RT.01 - DISSECTING THE INTERFACE BETWEEN HOST AND THE APICOMPLEXAN PARASITE *TOXOPLASMA GONDII*

RT.01.001 - EARLY CELLULAR AND HUMORAL BIOMARKER NETWORKS IN INFANTS WITH DISTINCT RETINOCHOROIDAL LESION STATUS OF CONGENITAL TOXOPLASMOSIS

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Ocular toxoplasmosis is a prominent and severe condition of high incidence in BRA. The current study provides new insights into the immunological events that can be associated with retinochoroiditis in infants with congenital-toxoplasmosis/(TOXO) as compared to non-infected-controls/(NI). TOXO were subgrouped according to the retinochoroidal lesion status as no-lesion/(NL), active-lesion/(ARL), active/cicatricial-lesion/(ACRL) and cicatricial-lesion/(CRL). Results demonstrated that whereas neutrophils and monocytes from TOXO display a combination of pro-inflammatory and regulatory cytokine profiles, NK-cells showed a predominantly pro-inflammatory profile upon *in vitro T. gondii* stimuli. The pro-inflammatory response of CD4+ and CD8+ T-cells was characterized by the enhanced production of IFN-□ and IL-17, whereas a robust monocyte-derived IL-10-mediated profile is observed in children with cicatricial ocular lesions. The analysis of serum biomarkers showed that TOXO displayed prominent chemokine storm mediated by IL-8/CXCL8, MIG/CXCL9, IP-10/CXCL10 and RANTES/CCL5. Additionally, TOXO was accompanied by mixed proinflammatory/regulatory cytokine pattern mediated by IL-6, IFN-γ, IL-4, IL-5 and IL-10. While TNF appeared as a putative biomarker for NL and IFN-γ/IL-5 as immunological features for ARL, IL-10 emerged as a relevant mediator in ACRL/CRL. IL-8/CXCL8 and IP-10/CXCL10 were broad-spectrum indicators of ocular disease, whereas TNF was a biomarker for NL, IFN-γ and MIG/CXCL9 for ARL and IL-10, a genuine serum biomarker for ACRL/CRL. The network analysis demonstrated a broad chemokine/cytokine crosstalk with divergences in the molecular signatures in patients with different ocular lesions during congenital toxoplasmosis. The reactivity of anti-*T. gondii* IgG subclasses resembles the clinical aspects of ocular lesions. IgG and IgG1 discriminate infants with cicatricial lesions (ACRL and CRL) from both ARL and NLR. IgG2 and IgG3 are particularly higher in ACRL and CRL as compared to NLR. Thus, the results indicated that the reactivity patterns of IgA, IgG and IgG subclasses are able to discriminate ARL, ACRL and CRL from NLR or NI. IgA and IgG subclasses are relevant serological biomarkers with diagnostic/prognostic applicability, respectively. Moreover, IgA and IgG1 were closely related to cytokine production by innate/adaptive immunity cells. IgA reactivity was directly associated to TNF-γ-derived from neutrophils, monocytes and CD8+ T-cells, while IgG1 was inversely correlated with IFN-γ-producing CD4+ and CD8+ T-cells but positively correlated with IL-10+ B-cells. These findings support the existence of a progressive immunological environment concomitant with the initial, apical and cicatricial phases in the process of retinochoroidal lesion formation in infants with congenital toxoplasmosis that may be relevant in the establishment of stage-specific clinical management. **Sup ported by:**CNPq, FAPEMIG, CAPES, NUPAD/UFMG and FIOCRUZ.
Keywords:) congenital toxoplasmosis; cellular/humoral immune response; retinochoroidal lesions

RT.01.002 - REFINING THE TOXOPLASMA HOST CELL INVASION MODEL : WHEN HIGH-SPEED MYOSINAMOTOR ACTIVITY COMPETES WITH HOST CELL COMPRESSIVE FORCES AND MEMBRANE STRETCHING TO OPTIMISE PRODUCTIVE INFECTION

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The several micron-sized Toxoplasma gondii protozoan achieves a remarkable feat in terms of cell biology. In less than a ten's of seconds it manages to successfully invade virtually any type of nucleated cells of a warm-blooded animal. Central to the mechanistic base of cell invasion is force production, which is thought applied by the parasite at the interface built between the two cells to shape a "door of entry". Toxoplasma then deforms and glides forward through it. While the prevailing dogma has emphasized the lack of host cell contribution, recent data indicate that host cell cortical occurs during entry and that motordeficient parasites retain invasiveness. We have addressed the force origin and features powering parasite entry using Toxoplasma that i) express the fluorescent core component of the door to perform kinematics and modeling of invasive behaviors, and ii) lack myosin motors and associated components to allow for the first time uncoupling of otherwise high speed coupled processes, namely, the formation of the door and the engagement of the myosin activity. Kinematics demonstrates that Toxoplasma engages a motor with and applies force on the junction molecular complex. This force efficiently tracts the parasite into a nascent vacuole only if the junction is properly anchored to the host cell membrane and cortex. This force unambisguously relies on the high-speed myosinA motor. In non phagocytic cells, real time simultaneous tracking of the plasma membrane and cortex reveal that ΔMyoA parasites assemble a jonction that triggers changes in membrane curvature and ruffle formation. These ruffles either encircle and push the parasite through the junction or fail to extend but in that case persistently apply compressive force that ends with Toxoplasma lethal membrane blebbing.
Therefore, we propose to refine the model of cell invasion by introducing the notion that Toxoplasma motor function competes with host cell membrane/cortex dynamics. Our work also suggests that shaping MyosinA-fast motor during evolution has optimized Toxoplasma invasiveness and fitness.
Keywords: Toxoplasma; cell invasion; motor

RT.01.003 - **IMAGING OF ACTIN DYNAMICS IN *TOXOPLASMA* REVEALS NEW FUNCTIONS AND MECHANISMS DURING THE ASEXUAL LIFECYCLE**

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Apicomplexan actin is well conserved and clearly important during the parasite's life cycle. Several studies assert that its polymerization kinetics are unusual, permitting only short, unstable F-actin filaments. However, it has not been possible to study actin in vivo, so its physiological role has remained obscure. This has led to functional models which are mutually conflicting, incompatible with actin behavior from other eukaryotes, and cannot explain actin's importance during basic processes such as parasite replication and egress. Here we use a chromobody that specifically binds F-actin to demonstrate that Toxoplasma forms stable actin filaments in vivo. F-actin is not only important for parasite replication, but also forms an extensive network that connects individuals both within and between parasitophorous vacuoles, and allows vesicles to be exchanged between parasites within a vacuole. During host cell egress, prior to motility, this network collapses in a calcium-dependent manner. This study demonstrates unexpected roles of Toxoplasma actin during the asexual life cycle, and proves that formation of F-actin depends on a critical concentration of G-actin, implying a polymerization mechanism similar to mammalian actin.**Supported by:**Wellcome Trust, European Research Council
Keywords: Apicomplexa; actin; motility and invasion

RT.01.004 - IDENTIFYING ESSENTIAL APICOMPLEXAN PROTEINS THROUGH GENOME-WIDE CRISPR SCREENS IN *TOXOPLASMA*

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Apicomplexan parasites are a leading cause of human and livestock diseases worldwide, yet most of their genes remain uncharacterized. Here, we present the first genome-wide genetic screen of an apicomplexan. Using CRISPR/Cas9, we assess the contribution of each *Toxoplasma gondii* gene to parasite fitness during infection of human fibroblasts. This analysis defined ~200 fitness-conferring genes unique to the phylum, from which 16 previously uncharacterized proteins were further investigated. Secondary screens identified the novel invasion factor claudin-like apicomplexan microneme protein (CLAMP), which displays similarity to mammalian tight-junction proteins and localizes to secretory organelles. CLAMP is found in all sequenced apicomplexan genomes, and its ortholog is essential during the asexual stages of the malaria parasite *Plasmodium falciparum*. These results provide broad-based functional information on *T. gondii* genes and will facilitate future genetic approaches, expanding the horizon of antiparasitic interventions. **Supported by:**National Institutes of Health (1DP5OD017892)
Keywords: Toxoplasma; invasion; crispr

RT.02 - CELL SURVIVAL AND DEATH IN PROTOZOAN PARASITES

RT.02.001 - CELL DEATH MECHANISMS IN PROTOZOAN PARASITES

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Since mammalian parasites have evolved effective means to escape the immune response, they are able to survive within the host's hostile environment. However, uncontrolled cell growth carries the risk to overgrow the host, leading to its early death that would rendering infection epidemiologically irrelevant. Thus, from the parasite’s point of view, a balanced population density would be ideal. In this way both would be ensured: unharmed survival within the host and a high chance for uptake by the transmitting vector. The detection of typical apoptosis markers in diverse protozoan parasites are indicative for an active contribution of these cells to control its population density, but raised a debate about the evolution of self destruction in single cell organisms. Although this problem seems to be solved by the concept of quorum sensing, clonal selection and stage conversion, the subdivision of cell death in accidental necrosis on the one hand and programmed cell death on the other hand, seems to be oversimplified. Thus in 2009 the nomenclature commission of Cell Death and Differentiation suggested to divide cell death in all organisms in three categories only: necrosis, apoptosis and autophagy related cell death. This concept reflects the recognition that in any case an affected cell takes genetically encoded measures to cope with harassments from intracellular or extracellular stimuli. All three cell death mechanisms have been observed and described in protozoa and especially in protozoan parasites. In case of apoptosis, one major problem was that caspases, the key molecules for the progression of this type of the so-called programmed cell death, are missing in protozoa and metacaspases, their equivalent in plants, are indeed found in protozoa, but are not involved in apoptosis here. However, caspase-independent

apoptosis is these days well established even in metazoa. In fact, the involvement of an endonuclease G (EndoG) seems to fulfil at least partly the function of an apoptosis inducing factor, as it is normally located in the mitochondrion, but released during cell death and involved in DNA degradation. Following this initial step, most of the canonical hallmarks for apoptosis are observed, such as ROS formation, loss of the inner mitochondrial membrane potential, phosphatidylserine exposure at the outer leaflet of the plasma membrane and DNA fragmentation. Interestingly, apoptosis is not only observed during several stress conditions, but can also be induced by staurosporine. Consequences of these mechanisms on the struggle for survival for African trypanosomes will be summarized and discussed with a main focus on kinetoplastid parasites. **Supported by:**German research foundation
Keywords: Cell death; protozoan parasites; apoptosis

RT.02.002 - APOPTOTIC MIMICRY AS AN EVASION STRATEGY FOR INTRACELLULAR PARASITES

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Apoptotic mimicry is a term coined to describe the mechanism by which intracellular parasites mimic a feature of apoptotic cells as a strategy to establish infection and avoid the immune response. Initially, it was described in the Leishmania amazonensis infection model and, since that, it was demonstrated as a tool for several protozoan parasites and viruses. In this context phosphatidylserine (PS) exposure is the most relevant mechanism involved in the mimicking of apoptotic cells. This molecule on the surface of parasites and viruses is able to signal for the efficient uptake of the infectious agent and to induce the alternative activation of the phagocyte, mainly by activating the secretion of anti-inflammatory cytokines. Regarding L. amazonensis infection model, we observed that both amastigote and promastigote forms expose PS as an evasion strategy. However, PS-positive promastigotes are indeed undergoing cell death while PS-positive amastigote forms are viable, infective and sustain PS exposure even inside the parasitophorous vacuole. Here we are going to discuss the modulation of PS-exposure on intracellular amastigotes in vivo and its consequences in parasite/host interplay.
Supported by:FAPERJ, CNPq
Keywords: Apoptotic mimicry; phosphatidilserine; intracellular infection

RT.02.003 - **STRICT CONTROL OF METACASPASE ACTIVITY IN TRYPANOSOMES: A MATTER OF LIFE OR DEATH**

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Apoptosis represents a form of programmed cell death that is critical for the development and homeostasis of multicellular organisms. In unicellular organisms, however, its existence as well as the possible role are still controversial. Despite the presence of morphological characteristics compatible with apoptosis, caspase orthologs are absent in the genomes of these organisms. About a decade ago, sequences with certain degree of similarity to those of caspases (including the conservation of the Cys-His catalytic dyad and a predicted common secondary structure) were identified in the genomes of plants, fungi and protozoans, and they were grouped into a new subfamily of peptidases named metacaspases.
The first years of research in the field of metacaspases were marked by vast contradictions, mainly due to different opinions about the biochemical and functional relationship between metacaspases and caspases. However, it is clear now that metacaspases possess a completely different substrate specificity compared to that of caspases. While caspases cleave peptides and proteins after aspartic acid residues, all metacaspases studied so far have specificity towards basic (Arg/Lys) aminoacids. Another important difference among caspases

and metacaspases is that the latter are absolutely dependent on calcium for activity, a property that has never been described for a caspase to date. In spite of this, they seem to share with caspases some functions, including the regulation of cell cycle and death although these observations could be related to the recently reported role in proteostasis through the removal of protein aggregates. However, since natural proteolytic substrates have scarcely been identified to date, the underlying signaling mechanisms remain an enigma.
Here, I will present our current work on Trypanosoma cruzi metacaspases. To start unveiling the molecular pathways modulated by these enzymes we assessed if metacaspase interactors (identified by mass spectrometry as pulled-down proteins from transgenic parasites expressing flag-tagged metacaspases using anti-Flag agarose resin) could also be substrates of these enzymes. Using a dual-vector E. coli system we evaluated proteolytic processing when co-expressing the potential substrate with the active peptidase and by this method we identified the proteasome adaptor protein Ddi1. The cleavage site, determined by N-terminal Edman sequencing of fragments produced in vitro with both recombinant purified proteins, presented an Arg residue upstream the hydrolyzed peptide bond matching perfectly the known metacaspase specificity. Moreover, replacement of this residue by Ala completely prevented cleavage. Similar results were obtained for T. brucei and budding yeast metacaspase orthologs on their respective substrates. Interestingly, in each case cleavage occurs at a linker region that connects different domains. The in vivo proteolytic event and its consequences are currently being studied. **Supported by:**CONICET, MINCYT
Keywords: Metacaspases; proteostasis; cell death

RT.02.004 - HOW STUDIES ON CHEMOTHERAPY OF LEISHMANIASIS CAN HELP US TO UNDERSTAND THE CELL DEATH PROCESS IN PROTOZOAN PARASITES

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Leishmaniasis is one of the most important neglected disease distributed throughout the world with high incidence of morbidity and mortality. It is caused by protozoan parasites of the Leishmania genus that are transmitted to human by sandflies from the genera Phlebotomus or Lutzomyia. This genus is divided in two subgenera, Leishmania and Viannia, which together are responsible for three groups of main clinical manifestations: cutaneous, mucocutaneous and visceral leishamaniasis. Nowadays, the chemotherapy is based on a few range of drugs that cannot be considered ideal due high toxic effects, long duration of treatment, high cost and low efficacy. Thus, there is an urgent need to new agents and/or safety therapeutic regimen that could be used in any regions of the world to treat the high diversity of clinical manifestations of leishmaniasis. Based on this, our group has studied in the last years promising classes of drugs against Leishmania parasites, trying to understand the cellular effects induced by treatments. Three classes of molecules have our attention: 1) Ergosterol biosynthesis inhibitors; 2) Phospholipid analogues; and, 3) Sirtuin inhibitors. For that, we have used several approaches to understand how these molecules interfere with the cell biology and metabolism of Leishmania promastigotes and intracellular amastigotes, such as: optical and electron microscopy, flow cytometry, fluorimetry, eletrophoresis and western blotting. Using these techniques, several alterations have been described in treated-parasites. Some of them have to be highlighted. Transmission electron microscopy revealed several ultrastructural alterations, such as: (a) mitochondrial swelling, vesiculation and disruption of its inner membrane; (b) presence of many lipid bodies randomly distributed through the cytoplasm; (c) abnormal chromatin condensation; (d) formation of blebs on the plasma membrane; (e) presence of autophagosome-like structures engulfing organelles as mitochondrion and portions of the cytoplasm; and, (f) plasma membrane disruption. On the other hand, physiological studies for mitochondrial function, flow cytometry with propidium iodide and TUNEL assay confirmed the alterations in the mitochondrial

metabolism, cell cycle, DNA fragmentation, and plasma membrane integrity Together, these findings indicate that in Leishmania sp., there are different ways of dying. Cell death in protozoan parasites could not be considered a canonical process, since the events are different from those observed in mammalian cells. Although cell death in these parasites remain controversy due the absence of a definition of the molecular basis involved, our results indicate the occurrence of several cellular effects that are similar to high eukaryote cells. Thus, we believe that cell death begin with mitochondrion energetic collapse followed by mitophagy; the mitochondrion failure seems to be induced by perturbation in the lipid metabolism, since all molecules induced lipid droplets accumulation. However, in culture cells of promastigotes, two different populations of cells can appear, one presenting apoptotic phenotype and the other one appearing like necrotic cells, both as late event during cell death. In these two group of cells, autophagy remains as a process to recycling organelles that lost the function during the treatment. Thus, our results indicate that understanding and better characterizing the different cell death mechanisms should be important for cell biology of protozoan parasites and help us in developing new chemotherapeutic approaches for leishmaniasis. **Supported by:**CNPq, FAPERJ and CAPES
Keywords: Leishmania; chemotherapy; cell death

RT.-3 - HOST-PARASITE INTERACTION IN THE GASTROINTESTINAL TRACT

RT.03.001 - ENTAMOEBA HISTOLYTICA-INDUCED INFLAMMASOME ACTIVATION INITIATES INTESTINAL AMEBIASIS

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Entamoeba histolytica (*Eh*) is an extracellular protozoan parasite of humans that invades the colon to cause life-threatening intestinal and extra-intestinal
amebiasis. Colonized Eh is asymptomatic, however, when trophozoites adhere to host cells there is a robust inflammatory response that is critical in the
pathogenesis of amebiasis. The host and/or parasite factors that trigger the inflammatory response to invading *Eh* are not well understood. We recently
identified that *Eh* adherence to macrophages induces inflammasome activation and in the present study we sought to determine the molecular events upon contact that coordinates this response. Here we report that *Eh* contact-dependent activation of α5β1 integrin is critical for activation of the NLRP3 inflammasome. *Eh* macrophage contact triggered recruitment of α5β1 integrin and NLRP3 into the intercellular junction, where α5β1 integrin underwent activation by an integrinbinding cysteine protease on the parasite surface, termed *Eh*CP5. As a result of its activation, α5β1 integrin induced ATP release into the extracellular space through opening of pannexin-1 channels that signalled through P2X7 receptors to deliver a critical co-stimulatory signal that activated the NLRP3 inflammasome. Both cysteine protease activity and integrin-binding domain of *Eh*CP5 were required to trigger α5β1 integrin that led to ATP release and NLRP3 inflammasome activation. Similar results were corroborated in animal models of disease and in human colonic tissues exposed to wild type and *Eh*CP5 deficient parasites. The results of this study have unlocked the key components on how *Eh* induces disease by engagement of *Eh*CP5 binding to α5β1 integrin at the parasite-host junction where NLRP3 functions as a pathogenicity sensor for invasive *Eh*.
Keywords: Entamoeba histolytica; macrophage; inflammasome

RT.03.002 - MECHANISM OF ACTION OF A MARINE COMPOUND WITH BROAD ACTIVITY AGAINST APICOMPLEXAN PARASITES.

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Cryptosporidium are ubiquitous gastrointestinal parasites that are an important cause of diarrheal disease world-wide. Currently, there is no effective treatment for cryptosporidiosis. Bioprospecting for natural products, historically a rich source of effective anti-microbials, has recently branched out into the marine environment. We identified a symbiotic bacterium from a marine mollusk that produces a compound with broad activity against apicomplexan parasites including *Cryptosporidium parvum*. This compound (Compound 1) was isolated by bioassay guided fractionation and was determined to have an ID50 in the low nanomolar range against *C. parvum*, *Toxoplasma gondii*, and *Babesia bovis* intracellular stages. Attempts to isolate parasites resistant to the compound were unsuccessful; therefore, to identify the mechanism of action we are analyzing both parasite and host transcriptomes of *T. gondii* infected fibroblasts treated with compound 1. Bioinformatics will be used to identify putative target homologs in *Cryptosporidium*. These studies have the potential to identify a drug target common to multiple apicomplexan parasites as well as reveal previously unknown aspects of apicomplexan host-parasite interactions. **Supported by:**NIH
Keywords: Cryptosporidium; drug discovery; natural products

RT.03.003 - **GIARDIA-AN INTESTINAL PARASITE OF TWO FACES**

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Giardia intestinalis is a major cause of diarrhea but half of the infected remain asymptomatic. The mechanisms of disease remain poorly defined but no major intestinal tissue destruction and inflammation is induced. The parasite has even been suggested to be protective against other intestinal infections that cause severe inflammation in the intestine. To better understand the crosstalk between G. intestinalis and human intestinal epithelial cells, and especially the low level of inflammation, we studied gene expression in human intestinal epithelial cells and the parasite secretome during interaction in vitro. RNA sequencing was performed upon in vitro interaction of human intestinal epithelial cells (IECs) and parasites. We used the G. intestinalis isolate WB (assemblage A) as well as the assemblage B isolate GS in order to detect putative assemblage-specific differences. Results were complemented with specific experiments using proteomics, RT-PCR, Western Blots and ELISA to verify the results from RNA sequencing. The two Giardia isolates lead to highly correlated response in human intestinal epithelial cells after 1.5 hours, dropping at later time points of 3 and 4.5 hours. Gene network analysis revealed that Giardia-infection leads to the immediate activation of chemokines (CCL2, CCL20, CXCL1, CXCL2, CXCL3) and cytokines (IL8) on the RNA level but the level of secreted cytokines is low. Most of the early induced genes were down-regulated on transcript-level before 3 hours. Data analyses suggested that this was due to RNA decay of AU-rich element-containing transcripts, induced by the human RNA binding protein TTP. The level of TTP expression remained high and the protein localized to P bodies as long as the parasite was present, inducing an anti-inflammatory state in the IECs. Several parasite proteins were specifically secreted during interaction with the host cells and three giardial cysteine proteases were shown to digest specific chemokines. Interactions between Giardia trophozoites and host intestinal epithelial cells induce specific responses in both cell types. These responses can explain the low levels of inflammation during Giardia infections and it suggests how the diarrhea-causing parasite Giardia can be protective against other diarrheal diseases. **Supported by:**Swedish Research Council
Keywords: Diarrhea; inflammation; rna binding

RT.03.004 - LESSONS FOR MUCOSAL IMMUNITY FROM MURINE MODELS OF GIARDIASIS

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The innate immune system has evolved several mechanisms for recognition of Pathogen Associated Molecular Patterns (PAMPS) including members of the Toll-like receptor family, intracellular NOD and RIG families, and the C-type lectin receptor family. *Giardia duodenalis* is a eukaryotic parasite which replicates in the lumen of the small intestine causing a variety of outcomes ranging from asymptomatic infection to diarrhea with severe cramps, nausea and fever. Data suggest that many of these outcomes are related to pathologic immune responses. It is unclear, however, how the immune system initially recognizes this parasite and how these early recognition events shape the development of immunity. *Giardia* was recently shown to glycosylate a subset of its proteome by the addition of N-acetyl glucosamine and we therefore hypothesized that C-type lectins would play a role in immune recognition of this parasite. We infected mice lacking the soluble mannose binding lectin (MBL) and showed that mice exhibited a delay in parasite elimination and that this correlated to delayed recruitment of mast cells to the small intestine, an immune response previously shown to contribute to parasite control and immunopathology. We then examined mice lacking complement C3aR and found these mice phenocopied the MBL knockouts. In addition, while C3aR deficient mice have normal production of anti-*Giardia* IgA, they have significant reductions in cytokine production when spleen cells were stimulated with parasite extracts ex vivo. Together these data indicate that activation of complement through MBL and innate recognition of parasite glycoproteins are important for development of immune responses to this eukaryotic pathogen and may contribute to the variable outcomes seen in human giardiasis. We have also investigated how the intestinal microbiota of mice can impact the development of immune responses. Previous work had shown that specific microbiota could inhibit colonization of mice with *Giardia*. We now show the converse, that infection with Giardia leads to changes in the intestinal microbiome of mice. Furthermore, treatment of mice with a cocktail of antibiotics that can help promote Giardia infections also reduces immunopathology. Specifically, reduced intestinal disaccharidase activity is a common outcome of giardiasis in mice and humans. Previous work has implicated CD8+ T cells in mediating this outcome, and we have now shown that antibiotics that target the microbiota and not the parasite can prevent the activation of CD8+ T cells in the lamina propria following *Giardia* infection and also block the development of disaccharidase deficiency. We are now seeking to understand how host genetics, the intestinal microbiota and parasite strain variation intersect to produce the spectrum of clinical outcomes observed in human giardiasis.
Supported by: National Institutes of Health **Keywords:** Giardia; immunology; lectins

RT.04 - NEUTROPHIL DYNAMICS IN *LEISHMANIA* INFECTION: WHAT'S NEW?

RT.04.001 - NEW INSIGHTS ON LEISHMANIA-INDUCED NETOSIS.

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Leishmania promastigotes are inoculated in the dermis into a blood pool formed during insect vector feeding, and parasites interact with blood and tissue cells and with extracellular matrix (ECM) proteins as well. Leishmania interaction with neutrophils triggers the release of Neutrophil Extracellular Traps (NETs), which are composed of decondensed chromatin associated with granular and cytosolic proteins. Importantly, NETs were detected in biopsies of cutaneous leishmaniasis, in close contact with the parasites.
The NET release process, named NETosis, may occur through the classical and the early/rapid ways, which are dependent and independent of reactive oxygen species (ROS) generated by

NADPH-oxidase, respectively. We showed that Leishmania amazonensis promastigotes trigger the classical NETosis by promoting redox imbalance, which is mediated by NADPH-oxidase and nitric oxide synthase derived ROS/RNS. The classical Leishmania-induced NETosis is dependent on PI3K, ERK, intracellular calcium mobilization, peptidyl arginine deiminase (PAD)-4 and elastase activity. Furthermore, promastigotes promote the early/rapid ROS-independent NET formation, occurring only 10 minutes after neutrophil-parasite interaction, which is dependent of elastase, but not on PAD4.
NETs trap and kill promastigotes, which can escape NET-mediated killing by means of 3`-nucleotidase/nuclease activity, by the lipophosphoglycan expressed on the promastigote membrane, and also by an endonuclease present in the vector saliva, which is inoculated into the host together with the parasite. NET released at the inoculation site may also modulate the function of different cells, such as the monocytes. We found that NETs reprogram monocyte differentiation into dendritic cells in response to IL-4 and GM-CSF, stimulating a pro-inflammatory macrophage differentiation.
We thank the Hemotherapy Service of Hospital Clementino Fraga Filho for buffy coats. **Supported by:**CNPq, CAPES and FAPERJ **Keywords:**Neutrophil; leishmania; netosis

RT.04.002 - ROS AND NEUTROPHILS DURING EXPERIMENTAL INFECTION WITH LEISHMANIA

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Neutrophils are recruited to the site of infection with Leishmania amazonensis, and are the first cells to bear parasites after injection in the skin. Parasites move to monocytes by a route not yet clarified, where they replicate intensively. Finally, parasites are found in macrophages, where their numbers are kept in check by a IFN-gamma- and NO-dependent mechanism. Neutrophil depletion led to faster lesion development, increased parasite numbers and higher arginase activity during the first week of infection in BALB/c mice, but not in C57BL/6 mice. Increased susceptibility was accompanied by higher levels of IgG and increased production of IL-10 and IL-17. Blockage of IL-10 signaling in neutrophil-depleted mice abrogated the increase in parasite loads, suggesting that parasite proliferation is at least partially mediated by IL-10. We tested the effect of IL-17 in inflammatory macrophages and found that IL-17 increased arginase activity and parasite growth. Thus, neutrophils control parasite numbers and limit lesions during the first week of infection in BALB/c mice. Infection of gp91 phox knockout (ko) mice on the C57BL/6 background shed a new light to the effect of neutrophils in L. amazonensis infection. Phagocytes from these mice do not produce phagocytose-triggered superoxide. In response to infection with L. amazonensis, neutrophils migrate to the site of infection and these cells persist longer. More wt neutrophils undergo apoptosis than ko, whereas ko neutrophils undergo necrosis. Coherently, ears from knockout mice underwent more necrosis than from wt mice. We conclude that neutrophils are the first cells to arrive at the site of infection with L. amazonensis and to harbor the parasite. They undergo apoptosis thereby promoting a non-inflammatory environment that favors parasite growth but also preserves tissues. Neutrophil apoptosis is dependent on a functional NADPH oxidase, and in its absence neutrophils persist longer in tissues and die of necrosis, causing severe damage. **Supported by:** FAPEMIG, CNPq, CAPES **Keywords:** Neutrophils; leishmania; reactive oxygen species

RT.04.003 - ROLE OF THE FIRST RESPONDERS TO LEISHMANIA BRAZILIENSIS INFECTION

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Neutrophils are the first line of defense against invading pathogens and are rapidly recruited to the sites of Leishmania inoculation. During Leishmania BRAiensis infection, depletion of inflammatory cells significantly increases the parasite load whereas co-inoculation of neutrophils plus L. BRAiensis had an opposite effect. Moreover, the co-culture of infected macrophages and neutrophils also induced parasite killing leading us to ask how neutrophils alone respond to

an L. BRAiensis exposure. Herein we focused on understanding the interaction between neutrophils and L. BRAiensis, exploring cell activation and apoptotic fate.Inoculation of serum-opsonized L. BRAiensis promastigotes in mice induced neutrophil accumulation in vivo, peaking at 24 h. In vitro, exposure of thyoglycollate-elicited inflammatory or bone marrow neutrophils to L. BRAiensis modulated the expression of surface molecules such as CD18 and CD62L, and induced the oxidative burst. Using mCherry-expressing L. BRAiensis, we determined that such effects were mainly observed in infected and not in bystander cells. Neutrophil activation following contact with L. BRAiensis was also confirmed by the release of TNF-α and neutrophil elastase. Lastly, neutrophils infected with L. BRAiensis but not with L. major displayed markers of early apoptosis. We show that L. BRAiensis induces neutrophil recruitment in vivo and that neutrophils exposed to the parasite in vitro respond through activation and release of inflammatory mediators. This outcome may impact on parasite elimination, particularly at the early stages of infection. **Keywords:**Leishmania BRAiensis; neutrophil; cutaneous leishmaniasis

RT.04.004 - SAND FLY GUT MICROBIOTA DRIVES PERSISTENT RECRUITMENT OF NEUTROPHILS AFTER INFECTED BITES THROUGH INFLAMMASOME-DERIVED IL1-β PRODUCTION

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Virulence of vector-transmitted *Leishmania major*, the etiological agent of cutaneous leishmaniais, has been associated to a persistence of recruited neutrophils where they promote parasite survival and successful establishment of the infection at the bite site. However, the mechanism driving persistent neutrophilic recruitment after sand fly bites has not been elucidated. Using a novel model of vector-transmitted *Leishmania donovani*, the etiological agent of visceral leishmaniasis, we report on a unique and sustained inflammatory response that is characterized by a marked induction of IL-1β and an intense and persistent neutrophilic infiltration up to 18h post-bite. Importantly, antibiotic treatment of sand flies harboring mature infections prior to transmission significantly reduced NLRP3, caspase1 and IL-1β protein levels in the skin 6 hours after infected bites implicating midgut microbiota of the vector in host inflammasome activation. Moreover, neutralizing the effect of IL-1β with anakinra abrogated neutrophil recruitment and severely impaired parasite visceralization after infected bites establishing that sand fly midgut microbiota are a key contributor to virulence of vector-transmitted visceral leishmaniasis.
Keywords: Leishmania; gut microbiota; nlrp3 inflammasome

RT.05 - PARASITE PROTEOMICS AND DRUG DEVELOPMENT

RT.05.001 - PHENOTYPIC SCREENINGS OF NOVEL CANDIDATES FOR CHAGAS DISEASE THERAPY

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Chagas disease (CD), described in 1909 by Carlos Chagas and caused by the protozoan Trypanosoma cruzi, makes up the group known as Neglected Tropical Diseases, eighteen infections that thrive mainly among the poorest populations. More than 15% of the world population suffers from at least one of them, but safe and effective therapies and prophylactic approaches are not available for most of these diseases. Since the introduction of NF and Bz,

despite the extensive list of classes of compounds assayed in vitro and in vivo against T. cruzi, only few compounds moved to clinical trials (e.g. allopurinol, itraconazole, fluconazole and recently posaconazole and a prodrug of ravuconazole), mainly due to the lack of a strong indication of their curative effect due to the use of low stringent protocols and experimental models, to their toxicity and/or teratogenic effects, emphasizing the importance to employ suitable experimental models and standardized protocols. In the last two decades, at the Cellular Biology Laboratory/IOC/Fiocruz, our group has focused on in vitro and in vivo studies related to anti-T cruzi candidates (both natural and synthetic compounds, under monotherapy and combination approaches) in collaboration with different academic and private groups from BRA as well as from other countries. Thus, presently a bulk of our findings related to different targets (such as DNA ligands, statins, CYP51 inhibitors, inhibitors of cAMP-phosphodiesterases among others) will be discussed as novel potential anti-*T.cruzi* candidates. These studies were conducted by standardized protocols and well-established experimental models relying on the incrementally increase of the stringency level of the experimental model in the hope of identifying new drug candidates for CD. Only the most promising molecules screened in vitro (screened against different parasite strains (e.g. Y, Tulahuen, Colombiana, among others)) and parasite forms relevant for mammalian infection (bloodstream trypomastigotes and intracellular amastigotes) were selected for in vivo studies, using a selective index (SI ≥50). For experimental models of acute and chronic models of *T.cruzi* infection, we will initially evaluate the effect upon acute infection with the Y strain and only those compounds that present greater activity than the reference drug (Bz) were advanced to the experimental models using naturally resistant *T.cruzi* strains (like Colombiana strain). The overall goal of the pre-clinical analysis is to contribute for the identification of more potent and safe drugs to treat the millions of chagasic patients that wait for new therapeutic options.

Keywords: Trypanosoma cruzi ; quimioterapia experimental ; doença de chagas

RT.05.002 - IS THE PHOSPHOLIPID MATRIX OF THE MEMBRANE A DESPISED DRUG TARGET IN LEISHMANIA?. A PEPTIDE APPRAISAL.

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The search of new targets in leishmaniasis is an urgent need, driven by the decreasing effectiveness of the current chemotherapy, and a pipeline for new leads scarcely populated.

The phospholipid matrix of the plasma membrane in Trypanosomatidae was mostly despised as a chemotherapeutic target when compared with other membrane components such as ergosterol or membrane proteins and polysaccharides, despite its essential role not only as a permeability barrier, but as a physical scaffold for the rest of the macromolecular components of the membrane, and modulation of the activity of integral proteins. This previous statement was not longer true in Nature, as antimicrobial peptides (AMPs) of the innate immunity exploited membrane disruption as their lethal hit to kill invading pathogens. Concerning human chemotherapy, there is a growing awareness of peptide-based therapies, once previous hurdles such as bioavailability and production costs have been progressively overcome.

The talk will provide an updated statement on the potentiality of these approaches against Leishmania infections under three different landscapes:

i) Membrane –active antimicrobial peptides with strong affinity for the phospholipid matrix, These will lead to an irreversible permeation of the plasma membrane with a lethal bioenergetic collapse ensuing, exemplified by the cecropin A-melittin hybrid peptides as well as others AMPs

ii) Antimicrobial peptides with intracellular targets, as the histidine-rich human saliva peptide Histatin 5, whose interaction with the membrane is mild and reversible, so to afford translocation into the intracellular milieu and access to its target, the mitochondrial ATP synthase.

iii) Cell-penetrating peptides, behaving similar to the previous group respect to their interaction with the membrane, but not necessarily endowed with antimicrobial activity. CPPs were used to smuggle a wide variety of cargo molecules across the membranes surpassing the presence of specific cognate transporters. This issue will be epitomized not only by the reversal of miltefosine resistance by its conjugation to Tat, but also by other cell penetrating leishmanicidal macromolecules, endowed with enzymatic activity, as a proof-of-concept for an enzybiotic-based therapy for *Leishmania*.

The state-of-art, potentiality, pros and cons, and limits of a peptide-based chemotherapy for Leishmania will be discussed. **Supported by:**FEDER RICET(RD12/0018/0007 & RD16/0027/0010), ISCIII PI12-02706, and SAF2015-165740-R

Keywords: Leishmania; membrane-active peptide; chemotherapy

RT.05.003 - TRYPANOSOMA CRUZI GLOBAL AND SUBCELLULAR PROTEOMICS

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The first manuscripts on Trypanosoma cruzi proteomics were published in 2004. At that time, proteomic approaches were mostly based on protein separation by two-dimensional gel electrophoresis (2-DE) followed by the identification of protein spots by MALDI-TOF. However, since then, gel-free bottom-up proteomics strategies have been applied to the comparative analysis of T. cruzi life forms. While some of the reports have described proteomes extracted from whole cells, others have focused on particular subsets of T. cruzi subproteomes present in specific organelles, or even fractions secreted to extracellular media. In this talk, an overview of global and subcellular proteomics of T. cruzi will be presented, including analysis of cell surface and nuclear subproteomes as well as the examination of exoproteome and phosphoproteome subsets. The presentation will try to show in which extent these analyses have permitted the comparative proteome analysis of T. cruzi epimastigote, trypomastigote and amastigote life forms and have been applied to the study of amastigogenesis and cell division. Also, based on the generated proteomic data, potential molecular targets for therapeutics and diagnosis of Chagas disease will be suggested. **Supported by:** CNPq, FAPEG, FINEP
Keywords: Proteome; subproteome; mass spectromety

RT.05.004 - PHOSPHOPROTEOMICS SHED LIGHT ON THE SIGNALING NETWORKS OF TRYPANOSOMA CRUZI METACYCLOGENESIS

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The metacyclogenesis is the process by which epimastigote forms of the parasite Trypanosoma cruzi differentiate into metacyclic trypomastigotes acquiring infective potential. The progression to the infective state is accompanied by a protein activities involving calcium binding, modification of other proteins as well as protein kinase activity, the latest being responsible for protein-phosphorylation events on maintenance and progression of life-cycle. With the understanding of the dynamics of phosphorylation, it is possible to understand what metabolic pathways or proteins are regulated during the stages that precedes metacyclogenesis of this protozoan pathogen. Within the current state of the phosphoproteomics techniques nowadays it is possible to highlight the phosphorylation event quantitatively, using high resolution mass spectrometry we aim to understand the dynamics of the signaling pathways regulated by phosphorylation during metacyclogenesis.
Keywords: Trypanosoma cruzi; proteomics; signaling

RT.06 - VECTOR-PARASITE INTERACTIONS

RT.06.001 - THE COSTS OF TRIATOMINE-TRYPANOSOME INTERACTION: A TWO SIDED CLASH?

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Triatomines and mammals are natural hosts of *Trypanosoma cruzi* and *Trypanosoma rangeli*. Both parasites are acquired by the insects when they take a blood meal from infected mammals. *T. cruzi* develops exclusively inside the intestinal tract of bugs and can be transmitted from these to mammals when contaminated feces are deposited on mucosa or injured skin or if an infected insect is eaten by the mammal. *T. rangeli*, in addition to its development in the intestinal tract, is able to invade the insect hemocoel, eventually colonizing the salivary glands where metacyclogenesis occurs. Parasites can then be transmitted through saliva during the bite, being inoculated inside the host skin. Here we show that these parasites present distinct strategies of development in the insect and promote different levels of

pathogenicity. Interestingly, they also induce changes in insect behavior that apparently modify their transmission rates to vertebrate hosts. Twenty four hours after an infective blood meal, 80% of the ingested *T. cruzi* are eliminated in the anterior midgut (AM). A factor present in the AM of recently fed insects seems responsible by the extensive parasite killing observed during this time interval. Few parasites reach the posterior midgut (PM) and transform into epimastigotes that will establish the infection. Even small *T. cruzi* populations, concentrated fundamentally on the PM and rectum, produce negative effects on the invertebrate host. These include delayed molt, decreased survival rates, as well as lower reproductive performances. *T. rangeli,* which normally is barely found in blood circulation of infected mammals, starts bug infection with a much reduced population. Within a few weeks, this parasite develops large populations in both the AM and PM. Nevertheless, the rates of hemolymph invasion vary among *Rhodnius* species, being generally low. As with *T. cruzi*, insects infected with *T. rangeli* present delayed molt, decreased survival and lower reproductive performances, but their negative effects are more pronounced. We have also shown that infection by *T. cruzi* and *T. rangeli* modifies triatomine behavior. Indeed, infection by each parasite alters bug locomotory activity patterns, negative phototaxis and shelter use in a species-specific manner. While *T. rangeli*-infected insects present increased activity levels independently of the context, the spontaneous activity of *T. cruzi*-infected bugs is lowered, but it is instead increased in the presence of host cues. Nevertheless, if predation by mammal hosts is allowed during experiments, infected bugs are more predated, independently of which *Trypanosoma* species infects them. The potential implications of these effects on trypanosome transmission rates are discussed.
Supported by: CNPq, Fiocruz, Fapemig
Keywords: Rhodnius prolixus; trypanosome; interaction

RT.06.002 - BEYOND METACYCLOGENESIS: THE SECRET BEHIND THE REMARKABLE SUCCESS OF *LEISHMANIA* TRANSMISSION BY A VECTOR BITE.

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The rise of metacyclic parasites is the most critical event in the life cycle of *Leishmania* parasites within the midgut of their vector sand fly enabling successful transmission to mammalian hosts. Nonetheless, there is a paucity of information on the conditions governing the development of these metacyclic promastigotes. Here, we use the naturally occurring vector/parasite species, *Lutzomyia longipalpis/Leishmania infantum* and *Phlebotomus papatasi/Leishmania major*, to reveal a novel developmental stage beyond metacyclics that fundamentally alters our perception of how *Leishmania* parasites are transmitted by bite. We report that metacyclic promastigotes have the ability to dedifferentiate into a dividing promastigote stage within the thoracic anterior midgut (Reverse Metacyclogenesis), a phenomenon never reported before in *Leishmania*-infected sand flies. This phenomenon resets the infection inside the vector allowing the parasites to significantly increase the number and homogeneity of the infective form through additional rounds of multiplication. Moreover, as a consequence of this late re-amplification cycle, we also describe a boost in the development of haptomonad promastigotes with the formation of a distinct spheroid structure (haptomonad plug) that physically blocks the stomodeal valve independently of, and in addition to the promastigote secretory gel. This increases the difficulty by which infected flies can feed and promotes parasite regurgitation into the host. Together, these data reveal a hidden part of the *Leishmania* life cycle that may begin to explain how sand flies are remarkably successful in transmitting this parasite to a mammalian host in nature.
Supported by:NIAID-NIH
Keywords: Leishmania; sand flies; metacyclogenesis

RT.06.003 - WHEN A VAMPIRE GETS SICK AND OLD: HOW IMMUNITY AND AGING REGULATE MITOCHONDRIAL FUNCTION IN THE MAJOR ARBOVIRUS VECTOR AEDES AEGYPTI.

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Adult females of Aedes aegypti are facultative blood sucking insects and vectors of many arboviruses, including Dengue and Zika viruses. Insect dispersal plays a central role in disease transmission and the extremely high energy demand posed by flight is accomplished by a very efficient oxidative phosphorylation process, which take place within the flight muscle mitochondria. These organelles play a central role not only in energy metabolism, interconnecting nutrient oxidation to ATP synthesis, but also represent an important site of cellular superoxide production. Given the importance of mitochondria to cell physiology, our laboratory aims to understand how mitochondrial physiology is modulated by different aspects of A. aegypti biology, focusing on the functional consequences to this organelle triggered by immunity and aging. We observed that respiratory rates in male insects were lower than females, regardless the substrates tested, which is connected to reported lower flight capacity of males. Activation of innate immune response by zymozan injection reduced flight capacity and proline-sustained respiration in flight muscle, as a result of specific complex I dysfunction. Reduced respiratory capacity in zymozan-challenged insects was negatively correlated with the degree of antimicrobial peptide expression, suggesting a systemic bioenergetic cost to assemble an efficient innate immune response against infection. Flight capacity and respiratory rates were consistently lower in flight muscle from older insects regardless the sex and substrates tested than young individuals. In older insects, midgut permeability was high, increasing tissue bacterial counts, promoting antimicrobial peptide expression in the fat body. Similar to zymozan-challenged insects, specific complex I dysfunction was a hallmark of mitochondrial functional changes observed in old insects. Together, these data suggest that immune activation, by either experimental sterile infection or naturally by aging, plays a central role in regulate A. aegypti flight capacity, mediating specific complex I dysfunction in flight muscle mitochondria with direct consequences to tissue bioenergetic capacity and insect dispersal. **Keywords:** Mosquito; mitochondria; immunity

RT.06.004 - THE TRIATOMINE-MICROBIOTA-TRYPANOSOMA CRUZI TRIPARTITE INTERACTION

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Chagas Disease is a potentially life-threatening illness caused by the protozoan Trypanosoma cruzi and mainly transmitted by the feces of insects of the Triatominae sub-family. It is the fourth most important infectious disease with approximately 8 million people infected Latin America. The insect microbiota has been shown to influence vector competence for pathogens through the elicitation of the insect immune system, pathogen sequestration by microbes, and bacteria-secreted anti-pathogenic molecules. These influences make the microbiota interesting to be used in the development of new strategy to control diseases transmitted by insects. In this work, we have evaluated the diversity of bacteria from the wild triatomine bug *Triatoma* sordida and tested the potential of its cultivable microbiota to inhibit *T. cruzi* development. First, we have analyzed the diversity of bacteria from different intestinal segments and developmental stages of the wild *T. sordida*. We found that the microbiota of the five stages of development of the *T. sordida*, determined through sequencing of ribosomal gene 16S, displays similar profiles qualitatively. We observed differences only in the less predominant bacteria. All triatomine developmental stages present bacteria of the phyla Actinobacteria, Firmicutes and Proteobacteria. However, males and females have quantitative differences in the three phyla in all intestinal segments. Then, we tried to isolate in laboratory conditions, bacteria from midgut of wild *T. sordida*. We were able to isolate seven bacteria, four Actinobacteria and three

Firmicutes. We assessed the ability of the bacteria isolates to inhibit T. cruzi. Regrettably, none had the desired effect. However, for our surprise, results showed that molecules secreted from the Bacillus sp. isolate caused remarkable increase in *T. cruzi* replication both in vitro and in vivo. We are now under the process of fractioning the supernatant in order to determine which are the molecules responsible for this pro-biotic effect. Under the assumption that this bacterium is naturally present in at least other *Triatoma* species (thus contributing for higher natural infection rates), envisioning a way to block its pro-biotic effects could result in the opening of new avenues in the control of Chagas Disease transmission.

Keywords: Triatomine; microbiota; trypanossoma cruzi