MC001 - **REDOX PARADOX: THE CONTRIBUTION OF REACTIVE OXYGEN SPECIES (ROS) IN THE PROLIFERATION AND DIFFERENTIATION OF *TRYPANOSOMA CRUZI*.**

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Trypanosoma cruzi is a protozoan that causes Chagas disease or American trypanosomiasis. The parasite has a heteroxenic biological cycle developing between a vertebrate host (mammal) and an invertebrate (insect vector). The first environment encountered by *T. cruzi* after the blood meal is the midgut of the insect, where large amounts of hemoglobin are degraded resulting in the release of huge concentrations of heme, a molecule known to increase the formation of ROS. Heme induces *T. cruzi* proliferation, and this phenomenon is accompanied by a marked time and concentration dependent increase in ROS formation and the modulation of epimastigotes mitochondrial physiology in an effort to increase ROS as a metabolic mechanism to maintain epimastigote survival and proliferation. Conversely, the antioxidants reverse the heme-induced ROS and dramatically impair epimastigotes proliferation. Following the cycle, when these forms are taken into the hindgut of the triatomine they return to the non-replicative, infective form, the metacyclic trypomastigotes. During the metacyclogenesis, antioxidants induce a significant increment of trypomastigotes. These data gives us the idea of a “shift” between proliferation to differentiation according to the influence of the redox status. Surprisingly, when metacyclic trypomastigotes are released into the insect feces the infection is again favored by ROS since inhibitors of Nox and antioxidants greatly decrease macrophage infection. Moreover, while the parasite needs oxidants for its proliferation inside the vector, the opposite occurs during its development inside the vertebrate host. H_2O_2 diminishes amastigotes proliferation and increases the trypomastigote differentiation into amastigotes. Taken together, our data reveal a redox paradox in which ROS plays antagonistic roles along *Trypanosoma cruzi* life cycle demonstrating a high capacity to adapt to its habitats. **Supported by:**FAPERJ, CNPq e INCT-EM
Keywords: Trypanosoma cruzi; ros; chagas disease

MC002 - **EXTRACELLULAR VESICLES IN PATHOGENIC PROTOZOANS: ROLE DURING THE HOST-PARASITE INTERFACE.**

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Trypanosoma cruzi and *Leishmania* are the causative agents of Chagas disease and Leishmaniasis, respectively. Those neglected diseases affect millions of people worldwide. Several studies have focused on understanding the role of parasite molecules during the immunopathological events in those diseases. In the past years, extracellular vesicles (EVs) have become an important field of interest due to their various functions in different cells. In pathogens, those structures are involved in several mechanisms during the host-pathogen interface. Those include communication, immunomodulation, pathology, invasion and many others. Trypomastigosted forms of *T. cruzi* release vesicles containing a wide range of surface molecules including GPI-anchored glycoconjugates. They are enriched with trans-sialidase (TS)/gp85 glycoproteins and α-galactosyl (αGal)-containing glycoconjugates, like mucins. Pre-treatment of BALB/c mice with EVs, followed by parasite challenge, significantly exacerbated parasite load and inflammation of the heart, and hastened animal mortality. Later, *T. cruzi* EVs were shown to induce NO and proinflammatory cytokines (TNF-α and IL-6) in murine macrophages via TLR2. Pre-treatment with EVs considerably enhanced invasion of host cells. In *Leishmania*, the role of EVs was first studied in *Leishmania donovani* during the interaction with macrophages. A distinguished feature of *Leishmania* EVs is the presence of the virulence factor gp63 in their surface. However, it is still unknown if other glycoconjugates highly expressed in the surface of *Leishmania* spp including lipophosphoglycan (LPG), glycoinositolphospholipids (GIPLs) are also present. Here, we determined if such molecules are also expressed in EVs of New World species *Leishmania infantum* and *Leishmania braziliensis*, the causative agents of visceral and cutaneous forms, respectively. Promastigote forms of both species were incubated in serum-free media for 2 hours, 37 oC for vesiculation. Parasites were fixed for conventional scanning electronic microscopy (SEM) for vesicle release demonstration. Culture supernatants were processed by

differential centrifugation and vesicles were isolated by Size Exclusion Chromatography (SEC) and quantitated by nanoparticle tracking analysis (NTA). NTA successfully detected the presence of EVs in the expected size range of exosomes with modal sizes of 125.2 (±15.8) nm and 133.6 (±15.8) nm for *L. infantum* and *L. braziliensis*, respectively. Immunodetection of glycoconjugates was performed in ELISA and dot-blot assays, SEC fractions were probed with anti-LPG (CA7AE) and anti-gp63 mAbs (1:1000). LPG was detected in same the gp63-exosome-containing fractions. Those data strongly support that LPG is also present in the surface of promastigote-derived vesicles. The LPG of *L. braziliensis* is very-pro-inflammatory via TLR2, whereas that of *L. infantum* is very immunosuppressive. Since this molecule is present in the EVs, our next step is to investigate whether those properties will also reflect in the immunomodulation of murine macrophages upon EVs stimulation. In conclusion, trypanosomatid EVs have an important role during the interaction with the vertebrate host. In *T. cruzi*, those structures seem to be more proiniflammatory, whereas in *Leishmania*, they exhibit a more immunosuppressive/immunomodulatory role. Together with other molecules, surface glycoconjugates are also expressed in EVs surface and may act as pathogen-associated molecular patterns (PAMPs) during the initial events of infection. **Supported by:**FAPEMIG/CNPQ
Keywords: Extracellular vesicles; trypanosoma cruzi; leishmania

MC003 - BLASTOCRITHIDIA, A TRYPANOSOMATID WITH ALL THREE STOP CODONS REASSIGNED

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Recently, the trypanosomatid Blastocrithidia and the ciliates Condylostoma and Parduczia were shown to reassign in their nuclear genome all three stop codons to code for amino acids. Blastocrithidia represents an ideal model system for studying this phenomenon. It belongs to the order Trypanosomatida, a well-studied protist group, which includes model species Trypanosoma and Leishmania, with available complete genomes and established methods of forward and reverse genetics. Unlike ciliates, which are well-known for genetic code reassignments, all known trypanosomatids except Blastocrithidia have a canonical nuclear genetic code. Consequently, the reassignment must have happened in this group relatively late on the evolutionary scale. Hence, we hope to be able to trace key steps leading to the emergence of such a system. We have sequenced and analyzed the genomes and transcriptomes of two cultivable Blastocrithidia species and also the genome of Leptomonas jaculum, a close relative of Blastocrithidia with a canonical genetic code. Besides, we have performed mass-spectrometry analysis of a subset of Blastocrithidia proteins to verify the predicted amino acid specified by the in-frame stop codons. We provide evidence that UGA codes for tryptophan, UAG and UAA code for glutamate, while only UAA is also used as a genuine stop codon in a context-dependent manner. We analyze the unique prerequisites of Blastocrithidia, which likely made the reassignment of all stop codons possible, and speculate about alterations of the translation termination in this remarkable flagellate.
Keywords: Genetic code; blastocrithidia; stop codon

MC004 - ***LEISHMANIA*** **DEVELOPMENT IN SAND FLIES: INSIGHT INTO SPECIFIC AND NON-SPECIFIC INTERACTIONS**

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Leishmania have evolved various mechanisms for surviving within the sand fly´s gut. Natural vectors are very susceptible and 1-2 parasites is enough to initiate mature infections. Blood taken by sand fly females is directed to the abdominal midgut and surrounded by peritrophic matrix (PM). The amount of the bloodmeal varies usually from 0,6 to 0.9 microliters. Amastigotes ingested along with a bloodmeal transform first into procyclic promastigotes. Some earlier studies have described that transforming parasites are susceptible to sand fly proteases, but our recent experiments did not show any direct negative effect. *Leishmania* are protected against proteolytic damage by surface phosphoglycans.

The PM represents an important mechanical barrier to promastigotes and prevents their escape from endoperitrophic space. At the end of digestion process, sand fly chitinases disintegrate the PM on its posterior end but *Leishmania* chitinase does not seem to participate in this process. The kinetics of PM synthesis and disintegration differs between sand fly species. After the PM disintegrates, the promastigotes must attach themselves to the midgut wall to avoid being expelled with the blood meal remnants. In some species this period is extremely short which results in this sand fly being refractory to various *Leishmania*.

In the midgut, promastigotes attach by flagella, inserting them between microvilli. While the early phase of infection in the vector is relatively non-specific, the attachment significantly differs between various sand flies. Some sand fly species display strict specificity for *Leishmania* species, for example *P. papatasi* supports late stage development of *L.major* and *L.turanica* only. Such sand flies are called, “specific” or “restrictive vectors”. On the other hand, the majority of *Phlebotomus* and *Lutzomyia* species support late stage development of multiple *Leishmania* species and are thus called “permissive vectors”. In addition, there is also a third category, sand flies refractory to all *Leishmania* tested. The midgut binding mechanism differs between specific and permissive vectors. In *P. papatasi* the attachment to midgut microvilli is controlled by galectin which serves as a receptor for terminal galactose present on L. major and L. turanica lipophosphoglycan (LPG). More recently, partial involvement of the flagellar protein FLAG1/SMP1 was demonstrated in P. papatasi-L. major pair. In contrast, in permissive sand flies the attachment does not require LPG, various *Leishmania* species attach due to unspecific binding to mucin-like molecules in sand fly midgut. Permissive sand fly species should be considered as potential vectors of various *Leishmania* species and they could be responsible for establishing new leishmaniases foci, the most important example is *Lutzomyia longipalpis*, the major New World vector of *L. infantum* (= *L. chagasi*).

Once attached and established in the midgut, parasite forms called leptomonads replicate vigorously and then migrate anteriorly to thoracic midgut. They accumulate in large numbers in the thoracic part of the midgut, produce promastigote secretory gel which, together with parasite masses, physically obstructs the gut. *Leishmania* also colonize the stomodeal valve, forms called haptomonad attach to cuticular lining of the valve through an expanded flagellum, cause its damage, interfering with its function and facilitating reflux of parasites from the midgut. Stomodeal valve of heavily infected sand flies seems to be permanently open, the shape of cells is changed and the chitin lining is destroyed due to the action of *Leishmania* chitinase. The so called “blocked sand flies” have problem to take a bloodmeal, bite repeatedly, increasing the chance of *Leishmania* transmission.

Keywords: Leishmania; phlebotomus; lutzomyia

MC005 - **UNEXPECTED PHENOTYPIC VARIABILITY OF *TRYPANOSOMA BRUCEI* PARASITES**

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Parasites have evolved sophisticated mechanisms of sensing and adapting to the environment. Understanding the cellular and molecular basis of such processes provide not only insight in the mechanisms of disease, but it may uncover novel therapeutic targets. *Trypanosoma brucei* is a protozoan parasite, responsible for sleeping sickness in humans and nagana in cattle. In my laboratory, we use a combination of tools to characterize how these extracellular pathogens adapt to the environment. We will present data showing that *T. brucei* adapts to time and space. By using genome-wide tools, we uncovered that *T. brucei* has a circadian clock that results in the 24 hour rhythmic expression of 10% of its genes. This rhythm is independent of the host and it is entrainable by temperature cycles, leading to phenotypic changes between parasites at different times of the day. We have also recently discovered that, in a mouse infection, *T. brucei* occupies the adipose tissue. Here parasites adapt to the “space” (tissue environment) and they change their gene expression both at the RNA and Protein level, including the activation of fatty acid beta-oxidation. We are currently testing how an extracellular parasite can scavenge lipids that are stored inside the adipocytes. Globally these findings show that the phenotypic variability of *T. brucei* is much larger than we originally expected. We found functional differences between parasites in the morning and in the evening and between parasites in the blood and in extravascular spaces. The implications in drug treatment will be discussed. **Supported** HHMI, FCT
Keywords: Circadian rhythm; adipose tissue; metabolism

MC006 - **MOLECULAR PATHOGENESIS OF CHAGAS DISEASE CARDIOMYOPATHY**

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The pathogenesis of Chagas disease cardiomyopathy (CCC) is still incompletely understood. Only 30% of infected individuals develop heart disease, and avaliable evidencie indicates there is a genetic component in CCC development. CCC can be fatal due to refractory heart failure or ventricular arrythmia in a significant proportion of patients. Inflammation in the heart plays a major pathogenic role, and the major cytokine produced locally in Interferon-gamma. The prognosis and survival of CCC patients is significantly worse than that of non-inflammatory cardiomyopathy. Paradoxically, IFN-gamma production stimulated by chronic persistent infection by *T. cruzi* plays a major role in holding parasitism in check after the acute phase, but also can induce direct heart damage in the chronic phase, both involving direct drestruction of cardiomyocytes as well as inducing gene expression and functional changes leading to disease progression. Over the last few years, increasing knowledge has been accumulating about the genetics and pathogenesis mechanisms downstream of the heart inflammatory response that lead to CCC development. I will review the advances on cardiac gene expression, proteomics and epigenetic regulation of gene expression, as well as knowledge recently acquired using advanced genetic approaches like whole exome sequencing and genome-wide association studies (GWAS) with large patient cohorts. Combined analysis provides new vistas as well as possible new therapeutic targets that could ameliorate current treatment of CCC.
Keywords: Chagas disease ; pathogenesis; immunology

MC007 - **DENSITY-DEPENDENT DEVELOPMENT AND SOCIAL INTERACTION BETWEEN AFRICAN TRYPANOSOME SPECIES IN THE MAMMALIAN BLOODSTREAM**

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African trypanosomes comprise a group of parasites that infect humans and animals in sub Saharan Africa, these being transmitted by tsetse flies. The most intensely studied is Trypanosoma brucei, which is characterised by exhibiting pleomorphism in the mammalian bloodstream. This describes the morphological heterogeneity of the parasites - represented by their possession of slender and stumpy morphotypes, of which stumpy forms are arrested and adapted for transmission. In contrast, other African trypanosomes (Trypanosoma congolense, Trypanosoma vivax) are monomorphic- i.e. they have a uniform morphology in hosts. Despite this, we have found that T. congolense and T. vivax share genes that are required in T. brucei for the slender to stumpy transformation, which is signalled by a density dependent quorum-sensing mechanism. Furthermore, T. congolense undergoes density-dependent growth arrest in the bloodstream. In recent work, we have found that T. congolense is able to manipulate the development of coinfecting T. brucei such that stumpy formation occurs prematurely with respect to a monoinfection with T. brucei alone. Furthermore, we have found that this is mediated through the same quorum sensing signalling pathway used by T. brucei when responding to its own density sensing signal. To explore the density-dependent mechanisms exhibited by T.congolense we have compared the transcriptome of ascending and peak parasitaemia parasites and compared these with the transcriptome of slender and stumpy form T. brucei. This has identified transcripts that might act as developmental markers in T. congolense. It has also highlighted the similarities and differences between the respect developmental programmes of these different trypanosome species as they prepare for transmission.
Keywords: Trypanosoma; differentiation; quorum-sensing

MC008 - **DEVELOPMENT OF POTENTIAL DIAGNOSTIC TESTS FOR THE CONTROL OF VISCERAL LEISHMANIASIS**

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The early diagnosis of the visceral leishmaniasis is one of the most effective measure of control as it allows the identification, early treatment of infected individuals and consequently amelioration of prognosis for treated individuals. Considering the advanced knowledge over the past few years in genomics, proteomics and peptide biology, here we presented data obtained by our group demonstrating the improvement of diagnostic performance for visceral leishmaniasis after selection of new candidates by several approaches. Finally, the current limitations and perspectives for the improved diagnosis of visceral leishmaniasis will be discussed.
Keywords: Diagnosis; visceral leishmaniasis; leishmania