLIFERATION, MIGRATION AND GLIOGENESIS IN MOUSE NEUROSPHERES.**

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Toxoplasmosis is a worldwide spread zoonosis caused by *Toxoplasma gondii*. Due to the severity of the congenital transmission and its effects in the central nervous system, the aim of this study is to evaluate the impact of *T. gondii* on neurogenesis and gliogenesis using *in vitro* models. Initially we used Neuro2a cells (N2a), a neuroblastoma cell line that have the potential to differentiate into neurons upon serum withdrawal. N2a were infected with *T. gondii* tachyzoites for 1 day and were induced to differentiate. As a second approach, a model of mouse neurospheres, composed of neural progenitor cells, was also employed. The effect of infection with *T. gondii* on cell host cell proliferation of floating neurospheres was analyzed until 96 hours post infection. For differentiation assays, neurospheres were plated in glass coverslips with differentiation medium. The migrated area was calculated until 120 hours and the immunoreactivity of different markers was analyzed by immunostaining. The infection of N2a cells with *T. gondii* decreased by 69% the rate of mature neurons, as observed by neurofilament-200-positive cells. With the neurospheres differentiation assays, we observed that infection reduced the cumulative rate of migration by 12% at 120 hours. We also verified reduction on gliogenesis, as observed by filaments of GFAP (an astrocyte marker) per perimeter (74 in uninfected and 54 in infected neurospheres). Additionally, we demonstrated that *T. gondii* infection interferes in the proliferation of neural progenitor cells in floating neurospheres. Immunostaining for Ki67, marker of proliferation, was reduced 14 and 11% at 48 and 72 hpi, when compared to controls. We conclude with the results found until this moment that *T. gondii* reduces neuronal and glial differentiation, proliferation and migration of neural progenitor cells. This data will contribute for the further understating of cellular and molecular mechanisms involved in the congenital toxoplasmosis. **Supported by:**CAPES, CNPq (Edital Universal 2014, PAPES VII) and Fiocruz (INOVA Fiocruz 2018). **Keywords:** Congenital toxoplasmosis; toxoplasma gondii; cellular differentiation

HP18 - **SEMI-QUANTITATIVE INTERPRETATION OF THE DPP®-CVL TEST FOR MANAGEMENT OF DOGS IN ENDEMIC AREAS OF VISCERAL LEISHMANIASIS**

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Visceral leishmaniasis (VL) in Brazil is a zoonosis caused by Leishmania (Leishmania) infantum and constitutes an important public health problem. In the urban areas, dogs are considered the main reservoir, and their presence at the domicile is associated with human VL. We proposed the use of the DPP®-CVL-Biomanguinhos in a semi-quantitative way, correlating the intensity of serological response with parasite load and a scale management of seropositive dogs based on the intensity of the reaction from semi-quantitative or visual reading of DPP®-CVL. For this, two groups of dogs were analyzed. Group 1 included 111 animals presenting different clinical scores; these animals were euthanized by the Zoonosis Control Center according the leishmaniasis control program, considering the result of the immunofluorescence test (IFAT-CVL Biomanguinhos). Group 2 consisted only of dogs with low clinical scores, including 146 animals. Forty-five dogs from Group 2 were reevaluated after three months aside from the first test. All animals were subjected to rapid testing (DPP®CVL), and the index of reactivity (IR), corresponding to the ratio between the intensity of the band test and control band, was used to interpret the results. Parasite load was estimated for all animals. Considering dogs from Group 1, we showed that the IR determined by DPP®CVL correlated with the splenic parasitic load and was more useful to determine the level of infection compared with the evaluation of the intensity of the test band only, as commonly undertaken. Evaluation of asymptomatic individuals (Group 2) indicated that animals with high IR tended to present an unfavorable prognosis. The use of IR based on the DPP®CVL test is a simple and low-cost tool that enables the management of seropositive dogs in endemic canine visceral leishmaniasis (CVL) areas. The data presented here support the use of IR based on DPP®CVL results as a tool to support the programs for controlling visceral leishmaniasis. **Supported by:** CAPES, CNPq, NIH **Keywords:** Canine visceral leishmaniasis; dpp®cvl; index of reactivity

HP19 - **INVESTIGATION OF NONCODING RNAS IN THE PROTOZOAN PARASITE *LEISHMANIA BRAZILIENSIS***

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Leishmania braziliensis species is the causative agent of mucocutaneous leishmaniasis, a deadly form of the disease in Central and South America. Gene expression in these parasites is regulated via post-transcriptional mechanisms comprising action of cis- and trans-regulatory elements and RNA binding proteins (RBPs). In this context, noncoding RNAs must be investigated in Leishmania transcriptome, as these are currently poorly explored as regulatory factors of parasite gene expression. We present here our developed bespoke pipeline for analyzing the whole transcriptome, both coding and ncRNAs, of L. braziliensis procyclic, metacyclic and amastigote lifecycle stages. Differential expression (DE) analysis and gene ontology enrichment between lifecycle stages confirmed known patterns of gene modulation during L. braziliensis development. Our computational pipeline identified 11,372 novel candidate ncRNAs in L. braziliensis; 3,266 ncRNAs displayed DE between procyclic (PCF) and metacyclic (META) stages, 3,058 between META and amastigote (AMA) and 4,380 between AMA and PCF stages. A select group of ncRNAs was subjected to Northern blot analysis to confirm size and DE and a subgroup was functionally assessed. Knockout parasites for ncRNAs enriched in META and AMA stages displayed reduced in vitro macrophage infection, suggesting ncRNAs may be implicit in leishmaniasis. RBPs which interact with these ncRNAs were isolated and are under investigation for possible regulation roles of these novel L. braziliensis ncRNAs. This work extends and outlines the complete L. braziliensis transcriptome; improving the understanding of both coding and noncoding RNA content and regulation key to parasite survival and infection.**Supported by:**FAPESP **Keywords:** Noncoding rnas; comparative transcriptomics; gene expression regulation

HP20 - **EIF2 KINASE REGULATES STRESS GRANULES FORMATION IN T*RYPANOSOMA CRUZI* SUBMITTED TO NUTRITIENT DEPRIVATION**

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Three eukaryotic initiation factor 2 (eIF2) specific kinases are predicted in the *Trypanosoma cruzi* genome, Tck1, Tck2 and Tck3. In most eukaryotes, these kinases phosphorylate the Ser51 of eIF2α in response to different types of stresses which arrest translation initiation and induce expression of factors that prevent cellular damage. The role of these kinases is unknown in trypanosomes and here we investigated the functions of Tck1, the orthologous of the eukaryote GCN2 kinase activated by amino acid starvation. TcK1 knockout lineages were generated by Crispr/Cas9. Whereas epimastigotes didn't present any striking phenotype, when starved, showed an increased capacity to differentiate into metacyclic trypomastigotes. Despite the higher metacyclogenesis, mammalian cell invasion by tissue culture trypomastigotes obtained from Tck1 knockout parasites was largely decreased as it was the proliferation in the host cells Epimastigotes, showed less eIF2α phosphorylation, increased amounts of polysomes, resistance to nutritional stress. In parallel the amount of DHH1 containing RNA granules were significantly reduced. These results suggest that TcK1 is involved in translation control and generation of appropriate parasite responses through the parasite life cycle stage **Supported by:**FAPESP / CNPq **Keywords:** Trypanosoma cruzi; translation; kinase

HP21 - ***LEISHMANIA (LEISHMANIA) AMAZONENSIS*** **PROMASTIGOTES MODULATE B-1 CELLS ACTIVATION**

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B-1 cells are a subtype of B lymphocytes with ability to produce natural and inducible immunoglobulins, cytokines and chemokines. Our lab showed that *L.amazonensis* promastigotes were phagocyted by B-1 cells, in vitro and in vivo, but some aspects of B-1 cells activation by Leishmania parasites are not completely understood. Herein, we evaluated in B-1 cells the expression of myeloid and lymphoid genes, Toll-like receptors (TLRs), activation markers, NO and ROS productions after their in vivo contact with the parasite. Total peritoneal cells were collected after 24 or 48 hours of intraperitoneal stimulating with *L. amazonensis* promastigotes. B-1 cells from uninfected mice were used as control. CD19 + CD23 - cells were gated and CD80, CD86, CD40, MHCII, F4/80, NO and ROS expression were analyzed by flow cytometry. We did not observed changes in expression of co-stimulatory molecules, MHCII, F4/80 in the gate of B-1 cells after stimulation with the parasites. After 24 or 48 hours of infection a significant decrease in NO production were detected in B-1 cells in infected group, as compared to uninfected animals. For ROS production, we observed a significant decrease in their production only after 48 hours of infection. Purified B-1 cells were used to analyze the expression of arginase, TLRs, myeloid and lymphoid genes. We detected higher expression in TLR2, TLR6, TLR9 and arginase gene in mice intraperitoneally stimulated for 24 and 48 hours with the parasite. There was an increase in the expression of myeloid genes in B-1 cells from infected mice as compared to uninfected, suggesting the differentiation of these cells to a myeloid profile. Thus, our study suggests that *L. amazonensis* promastigotes stimulated the differentiation of B-1 cells to a myeloid profile with M2 feature. Further studies to identify additional modifications in the B-1 cells status and their involvement with the parasite pathogenesis are in progress. **Supported by:**CAPES/ FAPESP / CNPQ **Keywords:** B-1 cells; leishmania amazonesis; differeciation

HP22 - **LEISHMANIA LYPOPHOSPHOGLYCAN TRIGGERS CASPASE-11 AND THE NON-CANONICAL ACTIVATION OF THE NLRP3 INFLAMMASOME**

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The activation of innate immune receptors by Leishmania parasites is critical for the outcome of leishmaniasis, a disease that affects over 12 million people worldwide. The innate immune response against Leishmania spp., including the activation of the family of nucleotide-binding and oligomerization domain-containing leucine-rich repeats (NLRs), plays an important role on the restriction of the parasite. Although many groups have demonstrated the importance of NLRP3 during Leishmania infection, the mechanisms by which the NLRP3 inflammasome is activated is unknown. Thus, the aim of this work is to evaluate the contribution of the caspase-11-mediated non-canonical inflammasome during Leishmania infection. We demonstrate that caspase-11 is activated in response to infection by Leishmania species and trigger the non-canonical activation of the NLRP3 inflammasome, a process that accounts for host resistance to infection in macrophages and in vivo. We also identified the parasite membrane glycoconjugate Lypophosphoglycan (LPG) as the molecule involved in caspase-11 activation. Intracellular delivery of Leishmania LPG in macrophages triggers caspase-11 activation. Accordingly, infections performed with Lpg1-/- parasites reduced the caspase-11-mediated non-canonical activation of the NLRP3. But different from bacterial LPS, LPG does not physically interact with caspase-11, suggesting the participation of additional molecules/receptors in LPG-mediated caspase-11 activation. LPG is extremely down regulated in the intracellular stage of the parasite, suggesting a mechanism to avoid caspase-11 activation and parasite killing. Collectively, our findings demonstrate an important role of caspase-11 in the control of Leishmania parasites and reveal the first non-bacterial Pathogen-associated molecular pattern (PAMP) molecule involved in caspase-11 activation (de Carvalho et al, Cell Rep, 2019). **Supported by:**FAPESP **Keywords:** Leishmania; inflammasome; caspase-11

HP23 - **MAP KINASE (MAPK) ACTIVATION PROFILING INDUCED BY TRYPANOSOMA CRUZI EXTRACELLULAR AMASTIGOTES (EAS) OF DIFFERENT INFECTIVITIES**

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Mitogen-activated protein kinases (MAPKs) phosphorylate their substrates at Ser and Thr residues sites to activate or de-activate their target. There are three well-stablished MAPK pathways: ERK1/2, c-JUN N-terminal kinase (1/2/3), and p38 (α,β,δ,γ). MAPK mediates the immune response formation through synthesis of anti- and inflammatory cytokines. Trypanosoma cruzi induces MAPK activation during cell invasion, favoring its entry and survival. Extracellular amastigotes (EAs) infective forms are generated by premature infected cells disruption or by extracellular trypomastigotes differentiation. EAs of G strain are more infective than EAs of CL strain (in vitro). This study aimed to elucidate EAs-mediated MAPK activation profile and its possible effects on host immune response modulation in Hela cells. ERK1/2, EGFR and p38 inhibition seemed to reduce the invasion rate of EAs of G strain. Western blotting showed that EAs of G strain induced a two-phase phosphorylation of MEK-ERK, possibly due to its higher infectivity. Conversely, EAs of CL strain did not exhibit two-phase activation of ERK-MEK. Moreover, CL strain induced phosphorylation of only MEK isoform 2 after 15 minutes of parasite interaction. EGFR was activated in early phases of cell invasion by CL strain (adhesion), and deactivated soon after, possibly due to parasite EGF sequestration. In contrast, EAs of G strain induced EGFR phosphorylation similar to MEK-ERK (two-phase manner). RT-PCR showed that EAs of both strains induced expression of IL-6, TGF-β and TNF-α after the first hour of infection whereas the G strain demonstrated greater mRNA production for all these cytokines. While neither strain positively regulated ELK1, cFos (AP-1) had its expression induced by only G strain at the first hour of infection. In sum, both strains induced differentially MAPK phosphorylation; however, G strain generated greater immune response development compared to CL strain, in agreement to its higher infectivity. **Supported by:**CNPQ, FAPEMIG, FAPESP e Capes
Keywords: Trypanosoma cruzi; extracellular amastigotes (eas); mapks

HP24 - **PROTEOMIC ANALYSIS OF BLOODSTREAM TRYPOMASTIGOTES AND THEIR EXTRACELLULAR VESICLES REVEALS A COMPLEMENT EVASION MECHANISM THROUGH EVS SECRETION IN *TRYPANOSOMA CRUZI***

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The trypomastigote (tryp) is the bloodstream circulating stage of *Trypanosoma cruzi*, the causative agent of Chagas disease. *In vivo*, parasites are highly adapted to survive into the mammalian host and to evade the immune system, in contraposition with tryps derived from *in vitro* cultures. Despite that the set of expressed proteins of *in vivo* bloodstream tryps should be differently enough to survive in this environment, we have little information about it. In this work, we carried out a proteomic analysis of bloodstream tryps (highly infective RA strain- TcVI) and their extracellular vesicles (EVs), to search for putative virulence factors and immune interaction proteins. Tryps were purified from infected mice and secreted EVs (shedding 6 hs, 37ºC in MEM) were obtained by ultracentrifugation (100000xg; 16 hs). The same procedure was performed on non-infected mice (control). Samples (triplicates of each condition) were processed in a Q Exactive HESI-Orbitrap coupled to a nano HPLC Easy-nLC 1000. MS/MS raw data were searched against both *T. cruzi* and *Mus musculus* databases, using PatternLab for Proteomics. Only a minor core of shared proteins between tryps and EVs (18/117) were found, including several HSPs. On the other hand, most of the *T. cruzi* proteins solely found in EVs were virulence factors and proteins related to immune system evasion (TS group II and V, GP63). Interestingly, mouse proteins, mostly involved in immune surveillance (i.e. complement (23/97) and antibodies (41/97)) were exclusively found associated to EVs. These data suggest that tryps are shedding mouse proteins through EV secretion, probably to avoid their action on the parasite surface during the acute phase of infection. The knowledgement of proteins differentially expressed on bloodstream tryp and their EVs may be helpful to understand the in vivo biology of *T. cruzi*, and also to identify novel targets for vaccine or drug development. **Supported by:** ANPCyT PICT 2014-1151, PICT 2016-0108. Argentina. **Keywords:** Trypanosoma cruzi; extracellular vesicles; proteomics

PV1 - **WHY IS THE LEISHMANIA GENOME SO PLASTIC? INVESTIGATING THE MOLECULAR MECHANISMS BEHIND GENOME FLEXIBILITY IN THE KINETOPLASTID PARASITE *LEISHMANIA MAJOR***

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Leishmania sp. have unusual genomes. DNA replication is thought to initiate from a single major origin per chromosome. Transcription, initiated from strand switch regions (SSRs), is bidirectional and genes are organised into large polycistronic units lacking RNA PolII promoters. The genome also exhibits extraordinary levels of plasticity manifesting as copy number variations including aneuploidy and gene amplifications. Though the underlying mechanisms are largely unknown, this flexibility has been attributed to DNA repair processes including components of a pathway directed by the ATR kinase which is activated by replicative stress. When single stranded DNA (ssDNA) lesions form, assembly of the Replication Protein A (RPA) complex onto ssDNA occurs. The heterotrimeric 9-1-1 complex (Rad9-Rad1-Hus1) is also recruited to direct lesion repair. Here, we examine the role of this pathway in Leishmania genome plasticity by determining the interactions between the 9-1-1 complex, RPA and the genome. Using CRISPR/Cas9 to endogenously tag Hus1 and RPA1 in L. major promastigotes, we studied their enrichment on the DNA using ChIPSeq in the presence and absence of replicative stress. Our data reveals two potential sources of genome fragility in Leishmania. Firstly, under replicative stress conditions, both Hus1 and RPA1 are associated with nucleosome depleted intergenic regions. Secondly, under acute stress, broad peaks of RPA1 originating from the single major origins and spanning bidirectionally ~ 50,000 kb from this site were noted. Given the coupling of replication and transcription in Leishmania, the major origins are likely prone to machinery clashes. Such instability at major sites of DNA replication suggests Leishmania are able to resolve such conflicts efficiently. As genome plasticity is often a consequence of fragility, examining the role of these fragile sites will further our understanding of the role the ATR pathway plays in Leishmania genome plasticity. **Supported by:**FAPESP Keywords: Atr pathway; gene amplification; leishmania

PV2 - **DEFINING THE STRUCTURE AND COMPOSITION OF TRANSITION FIBRES IN THE AFRICAN TRYPANOSOME FROM A WHOLE-GENOME TAGGING SCREEN**

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Trypanosoma brucei relies on a flagellum for a number of essential processes such as motility, sensory perception and cell division. At the base of the flagellum, the microtubule-based axoneme extends from a microtubule-organising basal body. Transition fibres (TFs) are appendage structures surrounding the distal end of the basal body and are indispensable for basal body to membrane docking, compartmentalisation of the flagellum and IFT protein recruitment. The genome-wide protein localisation study, TrypTag, has identified 267 proteins that localise to the basal body region of T. brucei. We tagged each of these proteins with the mNeonGreen fluorescent protein in a cell line expressing SAS-6::mScarlet – a basal body marker. From this screen we found 40 putative TF proteins, which will be confirmed to be TF proteins by co-localisation with CEP164A. We used automated image analysis to measure the dimensions of each putative TF protein signal and the offset of each signal from SAS-6 and CEP164a. We have generated a complete protein map of the transition fibres, indicating the relative position of each protein within the structure. The study provides evidence that the protein network of the TFs is far more complex and intricate than what has been previously described in mammals. **Supported by:**Nigel Groome studenship **Keywords:** Transition fibre; basal body; trypanosoma brucei

PV3 - **THE PROTEIN KINASE ATAXIA-TELANGIECTASIA AND RAD3-RELATED (ATR) OF LEISHMANIA MAJOR MODULATES THE PARASITE RESPONSE TO REPLICATION STRESS.**

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The protein kinase Ataxia-Telangiectasia and Rad3-related (ATR) is a master regulator of the eukaryotic response to DNA injuries that is activated in response to the accumulation of single stranded DNA (ssDNA) and orchestrates checkpoint activation, cell cycle arrest, replication fork stabilization and DNA repair recruitment providing genome maintenance and stability. The conservation of the ATR pathway has not been fully explored in Leishmania, whose plastic genome presents hallmarks of instability, such as gene and chromosome copy number variation, mosaic aneuploidy and chromosome rearrangements. In our attempt to characterize the conservation of the ATR pathway in Leishmania major, we sought to investigate the expression and functional relevance of the kinase ATR in the parasite DNA metabolism. The ATR locus has been highly resistant to manipulation and attempts to select ATR-null mutants were unsuccessful suggesting this is an essential gene in L. major. Genome editing allowed the selection of heterozygous cells in which the kinase domain was edited out of one of the two ATR alleles, generating an ATR-deficient cell line (ATR+/-). ATR deficiency significantly affected not only the response to hydroxyurea-mediated replication stress, but also the pattern of accumulation and resolution of ssDNA and phosphorylated histone H2A. Endogenous tagging of ATR revealed that the kinase is mostly located in, but not restricted to, the nuclear compartment, being also found in the cytoplasm. We are currently investigating this unusual pattern of subcellular localization. We also used the ChIP-Seq approach to investigate the enrichment of ssDNA binding protein RPA1 in the presence or absence replication stress. We found that ATR kinase deficient cells had a defective recruitment of RPA1 to ssDNA, which suggested that ATR activity is required for proper RPA1 recruitment and/or stabilization of RPA1 interaction with ssDNA. **Supported by:**FAPESP **Keywords:** Dna damage response; genome stability; leishmania

PV4 - **POLYOMIC CHARACTERISATION OF POLYENE DRUG RESISTANCE IN *LEISHMANIA SPP.***

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Polyomic approaches were used to characterise polyene resistance lines of Leishmania spp. Previous work has studied multiple resistant lines against amphotericin B (AmBR) in amastigotes and promastigotes (Al-Mohammed et al. 2005). We identified genomic and transcriptomic changes related with resistance to both polyenes AmB and nystatin in AmBR lines of L. mexicana and L. infantum. Previous work from our Lab identified three genes of the sterol pathway related with AmB resistance (Mwenechanya et al., 2017; Pountain et al., 2019). Here, genomics coupled with untargeted and targeted metabolomics, identified molecular changes related with AmBR. Additionally, this study characterized mutations conserved across AmBR lines (n=12), and correlated with their sterol profiling and with their phenotype in vivo. Additionally, we screened all polyene resistant lines for cross-resistance against other antileishmanials (miltefosine, pentamidine, antimonials and paromomycin), and a new library of sterol inhibitors that were proved sterol 24-methyl-transferase-specific using enzymatic assays. **Keywords:** Drug resistance; amphotericin b; leishmania

PV5 - **NEW INSIGHTS ABOUT THE STEROL BIOSYNTHESIS PATHWAY IN TRYPANOSOMATIDS**

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Human Kinetoplastid protozoans, such as T*rypanosoma cruzi, T. brucei* and *Leishmania* spp. produce ergostane-derived sterols instead of cholesterol. However, ergosterol biosynthesis is not fully elucidated for the Trypanosomatidae family. Thus, it is assumed that other trypanosomatids, such as *Phytomonas* spp., are also able of producing ergosterol. Then, the aim of the present work is to elucidate and describe the sterol biosynthesis pathway (SBP) in P. serpens, a trypanosomatid that parasites tomatoes. For this purpose, a bioinformatics strategy were established.The KEGG database was downloaded, and the SBP genes were identified and searched using BLAST against the predicted proteins of *P. serpens*. Using this approach, ,we noted that some of the expected genes based in the reference pathway were absent. For the identification of the sterols profile, we used GC-MS in three experimental conditions: with fetal bovine serum (FBS), with delipidated FBS and without FBS. Growth curves were performed under the same described conditions. As a result, it was noted that the *P. serpens* is a very well adapted trypanosomatid in the presence and absence of exogenous sterol source. The GC-MS results demonstrated that *P. serpens* has a different sterol profile compared to other trypanosomatids, presenting ergostane-derived sterol in all conditions but, in absence of FBS, we noted the presence of C14-methylated sterols as major constituents, such as lanosterol. As a proof of concept, known inhibitors of SBP were used, such as simvastatin (HMG-CoA reductase), terbinafine (squalene epoxidase), miconazole (C14-demethylase) and amphotericin B (binds to ergosterol). Only simvastatin was able to inhibit the growth of *P. serpens.*These results suggest a paradigm change, where it is believed that all trypanosomatids depend of ergosterol for maintaining the integrity of their membranes, once P. serpens was able to survive even with methylated sterols, which are toxic for other trypanosomatids. **Supported by:**CAPES, FAPERJ, CNPq **Keywords:** Trypanosomatidae; phytomonas; sterol

PV6 - **KNOCKING OUT HISTIDINE AMMONIA-LYASE BY USING CRISPR-CAS9 ABOLISH ITS ROLE IN *TRYPANOSOMA CRUZI* BIOENERGETICS**

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The metabolism of amino acids is a major player in the survival game of trypanosomatids. Once *T. cruzi* epimastigotes metabolise the glucose available in the extracellular environment, it switches to use amino acids as the main carbon and energy source. Our lab has previously characterised the ability of *T. cruzi* epimastigotes to uptake and fully oxidize His to CO_2, which points to the existence of an active His degradation pathway in these parasites. Putative coding sequences for the enzymes responsible for the four steps of the His degradation pathway are present in *T. cruzi*'s genome. The first step of this pathway consists in non-oxidative deamination of His to urocanate, which in other organisms is catalysed by histidine ammonia-lyase (HAL). Aiming to investigate the functionality of the putative HAL coding sequence in this organism (*Tc*HAL), we generated *T. cruzi* CL Brener epimastigotes *Tc*HAL null mutants using CRISPR-Cas9 technology and characterised the impact of *Tc*HAL double knockout on parasite's survival and bioenergetics. *Tc*HAL null mutant epimastigotes showed no differences in the proliferation rate when compared to the wild type and Cas9-expressing controls. Additionally, when TcHAL null parasites were incubated with [14C(U)]-His, they did not produce considerable levels radiolabelled CO_2 when compared with the control cell lines, indicating the successful disruption of the His degradation pathway. Differently from the wild type and Cas9-expressing controls, when *Tc*HAL null mutants were recovered from nutritional stress with His, they did not show restoration of $\Delta\Psi m$ and were not able to consume O_2. However, recovery of *Tc*HAL null mutants from starvation with urocanate, promoted restoration of $\Delta\Psi m$ and O_2 consumption. Together, our data show that disruption of the putative *Tc*HAL leads to inactivation of the first step of His oxidation pathway with major consequences to the ability of the parasite to metabolise and use His for mitochondrial ATP synthesis. **Supported by:**FAPESP **Keywords:** Trypanosoma cruzi; metabolism; histidine

PV7 - **DISRUPTION OF GENES ENCODING ACTIVE TRANS-SIALIDASES USING CRISPR/CAS9 TECHNOLOGY GENERATES ATTENUATED TRYPANOSOMA CRUZI MUTANTS**

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Trans-sialidases (TSs) are Trypanosoma cruzi surface proteins responsible for transferring sialic acid from host glycoconjugates to parasite mucins. Known as a virulence factor, TS are involved in host cell invasion as well as in host immune evasion mechanisms. Besides enzymatically active TSs, the T. cruzi genome encodes more than 1,000 copies of highly polymorphic inactive TS (iTS). Active TSs (aTS) are shed by trypomastigotes and most of them have a repetitive amino acid domain at their C terminal portion, which enhances TS half-live in the blood and strongly induces anti-TS antibodies. We investigated the role of active TS by generating T. cruzi cell lines in which several aTSs genes were disrupted using CRISPR/Cas9. Epimastigotes constitutively expressing Cas9 were transfected with two different single guide RNAs targeting aTS specific sequences together with a donor DNA fragment that inserts stop codons within aTS genes. PCR analyses identified several transfected cloned cell lines in which the donor DNA was inserted at multiple TS genes, a result confirmed by sequence analyses of one cell line. Quantitative RT-PCR and western blot analysis with anti-SAPA antibodies as well as enzymatic assays showed that aTS expression was abolished in trypomastigotes from one cloned cell line (TSKO7). In vitro infection of LLCMK2 cells with TSKO7 showed decreased numbers of infected cells as well as a 4-fold reduction in the number of trypomastigotes released in the culture supernatants compared to infection with WT parasites. Infection of IFN-KO mice resulted in almost undetectable parasitemia at much later time point compared to WT parasites. Thus, a highly attenuated T. cruzi mutant, showing reduced capacity of spreading within the host tissues, were generated. Most importantly, BALB/c mice immunized with these attenuated parasites where protected against a challenge with T. cruzi Y strain, while mice injected only with PBS showed high levels of parasitemia. **Supported by:**CNPQ/FAPEMIG **Keywords:** Trans-sialidases; crispr/cas9; attenuated parasites

PV8 - ***IN VITRO* CHARACTERISATION OF THE NOVEL TRYPANOSOME MRNA DECAPPING ENZYME ALPH1**

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The 5′ ends of eukaryotic mRNAs are modified with a m7G cap as a protection against uncontrolled decay. mRNA decay is typically initiated by the shortening of the poly(A) tail, followed by degradation of the mRNA in either 5′ to 3′ or 3′ to 5′ direction. In the 5′-to-3′ decay pathway, the m7G cap is removed by the nudix domain protein Dcp2 along with a specialized multiprotein factory called the decapping complex. Several further decapping enzymes were identified, but all have nudix domains and are thus similar to Dcp2. Trypanosomes lack Dcp2 homologues and we have recently identified an ApaH-like phosphatase (TbALPH1) as the major mRNA decapping enzyme of trypanosomes. TbALPH1 is essential and fulfils all in vitro and in vivo criteria of a decapping enzyme. The trypanosome enzyme is the first ApaH like phosphatase with an assigned function and bioinformatics indicates that this mRNA decapping function is likely restricted to Kinetoplastida.
To investigate the mechanism of mRNA decapping in trypanosomes, we have started a full biochemical characterization of TbALPH1 in vitro. We demonstrate that ALPH1 is active in a wide range of buffer conditions with a preference of Mg2+ as the main divalent cation. Moreover, the decapping activity of TbALPH1 is largely independent on the exact cap structure, as ALPH1 can decap both the trypanosome-unique type 4 cap and the type 0 cap. ALPH1 has no activity towards p-Nitrophenyl Phosphate indicating that the enzyme has no phosphatase activity. Surprisingly, we found that the catalytic domain of ALPH1 is sufficient for mRNA decapping *in vitro*, suggesting that the N- and C termini, which are unique to Kinetoplastida, are not required for the enzyme activity, but may have regulatory functions *in vivo*.
Our in vitro data suggest a rather general mRNA decapping activity of TbALPH1 that is not too dependent on the exact conditions and substrates. We are currently investigating how the enzyme activity of ALPH1 is regulated in the cell. **Supported by:**Deutsche Forschungsgemeinschaft (DFG) **Keywords:** Mrna decapping; tbalph1; trypanosoma brucei

PV9 - **ANALYSIS OF BASAL BODY GENES FROM A GENOME-WIDE TAGGING SCREEN IN TRYPANOSOMA BRUCEI**

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A genome-wide protein localisation study called TrypTag has identified 350 proteins localised to the basal body region of *Trypanosoma brucei*. In order to characterise this cohort further and identify the precise location and cell cycle timings a further screen is in progress using a marker cell line expressing SAS-6:mScarlet. SAS-6 is a protein which localises to the cartwheel structure of basal bodies. Each of the candidate proteins is expressed with an mNeonGreen tag. Live cell imaging and extracted cytoskeletons of each cell line are being analysed to identify cytoskeletal and soluble basal body proteins. All proteins are being categorised depending on their localisation to structures in the basal body region. These include mature and pro-basal bodies, tether, transitional fibres, and accessory structures. To date, 192 proteins have been analysed. There are 26 transitional fibre proteins, 2 proteins which are present between the mature and pro-basal body, and 15 proteins that show a brighter signal in the mature or pro-basal bodies. Furthermore, 31 proteins display a cell-cycle dependent localisation to basal bodies. The cell-cycle regulated proteins can be further divided into categories: 4 proteins are present on the mature basal body only, 4 proteins are present on the pro basal body only, and 22 proteins are present on both the mature and pro basal body with the signal intensity changing through the cell cycle. This study provides a detailed analysis of basal body genes in *T. brucei* and identifies candidates suitable for more in-depth analysis of the biogenesis of the basal body. **Supported by:**Biotechnology and Biological Sciences Research Council (BBSRC) **Keywords:** Basal bodies; sas-6; co-localisation screen

PV10 - **IN VITRO ACTIVITY AND MODE OF ACTION OF NEW ENDOPEROXIDES AGAINST LEISHMANIA**

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Leishmaniasis is still one of the 10 Neglected Tropical Diseases and its treatment remains challenging due to the absence of satisfactory, non-toxic and unexpansive drugs. In the search for leishmanicidal compounds, a chemically diverse library of endoperoxides was designed and synthesized. This class of compounds has been previously tested against *Plasmodium* and *Leishmania* presenting promising efficacy and safety profiles. The objectives of this study were to deepen previous results in terms of cytotoxicity (J774A.1 macrophage cell line) and susceptibility of *L. infantum* and *L. donovani* parasites with an improved group of 12 synthetic endoperoxides (tetraoxanes and trioxolanes) and to provide an insight into the possible mode of action by analyzing morphological alterations, oxidative stress and events that lead to parasite death.
Results have shown that peroxides exhibited micromolar anti-*Leishmania* activity (IC50 13-792 µM for promastigotes and 9,4-425 µM for amastigotes) with low cytotoxicity towards mammalian cells. Compounds LC132, LC137, LC138 presented good activity and alterations on the morphology of *L. infantum* promastigotes with changes in flagellum and body size and cytoplasmic disorganization. LC132 showed to be active against intracellular amastigotes of both *Leishmania* species. LC138 showed the best efficacy and safety profile against intracellular amastigotes of *L. infantum* (IC50 = 23,19 µM), with a selectivity index of 22,8 and apoptosis seems to play a role on the effect of this compound. As main conclusions, these encouraging results obtained for the activity and safety of some of these endoperoxides, together with their easy access through chemical synthesis, support the relevance for further investigations, namely in vivo testing, of this class of compounds in the context of leishmaniasis therapy. **Supported by:**FCT-MCTES, Portugal: GHTM (UID/Multi/04413/2013), IF/0773/2015 (S.Cortes), CCMAR (UID/Multi/04326/20 **Keywords:** Leishmania; in vitro susceptibility; endoperoxides

TB1 - **NON-INVASIVE MONITORING OF DRUG ACTION: EXPLORING A NEW ASSAY DESIGN FOR CHAGAS' DISEASE IN VITRO DRUG DISCOVERY**

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The high number of relapses in posaconazole-treated Chagas' disease patients in the clinical phase II trial published in 2014 posed a number of questions to Chagas' disease drug discovery research: How does Trypanosoma cruzi escape the host's immune response or survive drug pressure? Are there inert, non-replicating - dormant - stages? Is replication deterministically regulated or does it happen stochastically? It became clear that the regulation of replication of T. cruzi amastigotes needs to be studied in more detail. Currently, we are exploring a new assay design. We are employing a green fluorescent T. cruzi line for live imaging. An innovative plate setup enables us to follow amastigote replication in four hour intervals over a period of 6 or 10 days. Indeed, we can follow the change in parasite numbers per host cell over time after infection. The results from the live imaging are compared to the results from imaging after fixation and nuclear staining. With these results, we can model the influence of several parameters on in vitro intracellular replication of T. cruzi. Additionally, we can follow parasite numbers per host cell over time of drug exposure. This enables us to determine the time to kill. We can also monitor the decrease of the IC50 value over time of drug exposure. This innovative assay design provides data to enrich the pharmaco-dynamic profile of drug candidates. Moreover, it builds a basis for a new approach to develop and test new models of the regulation of replication in T. cruzi. **Supported by:**Swiss National Science Foundation, Burckhardt-Bürgin-Stiftung, Freiwillige Akademische Gesellschaft **Keywords:** Trypanosoma cruzi; in vitro assay; statistical modelling