RT.01 - OXIDATIVE STRESS IN THE PARASITE-HOST INTERPLAY

RT.01.1 - *LEISHMANIA*-INDUCED NETOSIS IS DEPENDENT OF CELLULAR REDOX IMBALANCE MEDIATED BY NITRIC OXIDE SYNTHASE AND NADPH OXIDASE ACTIVITIES.

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Neutrophils are the most abundant leukocytes in the blood and are the first cells recruited to inflamed tissues. These essential cells to the innate immunity have a pivotal role in infection not only by ingesting and destroying microbes, but also by secreting various cytokines and chemokines. A novel microbicidal mechanism has been described for neutrophils and other granulocytes in which, chromatin decorated with granular and some cytoplasmic proteins are released to the extracellular milieu, forming a web and named neutrophil extracellular traps (NETs). Moreover their microbicidal properties, NETs trap microbes facilitating their phagocytosis and avoiding their spreading. This mechanism, known as netosis, can be triggered by an increasing list of synthetic and physiological molecules and by microorganisms such as bacteria, fungi, protozoa and virus as well.
It has been shown that NET release by the majority of stimuli described to date, depends upon reactive oxygen species generated by NADPH oxidase complex. Moreover, one of the netosis hallmarks is chromatin decondensation, which depends on both elastase and peptidyl arginine deiminase (PAD4) activities and to an unknown mechanism mediated by myeloperoxidase.
We reported that *Leishmania* promastigotes and amastigotes are able to induce NET release from human neutrophils and that these webs possess leishmanicidal activity (Guimarães-Costa et al., 2009). Additionally, released NETs exert an extracellular leishmanicidal activity caused, at least in part, by the histones present in the meshes. Moreover, NETs were observed in lesions from human cutaneous leishmaniasis.
Here we dissect the redox mechanisms involved in the netosis induction by this protozoan parasite, evidencing the role of NADPH oxidase and nitric oxide synthase.
Supported by:CNPq, FAPERJ, and we thank the Hemotherapy Service of Hospital Clementino Fraga Filho

RT.01.2 - ANTIOXIDANTS AND CARDIOPROTECTION IN CHAGAS' HEART DISEASE

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The protection against tissue damage, disease tolerance, is a strategy that allows the host to survive infection with minimal cost. It is successful against disease caused by pathogens that can not be eliminated by the immune system and explains why individuals with a similar pathogen burden can present varying disease gravities. The mechanisms that allow disease tolerance are starting to emerge and include activation of Nrf2 and SIRT1, pathways also involved with antioxidant defenses. Heme oxygenase 1 (HO-1) is a heme-degrading enzyme controlled by Nrf2 pathway and capable of protecting the heart against oxidative tissue damage in diabetes and hypoxia. Cobalt proto-porphyrin (CoPP) is an HO-1 inducer that acts by activating Nrf2. The activation of SIRT1 is also an important pathway to cardioprotection, both in oxidative stress related and unrelated diseases. Resveratrol, a polyphenol present in grapes, is thought to mediate grapes' cardioprotective effect, and is both an Nrf2 and a SIRT1 activator, promoting activation of antioxidant defenses, stimulating mitochondrial biogenesis, blood flow, angiogenesis, and the use of lipids as fuel. Resveratrol decreases heart damage in various models, prevents atherosclerosis and interferes with Ca2+ handling in cardiomyocytes, restoring heart contractibility in cardiomyopathies.Chagas' heart disease is caused by the protozoan Trypanosoma cruzi, which causes a chronic infection and heart inflammation. The prevention or reversal of tissue damage with cardioprotectors have never been attempted in Chagas' heart disease. We have tested whether cardioprotectors could prevent (acute phase, Y

strain) or reverse (chronic phase, Colombian strain) functional Chagas' heart disease in the susceptible BALB/c mice. Treatment with CoPP prevented the electrical abnormalities of the heart during acute Y strain infection (increase in PR interval, P duration and QTc) along with inflammation and tissue parasitism. CoPP was more effective to prevent electrical abnormalities than benzonidazole, despite being less effective to reduce parasite burden, indicating that it has cardioprotective effects. Resveratrol was not as effective as CoPP to prevent Y strain acute disease. Treatment with CoPP failed to reverse an established functional heart disease (Colombian strain). Treatment with resveratrol efficiently reversed electrical and mechanical abnormalities in chronic Chagas' heart disease and also reduced heart parasitism (Colombian strain), but did not reduce inflammation, altered angiogenesis or interstitial fibrosis. Resveratrol efficiently reduced ROS in extravascular heart areas. None of the drugs were able to directly kill trypomastigotes, while benzonidazole was effective. Our results also offer some clues to the molecular mechanism of action of CoPP and resveratrol.

RT.01.3 - ROLE OF MITOCHONDRIA IN TRYPANOSOMATIDS SURVIVAL

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Trypanosomatids are responsible for major infectious disease in humans, among which Chagas disease and leishmaniose are the most relevant ones. In these parasitic infections, the need for a more specific treatment is a priority since the available ones lead to toxicity, resistance and are far from being successful. In this sense, the search for more efficacious therapeutic treatments has been the subject of intense research. In this regard, trypanosomatids mitochondrion has attracted interest due to its unique features and involvement in many cellular events, such as ATP and reactive oxygen species (ROS) production, calcium homeostasis, proliferation, redox balance and programmed cell death, i.e., an organelle essential for parasite survival. Regarding mitochondrial bioenergetics parameters, besides the differences among *Trypanosoma cruzi* and their mammalian host, there are also significant differences among strains and also among isolates from *T. cruzi* infected individuals with distinct clinical manifestations. Furthermore, in their host, trypanosomatids have to deal with ROS and reactive nitrogen species to ensure their survival. In other to achieve this goal, the parasite antioxidant system plays an important role and has been pointed out as a source of potential drug targets. Until now, *T. cruzi's* mitochondrion "pathway" has not been characterized, and the mitochondrial tryparedoxin peroxidase interactome provided insights regarding its mode of action. In the meantime, two tryparedoxins, one cytosolic (TcTPNI) and another one associated with endomembranes (TcTPNII), have been characterized in *T. cruzi*. TcTPNI is involved in the cytosolic pathway, while the interactome of TcTPNII also suggests its interaction with the cytosolic pathway. Taken all this into consideration, the mitochondrion contribution to these different events will be discussed. **Supported by:**FAPESP, CNPq and FINEP (CT/INFRA)

RT.01.4 - SENSE OR NOT SENSE THE OXIDATIVE STRESS: THAT IS THE QUESTION

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The main consequence of oxidative stress is the formation of DNA lesions, which might result in genomic instability and lead to cell death. Guanine is the base that is most susceptible to oxidation due to its low redox potential, and 8-oxoguanine (8-oxoG) is the most abundant lesion. This characteristic makes 8-oxoG a good cellular biomarker to indicate the extent of oxidative stress. If not repaired, the 8-oxoG could pair with adenine and cause G:C to T:A transversion. When 8-oxoG is inserted during the DNA replication it could generate double strand breaks, which makes this lesion particularly deleterious. *Trypanosoma cruzi* needs to deal with various oxidative stress situations that it is exposed to, such as the mammalian intracellular environment and the triatomine insect gut where it replicates. We focus on the enzymes that are responsible to deal with the 8-oxoG. In order to investigate the importance of the 8-oxoG for the parasite, we generated T. cruzi strains expressing genes involved in the 8-oxoG metabolism. We were able to show that these different enzymes were more resistant to hydrogen peroxide treatment. Some of these recombinant strains also showed statistically significant increased

growth after 48 hours of infection in fibroblasts when compared to wild type cells, as well as increased parasitemia in Swiss mice. Additionally, we demonstrated by western blotting experiments that MutT (enzyme responsible to remove the 8-oxoG from the nucleotide pool) heterologous expression can influence the parasites' antioxidant enzymes protein levels and the response to oxidative stress. On the other hand, the cells that overexpress the E. coli catalase enzyme (transform hydrogen peroxide in oxygen and water) were unable to respond to the oxidative stress. These results show the importance of the 8-oxoG to permit the cells to sense the oxidative stress and suggest that this organism lost the catalase gene to improve the signalization of the oxidative stress.

RT.02 -PAVING THE WAY FOR ANTI-PARASITIC CHEMOTHERAPY

RT.02.1 - A PUBLIC-PRIVATE PARTNERSHIP TO FIGHT VISCERAL LEISHMANIASIS: STUDY OF A NOVEL LIPOSOMAL FORMULATION OF BUPARVAQUONE

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Public-private partnerships (PPPs) can be an efficient strategy for connecting basic research and clinical development to fight neglected parasitic diseases. Joining the expertise from pharmaceutical industry and the public sector, Drug Discovery & Development programs can beneficiate by sharing the elevated costs and risks. In the search for new uses for clinically approved drugs, buparvaquone (BPQ), a hydroxynaphtoquinone used in the veterinary treatment of Theileriosis, was considered a promising in vitro compound against Leishmania sp. Despite the lack of efficacy in rodent and canine models, BPQ is highly selective and could be considered a drug candidate if formulated in drug delivery systems (DDS). Although the elevated costs of liposomal drugs as liposomal amphotericin B (Ambisome) are sometimes prohibitive for most affected countries, cost-benefit analysis has been shown the higher prices of more sophisticated pharmaceutical formulations compensates the expenses with hospitalization and clinical monitoring of patients treated with conventional drugs. Furthermore, reduced doses and time of treatments with DDS, result in higher safety and patience compliance, also contributing to reduce resistance. In this talk, we will explore a PPP to improve the efficacy of BPQ by using phosphatidylserine-liposomes in a pre-clinical study with Leishmania (L.) infantum.

RT.02.2 - EXPLOITATION OF AUXOTROPHIES AND METABOLIC DEFECTS IN TOXOPLASMA AS THERAPEUTIC APPROACHES

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Membrane trafficking, a basic and essential cellular activity, plays pivotal roles not only for mammalian cell functions but also in interactions between mammalian cells and microorganisms. Upon infection with the intracellular protozoan Toxoplasma gondii, a mammalian cell acquires a novel dynamic compartment – the parasitophorous vacuole (PV). T. gondii designs the composition of the PV membrane by incorporating its own proteins and lipids, and selected components from the host cell's plasma membrane, which makes the PV a unique, specialized 'organelle'. Consequently, the PV is impervious to hostile cytosolic innate immune-surveillance pathways and inflammatory signaling cascades, and does not fuse with any organelles. However, the PV is not isolated from mammalian structures as microtubules, endosomes, the ER, mitochondria and Golgi vesicles closely associate with the vacuole, suggesting interplay between the mammalian cell and the PV. T. gondii has lost its capacity for living independently of another organism. It lacks many genes that encode for entire metabolic pathways and has, in return, expanded genes that promote nutrient scavenging to meet its basic metabolic requirements. Our talk will explore the natural auxotrophies and nutrient scavenging activities of the parasite, emphazising unique transport systems and salvage pathways. Previously, we showed that T. gondii is auxotrophic for low-density lipoprotein-derived cholesterol and retrieves this lipid from mammalian endocytic organelles. Since the PV

membrane is nonfusogenic, how T. gondii obtains its nutrients from the cell's organelles has been an intriguing question in the field. We analyzed the vesicular trafficking along the endocytic, recycling and secretory pathways in infected mammalian cells. We demonstrated that T. gondii intercepts Rab11 and Rab4 recycling vesicles, Rab1 vesicles originating from the ER, and the Rab14, Rab30 and Rab43 vesicles from the Golgi. These latter vesicles contained sphingolipids that were transferred to the parasite. All of these Rab vesicles were sequestered within the PV as intact vesicles. Our findings point to a general mechanism of nutrient acquisition that involves the macroendocytosis of organelles in the PV without the need for membrane fusion. From a therapeutic perspective, we will survey the different possibilities to starve T. gondii by nutrient depletion or disruption of salvage pathways.

RT.02.3 - THE EPIGENETIC ROAD TOWARD ANTIPARASITIC DRUG DISCOVERY

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The disease burden of infectious diseases is widely studied and is an important instrument to plan a strategy to control or prevent this disease. One featured scenario is the lack of innovation in drug discovery toward novel anti-parasitic agents to control and prevention. This is an important concern, and the genome sequencing of several parasites is able to accelerate the identification of new drug targets. In this context, the parasites epigenome rises as an interesting target to drug discovery programs. Epigenetics comprises a series of chemical modifications of DNA and their associated histone proteins and it is known to be an especially important aspect of parasite biology, although it is underexplored in drug discovery programs. In this context, it will be shown the epigenome of parasites as promising targets toward innovative drug candidates. Focusing on trypanosomatids, many well-conserved post-translational modifications of histones seem to be not present, while unusual histone sequences, histone modifications and modifiers exist in this family. Exploring these features is a new multidisciplinary approach for finding selectivity against parasites, bringing together molecular parasitology, enzymology, medicinal chemistry, organic synthesis and pharmacology toward the development of a versatile platform to to find drug candidates to infectious diseases. **Supported by:**Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP)

RT.02.4 - YEAST AS A VEHICLE FOR ANTI-KINETOPLASTID DRUG DISCOVERY

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High throughput screening (HTS) efforts for anti-kinetoplastid drug discovery have recently received increased attention as several initiatives have begun to attempt to reduce the deficit in new and clinically acceptable therapies for the Neglected Tropical Diseases caused by these protozoa. HTS primarily utilizes two basic approaches, cell-based and in vitro target-directed screening. Both of these have problems, for example cell-based screening does not reveal the target or targets that are hit, whilst in vitro methodologies lack a cell context. Furthermore, both can be technically challenging, expensive and difficult to miniaturize for ultra (u)HTS. The application of yeast-based systems may overcome some of these problems and offer a cost-effective platform for target-directed screening within a eukaryotic cell context. Here I will describe the successful use of such a system to identify inhibitors of the kinetoplastid Leishmania inositol phosphorylceramide synthase, an enzyme with multiple transmembrane domains making conventional biochemical assays challenging. Primary yeast-based uHTS with the GSK library (1.8 million compounds) identified 500 selective inhibitors and following secondary, cell-based screening a single compound class was selected on the basis of anti-leishmanial activity, low cytotoxicity and physicochemical properties. This compound class demonstrated potency against the inositol phosphorylceramide synthase in a cell-free biochemical assay, thereby validating the primary yeast-based uHTS platform. The success of this approach indicated that similar platforms could be developed for other targets from the kinetoplastids or other pathogens. **Supported by:**Open Lab Foundation

RT.03 - FROM GENOMIC PLASTICITY TO CELL INFECTION: A MOLECULAR VIEW OF PROTOZOA

RT.03.1 - DRIVING LICENSING, DIRECTION, AND ROAD CONDITIONS OF DNA REPLICATION IN TRYPANOSOMES

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The precise maintenance of genetic material requires the well-engineered control of DNA duplication. During the cell cycle in eukaryotes, DNA replication occurs only during S phase, and S phase DNA may only be replicated one time. The pre-replication machinery is a protein complex that recognizes and licenses replication origins, which recruits the replication machinery to initiate DNA duplication. Eukaryotic genome duplication relies on hundreds to thousands of origins of replication, distributed over multiple chromosomes, to initiate DNA replication. These origins are distinct in terms of efficiency and activation, and individual origins fire at characteristic times. A recent genome-wide analysis of Trypanosoma brucei, the etiological agent of sleeping sickness, localized its replication origins to boundaries of large transcription units. In this talk I will discuss the pre-replication machinery of trypanosomes, highlighting the differences between them and pre-replication machinery of other eukaryotes. Also, I will show some aspects of DNA replication fork progression that we found examining DNA replication by single molecule analysis of replicated DNA (SMARD). The speed of replication forks as well as the presence of dormant origins activated after replicative stress in Trypanosome brucei will be also discussed. **Supported by:**FAPESP

RT.03.2 - A DIVERGENT 9-1-1 CLAMP IS POSSIBLY INVOLVED IN *LEISHMANIA* GENOME PLASTICITY

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Chromosome copy number variation (CNV), gene amplification and arrays of duplicated genes are traits that clearly demonstrate the genome plasticity of *Leishmania*. This apparently loose control of gene CNV hints at a peculiar DNA metabolism. Uncontrolled CNV is commonly observed in mammalian cells with a defective cell cycle checkpoint response. In mammalian cells and also in yeast, a three-protein complex (the 9-1-1 clamp) plays a pivotal role in the detection of DNA damage structures and in the signaling events that lead to the cell cycle arrest required for proper DNA repair. We have been studying the peculiar homologues of the 9-1-1 components in *Leishmania*. We found two parasite proteins, LmHus1 and LmRad9, which are phylogenetically related to the 9-1-1 complex subunits from other eukaryotes. Interestingly, conventional searches suggested that the parasite genome does not encode a homologue for the third component of the clamp, Rad1. LmHus1 and LmRad9 not only are recruited to the chromatin upon genotoxic stress, but also forms a DNA damage responsive complex in vivo. Moreover, reduced levels of LmHus1 and LmRad9 affected the parasite ability to cope with the genotoxic stress caused by hydroxyurea, camptothecin or methyl methanesulfonate treatment. The clear involvement of LmHus1 and LmRad9 in DNA metabolism led us to speculate not only on the architecture of a possible 9-1-1 clamp in trypanosomatids, but also on the participation of these proteins in the molecular mechanisms underlying genome plasticity in *Leishmania*. Our current work is focused on the investigation of the interaction between possible components of a trypanosomatid clamp involved in DNA damage response. We are also investigating the molecular mechanisms underlying the role of LmHus1 and LmRad9 in the control of gene CNV in *Leishmania*. We believe these studies will contribute to our understanding of the way *Leishmania* maintain and express its genome. **Supported by:**FAPESP

RT.03.3 - IMMEDIATE EARLY RESPONSE GENES IN *TRYPANOSOMA CRUZI* INFECTION

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Trypanosoma cruzi has the peculiarity, when compared with other intracellular parasites, to invade almost any type of cell. Trypomastigotes must interact, through their surface, with host-surface molecules, in order to generate signaling and/or metabolic changes that favor infection. When parasites enter their host through a skin lesion, by contact with mucous tissue, or by ingestion, the establishment of the infection depends on its ability to rapidly invade epithelial cells that constitute the first barrier against infections. The epithelium provides both a physical barrier and a variety of antimicrobial factors to avoid microbial entry. On the other hand, trypanosomes invade macrophages, with consequent relevance both in innate and adaptative immunity. Once the infection becomes chronic, a significant percentage of patients evolve to cardiac damage, which involves the invasion of cardiomyocytes by T. cruzi. In this work we focused our study in the early response of human cells to Trypanosoma cruzi infection, in epithelial cells, macrophages and cardiomyocytes, through the study of transcriptomic changes. In the three cell types studied, hundred of genes are up and down regulated immediately after infection: cells are literally reprogramed. However, a detailed analysis of the altered pathways clearly indicate that each cell type has extremely different responses. Epithelial cells are mostly altered in pathways related to inflammatory and lipid metabolism genes; in cardiomyocytes the glycolysis, oxidative phosphorylation and protein synthesis related genes are the most affected pathways. In macrophages, although as expected, immune response related genes are the most affected, a more in deep analysis at the level of alternative splicing patterns indicates that the most up regulated genes are related to autoimmune diseases.

RT.03.4 - DIFFERENT CAPS FOR DIFFERENT OCCASIONS – THE RNA CAP BIOLOGY OF *TRICHOMONAS VAGINALIS*

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The phylogenic position of the ‘deep-branching’ or ‘fast-evolving’ eukaryotes, such as Trichomonas vaginalis, is under debate. This gives us the opportunity to examine basic mechanisms of gene expression that are evolutionarily conserved among eukaryotes. The observation that this organism contains structurally conserved spliceosomal snRNAs lacking a guanosine cap nucleotide leaded us to investigate RNA cap biogenesis. Although our findings indicate that splicing and capping have been conserved throughout eukaryotic evolution, T. vaginalis is unique in some aspects. It makes use of a metazoan/plant-like capping machinery and, different than yeast and metazoans/plants, it has a conserved cap 1 structure. This organism harbours a cap hypermethylase or Tgs and it contains all three methylated forms of guanosine caps. Contrary to other eukaryotes, snoRNAs box H/ACA are solely the preferred substrates for this enzyme in T. vaginalis. Despite the evolutionary conservation, our study illustrates that RNA cap evolution in eukaryotes was hallmarked by multiple events of gene loss/gain and appears to have continuously evolved after lineages diverged.

RT.04 - IMMUNE RESPONSE TO LEISHMANIA

RT.04.1 - AN OVERVIEW OF THE SYSTEMIC AND COMPARTMENTALIZED IMMUNE RESPONSE IN CANINE VISCERAL LEISHMANIASIS.

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Human visceral leishmaniasis (VL) and canine visceral leishmaniasis (CVL) are the most important emerging diseases with high prevalence in Latin American countries and are mainly caused by Leishmania (L.) infantum. CVL has a great impact on Brazilian public health because domestic dogs are the most important VL peri-domicile reservoirs in both urban and peri-urban areas. Our findings highlight the complexity of cellular immunological events related to the natural infection from dogs by L. infantum, additionally correlating major peripheral blood phenotypic markers with clinical status and tissues parasite density. Regardless the systemic immune response, our main results demonstrated that lower frequency of circulating B cells and monocytes are important markers of severe CVL, whereas increased levels of CD8+ lymphocytes appear to be the major phenotypic feature of asymptomatic disease. Pioneer findings obtained by our group showed a correlation between clinical status of CVL with degree of tissue parasite density. This data demonstrated that asymptomatic dogs presented low parasitism while symptomatic dogs are associated with high parasite load in various tissues such as skin, bone marrow and spleen. We have also investigated the association between tissue parasitism and CVL clinical forms. Despite the consequences of clinical status, skin and spleen are the major sites of high parasite density during ongoing CVL. Cytokine and transcription factor profiles in the skin of dogs naturally infected by L. infantum presenting distinct cutaneous parasite density and clinical status. A mixed cytokine profile with high levels of expression of IFN-γ, TNF-α and IL-13 was determined in asymptomatic dogs. Additionally, the levels of transcription factors GATA-3 and FOXP3 were correlated with the asymptomatic disease. A mixed cytokine profile was also observed during active CVL. Moreover, high levels of IL-10 and TGF-β1, concomitant with the low expression of IL-12, may represent a key condition that allows persistence of parasite replication in the skin. In addiction these results indicate that in asymptomatic disease or lower levels of skin parasite density, a mixed inflammatory, regulatory immune response profile may be of major relevance for both the maintenance of the clinical status of the dogs as well as for parasite persistence and replication at low levels. In this sense, we demonstrated that higher expression of CCL2, CCL4, CCL5, CCL21, and CXCL8 chemokines in the skin associated with parasite density in CVL. These findings represent an advance in the knowledge about skin inflammatory infiltrates in CVL and the systemic consequences. Furthermore, we demonstrated that spleen parasitism is the most reliable parasitological markers to decode the clinical status of CVL. We evaluate the cellular immunophenotypic profile in the splenic compartment during ongoing CVL demonstrating that the role of CD8+ T splenocytes seems to be important for an effective immunological response, a hallmark of asymptomatic CVL, whereas the pronounced loss of MHC-II expression upon LSA stimulation is a biomarker of symptomatic CVL. Moreover, a positive correlation between the levels of expression of IL-10 with respect to the progression of the disease and the levels of expression of IL-10 and INF-gamma increase in higher parasitism were observed. Such data suggest that CVL is marked by a balanced production of Th1 and Th2 cytokines, with a predominant accumulation of IL-10 as a consequence of an increase in parasitic load and progression of the disease. Herein, we have reviewed some aspects of the immunopathological events occurring in natural L. infantum infection, pointing out the main L. infantum-parasitized tissue. We have discussed the importance of the association between parasite density, immunopathological aspects and clinical status of the CVL, their current applications, challenges for the future and potential opportunities in CVL research. **Supported by:**CNPq, FAPEMIG, CAPES

RT.04.2 - THE ROLE OF NEUTROPHILS DURING VISCERAL LEISHMANIASIS: EFFECTOR AND REGULATORY MECHANISMS

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Visceral leishmaniasis (VL) is a chronic and fatal disease caused by *Leishmania infantum* in Brazil. Leukocyte recruitment to infected tissue is a crucial event for the control of infections such as VL. Among inflammatory cells, neutrophils are recruited to the site of Leishmania infection and may control parasites replication through oxidative or non-oxidative mechanisms. The neutrophils recruitment, activation and functions are coordinate by pro-inflammatory cytokines, chemokines, and lipid mediators released during parasites recognition by pattern recognition receptors (PRRs). TLR9 contributes to the development of the innate immune response developed during infection by *L. infantum*. The protective mechanism is related to the recruitment of neutrophils to the appropriate inflammatory site. This mechanism is directly coordinated by DCs which, when activated via TLR9, induce the release of chemotactic mediators of neutrophils to infectious focus and thus control parasites. In addition, Leucotrienes induces neutrophils recruitment to infectious foci by providing a prototypal Th17 environment. Neutrophils being important sources of substances that may promote tissue damage, its regulation must be finely controlled in order to eliminate the pathogen but not causing immunopathology. The modulation of neutrophils by adenosine and its A2A receptor seems to be an important mechanism to prevent tissue damage developed during VL. Furthermore, IL-27 may also promote a regulation in the inflammatory process. Understanding the mechanism involved in infectious process caused by L. infantum focusing on neutrophils functions may elucidate, at least in part, the immunopathogenesis of VL. **Supported by:**SBPZ

RT.04.3 - HOW AND WHEN A CUTANOEUS LEISHMANIASIS PATIENT ACHIEVES AN IMMUNOLOGICAL PROFILE ASSOCIATED WITH CURE OR PROTECTION AGAINST *LEISHMANIA (VIANNIA) BRAZILIENSIS* INFECTION?

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Leishmania reactive CD4+ and CD8+ T cells are expanded in long-term healed cutaneous leishmaniasis (hCL) patients but their functional characteristics remain to be determined. Indeed, transcription factors (TF) are important to regulate cytokines involved in the maintenance of clinical cure or parasite control. This study investigates antigen-specific recall in long-term hCL caused by L. braziliensis, and if the healing time affects the degree of lymphocyte differentiation and activation, TF induction and cytokine profile. Healed CL according to the time elapsed since the end of therapy: less than 2 years (CCL<2y) and 2 to 5 years (CCL2–5y), spontaneous healing (SH>10y). Activation phenotype (CD69+/CD25+) and central memory (TCM: $CD45RO^{+}CCR7^{+}$) or effector memory (TEM: $CD45RO^{+}$ $CCR7^{-}$) were quantified in ex vivo blood mononuclear cells and after *Leishmania* antigens stimuli. A reduction in the percentage of activated *Leishmania*-responder $CD4^{+}$ and $CD8^{+}$ T cells in hCL was associated with the time elapsed since clinical cure. Ex vivo analyses showed contracted TEM CD4+ and TEM CD8+ compartments, although they were renewed following in vitro exposure to leishmanial. There was no difference in T-bet, GATA-3, and Foxp3 RNA copies between CCL. However, a correlation matrix showed that CCL2–5y presented higher number of positive correlations between pro- and anti-inflammatory/regulatory molecules than SH and CCL<2y, indicating that CCL2–5y achieved higher immune response homeostasis. Our results show that healed *L. braziliensis* infected patients exhibit a recall response to *Leishmania* antigens with evident expansion of effector memory T cells. Even though all participants had the same clinical outcome, the induction of cytokines and regulatory factors may be related to stable cure of CL, a process that nevertheless takes a long time to be achieved. Then, regulated leishmanial-specific response seems to emerge only about two years after initial contact with the parasite antigens.

RT.04.4 - CYTOTOXIC RESPONSES ARE RESPONSIBLE FOR TISSUE INJURY IN THE LESIONS OF LEISHMANIASIS CUTANEOUS PATIENTS

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The role played by CD8T cells in cutaneous leishmaniasis is not well established. Recently, we showed CD8 Tcell accumulate in cutaneous leishmaniasis (CL) lesions. Most of these cells express cytolitic markers, CD107a and granzyme B. Granzyme B expression in lesions positively correlated with lesion size and percentage of TUNEL-positive cells. It is also observed a significantly higher percentage of TUNEL-positive cells and granzyme B expression in the biopsies of patients showing a more intense necrotic process. Using different experimental approaches of co-culture of infected macrophages and CD8 and CD4 T cells, it was noticed the all the mediators released by CD8T cells did not have any effect on parasites killing. On the other hand, IFN-g produced by CD4 T cells is responsible for Leishmania killing. Recently, we used proteomics and biological networks analysis to identify potential biological processes and components present in the proteins identified in biopsies from CL patients infected by L. braziliensis. There were 59 different proteins differently expressed in samples from infected and normal skin. Biological networks analysis performed by identified proteins showed the presence of networks that amy be involved in the cell death mediated by cytotoxc T lymphocytes. After immunohistochemistry analyses, the expression of caspase9, caspase3 and grnazyme B were validated in the tissue and positively correlated with the lesion size in CL patients. These studies identified differentially expressed proteins in the inflammatory site of CL and highlighted mechanisms associates with the progression of tissue damge observed in lesions.

RT.05 - CELLULAR AND MOLECULAR INTERACTIONS OF APICOMPLEXAN PARASITES AND THEIR HOSTS

RT.05.1 - *TOXOPLASMA GONDII* USES APOPTOTIC MIMICRY TO EVADE THE NITRIC OXIDE MICROBICIDAL DEFENSE SYSTEM

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Toxoplasma gondii is the agent of toxoplasmosis a disease that infects around 30% of the human population. This parasite has many microbicidal evasion mechanisms, including “apoptotic mimicry” that is based on the exposure of phosphatidylserine (PS) and the mimicking of the anti-inflammatory response of apoptotic cells. Nitric oxide (NO) is a microbicidal agent produced by inducible NO synthase (iNOS) that controls *T. gondii* growth. However, it has been shown that infection of *T. gondii* in activated macrophages causes the degradation of iNOS, and reduction of NO production, allowing the parasite to persist in these cells. Here we show what the molecular mechanism involved in the degradation of iNOS is, how apoptotic mimicry is involved, and if it is a general mechanism within different strains of *T. gondii*. The described degradation mechanisms that normally control iNOS expression are: proteasome, calpain and lysosomal. Activated macrophages were pharmacologically inhibited and iNOS expression and NO production assayed. Only the inhibitor of the proteasome pathway was able to revert degradation of iNOS and NO production inhibition after *T. gondii* infection. To verify if apoptotic mimicry was involved in this phenomenon, four strains of *T. gondii*, with different virulence, were used to infect activated macrophages. Only the RH strain (more virulent) was capable of persisting in activated macrophages, sustaining the degradation of iNOS up to 48h. The other three strains (ME-49, VEG, PBr) initially degraded iNOS and, thus, inhibited NO production. However, after 6-12h, they were destroyed by the macrophage, and iNOS expression and NO production returned. In all four strains transforming growth factor-beta1 signaling was involved in the degradation of iNOS but only the RH strain sustained this response for the examined period. All four strains exposed PS, but, surprisingly, the less virulent strains did it in a higher level. Taken together, these results suggest that the exposure of PS is a general mechanism, important for this parasite to persist in activated macrophages, indicating that the evasion of the NO microbicidal defense system is probably imperative to all strains of *T. gondii*. **Supported by:**FAPERJ, CNPq, CAPES

RT.05.2 - MRNA EXPORT IN PARASITES AND MAMMALS: ARE THEY USING THE SAME PATHWAYS?

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In eukaryotes, the mRNA is exported by a specific pathway that is dependent on the nuclear receptor TAP in mammals (Mex67 in yeast). Excluding model eukaryotic organisms, the mRNA export machinery of several species of parasites remains unknown. Previous results from our group indicate that mRNA export pathways are the less conserved, suggesting a divergence during evolution (Serpeloni et al, 2011a). We are currently investigating the function of some proteins in the protozoans Trypanosoma cruzi and Toxoplasma gondii to better understand mRNA export in early diverging eukaryote lineages. The most conserved protein throughout the eukaryotic philogeny is UAP56, a specific component of mRNA transcription/export complex (TREX). We demonstrated that this protein is involved in export of mRNA in trypanosomes (Serpeloni et al, 2011b) and T.gondii (in preparation). The conserved function of UAP56 in parasites from different phyla indicates that it is a core component of mRNA export in protozoa species. So far, we cannot find homologues of other components from mRNA export that have been already described in mammals and yeast. However, we have identified a RNA Helicase that shuttles between the nucleus and cytoplasm and is located near to Nuclear Pore Complex (Inoue et al. under revision). These data show that the shuttling of this helicase is dependent on the homologue of Mex67 receptor in trypanosomes. It indicates that components of the mRNA export pathway in parasites present distinct features and that the function of specific components needs to be dissected within the context of these particular organisms. For this purpose, we have obtained a T.gondii stable cell line for inducible expression of the yellow fluorescent protein (YFP) fused to MS2 protein that resulted in specific labelling of a tagged-mRNA in the cytoplasm. We aim to generate parasite lines where the MS2 system will be used to evaluate phenotypes that affect mRNA export. The establishment of this system will provide an important tool for analysis of genes involved in mRNA export as well as for studies into other molecular biology events, such as mRNA localization and/or translation. **Supported by:**Fiocruz, Fundação Araucária, CAPES

RT.05.3 - HOTSPOTS OF BLOOD VESSEL INVASION BY MALARIA SPOROZOITES

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After being inoculated into the extravascular regions of the host skin by an infected mosquito, malaria sporozoites are obliged to cross the endothelial barrier of cutaneous blood vessels to enter into the systemic circulation, and ultimately invade hepatocytes. Here we describe when, where and how sporozoites intravasate in the host skin. Using multimodal in vivo imaging, we show that most sporozoites leave the inoculation site and invade the liver in the first hour post-inoculation. Surprisingly, blood vessel invasion events are not random. Instead, multiple events of invasion are clustered in space and time at preferential sites on the skin microvasculature. These hotspots of invasion are associated with the presence of CD146+CD31-Flk1- pericytes, which are contractile perivascular cells closely apposed to the endothelial cells. These perivascular cells are the skin counterparts of hepatic stellate cells, which are thought to play a role in the arrest of sporozoites in the liver. Using functional imaging, we also observe that the majority of the cutaneous endothelial crossing events are cell traversal independent, i.e., are not linked with the sporozoite capacity of breaching the host cell membrane and migrate through the endothelial cell cytoplasm. Indeed most invasions are occurring at the extravasation sites of intravenously injected nanoparticles, suggesting that sporozoites are using a paracellular route to get access to the blood circulation. In summary, most sporozoites leave rapidly the site of inoculation by intravasating at hotspots associated with the presence of pericytes, presumably passing through inter-endothelial junctions in a cell traversal-independent manner.

RT.05.4 - INFLAMMASOME ACTIVATION DURING PLASMODIUM INFECTION

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Together Plasmodium falciparum and P. vivax infect approximately 250 million individuals, reaping life of near one million children every year. Extensive research on malaria pathogenesis has funneled into the consensus that the clinical manifestations are often a consequence of the systemic inflammation. Importantly, secondary bacterial and viral infections potentiate this inflammatory reaction being important co-factors for the development of severe disease. We dissected the mechanisms of induction and the importance of the pyrogenic cytokine, IL-1β in the pathogenesis of malaria. Our results demonstrate the critical role of the innate immune receptors named Toll-Like Receptors and inflammasome on induction, processing and release of active form of IL-1β during malaria. Importantly, we provide evidences that bacterial superinfection further potentiates the Plasmodium-induced systemic inflammation, leading to the release of bulk amounts of IL-1β and severe disease. We have shown that NLRP12/NLRP3-dependent activation of caspase-1 is likely to be a key event in mediating systemic production of IL-1β and hypersensitivity to secondary bacterial infection during malaria. Hence, thus data elucidated new checkpoints that could be targeted for preventing systemic inflammation and severe malaria.

RT.06 - INSIGHTS INTO VECTOR PATHOGEN INTERACTIONS

RT.06.1 - NEW INSIGHTS INTO THE PROTEIN AND GLYCAN COMPOSITION OF TRYPANOSOME-INFECTED TSETSE SALIVA

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During the tsetse life cycle, all Trypanosoma brucei sub-species colonize the fly's salivary glands (SG) to develop into the infectious metacyclic form. Establishment of a trypanosome infection in SGs is accompanied by a severe down-regulation in the overall expression of salivary proteins, which causes a feeding phenotype. Despite the importance of tsetse saliva in disease transmission, little is known about its composition during a trypanosome infection. We reasoned that saliva from trypanosome-infected flies contain soluble factors that may be important for parasite transmission into the mammalian host. Here, we compared the proteome and glycome of naïve and trypanosome-infected tsetse saliva.

Salivary proteins from both naïve and trypanosome-infected saliva were either subjected to an in-solution analysis or fractionated on 1D SDS-PAGE, followed by trypsin digestion. nLC-MS/MS analysis of the tryptic fragments from both preparations suggest that the overall protein composition of trypanosome-infected saliva does not change upon an infection. Furthermore, saliva from infected flies contains abundant trypanosome GPI-anchored glycoproteins, including several members of the large Fam50 protein family metacyclic-specific variant surface glycoproteins (VSG). Interestingly, tsetse saliva also contains factors from bacterial symbionts.

As N-glycosylation is important for protein secretion it was investigated whether T. brucei infection alters the glycosylation of salivary proteins. Tsetse saliva glycans were enzymatically released and their structures determined by LC-MS/MS. A trypanosome infection does not alter protein glycosylation, which mainly consists of high mannose- and hybrid-type species.

In summary, trypanosome-infected tsetse saliva is a cocktail comprised of tsetse, parasite and bacterial molecules, which may be important for parasite transmission. These could potentially lead to novel anti-disease vaccine candidates and biomarkers discovery. **Supported by:**Wellcome Trust

RT.06.2 - EXPLORING THE TICK RESPONSE TO RICKETTSIAL INFECTION

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Ticks are blood-feeding parasites transmitting a large variety of pathogens to their vertebrate hosts. The vector competence of ticks is firmly linked with their immune system. However the knowledge of this system in ticks is still scarce. In order to characterize the molecular mechanisms driving the immune response of ticks, we have initially done an in silico search for homologous sequences of the main cell signaling pathways involved in immunity (Toll, Imd and Jak/Stat) as well of immune effectors followed by transcriptomic analysis of Anaplasma marginale -infected tick cell line BME26. The in silico analyses demonstrated that both Toll and Jak/Stat pathways are highly conserved across arthropods. However, important components of the Imd pathway were not found in ticks, including Imd and its associated proteins FADD, DREDD, Pirk and Dnr1. High-throughput qPCR analyses revealed that while the encoding genes of certain components of the three signaling pathways were repressed in A. marginale-infected cells, those were induced after stimulation with M. luteus, E. cloacae and S. cerevisiae and to a lesser extent in R. ricketsii-infected cells. Moreover, in both A. marginale and R. rickettsii-infected cells, the transcript abundance of antioxidants was significantly induced, including peroxidase and catalase. Conversely, the expression of genes involved in ROS production, as dual oxidase 1 and 2, was down-regulated by the A. marginale infection. Taking together our data suggest that A. marginale induces an immunosuppression of the tick genes. In order to go further on the immune mechanisms involved in tick-rickettsia interactions, functional studies are underway. **Supported by:**FAPESP, CNPq, CAPES e INCT-EM

RT.06.3 - CHARACTERIZATION OF *LUTZOMYIA LONGIPALPIS* INTERACTION WITH *LEISHMANIA* AND VIRUS

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Lutzomyia longipalpis is the major vector for visceral leishmaniasis in Brazil and also transmits some viruses. We are studying immune responses both in the insect vector and in the *L. longipalpis* LL5 embryonic cell line. Insect innate immune response pathways are Toll, IMD and JAK-STAT. We have identified an IMD and Toll response in LL5 cells through the silencing of the respective repressor genes caspar and cactus. This silencing increased the expression of the antimicrobial peptides (AMPs) defensin and cecropin, thus establishing LL5 cells as a valid model to study sandfly immunity. We also investigated the effect of these repressors in female adult insects. We have previously determined a role for the IMD pathway in the vector infection by *Leishmania* through the silencing of the repressor caspar, which decreased infection. We now silenced cactus and, surprisingly, as opposed to LL5 cells, this led to the decrease of AMPs production. As a possible explanation, we verified that WntD, an inhibitor of the Toll pathway identified in Drosophila, had increased expression upon cactus silencing. This might be related to the preservation of the insect microbiota, which was actually decreased when cactus was silenced. We have also studied the effects of *Leishmania* infection on *L. longipalpis*. Infection caused an early increase of cactus expression followed by a return to normal levels that was accompanied by a higher expression of AMPs. Interestingly the infection by *Leishmania* also caused an early growth of the microbiota, probably related to the increase of cactus expression. We were intrigued by the apparent capacity of *Leishmania* to increase the expression of cactus and thus inhibit the Toll pathway. It is known that *Leishmania* Gp63 activates a macrophage tyrosine phosphatase (SHP-1), capable of inhibiting the Toll and Jak/STAT pathways. *L. longipalpis* has a homoloque of SHP-1, which has increased expression during *L. longipalpis* infection by L. i. chagasi, indicating the possible conservation of a mechanism of *Leishmania* infection control in mammals and insects.

We have previously identified a nonspecific antiviral response in LL5 cells in response to transfection with double stranded RNA (dsRNA). This was the first report of this kind of response in an insect cell line. We are presently identifying the mechanisms by which LL5 cells recognize dsRNA, using various approaches. We have performed deep-sequencing of transcripts from cells transfected with dsRNA or mock-transfected (control). These data are

under analysis. We have also investigated the involvement of exosomes in the antiviral response. Exosomes isolated from dsRNA transfected cells induced an antiviral response in LL5 cells. The exosomes protein composition was determined by mass spectroscopy and these data are under analysis. Among interesting proteins encountered specifically in dsRNA transfected exosomes was vir, a virus induced protein. miRNAs were detected in the exosomal fractions. These are presently being sequenced in search for differential small RNAS expression in cells transfected with dsRNA. We also prepared conditioned media from cells mock or dsRNA transfected. Conditioned medium from cells transfected with dsRNA was able to reproduce the antiviral response in LL5 cells. We performed proteomics on the secreted proteins. Among candidates for antiviral response we found a scramblase, which in humans is an interferon-stimulated gene with an antiviral function. These and other candidates of interest are presently being validated.

RT.06.4 - *WOLBACHIA* IN A DENGUE CONTROL STRATEGY

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Vector-borne diseases such as dengue, malaria and other arboviruses impose serious burden towards public health in tropical regions of the world. Current control methods mainly rely on insecticides for mosquito control and because of that, resistance against commonly used chemicals is increasingly widespread. Our project involves the use of a naturally occurring bacterium called *Wolbachia* as a novel biological control agent. *Wolbachia* manipulates the reproduction of their host in order to be vertically transmitted from the mother to offspring. This bacterium is believed to be present in up to 70% of all insect species worldwide but it has never been found in *Aedes aegypti*, the main vector for dengue. When stably introduced into *A. aegypti*, *Wolbachia* was able to block dengue virus transmission and significantly reduced Chikungunya virus and Plasmodium gallinaceum load in these mosquitoes. Currently, field tests are been carried out in Australia, Indonesia and Vietnam (as part of the Eliminate Dengue Program), where Wolbachia-containing mosquitoes were able to invade local populations of *A. aegypti*. Since 2012 we have been working to implement this project in Brazil, involving the introgression of Wolbachia into Brazilian *A. aegypti* background, field entomology activities to study mosquito abundance and population structure as well as community engagement activities. Here we will discuss several aspects towards the implementation of the Eliminate Dengue Project in Brazil, showing recent results of mosquito vector competence, field entomology, community engagement and communication. This strategy has the potential to significantly reduce the burden of dengue in Brazil and in other endemic countries and is perfectly complementary to current control methods, including vaccines.

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