RT.01 – Translational Biology

RT01-01 - **Role of miR146a-5p in regulating macrophage plasticity during *Leishmania donovani* infection**

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Visceral leishmaniasis (VL) caused by *Leishmania donovani*, is fatal if left untreated. Therapeutics currently used have severe side effects and there is a risk of development of resistance. Thus, identification and characterization of new host directed drug therapy could be a better option. During VL, the parasite induces upregulation of M2 macrophage polarisation genes such as IL-10, arginase 1, etc., concomitantly downregulating M1 marker genes such as 1L-12a, STAT1 and NF-kB for establishing infection in the macrophage. Recently, a role of small non-coding RNAs called microRNAs in regulating such plasticity has been highlighted. However, the mechanistic insight of microRNA regulation and macrophage polarisation during *Leishmania* infection remains elusive. Here, in this study we have undertaken small RNA sequencing of BALB/c mice BMDMs infected with virulent and nonvirulent parasites. We found a differential enrichment of macrophage polarity regulating micro-RNAs including miR146a-5p during infection. miR146a encoding genes have been reported to be regulated by unique epigenetic elements called as super enhancer (SE). To our surprise, we found prolonged expression of miRNA146a-5p along with SE complex such as BRD4, P300 Acetyl transferase and RNA polymerase II during infection. Infection induced miR146a-5p promoted the M2 polarisation of macrophage by targeting TRAF6-IRAK-1-NF-kB signalling axis. Depletion of SE element, bromodomain-containing protein 4 (BRD4), in infected BMDMs, using esiBRD4 RNA showed downregulation of miRNA146a and M2 marker genes such as IL-10 and Arginase 1. Finally, through BRD4 CHIP assay, we confirmed the enhanced occupancy of miR146a-5p enhancer with SE element. Together, we found a unique regulation of M2 macrophage polarity mediator miRNA146a-5p through SE. Our study highlights the miRNA inhibitor-based therapy to overcome the issues of immune suppression which may be further developed for therapeutic interventions.

RT01-02 - **From sampling procedures to parasite identification: experiences on diagnosing leishmaniases through real time PCR / HRM**

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The relevance of leishmaniases as a public health problem in several developing countries with around 350 million people at risk of infection and around 2 million new cases every year clearly indicates the need of accurate diagnostic procedures. More than 20 *Leishmania* species are involved in human infections with a wide range of clinical manifestations and, therefore, a precise identification of the parasite is important for clinical diagnosis, treatment and follow-up management. Additionally, diagnostic tests are critical in conducting active surveillance and identifying risk factors.
PCR-based assays are the main approaches among molecular techniques currently used for the diagnosis of leishmaniases. These protocols have increased the sensitivity and specificity compared to those of the conventional approaches based on parasite culture and microscopy. The purity and integrity of DNA samples are factors that strongly influence the results of PCR-based assays. Therefore, choosing suitable procedures for obtaining DNA from patients' biopsies for diagnostic purposes is paramount, from sample collection to extraction methods. Different targets and strategies can be employed in PCR-based assays to detect, quantify and identify *Leishmania* spp. in biological samples. HRM – High Resolution Melting analyses have been described as a useful tool for diagnosing leishmaniases, with all advantages inherent to the real time PCR methodology: target detection / discrimination is achieved by monitoring the accumulation of specific products within a closed tube, with no need for sequencing or gel fractionation,

minimizing risks of environment contamination; it is also a relatively rapid, simple, sensitive, specific and low-cost method.
Here we report over 20 years of experience in diagnosing leishmaniases with the tools available in our laboratory since the advent of PCR, showing which procedures were more accurate from sample collection to the final results of molecular tests. Sampling protocols were systematically evaluated, including storage procedures and DNA purification methods for *Leishmania* spp. detection and quantification in biological samples. The efficiency of three preservation solutions, a phosphate buffer solution, an EDTA buffer solution, and 70% ethanol were compared in combination with three DNA extraction protocols: a commercial silica column kit, salting-out protein precipitation and organic extraction with phenol-chloroform. The resultant DNA was used in real-time PCR assays for the detection and quantification of parasite and host targets. The results of the optimized protocols showed that the DNA extraction method did not influence the test quality, but DNA from samples preserved with the EDTA buffer solution produced higher amounts of target amplicons. Based on these results, we concluded that samples from suspected cases of leishmaniasis to be submitted to molecular diagnostic procedures should be preferentially preserved in EDTA followed by any DNA purification. Exploring the HRM profiles of amplicons from real time PCR assays targeting heat shock protein 70 or amino acid permease 3 coding sequences revealed differences that allowed the discrimination of *Leishmania* species found in the Americas, Eurasia and Africa in samples obtained from standard promastigote culture, naturally infected phlebotomines, experimentally infected mice and clinical human samples, including fresh biopsies, paraffinized samples and smears.
The knowledge accumulated on the bench could be applied in practical situations, in which the precise identification of the parasite was a useful tool for physicians in indicating an appropriate treatment and prognosis. Here we would like to exemplify how the knowledge generated by basic research can be extrapolate from the laboratory routine to the understanding of eco-epidemiological scenarios, planning of public health actions and determining practical clinical outcomes.
***Supported by:*:** FAPESP 2018/23512-0 ***Keywords*:** Leishmania;Diagnosis;DNA.

RT01-03 - **Exploring old and new compounds in the search for new treatments for acute and chronic Toxoplasmosis**

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Toxoplasma gondii is the protozoan responsible for causing toxoplasmosis, a disease commonly associated with congenital malformations, retinochoroiditis, and encephalitis in immunocompromised individuals. It is estimated that 30% of the world's population is infected with *T. gondii*, however, the rates of infection vary according to each region. For example, Brazil is one of the countries with the highest infection rates and it is estimated that at least 50-60% of the population is infected with *T. gondii*. In addition to its high prevalence, Brazil and other South American countries also show a higher incidence of symptomatic cases and sequelae than other regions. Lesions from retinochoroiditis due to congenital toxoplasmosis are more severe and frequent in the Americas than in Europe. In Brazil, ocular toxoplasmosis is the most common cause of childhood blindness and adult posterior uveitis. Despite its high prevalence and importance, treatment for toxoplasmosis is far from ideal. For more than 60 years, the therapy of choice has been based on the administration of the combination of sulfadiazine (SDZ) and pyrimethamine (PYR). Although this combination is generally effective in the acute phase of the disease, it is related to several side effects and is ineffective in the chronic form of the disease. As well as the alternatives available to replace SDZ and PYR are also related to side effects. Indeed some patients show a reduced response to treatment, suggesting the existence of strains with less susceptibility or even resistance to the current treatment drugs. Thus, new compounds or drugs with low toxicity and active against the acute and, especially, the chronic stage of *T. gondii* are of utmost importance. With this objective, our group has been using different strategies such as drug repositioning and the investigation of new molecules to discover new potential chemical entities against acute and chronic toxoplasmosis. ***Supported by:*** CNPq Universal 408964/2018-9; UFMG PRPq ***Keywords*:** Toxoplasma gondii;repositioning;phenanthrolines.

RT01-04 - **Interferon-Gamma and Mitochondrial dysfunction in the pathogenesis of Chagas disease cardiomyopathy: therapeutic targets**

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Mitochondria are the central organelles of metabolism, and the main source of ATP. Mitochondrial dysfunction triggers severe disease, including cardiomyopathy, chronic inflammation and neurodegeneration. We will here spotlight the interplay of inflammation and mitochondrial dysfunction in Chagas disease cardiomyopathy (CCC), which has a worse prognosis than other cardiomyopathies. IFNγ -mediated inflammation and mitochondrial dysfunction play a pathogenic role. CCC patients display and increased number of IFNγ-producing T cells in peripheral blood as compared to indeterminate form patients. IFNγ is the most highly expressed cytokine in the CCC heart, and is the top upstream regulator of the transcriptome. Proteomics in CCC myocardium disclosed mitochondrial dysfunction and reduced lipid beta-oxidation pathways.Exome sequencing in families with multiple CCC cases has disclosed heterozygous rare pathogenic variants in mitochondrial and inflammatory genes segregating in CCC cases. IFNγ and TNFα stimulation of AC16 cardiomyocytes recapitulated myocardial oxidative/nitrosative stress and mitochondrial dysfunction found in CCC myocardium acting through the STAT1/NF-kB/NOS2 pathways. Cytokine-stimulated cardiomyocytes further display decreased mitochondrial ATP production and dependency of fatty acid oxidation. Treatment of cardiomyocytes with mitochondria-sparing agonists of AMPK, NRF2 and SIRT1 rescues mitochondrial membrane potential in IFNγ/TNFa-stimulated cells. Our results suggest that IFNγ and TNFa cause direct damage to cardiomyocytes' mitochondria by promoting oxidative and nitrosative stress and impairing energy production pathways, which can act as the second hit on genetically susceptible individuals and induce overt mitochondrial dysfunction with progression to CCC. Treatment with mitochondria-sparing agents might be an approach to ameliorate the progression of CCC. ***Supported by:*** FAPESP 2013/50302-3 ***Keywords*****:** Chagas disease;Interferon-gamma;Mitochondria.

RT.02 – Interactions of pathogenic trypanosomatids with their hosts

RT02-01 - **The Antioxidant systems of *Leishmania* and their role in parasite infectivity: a tale of fascinating enzymes**

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In spite of over 100 years of research, infections by *Leishmania* species continue to inflict a heavy burden on human health, affecting approximately 12 million people worldwide and resulting in severe disfigurement, disability and death. To overcome this situation, better treatment regimens are needed. Because these parasites are exposed to oxidants of both endogenous and exogenous origin, and yet they are devoid of catalase and classical glutathione-peroxidase activity, their antioxidant capacity has long been viewed as a possible target of therapy. The fact that such defenses depend on the unique thiol trypanothione, instead of the ubiquitous glutathione, further suggested that the parasite antioxidant systems could be selectively inhibited.
Inspired by the seminal work by Leopold Flohé and colleagues, back in 1997 (Nogoceke et al, Biol Chem, 378:827,1997), our group has been dissecting the mechanisms used by *Leishmania* to reduce hydroperoxides. We focused mainly on the tryparedoxin-tryparedoxin peroxidase systems, investigating their essentiality and role in *Leishmania* infectivity. This talk will provide and overview of our main findings. We will i) discuss the present picture regarding the antioxidant machinery of *Leishmania*, ii) inform on the pertinence of the different enzymes to survival of the parasite disease-causing stage, and discuss which have a higher prospect of being inactivated by drugs. Finally, we will iii) highlight unanticipated aspects of some of the components of the parasite antioxidant machinery as we found them to be much more than mere antioxidant weapons.

RT02-02 - **Studies on paromomycin resistance in *Leishmania amazonensis***

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Paromomycin (PM) is an aminoglycoside antibiotic used in the treatment of visceral leishmaniasis and also as topical agent against cutaneous leishmaniasis. This drug inhibits the translation machinery, although the mechanism of action is not completely understood in *Leishmania*. Due to the limitations of the treatment specially in Brazil and the potential of PM as an alternative drug, our aim is to identify potential genes associated with PM susceptibility and resistance in *L. amazonensis,* in order to understand the mechanism of action and identify the main targets of PM. To achieve this goal, we have generated resistant lines *in vitro* as promastigotes and intracellular amastigotes. The selection of PM resistant lines was done through three strategies: *in vitro* mutagenesis and stepwise selection in both forms of the parasite. Additionally, we have characterized *L. amazonensis* clinical isolates with differential susceptibility to PM that are intrinsically resistant and susceptible to PM. These resistant and susceptible lines to PM have their whole genome sequenced by next generation sequencing and single nucleotide polymorphisms, mutations, insertions and deletions were identified. Currently, potential genes involved in PM resistance are being functionally validated by gene knockout using CRISPR/Cas9 technology and/or gene overexpression. Among these genes, *CDPK1*, a gene that codes for a protein kinase involved in the control of translation and previously involved in PM resistance in *L. infantum*, was mutated in resistant lines selected by *in vitro* mutagenesis, but not in clinical isolates that are intrinsically resistant to PM. Gene inactivation of *CDPK1* gene confirmed its role in PM resistance in *L. amazonensis.* PM accumulation in isolates with differential susceptibility was evaluated by fluorescence microscopy and flow cytometry, using a fluorescent analog of PM. We found a direct correlation between PM susceptibility and accumulation of this drug in this species, indicating that a transporter may be involved in the resistance phenotype. Moreover, *in vivo* studies in mice infected with isolates susceptible and resistant to PM showed that the effectiveness of this drug is directly correlated with the susceptibility of the parasite *in vitro*. ***Supported by:*** FAPESP: 2016/21161-7; CNPq: 405235/2021-6; UK Research and Innovation: MR/P027989/1
***Keywords*:** Paromomycin;Drug resistance;Leishmania amazonensis.

RT02-03 - ***Trypanosoma cruzi* modulates host transcription and splicing machinery during infection**

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Parasites depend on host factors for every step of their life cycle. During infection, parasite-associated modifications occur to the host cell metabolism and morphology. *Trypanosoma cruzi*, the causative agent of Chagas Disease, lives intracellularly within host cells. However, little is known about the effect of *T. cruzi* infection on the host cell nucleus and nuclear functionality. We recently showed that *T. cruzi* modulates host transcription and splicing machinery in non-professional phagocytic cells during infection. We found that *T. cruzi* regulates host RNA polymerase II (RNAPII) in a time-dependent manner, resulting in a drastic decrease in RNAPII activity. Furthermore, host cell ribonucleoproteins associated with mRNA transcription (hnRNPA1 and A2B1) are downregulated concurrently. We reasoned that *T. cruzi* might hijack the host U2AF35 auxiliary factor, a key regulator for RNA processing, as a strategy to affect the splicing machinery activities directly. Using an adenovirus E1A pre-mRNA splicing reporter, we performed *in vivo* splicing assays revealing that intracellular *T. cruzi* directly modulates host cells by appropriating U2AF35. For the first time, our results provide evidence of a complex and intimate molecular relationship between *T. cruzi* and the host cell nucleus during infection. **Supported by:** FAPESP 2018/03677-5, CAPES, FAEPA
***Keywords*:** Trypanosoma cruzi;Splicing;host-parasite interactions.

RT02-04 - **New insights regarding the regulation of translation in trypanosomatids derived from the study of two eIF4F-like initiation complexes**

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Regulating the initiation stage of the mRNA translation, during protein synthesis, is critical for proper regulation of gene expression in most organisms and even more relevant in trypanosomatids, due to their general lack of regulation during mRNA synthesis. Most known mechanisms regulating translation involve the Initiation Factors (eIFs in eukaryotes), with several targeting the eIF4F complex, required for mRNA recognition and ribosome recruitment. eIF4F is formed by the union of eIF4E, the cap binding protein, with the large eIF4G subunit, which binds to several other eIFs and proteins partners. One or two eIF4F complexes are seen in most eukaryotes. Within trypanosomatids, however, five eIF4F-like complexes were identified by us and others, based on distinct eIF4Es and eIF4Gs. Their presence implies specific functions regulating translation and gene expression, but until recently no clear roles for specific complexes were defined. Focusing on the two best studied complexes from *Trypanosoma brucei* and *Leishmania*, based on the EIF4E4/EIF4G3 and EIF4E3/EIF4G4 subunits, we have been able to define distinct protein partners and mRNAs targets for each complex which indicate relevant differences in their mode of action. We found a limited but specific set of RNA binding proteins (RBPs) with EIF4E4/EIF4G3, with the complex preferentially supporting the translation of mRNAs encoding ribosomal proteins. Conserved phosphorylation of EIF4E4 by cell cycle regulated kinases links the regulation of translation of these mRNAs with the trypanosomatid cell-cycle and growth phase. A more diverse set of RBPs and mRNA targets was found associated with EIF4E3/EIF4G4, with EIF4E3 being targeted by post-translational modifications which vary between species and need to be better understood. Our results are compatible with both complexes being impacted by multiple signals which help define the fate of bound mRNAs and require the action of different RBPs and other proteins. ***Supported by***: FACEPE (APQ-1662-2.02/ 15), CNPq (401888/2013-4; 400789/2019-1; 310032/2019-9), CAPES/FACEPE/PNPD (APQ-0876-2.02/16).
Keywords**:** PROTEIN SYNTHESIS;GENE EXPRESSION;REGULATION.

RT.03 – Host-parasite relashionship

RT03-1 - ***In vivo* CRISPR screens identify GRA12 as a transcendent virulence factor of *Toxoplasma gondii*, including in highly virulent South American strains**

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Toxoplasma gondii is a ubiquitous Apicomplexan parasite with an incredibly vast host range that granted it the reputation of most successful parasite on Earth. *Toxoplasma* infects any nucleated cell type and any warm-blooded animal, including one third of the human population. The virulence varies widely, ranging from strains considered mildly pathogenic to virulent ones that caused several outbreaks. Isolates identified in South America (SA), particularly in Brazil, are highly associated with severe clinical symptoms in humans, including vertical transmission and ocular Toxoplasmosis, and even caused fatalities in immunocompetent individuals. The virulence factors specifically responsible for the higher virulence of SA isolates compared to strains from Europe and North America is currently unknown. *Toxoplasma*'s ability to survive within the infected cell and to protect its replicative niche, the parasitophorous vacuole, is secured by proteins secreted from the parasite into the host cell after invasion. The secretome includes dense granules (GRA) and rhoptry proteins (ROP). Recent work

has estimated their number to be over 200, the vast majority of which remains uncharacterised. Most effectors important for parasite survival within the infected cell have been shown to be either host- or parasite strain specific, such as the secreted rhoptry kinase ROP18. However, effector proteins required to colonize multiple if not all hosts, or that are in common between *Toxoplasma* lineages are largely unknown. We performed CRISPR-Cas9 genetic screens targeting all putative secreted proteins to identify factors contributing to parasite survival *in vivo* in 4 *Toxoplasma* strains of different pathogenicity, including the strain VAND which was isolated form a lethal *Toxoplasma* infection in French Guyana. We identified GRA12 as a key protein required for parasite growth in the mouse peritoneum, regardless of the host or the parasite genetic backgrounds. A CRISPR screen in IFNγ-treated murine bone marrow-derived macrophages supports a most critical function of GRA12 for promoting parasite survival within the infected cell. To confirm the results, we engineered a VAND strain that lacks the ability for non-homologous end-joining (Dku80) but retains high virulence *in vivo*. Targeted deletion of *Gra12* in the VANDDku80 strain confirms GRA12 as virulence factor *in vivo* and important to protect *Toxoplasma* from the cell-autonomous immune response, independently from the known strain-specific virulence factors ROP18. Importantly, VANDDku80 is a novel tool for research on the chronic infection, as it retains the capacity to develop tissue cysts both *in vitro* and *in vivo*. Targeted deletion of *Gra12* in other 2 Toxoplasma strains confirms its role as a virulence factor in macrophages of mice, rat and human origin, indicating a parasite strain- and species-transcendent function. Preliminary results show that GRA12 orthologs from the closely related parasites *Hammondia hammondi* and *Neospora caninum* can complement the function of TgGRA12 demonstrating a likely role beyond *Toxoplasma*. GRA12 resides inside the parasitophorous vacuole but affects recruitment of host autophagic markers, such as p62. Pull down of GRA12 identified host proteins involved in known cell autonomous restriction pathways, such as lysosome clearance and autophagy. Current investigation focuses on how GRA12 functions at the host-pathogen interface. In summary, we show that GRA12 is a strain- and species- transcendent virulence factor critical for the cell-autonomous survival of *Toxoplasma*. We also present a novel transgenic parasite strain, VANDDku80, as a research tool to study host-pathogen interaction in clinical strains from South America.

RT03-2 - ***Leishmania*-Induced Dendritic Cell Migration and Its Potential Contribution to Parasite Dissemination**

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Leishmania, an intracellular parasite species, causes lesions on the skin and in the mucosa and internal organs. The dissemination of infected host cells containing *Leishmania* is crucial to parasite survival and the establishment of infection. Migratory phenomena and the mechanisms underlying the dissemination of *Leishmania*-infected human dendritic cells (hDCs) remain poorly understood. The present study aimed to investigate differences among factors involved in hDC migration by comparing infection with visceral leishmaniasis (VL) induced by *Leishmania infantum* with diverse clinical forms of tegumentary leishmaniasis (TL) induced by *Leishmania braziliensis* or *Leishmania amazonensis* in 2D and 3D environments. Following the infection of hDCs by isolates obtained from patients with different clinical forms of *Leishmania*, the formation of adhesion complexes, actin polymerization, and CCR7 expression were evaluated. We observed increased hDC migration following infection with isolates of *L. infantum* (VL), as well as disseminated (DL) and diffuse (DCL) forms of cutaneous leishmaniasis (CL) caused by *L. braziliensis* and *L. amazonensis*, respectively. Increased expression of proteins involved in adhesion complex formation and actin polymerization, as well as higher CCR7 expression, were seen in hDCs infected with *L. infantum*, DL and DCL isolates. Together, our results suggest that hDCs play an important role in the dissemination of *Leishmania* parasites in the vertebrate host. **Supported by:** CNPq (435536/2018-4), Programa Inova Fiocruz (1131778468), NIH (AI136032) ***Keywords*:** dendritic cell;Leishmania;migration.

RT03-3 - **Innate immunity in the vasculature and the CNS during *Toxoplasma gondii* infection**

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Innate immune cells, such as monocytes, are among the first cells recruited to sites of infection and play a key role in host defense against *Toxoplasma gondii* infection. We have previously shown that *T. gondii*-infected primary human peripheral blood monocytes produce IL-1β through a Syk-PKC-δ-CARD9-MALT1-NF-κB signaling pathway, and IL-1β release requires the NLRP3 inflammasome and caspase-1 activity. To investigate a potential role of other caspases in IL-1β release, we conducted CRISPR/Cas-9 genome editing to knock out caspase-1, -4, -5, or -8 in THP-1 cells. Genetic ablation of caspase-1 or -8, but not caspase-4 or caspase-5, decreased IL-1β release during *T. gondii* infection. Furthermore, dual pharmacological inhibition of caspase-8 with IETD and RIPK1 with necrostatin-1 in primary human peripheral blood monocytes decreased IL-1β release without effecting cell viability or infection efficiency. Caspase-8 was not required for the production or cleavage of IL-1β but rather, caspase-8 inhibition led to the retention of mature IL-1β within the cells. Our data suggest that during type II *T. gondii* infection of human monocytes, caspase-8 functions in a novel gasdermin D-independent mechanism controlling IL-1β release from viable cells. This study expands on the molecular mechanisms of IL-1β release from human immune cells and on the inflammatory role of caspase-8 in host defense. Additional research from our laboratory has investigated the role of monocytes in innate immunity at the blood-brain barrier and in the central nervous system and the molecular cues guiding these cells to sites of *T. gondii* infection.

RT03-4 - **Cell invasion by Leishmania infective forms: unveiling hidden routes of infection and their possible impacts**

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Parasites of the genus Leishmania, the causative agents of the different forms of Leishmaniasis, present two distinct evolutive forms: the extracellular promastigotes and the intracellular amastigotes. During the chronic phase of the disease in mammals, the amastigotes are mainly found inside macrophages. However, several other cell types, including non-phagocytic cells, have been found harboring intracellular amastigotes *in vivo*. Regarding their invasion processes, these parasites were long considered as passive players, relying only on the phagocytic abilities of phagocytes to invade host cells. However, the fact that amastigotes are also found within non-phagocytic cells shows that infection routes other than classic phagocytosis must occur. Therefore, we tackle to investigate the process of cell entry by both promastigotes and amastigotes of *Leishmania amazonensis* in non-phagocytic cells in order to define the cell biology basis of invasion. Our results show that both evolutive forms are able to actively induce cell entry in non-phagocytes where they live, replicate and persist within typical acidic compartments rich in lysosomal markers. Nonetheless, the route of cell invasion depends on the evolutive form of the parasite involved. Our data show that promastigotes can actively induce their own entry into these cells independently of host cell actin cytoskeleton activity, thus by a mechanism that is distinct from phagocytosis. Invasion involves subversion of host cell functions such as calcium signaling and recruitment and exocytosis of host cell lysosomes involved in plasma membrane repair, similarly to what was observed during *Trypanosoma cruzi* invasion, pointing to the conservation of this mechanism in these intracellular Trypanosomatids. On the other hand, cell entry by amastigotes in non-phagocytic cells is highly dependent on host cell cytoskeleton and involves an intense, but localized, re-arrangement of host cell F-actin at the entry site. Future studies about the impact of these new infection routes allowing these parasites to hide within non-phagocytic cells and on the role of these cell types in the course of infection may provide important information about the biology of these parasites and the diseases they cause. The presence of *Leishmania* spp. amastigotes within non-phagocytic cells is an overlooked aspect of the biology of these parasites that may contain key elements to understand unsolved gaps of their biology such as drug resistance and late infection reactivation. ***Keywords*:** Leishmania spp; - Cell Invasion ;Parasite;Host Cell Interaction.

RT.04 – Next generation (not sequence!) of protozoologists

RT04-1 - Regulation of Transcription in *Leishmania* by Bromodomain Factor 5 and the CRKT complex

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Alongside other organisms in kinetoplastida, *Leishmania* have evolved an unusual genome architecture that requires RNA polymerase II transcribed genes to be expressed from polycistronic transcription units, with broad transcriptional start regions defined by histone variants and histone lysine acetylation. However, the way these chromatin marks are interpreted by the cell is not understood. Seven predicted bromodomain factors (BDF1-7), the reader modules for acetyl-lysine, were identified in the *Leishmania mexicana* genome. Cas9-driven gene deletions indicate that BDF1-5 are essential in promastigotes, whilst DiCre inducible gene deletion of the dual-bromodomain factor BDF5 identified it to be essential for both promastigotes and amastigotes. ChIP-seq assessment of BDF5s genomic distribution revealed it as highly enriched at transcriptional start sites. Using an optimised proximity proteomic and phosphoproteomic technique, XL-BioID, we defined the BDF5-proximal environment to be enriched for other bromodomain factors, histone acetyltransferase 2, and proteins essential for transcriptional activity and RNA processing. Inducible deletion of BDF5, led to a disruption of pol II transcriptional activity and global defects in gene expression. The proteins that form stable interactions with BDF5 likely represent a conserved regulator of kinetoplastid transcription (CRKT) complex, as orthologs are found in most other kinetoplastid species. Our results indicate the requirement of *Leishmania* to interpret histone acetylation marks for normal levels of gene expression and thus cellular viability. Furthermore, we have identified acetylated peptides capable of binding BDF5 bromodomains which have enabled the development of fluorescent polarisation assays to characterise binding specificity and to provide a platform for inhibitor screening assays, indicating that BDF5 might represent a potential drug target. ***Supported by:*** This work was supported by funding from GSK through the Pipeline Futures Group and a Fellowship from a Research Council United Kingdom Grand Challenges Research Funder under grant agreement 'A Global Network for Neglected Tropical Diseases' grant number MR/P027989/1. to Nathaniel Jones. This work was part-funded by the Wellcome Trust [ref: 204829] through the Centre for Future Health (CFH) at the University of York. ***Keywords*****:** Leishmania;epigenetics;bromodomains.

RT04-2 - *Giardia duodenalis* reshapes intestinal mucosal immunity to prevent tissue damage and attenuate *Toxoplasma gondii*-driven inflammation

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Enteric infections that cause diarrheal disease are the second-leading cause of death in children worldwide. Recent epidemiological studies identified a decreased incidence and severity of life-threatening diarrhea in those children co-infected with the intestinal protozoan parasite *Giardia duodenalis.* To determine precisely how *Giardia* infection ameliorates tissue damage and reshapes mucosal immunity to confer a host protective effect, we show that *Giardia* induces a robust type-2 associated cytokine response, an antigen specific Th2 immune response within the lamina propria and causes a precipitous expansion of IL-10-producing CD4 T cells, that protects the host from tissue damage. To demonstrate that this "protist" can function as a potent anti-inflammatory agent, we induced a Th1-driven/IFN-gamma-mediated lethal ileitis by co-infecting *Giardia*-infected mice with *Toxoplasma gondii*, an intestinal protozoan parasite that causes a Crohn's disease-like enteritis. We found that the presence of *Giardia* significantly reduced *Toxoplasma*-mediated inflammation in the small intestine, by downregulating the frequency of $Tbet^{+}IFN\text{-}y^{+}Foxp3^{+}IL\text{-}10^{+}$ Th1 cells. Moreover, *Giardia* induced the expansion of IL-10 producing $ST2^{+}CD4+$ T cells expressing GATA-3, which downregulated *Toxoplasma*-driven IFN-y. Recombinant IL-10 injection only reduced the frequency of Th1 cells but was not sufficient to completely revert the histopathology induced during *Toxoplasma* infection, suggesting the importance of a cellular component. We are currently examining whether *Giardia* colonization can confer protection from other inflammatory driven processes associated with, for example, DSS-induced colitis.

RT04-3 - **Disruption of a single IP6K allele alters cell morphology and leads part of the *Trypanosoma cruzi* population to quiescence**

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Inositol pyrophosphates (PP-IPs) – mainly IP_7, and IP_8 – are involved in a wide range of processes in eukaryotes. However, the mechanism of action of PP-IPs is not yet fully understood. IP_7 and IP_8 are synthesized by pathways involving the participation of IP6K and PP-IP5K kinases, respectively. Trypanosomatids have an ortholog gene for IP6K. However, our analyses suggests that these organisms lost orthologs for PP-IP5K in the evolutionary course, possibly during the transition from free-living to a parasitic lifestyle. Using the CRISPR/Cas9 approach and two rounds of 'sgRNA' transfection, we were able to disrupt the single and double alleles of IP6K in *Trypanosoma cruzi* (the causative agent of Chagas Disease), generating $IP6K^{-/+}$ and $IP6K^{-/-}$ lineages, respectively. IP6K inactivation causes several morphological effects in both lineages, such as rounding and wrinkling of the cell body, increased number of glycosomes, and mitochondrial enlargement. Notably, $IP6K^{-/-}$ lineage was unable to proliferate, and most *T. cruzi* cells died a few days after transfection, suggesting IP6K is essential to this organism. Curiously, $IP6K^{-/+}$ lineage showed a slight cell cycle arrest at G0/G1 phase. However, the arrested population showed no DNA damage. Then, after estimate the cell cycle phases length and the doubling-time, we developed a pioneering assay to measure quiescent cells based on negative EdU (5-Ethynyl-2′-deoxyuridine) labeling. The result of this assay suggests that the presence of IP6K is important to keep *T. cruzi* committed with the cell cycle. Together, our preliminary data suggests that the loss of IP6K has harmful consequences for *T. cruzi*, which points this kinase as a potential target for drug development, given that its identity relative to its human homolog is ~ 15%. Furthermore, these findings can contribute to a better understanding of the pyrophosphorylation performed by IP_7, an apparent non-enzymatic post-translational modification still little studied. **Supported by:** São Paulo Research Foundation (FAPESP)
Keywords: Inositol pyrophosphates;inositol heptakisphosphate;quiescence, Chagas disease, potential drug target.

RT04-4 - **Transcriptomic analysis revealed a conserved protein regulated by arginase activity in *Leishmania amazonensis***

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Arginase is an enzyme that uses L-arginine to produce urea and ornithine, being this last one a precursor of polyamines pathway. The importance of polyamines pathway in *Leishmania* is related to parasite replication and infectivity. Using RNA-seq technology to compare both promastigotes and axenic amastigotes of *L. amazonensis* arginase knockout and *L. amazonensis* wild type, we identified a differential transcriptomic profile and coordinate responses in the absence of arginase activity. The Top 5 list among the most differentially expressed genes revealed that the transcript of a hypothetical protein is increased 3.98-fold in parasites in the absence of arginase. The identification and characterization of hypothetical proteins is important not only for functional genomics, but also to improve the knowledge about signaling pathways, metabolism, stress response, drug resistance, as well as for the identification of new therapeutic targets. According to *in silico* analysis, this hypothetical protein is conserved among several *Leishmania* species, presents a signal peptide in the N-terminal region, no conserved domains, no transmembrane domain and an intrinsically disordered region. Despite the lack of a stable 3D structure, intrinsically disordered proteins comprise a large and functionally important class with highly flexible conformation and role in many biological processes, such as regulation of transcription, translational and signal transduction. The characterization of this protein comprises the generation of a null mutant that can provide important insights on how *Leishmania* is able to modulate the mechanisms of gene expression regulation to allow the parasite survival. **Supported by:** FAPESP (#2021/04422-3 and #2018/23512-0) and CAPES (#88887.502799/2020-00). **Keywords:** RNA-seq;differentially expressed genes;disordered protein.