# RT.01 – "OMICS" AND METABOLISM TO UNDERSTAND TRYPANOSOMATIDS BIOLOGY

## RT01.001 - **METABOLIC ADAPTATION TOWARDS METACYCLOGENESIS IN *TRYPANOSOMA CRUZI*: A TARGETED METABOLOMICS APPROACH**

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*Trypanosoma cruzi*, the etiological agent of Chagas' disease, presents a complex life cycle, involving a triatominae insect and mammals, through which it faces several alternated events of cell division and cell differentiation. Along its life cycle, the parasite is exposed to different environmental conditions, and the variation in nutrients availability is probably one of the most challenging. As for most unicellular organism, epimastigote forms of *T. cruzi*, presents both, in natural environments or in vitro culture, two growth phases: the exponential, where cells divide at a roughly constant rate in a rich-nutrient environment, and stationary, where cells faces a poorer nutrient condition and slow down or stop the cell cycle and division. Importantly, it was described that the arrest in cell division is required to metacyclogenesis, and that stationary phase can be considered as a pre-adaptative stage to the metacyclic trypomastigotes differentiation. With regard to its energy metabolism, epimastigotes can use either glucose or amino acids as energy source. During exponential growth phase, glucose is preferentially consumed, while amino acids are more consumed at the stationary phase. In this work, we initially hypothesized that *T. cruzi* epimastigotes perform a metabolic switch, when exposed to nutritional starvation. To assess this, we applied a targeted metabolomics approach to evidence the possible metabolic changes occurring in the parasites during the transition between exponential-phase epimastigotes (EPE) and stationary-phase epimastigotes (SPE). We observed that both, EPE and SPE, constitute two different populations with regard to metabolic composition, related to the parasite adaptation to variable nutrients availability. Glycolysis and most of the Krebs Cycle-related metabolites were decreased during the transition from EPE to SPE, while amino acids and related metabolites increased their levels at SPE. Histidine, a relevant amino acid in nutritional stress conditions, increases its uptake and degradation rates at SPE. Proline synthesis was increased during EPE, driving the metabolism to a pre-adaptive accumulation of this amino acid that further support the transition from SPE to metacyclic trypomastigotes. Thiol containing amino acids are important metabolites related to energy metabolism and defense against oxidative unbalance. When compared the levels of intermediate metabolites involved in different routes for cysteine biosynthesis between EPE and SPE, we found the co-existence of intermediates related with de novo synthesis of Cys and reverse transsulfuration pathway, and that intermediates were increased in SPE as compared with EPE. The results herein presented show how parasites adapt their metabolism in the transition between exponential and stationary phase of growth and how prepare it towards metacyclogenesis. This metabolic plasticity appears to be crucial for survival of the parasite in the myriad different environmental conditions to which it is exposed during its life cycle. A more detailed knowledge of their metabolic abilities will reveal key metabolic checkpoints as novel targets for future therapeutic approaches against Chagas' disease.

**Keywords:**Trypanosoma cruzi metabolism; targeted metabolomics; metabolic adaptation

RT01.002 - **QUANTITATIVE PROTEOMICS UNRAVELS TRYPANOSOMA CRUZI CHROMATIN AND HISTONE POST TRANSLATIONAL MODIFICATIONS**

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Trypanosoma cruzi alternates between replicative and non-replicative forms accompanied by a shift on global transcription levels and by changes in its chromatin architecture. As chromatin associated proteins are key regulators of many important processes in the cell, we investigated the T. cruzi chromatin proteome and compared it between replicative (epimastigote) and non-replicative (trypomastigote) forms by high-resolution mass spectrometry. Strikingly, the two parasite forms differ greatly regarding their chromatin-associated factors composition and amount. Although the nucleosome content is the same for both life forms (as seen by MNase digestion), the remaining proteins were much less detected in non-replicative forms, suggesting that they have a naked chromatin. Proteins associated to DNA proliferation, such as PCNA, RPA and DNA topoisomerases were exclusively found in the chromatin of replicative stages. On the other hand, the non-replicative stages have an enrichment of a histone H2B variant. We are currently investigating the role of this histone variant in T.cruzi by identifying its interaction patterners using both pulldown and immunoprecipitation assays. Regarding histones PTMs, we also identified and quantified many post-translational modifications in replicative and non-replicative stages of T.cruzi. We detected 44 new modifications including acetylations, monomethylations, dimethylations, trimethylations and phosphorylations. We found that replicative (epimastigote stage) contains more histone modifications than non-replicative and infective parasites (trypomastigote stage). Acetylations of lysines at histone H2A C-terminus and methylations of lysine 23 of histone H3 were found enriched in trypomastigotes. In contrast, methylations of lysine 76 of histone H3 predominates in proliferative states. Our findings provide new insights into the role of chromatin associated proteins as well as histone modifications related to the control of gene expression and cell cycle regulation in this parasite.
**Supported by:**FAPESP,CNPQ e CAPES
**Keywords:**Proteômica; cromatina; histona

RT01.003 - **CHANGES IN THE PROTEOME OF LEISHMANIA-INFECTED MACROPHAGES**

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Understanding the molecular interactions between Leishmania parasites and the host is critical to the design of strategies to control infection or to reduce pathology. The intimate and protracted nature of the amastigote:macrophage interaction implies a complex communication that will be challenging to interpret.
We are developing methods to characterise proteomic changes in macrophages that are elicited upon Leishmania infection. We have employed a pulse-chase metabolic labelling approach to highlight macrophage proteins that are induced, repressed or that show altered turnover upon infection with Leishmania.
We are exploiting this system to investigate changes in the macrophage proteome over time after infection. Comparing the proteomes of THP-1 macrophages before infectionand 24 hours after infection with Leishmania mexicana, we identified approximately 2000 proteins and collected quantitative expression data for approximately 800 of these. Amongst diverse changes in protein expression, we observed a general down-regulation of metabolic enzymes and of several HLA antigens, and an upregulation of signalling pathways and pro-apoptotic proteins. Many of the metabolic changes were seen to be reversed after 48 hours of infection but HLA antigen levels were further reduced. Our data support some previous published observations and highlight some previously unreported responses to infection. We are currently applying this approach to look at different time points, and extending our data analyses to resolve the effects of protein degradation from from protein synthesis.
In parallel, we are characterising proteins that are secreted by Leishmania promastigotes and amastigotes. We are currently aiming to exploit pulse-chase labelling to assess the impact of the Leishmania secretome on macrophage protein expression.
**Supported by:**Malaysian Government **Keywords:**Leishmania; macrophage; proteome

RT01.004 - **PURINE AND PYRIMIDINE TRANSPORTERS OF KINETOPLASTID PARASITES**

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Purine and pyrimidine nucleotides have a multitude of essential functions, in all living cells; these include the formation of the nucleic acids DNA and RNA, but also cell signalling (cAMP, cGMP, GTP), energy (ATP, NADH, NADPH, Acetyl-CoA), metabolism, glycosylation etc. Conversely, any interference with nucleotide metabolism or availability has serious consequences for the cell, and this why nucleoside analogs are highly successful chemotherapeutic agents against cancer and viral infections for instance. However, purines and pyrimidines need to cross the plasma membrane in order to enter the target cell, and in the case of intracellular parasites, must enter the host cells first. This process depends on nucleoside and nucleobase transporters, which are therefore critical both for parasite biochemistry, and for anti-parasite pharmacology. We have studied protozoan parasites for more than 20 years, including most of the major human pathogens (T. brucei, Leishmania species, T. gondii, P. falciparum, T. vaginalis and most recently T. cruzi). These display generally much higher substrate affinity than the equivalent human transporters, and many utilise the proton-motive force across the plasma membrane to energize the process. All protozoan nucleoside/base transporters that have been cloned to date belong to the Equilibrative Nucleoside Transporter (ENT) family but it is clear that some transporters belong to one or more other, unknown, transporter families. Part of our current effort is to identify these transporter families, but we are mostly working to exploit our understanding of protozoan transporters to develop new anti-parasite nucleoside analogs that are efficiently and selectively taken up by the parasites. It is hoped that this will extend nucleoside-based chemotherapy to protozoan targets.

**Keywords:**Nucleoside transporter; purine antimetabolite; chemotherapy

# RT.02 – DISSECTING CELLULAR AND MOLECULAR ASPECTS OF APICOMPLEXAN PARASITES

RT02.001 - **USING COMPARATIVE APPROACHES TO UNDERSTAND LIFE CYCLE EVOLUTION IN TOXOPLASMA GONDII**

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Hammondia hammondi is the nearest relative of Toxoplasma gondii, but unlike T. gondii is obligately heteroxenous. We have compared H. hammondi and T. gondii development in vitro and identified multiple H. hammondi-specific growth states. Despite replicating slower than T. gondii, H. hammondi is resistant to pH-induced tissue cyst formation early after excystation. However, in the absence of stress H. hammondi spontaneously and fully converts to a terminally differentiated tissue cyst stage while T. gondii does not. Despite previous reports that H. hammondi cannot be subcultured, we found that it is capable of infect new host cells in vivo and in vitro for up to 8 days following excystation. We exploited this period of time to generate the first stably transgenic H. hammondi parasites expressing a dsRED transgene. Based on the first RNAseq analysis of H. hammondi parasites during in vitro development, we find that H. hammondi expresses a number of typical bradyzoite genes at higher levels than T. gondii at early and late time points post-excystation, and also expresses a subset of merozoite-specific transcripts that are not found to be expressed in T. gondii asexual stages. Coupled with data also showing dramatic differences in the host response to H. hammondi compared to T. gondii, our data clearly show that H. hammondi zoites grow as stringently regulated life stages that are fundamentally distinct from T. gondii tachyzoites and bradyzoites. We are now investigating the genetic basis for these phenotypic differences using comparative and functional genomic approaches. **Supported by:**National Institutes of Health (*USA*)
**Keywords:**Toxoplasma ; hammondia hammondi; life cycle

RT02.002 - **UNRAVELING THE COMPOSITION AND FUNCTION OF THE TOXOPLASMA GONDII INNER MEMBRANE COMPLEX**

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The Toxoplasma inner membrane complex (IMC) is a peripheral membrane system that is composed of flattened alveolar sacs that underlie the plasma membrane, coupled to a supporting cytoskeletal network. The IMC plays important roles in parasite replication, motility, and host cell invasion. Despite these central roles in the biology of the parasite, the proteins that constitute the IMC are largely unknown. We have adapted a technique named proximity-dependent biotin identification (BioID) for use in T. gondii to identify novel components of the IMC. Using IMC proteins in both the alveoli and the cytoskeletal network as bait, we have uncovered over 45 new IMC proteins in both of these suborganellar compartments. Importantly, labeling of IMC proteins using this approach has revealed a group of proteins that we named IMC suture components (ISCs) that localize to the transverse and longitudinal sutures of the alveolar sacs, which have only been previously seen in Toxoplasma by freeze fracture electron microscopy. One of these ISC proteins (ISC6) is predicted to have severe phenotypic defects by a Toxoplasma genome wide CRISPR screen. Consistent with this data, genetic knockout of ISC6 by CRISPR/Cas9 induced homologous recombination results in gross defects during parasite replication and significant extracellular morphological defects. Additionally co-localization experiments with other membrane ISC proteins suggest that ISC6 is a key player in organizing membrane suture proteins in this IMC subcompartment. Collectively, our study greatly expands the repertoire of known proteins in the IMC and provides new insight into the function of the IMC sutures, a novel subcompartment of the Toxoplasma IMC. **Supported by:**National Institutes of Health **Keywords:**Toxoplasma gondii; inner membrane complex; bioid

RT02.003 - **HISTONE VARIANTS AND EPIGENETIC MODULATION OF CHROMATIN IN TOXOPLASMA GONDII**

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Toxoplasma gondii is a coccidian protozoan parasite that belongs to the phylum Apicomplexa. It is estimated that toxoplasmosis exists as a chronic asymptomatic form in 5 hundred million to 1 billion of the world human population. Although infection with T. gondii is usually asymptomatic in most individuals, it is of great medical significance for pregnant women and immunocompromised patients. In human and other mammals, T. gondii infection is characterized by two stages, the rapidly growing tachyzoites, and the latent bradyzoite tissue cysts. These two developmental stages are essential for disease propagation and causation. Tachyzoite to bradyzoite conversion, and vice-versa, includes a high number of gene expression modifications. It is believed that the epigenetic control of gene regulation is crucial for parasite development, a process that relies on the post-translational modification (PTM) of histones and histone variant exchange. T. gondii possesses the four canonical histones H2A, H2B, H3 and H4 and variant histones of H3 (H3.3) and H2A (H2A.Z and H2A.X) families. Interestingly, T. gondii has a variant of H2B, that has been named H2B.Z since it dimerizes mainly with H2A.Z. Double variant H2A.Z/H2B.Z nucleosome and H2A.X/H2Ba are not present in the same genomic regions as it was observed by ChIP-qPCR and ChIP-seq. Moreover, H2A.X is enriched at Telomeric Associates Sequences. These findings reveal that nuclesomal arrangements are not random in protozoa, highlighting their relevance in chromatin composition and regulation. H2A.Z and H2B.Z have shown to be highly acetylated at their N-terminal tails, a marker of active chromatin. The over-expression of different H2B.Z mutants, that are unable to acetylate the N-tail, have shown an alteration in the differentiation process. On the other hand, proteomic analysis confirms the presence of □H2A.X in normal conditions suggesting that tachyzoites may be subjected to fork collapse and DSB, situations that activate the homologous recombination repair machinery. H2A.X is phosphorylated at its SQE motif by ATM kinase at the initial step of HRR pathway. The treatment of intracellular tachyzoite with KU55933, an ATM inhibitor, produced a significant effect on parasite replication, suggesting their inhibition effect

may be blocking T. gondii DNA replication and/or activating cell cycle. Taken together the results show that histone variants and their PTM are important epigenetic regulators in different processes of the parasite life cycle. **Supported by:**NIH 1R01AI083162-01; MINCyT PICT 1288, CONICET PIP 0145 **Keywords:**Toxoplasma; epigenetic; dna repair

RT02.004 - **NEW MOLECULAR TOOLS TO STUDY NEOSPORA CANINUM AND OTHER APICOMPLEXAN PARASITES**

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The development of molecular genetics has greatly enhanced the study of the biology and pathology associated with parasites of the phylum Apicomplexa. We have established a system specifically designed for Neospora caninum, and used this system as a heterologous platform for the expression of foreign genes. Plasmid constructs containing fluorescent proteins or targeted genes of Toxoplasma gondii, driven by N. caninum promoters, have yielded robust expression and correct trafficking of target gene products as assessed by immunofluorescence assays and Western blot analyses. Using this approach, we here demonstrated that N. caninum expressing T. gondii's GRA15 and ROP16 kinase are biologically active and induced immunological phenotypes consistent with T. gondii strains. N. caninum expressing TgGRA15 differentially disturbed the NF-kB pathway, inducing an increased IL-12 production. On the other hand, N. caninum expressing TgROP16 induced host STAT3 phosphorylation and consequent reduction of IL-12 synthesis. These results indicate that heterologous gene expression in N. caninum is a useful tool for the study of specific gene functions and may allow the identification of antigenic targets responsible for the phenotypic differences observed between these two closely related apicomplexan parasites. Additionally, these observations may prove to be useful for the development of vaccine protocols to control toxoplasmosis and/or neosporosis. **Supported by:**CAPES, CNPq, FAPEMIG **Keywords:**Neospora caninum; toxoplasma gondii; innate immunity

# RT.03 – GENOME EDITING AND THE NEW ERA IN THE STUDY OF PROTOZOAN PARASITES

RT03.001 - **TWO ESSENTIAL THIOREDOXINS MEDIATE PROTEIN IMPORT AND GENE EXPRESSION OF THE TOXOPLASMA GONDII PLASTID**

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Thioredoxins are ascribed an increasing number of essential cellular functions, including redox control of protein function and trafficking in the secretory system. Parasites must adjust to redox insults to survive, yet the roles of parasite thioredoxins remain largely understudied.
Apicomplexan parasites are global killers. The apicoplast, an essential plastid organelle, is a verified apicomplexan drug target. Nuclear-encoded apicoplast proteins traffic through the ER and multiple apicoplast sub-compartments to their place of function. We propose that thioredoxins contribute to the control of trafficking and function within these compartments.
We studied the role of two Toxoplasma apicoplast thioredoxins (ATrxs). Both are essential for apicoplast biogenesis and this is dependent on their disulphide exchange activity. We provide evidence for ATrx1's involvement in ER to apicoplast trafficking and ATrx2 in the control of apicoplast gene expression components. ATrx2 is divergent from human thioredoxins. We demonstrate its activity in-vitro providing scope for drug screening.
**Supported by:**BBSRC, RSE **Keywords:**Toxoplasma; apicoplast; thioredoxin

RT03.002 - **DEVELOPMENT OF ATTENUATED VACCINE STRAINS OF *LEISHMANIA* USING CRISPR-CAS GENOME EDITING**

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There are ample examples from animal models and human infections that prior exposure to *Leishmania* provides immunological protection against re-infection. This provides evidence that a vaccine against Leishmaniasis is possible and that live attenuated strains unable to cause pathology deserve consideration for vaccine development. Several attenuated *Leishmania* strains have been previously developed using traditional gene replacement with antibiotic resistance genes and these strains have been shown to be protective in animal models. The presence of the antibiotic resistance genes in the attenuated strains however precludes their use in humans. The newer CRISPR technology enables gene deletion or editing with or without gene replacement with antibiotic resistance gene markers. It was however originally thought that since *Leishmania* goes not have DNA repair mechanisms involving non-homologous end joining (NHEJ) that CRISPR technology would not be possible in *Leishmania*. However, we demonstrate that CRISPR gene editing in *Leishmania* is possible through either microhomology end joining (MHEJ) or homologous DNA repair (HDR) mechanisms. This presentation will describe the development of CRISPR technology in *Leishmania* and the engineering of attenuated *Centrin* gene deleted *Leishmania* strains without the presence of antibiotic resistance genes. This presentation will also demonstrate the ability of CRISPR to delete multi-gene families including the A2 virulence gene family and the precise targeted integration of GFP gene into the genome of *Leishmania*.
**Keywords:**Vaccine; crispr; attenuation

RT03.003 - **A CRISPR-CAS9 TOOLKIT FOR LOSS-OF-FUNCTION SCREENS IN *LEISHMANIA***

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CRISPR technology provides the opportunity to develop scalable methods for high-throughput production of mutant phenotypes. We developed a CRISPR-Cas9 toolkit that allows rapid tagging and gene knockout in kinetoplastids. A set of plasmids allows easy and scalable generation of DNA constructs by PCR for transfections in just a few hours. The single guide RNA is transcribed in vivo by T7 RNA polymerase from PCR-generated DNA templates and an online resource (LeishGEdit.net) facilitates automated primer design. We show how these tools enable for the first time rapid and large-scale knockout screens to study gene function in *Leishmania*: we used these tools to study *Leishmania mexicana* genes identified through proteomic analysis of the flagellum. Using label-free mass spectrometry, 620 flagellar and 1871 cell body enriched proteins were identified. In situ tagging validated flagellar localisations for 78 of 90 selected proteins; 74 of these were targeted for single-step gene deletion, resulting in 63 viable knockout mutants. Analysis of individual mutants revealed diverse phenotypes including loss of motility, disruption of the paraflagellar rod and defects in flagellar assembly. Tagging each mutant with a unique barcode enables screens of large pools of diverse mutants and opens up the possibility to test the relative fitness of mutants in culture, in the mammalian host and in the insect vector.
**Supported by:**Royal Society, MRC **Keywords:**Leishmania; crispr; flagellum

RT03.004 - **EXTENDING THE *TRYPANOSOMA CRUZI* EXPERIMENTAL TOOL BOX BY COMBINING BIOLUMINESCENCE, FLUORESCENCE AND HIGH-THROUGHPUT GENOME EDITING TECHNOLOGY**

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*Trypanosoma cruzi* causes a chronic life-long infection. However, parasites are difficult to detect in blood and tissues, and infected patients may routinely be PCR-negative. Highly sensitive bioluminescence imaging (BLI) has allowed us to establish the GI tract, particularly the colon and stomach, as the major reservoir site in murine models of chronic *T. cruzi* infections. However, BLI does not readily allow the localisation of parasites at a cellular level, or the characterisation of the local immune environment. To address these issues, we generated trypanosomes expressing a luciferase-mNeonGreen fusion protein, so that parasites were both highly bioluminescent and fluorescent. This has enabled us to identify the precise location of parasites within tissues and organs during chronic stage infections, and to assess their replicative status. In addition, it has provided a platform to study tissue-specific immune responses. The bioluminescent/fluorescent parasites have been engineered further to incorportate genetic machinery that facilitates high-throughput CRISPR/cas9-mediated genome editing. In combination, these tools should find widespread applications in the study of parasite biology and disease pathogenesis.**Supported by:**DNDi, British Heart Foundation, GSK Cofund
**Keywords:**Chagas; imaging; crispr/cas9

# RT.04 – IMMUNE RESPONSE TO PROTOZOANS

RT04.001 - **INTERLEUKIN 32: A NEW PLAYER IN LEISHMANIASIS**

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Interleukin 32 (IL-32) is produced by different cell types in higher mammals but not in rodents. This pro-inflammatory cytokine is predominantly expressed intracellularly as at least nine isoforms that can interact with each other to control their biological activities. IL-32γ has been detected in American tegumentary leishmaniasis lesions caused by Leishmania (Viannia) sp. or L. amazonensis. The expression of IL-32γ is associated with tumor necrosis factor alpha (TNFα) production in mucosal leishmaniasis. In THP-1-derived human macrophages, L. braziliensis or L. amazonensis induced IL-32γ in a TNFα-dependent manner. Silencing of IL-32γ expression increased the infection whereas decreased cytokine and microbicidal molecule production. Opposite results were found during overexpression of IL-32γ in these cells. Especially TNFα and IL-8 were closed associated with IL-32γ as well as cathelicidin and β-defensin 2 antimicrobial peptides. Although mice do not present IL-32 their cells can respond to this human cytokine. Thus, in human IL-32γ transgenic mice (IL-32γTg; C57BL/6 background) we have shown that IL-32γ promotes the healing of cutaneous lesions caused by L. braziliensis but not by L. amazonensis. However, this cytokine improved the control of L. amazonensis dissemination to spleens. This raised the hypothesis that IL-32γ can contribute to control the parasites in visceral leishmaniasis. In fact, IL-32γTg mice presented less parasitism in the spleen and liver compared to wild-type (WT) mice during L. infantum chagasi infection. Protection could be associated with higher number of granulomas, pro-inflammatory cytokines (Th1/Th17 profiles) and nitric oxide production in IL-32γTg than in WT mice. Data suggest that IL-32 is an important player in immune response against Leishmania parasites. Differential effects of IL-32γ on immune responses after infection with distinct Leishmania sp. indicate that IL-32γ can differently influence the outcome of leishmaniasis. **Supported by:**CNPq; CAPES
**Keywords:**Il-32; tegumentary leishmaniasis; visceral leishmaniasis

RT04.002 - **T FOLLICULAR HELPER CELLS REGULATE THE ACTIVATION OF B LYMPHOCYTES AND ANTIBODY PRODUCTION DURING PLASMODIUM VIVAX INFECTION**

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Although the importance of humoral immunity to malaria has been established, factors that control antibody production are poorly understood. Follicular helper T cells (Tfh cells) are pivotal for generating high-affinity, long-lived antibody responses. While it has been proposed that expansion of antigen-specific Tfh cells, interleukin (IL) 21 production and robust germinal center formation are associated with protection against malaria in mice, whether Tfh cells are found during Plasmodium vivax (P. vivax) infection and if they play a role during disease remains unknown. Our goal was to define the role of Tfh cells during P. vivax malaria. We demonstrate that P. vivax infection triggers IL-21 production and an increase in Tfh cells (PD-1+ICOS+CXCR5+CD45RO+CD4+CD3+). As expected, FACS-sorted Tfh cells, the primary source of IL-21, induced immunoglobulin production by purified naïve B cells. Furthermore, we found that P. vivax infection alters the B cell compartment and these alterations were dependent on the number of previous infections. First exposure leads to increased proportions of activated and atypical memory B cells and decreased frequencies of classical memory B cells, whereas patients that experienced multiple episodes displayed lower proportions of atypical B cells and higher frequencies of classical memory B cells. Despite the limited sample size, but consistent with the latter finding, the data suggest that patients who had more than five infections harbored more Tfh cells and produce more specific antibodies. P. vivax infection triggers IL-21 production by Tfh that impact B cell responses in humans. **Supported by:**CNPq, FAPEMIG, Fiocruz-MG
**Keywords:**Follicular helper t cells; plasmodium vivax; b cells

RT04.003 - **ROLE OF PURINERGIC RECEPTORS IN CONTROLLING OF T. GONDII INFECTION**

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The mechanisms of host resistance against T. gondii infection still not completely resolved even though almost a century of research. T gondii is an intracellular parasite known to survive and replicate within various cells type including macrophages through a variety of strategies such as blocking the production of reactive oxygen species or inhibiting phagolysosomes formation.
Purinergic signaling is involved in intracellular parasite control in infections with a variety of parasites from bacteria to protozoan. We and others have exploited the possible contribution of this much conserved mechanism of signaling in the context of infection with T. gondii.
Extracellular nucleotides are alarmins involved with natural resistance to infection with intracellular parasites. They activate P2 receptors responsible for diverse physiological responses such as cell death, pro-inflammatory cytokines secretion, production of lipid mediators and reactive oxygen species. Here we discuss the function of purinergic receptors: in vitro using macrophages and intestinal epithelial cells as cargo, and in vivo: evaluating systemic compromise due to infection and the extension of infection in brain. We showed the importance of P2X7 receptors to protect the shock organs such as liver, spleen, mesenteric lymph nodes and brain. In vitro, activation of P2X7 receptor confers resistance to infection via ROS production, IL-1β secretion and induction of fusion between lysosomes and the parasitophorous vacuole, with consequent parasite elimination in murine and human macrophages and intestine epithelial cells. Thus, the study of how purinergic signaling participates during intracellular parasite infection might represent a new avenue to the development of new strategies against infections of intracellular parasites. **Supported by:**CNPq, FAPERJ
**Keywords:**Extracellular atp; p2x7 receptor; t. gondii

RT04.004 - **USING TRYPANOSOMA CRUZI-DERIVED COMPONENTS AS STRATEGIES TO MODULATE THE HOST'S IMMUNE RESPONSE: TOWARDS THE PREVENTION OF CHAGAS DISEASE CARDIOMYOPATHY**

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The control of inflammatory responses to prevent the deadly cardiac pathology in human Chagas disease is a desirable and currently unattained goal. Double-negative T-cells (DN T) are important sources of inflammatory and anti-inflammatory cytokines in cardiac and indeterminate Chagas disease patients, respectively. These cells do not express the co-receptors CD4 and CD8, but express T cell receptors (TCR) αβ or γδ and can recognize glycoconjugates presented by molecules of the nonclassical histocompatibility complex family CD1. Given the immunodominance of glycoconjugates on the surface of Trypanosoma cruzi - the causative agent of Chagas disease, the importance of DN T-cells in immunoregulatory processes, and their potential use as targets for controlling inflammation-induced pathology, we studied the involvement of CD1 molecules in the activation and functional profile of T. cruzi-specific DN T-cells. We evaluated the expression of CD1a, b, c and d molecules by CD14+ cells from Chagas disease patients with well-defined indeterminate and severe cardiac clinical forms, as well as non-Chagas individuals. We also determined the association between the expression of CD1 molecules and the activation status and cytokine expression by DN T cells. We observed that parasite stimulation increased the expression of CD1 a, b, c and d by CD14+ cells from Chagas disease patients. Importantly, despite the increased expression of all analyzed CD1 molecules in CD14+ cells upon parasite stimulation, only CD1d expression showed an association with the activation of DN T-cells, as well as with worse ventricular function in Chagas disease patients. Blocking of CD1d-mediated antigen presentation, led to a clear reduction of DN T-cell activation and a decrease in the expression of IFN-gamma by DN T-cells, shifting the cytokine environment towards a less inflammatory one. Thus, our results showed that antigen presentation via CD1d is associated with activation of DN T-cells in Chagas disease, and CD1d blocking leads to downregulation of IFN-gamma by DN T-cells from cardiac patients. In order to determine which T. cruzi trypomastigote fraction was responsible for the activation of DN cells, we fractionated trypomastigote forms into lipid (LIP), protein (PRO) and glicoconjugate (GCL) components. Our analysis showed that GCL but not LIP or PRO leads to activation of DN T cells. Interestingly, PRO leads to activation of B1 B cells, which is associated with a protective response. These data suggest that preventing GCL-mediated activation of DN T cells, and activation of B1 B cells by PRO, may be potential targets for preventing progression of inflammation-mediated dilated cardiomyopathy.

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