MC001 - BIOGENESIS OF BASAL BODY CONNECTIONS IN *TRYPANOSOMA BRUCEI*

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Basal bodies are crucial for flagellum function and exist as a pair that are physically connected to each other. During the cell cycle, basal bodies duplicate and segregate to the two daughter cells and connections between the basal bodies must re-organise and sever in order for the basal body pairs and two flagella to segregate. The structural organisation of the connections and the molecular charactisation are poorly understood. We have used Electron tomography to characterise the connections through the cell cycle. In addition we have discovered a hypothetical protein that localises between the basal bodies and is important for basal body segregation. **Supported by:**BBSRC
Keywords: Basal bodies; flagellum; microtubules

MC002 - GLOBAL TRANSCRIPTOME PROFILING OF PRIMARY HUMAN MACROPHAGES (AND THP-1) CELLS INFECTED WITH LEISHMANIA

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Until recently, most studies of Leishmania immunobiology and genetics, as well of host parasite interactions, have made use of murine models of infection to mimic human disease. We have recently conducted a high resolution transcriptomic analysis of the global changes of gene expression in human macrophages infected with metacyclic forms of L. major (cutaneous self-healing) and *L. amazonensis* (cutaneous self-healing/ cutaneous diffuse). In addition, we compared these results to the transcriptome profile of latex bead-loaded macrophages to be able to characterize the biological response of the macrophage towards the parasite, while taking into account the generic phagocytic response to inert particles. Simultaneously, we interrogated the transcriptome of the parasites at different time points of interaction to obtain a comprehensive profile of the changes in the steady state RNA during the course of infection.
Our results revealed a vigorous, parasite-specific response of the human macrophage early in the infection that was greatly tempered at later time points. An analogous temporal expression pattern was observed with the parasite, suggesting that much of the reprogramming that occurs as parasites transform into intracellular forms generally stabilizes shortly after entry. Following that, the parasite establishes an intracellular niche within macrophages, with minimal communication between the parasite and the host cell later during the infection. No significant difference was observed between parasite species transcriptomes or in the transcriptional response of macrophages infected with each species. A comparative analysis of gene expression changes that occur as mouse and human macrophages are infected by Leishmania spp. points toward a general signature of the Leishmania-macrophage infectome. The results of a THP-1 cell infection with L. major will be described.
We will discuss our results with an emphasis on experimental design, statistical rigor and reproducibility of results.
Keywords: Transcriptome; leishmania; macrophage

MC003 - ESCRT-III COMPLEX MEMBERS IN ENTAMOEBA *HISTOLYTICA* TROPHOZOITES: GENES AND PROTEINS

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Phagocytosis is one of the most important mechanisms of pathogenesis in Entamoeba histolytica infection. This is a complex process that involves the endosomal-sorting complex required for transport (ESCRT) machinery, described in other organisms as fundamental for endocytosis. Our group has already described the presence of EhVps2, EhVps20, EhVps24 and EhVps32 during endocytosis and phagocytosis, as well as their association with lipids and proteins. Here we present data to confirm that in live trophozoites, localization of these proteins changes during erythrophagocytosis. Then, we discuss several in vivo and *in vitro* experiments to delimitate the function of each one of the ESCRTIII proteins during the multivesicular bodies' formation in the trophozoites. We also will analyze the gene expression of each ESCRT-III complex member through erythrophagocytosis from 0 to 90 min and the mRNA levels. Differential expression was calculated according to the 2-□□Ct method. Our results show unlike responses in gene expression, EhVps20 and EhVps24 mRNA levels decreased in early erythrophagocytosis (before 10 minutes), while in late erythrophagocytosis (after 30 minutes), these mRNAs were recovered at nearly basal levels. On the other hand, EhVps2 and EhVps32 mRNA levels did not change significantly through the erythrophagocytosis. Our results suggest that gene transcription regulation and/or mRNA stability mechanisms are involve along this process. **Keywords:**Entamoeba histolytica; escrt iii proteins and genes; phagocytosis

MC004 - GENOME-WIDE IDENTIFICATION AND CHARACTERIZATION OF FUNCTIONAL PROMOTER SEQUENCES FOR RNA POLYMERASE II IN TRYPANOSOMES

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Evolution since the separation of kinetoplastids from other eukaryotes has resulted in novel molecular and cellular features. In particular, the transcription of protein coding genes is polycistronic and an understanding of the initiation of transcription of these genes clusters by RNA polymerase II (pol II) has been elusive. Previous work has suggested that transcription might start in Strand Switch Regions (SSRs) between transcription units, however SSRs are large and it is not clear whether or how transcription starts in SSRs.

To identify possible promoter sequences we examine the occupancy of RNA pol II in the T. brucei genome by chromatin immunoprecipitation combined with massive parallel sequencing (ChIP-Seq) assays. The high resolution of this technique allowed us to accurately define regions where RNA pol II accumulates. Our results revealed a significant accumulation of RNA pol II in 306 peaks along the genome, most of them associated with SSRs (52.6%), compared to intergenic regions (27.4%) and coding sequences (19,9%). All the sequences enriched by RNA pol II within the chromosome 7 were functionally analyzed for promoter activity using a reporter gene assay after transient transfection. Genuine promoters sequences were identified mainly within the SSRs as two independent promoters driving each one the polycistronic transcription units. The sequences identified comprised less than 200bp in most cases and drive transcription in a directional manner in both, transient and chromosomally inserted reporter constructs. One of the sequences was further analyzed first by sequential deletions to define the smallest region necessary for fully transcription efficiency and then by mutations across this fragment. We determined the transcription start site (+1) by primer extension and found that main regulatory elements were located downstream of the +1. Thus, the potential of these regions as promoter sequences is being finally recognized and sequence-defined promoters within the SSRs are demonstrated.

MC005 - KINETOPLASTIDS AS A PLAYGROUND OF MOLECULAR EVOLUTION: LESSONS WE LEARNT FROM TRYPANOSOMATIDS' GENOMES

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Kinetoplastid protists are considered a unique playground for molecular evolution. It appears that a plethora of molecular innovations has been "field-tested" in this group. These include RNA editing, trans-splicing, nucleotide modifications, and many others. In this presentation, Dr. Yurchenko will discuss a progress his group has recently made in identification of the novel virulent factors in Leishmania infection, and discovery of the unique genetic code in trypanosomatids, in which all three stop codons can be used as sense ones.
Kinetoplastids also offer a rare opportunity for characterizing the evolution of parasitism and endosymbiosis. While their close relatives are either photo- or phagotrophic, a number of kinetoplastid species are facultative or obligatory parasites, supporting a hypothesis that parasitism emerged within this group of flagellates. Genes and pathways potentially involved in the establishment of parasitism will also be discussed.
Keywords: Leishmania; virulence; novel genetic code

MC006 - LESSONS LEARNED ON THE OUTCOME OF LEISHMANIA INFANTUM INFECTION WITH URBANIZATION

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Manifestations of Leishmania infantum infection range from asymptomatic to visceral leishmaniasis (VL), which is fatal in 5-10% of the cases even with treatment. Individuals with VL have suppressed immune responses, whereas treatment-induced cure of disease requires antigen-specific Type 1 immunity. Asymptomatic infection is also associated with protective immunity and a Type 1 response. The intradermal Leishmanin skin test (LST) is a delayed type hypersensitive test that has been interpreted as a marker of protective immunity, and is used as a marker of asymptomatically infected persons or of protection against relapse of symptomatic VL. In this presentation, we will discuss how the urbanization of visceral leishmaniasis in Brazil has allowed us to ask questions related to the epidemiology of L. infantum infection including the role of dogs and humans as potential reservoirs. In addition, the number of humans in urban areas has allowed us to study host genetic factors involved in the ranges of outcomes of L. infantum infections.
VL has become a disease of urban and periurban areas in Brazil over the past 20 years. Our cohort studies of people exposed to L. infantum in Natal, where L. infantum transmission continues to occur, has allowed a better understanding of the natural history of infection. Between 1990 and 2000, VL was primarily found along the eastern coast of the State of Rio Grande do Norte, spreading thereafter to other regions. A continuous increase in the proportion of VL cases in males has been documented ($p=0.05$, $\beta=0.003$ per year and $R2=0.398$). Simultaneously, the incidence of VL has significantly decreased in children in age groups 0 to 4 years ($p<0.000$; $\beta=-0.0117$; $R2=0.6564$) and 5 to 9 years ($p<0.0001$; $\beta=-0.0042$; $R2=0.4740$). At the same time there has been an increase in VL in adults belonging to age groups 20-39

(p<0.0071; β=0.0071, R2=0.5636) and over 40 years (p<0.0001; β=0.0105, R2=0.8309). These findings have been associated with improved nutritional status in children under 5 years, and vaccination with BCG (p<0.0001), polio vaccine (p=0.0036) and measles vaccine (p=0.0276). Similar to prior reports by our group and others, the majority of people infected with L. infantum had immunologic evidence of infection without a history of clinical symptoms, indicating they had controlled the infection.
Domestic dogs are a reservoir for L. infantum infection in northeast Brazil. Dogs also provide a means of studying immune responses to the parasite in a non-human, naturally infected host. By studying transcriptional profiles of blood and spleens of dogs with VL versus dogs with no evidence of disease, we found marked difference between responses of symptomatic versus asymptomatic dogs. We found with higher expression of transcripts indicating NK cell activation, toll-like receptor and NOD-like receptor pathways. In contrast, transcripts belonging to GPI-anchor synthesis and immunoproteasome activity were down-regulated in the spleens of dogs with symptomatic disease, and upregulated in animals with asymptomatic infection.
Our observations underscore a change in the demographic of human visceral leishmaniasis in the state of Rio Grande do Norte, and document differences in inflammatory gene expression in local dogs with symptomatic versus asymptomatic infection, but also in humans. These combined data will potentially aid development of new strategies to treat visceral leishmaniasis. These data will also help tease out the need to treat a subgroup of people with asymptomatic L. infantum infection who have a risk of developing disease. **Supported by:**National Institutes of Health (AI-30639), Conselho Nacional de Desenvolvimento Científico e Tecnológ
Keywords: Visceral leishmaniasis; gene expression; susceptibility/resistance

MC007 - DEVELOPMENT OF ATTENUATED VACCINE STRAINS OF LEISHMANIA BY GENOME EDITING USING CRISPR-CAS9

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

MC008 - BIOCHEMICAL AND BIOPHYSICAL CHARACTERIZATION OF SPECIFIC PROTEINS INVOLVED IN TRYPANOSOMA BRUCEI SL TRANS-SPLICING

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The spliced-leader (SL) trans-splicing catalyzed by the spliceosome in Trypanosoma brucei is responsible for processing polycistronic pre-mRNAs into mature mRNAs. The spliceossome machinery is assembled by small nuclear ribonucleoproteins (snRNPs) U1, U2, U4/U6 and U5 that are composed by small nuclear RNAs (snRNAs), a canonical complex of Sm proteins (SmB, SmD3, SmD1, SmD2, SmE, SmF, SmG) and specific factors. The Sm core varies in T. brucei, where SmD3/SmB are replaced by Sm16.5K/ Sm15K in U2 snRNP and SmD3 is substituted by SSm4 in U4 snRNP. We are investigating the interaction of the different recombinant Sm cores with U2, U4 and U5 snRNAs and will describe the first structural investigation of the spliceosomal complexes from a human parasite. The crystallographic structure of *T. brucei* U2A'/U2B'' shows an overall organization similar to the one observed in the human counterpart. However, a 6 Å deviation in the medium point of a positively charged turn in the RRM motif of U2B'' is observed to accommodate U2 snRNA and a long α-helix is observed in the C-terminal region of U2A'. Structural analysis of Sm core variations in *T. brucei* was proceeded using molecular modelling techniques associated with small angle X-ray scattering. The quaternary structure models show seven Sm proteins as β-barrels with positively charged interior for cognate snRNA interaction. The main difference among these Sm core structures resides in the C- and N-terminal regions of the variant proteins, probably enabling the interaction of Sm15K/Sm16,5K with U2A' in the arm 1 of the spliceosome, and the association of SSm4 with U5-220K and U5-200K in the spliceosome's body. The results presented contribute with the first structural study of spliceosomal proteins of a human parasite and give new insights on its biogenesis in T. brucei.
Keywords: Sl trans-splicing; spliceossome; sm proteins, trypanosoma brucei

MC009 - SKIN-RESIDENT CD4+ T CELLS PROMOTE RAPID CONTROL OF *LEISHMANIA MAJOR* BY RECRUITING ACTIVATED INFLAMMATORY MONOCYTES

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Leishmania-infected patients as well as experimentally infected mice become refractory to reinfection following disease resolution, but an effective vaccine has not been developed. Using a combination of skin grafting and parabiosis experiments, we identified a population of skin-resident Leishmania-specific memory CD4+ T cells in mice that had resolved a primary infection. Importantly, we found that these resident memory T cells (Trm cells) could be maintained in the absence of parasites. Furthermore, they were not only present at the site of the primary infection, but were found at distant skin sites in immune mice indicating that they were poised to respond to a dermal challenge anywhere in the immune animal. Upon challenge, they produced IFN-γ and recruited circulating T cells to the skin in a CXCR3 dependent manner, resulting in better control of the parasites when assessed several weeks following challenge. Since Trm cells are present in the skin at the initiation of the infection, we investigated how rapidly they might be able to control a challenge infection. We found that the delayed-type hypersensitivity (DTH) response observed in immune mice was dependent upon CD4+ Trm cells and led to a significant decrease in the parasite burden by 72 hr after challenge infection. This protection was dependent upon the recruitment of inflammatory monocytes, the production of both reactive oxygen species and nitric oxide, and was independent of the recruitment of T cells from the circulation. Thus, following infection with leishmania a population of protective CD4+ Trm cells globally populate the skin, are maintained in the absence of persistent parasites, and can provide rapid protection even in the absence of circulating leishmania-specific T cells. Therefore, the development of a successful leishmaniasis vaccine that recapitulates the strong immunity seen following a primary infection may depend upon generating a population of skin resident memory T cells.
Keywords: Leishmania; vaccine; resident memory t cells

MC010 - THE UPS AND DOWNS OF CALCIUM SIGNALING IN *TOXOPLASMA GONDII*

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Toxoplasma gondii is an obligate intracellular parasite that is estimated to infect nearly 30% of the world's population. Toxoplasmosis is largely asymptomatic in healthy individuals but can result in serious disseminated disease in immunosuppressed patients. *T. gondii* pathogenesis is directly linked to its lytic cycle, which starts when the parasite invades a host cell, replicates, and exits to find another host cell. Ca2+ signaling forms part of the regulation of each of these steps. The genome of *T. gondii* reveals many genes involved in Ca2+ signaling. Major Ca2+ stores have been characterized, such as the endoplasmic reticulum (ER), the acidocalcisome, and the plant-like vacuole. There is also evidence for the presence of an active IP3 signaling pathway. A phosphoinositide phospholipase C (PI-PLC), the IP3 producing enzyme, has been characterized and localized to the plasma membrane of the parasite. Ryanodine and cyclic ADP ribose (cADPR) stimulate Ca2+ release from an undefined store. However, there is no genomic evidence for the presence of an IP3R and/or ryanodine receptor. We have expressed Genetically Encoded Calcium Indicators (GECIs) in the cytoplasm of *T. gondii* tachyzoites to generate GCaMP6-expressing parasites to study Ca2+ signaling. We studied Ca2+ signaling during egress and invasion and also its role in motility of parasites. We were able to look at Ca2+ fluctuations in real time and found that there is a correlation between the amplitude of the Ca2+ signal and downstream biological responses like motility, egress and invasion. We are studying the mechanism of Ca2+ influx and its role in signaling. Our data indicates that changes in the membrane potential, maintained by a proton gradient, regulate Ca2+ influx and downstream biological features like motility. We are also interested in Ca2+ release from intracellular stores and its role in regulating Ca2+ entry and downstream signaling. We have expressed GECIs in organellar compartments like the mitochondria, the apicoplast and the plasma membrane. In addition, we have developed a protocol to measure Ca2+ in the endoplasmic reticulum using Mag-Fluo4-AM. Our data shows that the endoplasmic reticulum is important for signaling and the mitochondria is important for buffering Ca2+. We are characterizing several potential molecules that could be part of the Toxoplasma Ca2+ toolkit involved in signaling and regulation of critical virulence traits of *Toxoplasma gondii*.
Supported by: National Institutes of Health
Keywords: Toxoplasma gondii; calcium signaling; lytic cycle

MC011 - BREACHING BORDERS WITHOUT A VISA: UNDERSTANDING TRYPANOSOMES MIGRATION WITHIN THE TSETSE

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Trypanosoma brucei parasites undergo a complex life cycle within the tsetse vector, which involves migration and colonization of different fly tissues before becoming transmissible to another vertebrate host. Once 'stumpy' (blood) forms successfully transform into procyclic trypanosomes within the fly's gut, establishment of an infection only occurs after the parasites reach the ectoperitrophic space (ES), a cavity that is in direct contact with the gut epithelium and is separated from the lumen by the peritrophic matrix (PM). The tsetse PM is an acellular tissue whose main functions are to compartmentalize the blood meal and contain oral pathogens (i.e trypanosomes and bacteria) within the midgut lumen. Although unproven, the most widely accepted hypothesis on how T. brucei procyclics reach the tsetse ES from the midgut lumen is by direct crossing of the PM in three steps: 1) parasite binding to and 2) degradation of the 1st PM layer, and 3) direct degradation of the PM 2nd and 3rd layers. However, PM degradation by trypanosomes is unlikely considering that this is a tissue rich in chitin fibers and T. brucei lacks chitinase activity. Furthermore, while previous microscopy experiments suggests trypanosomes can be found confined between PM layers, evidence of degradation from the midgut lumen (step 2) or towards the ES (step 3) have so far been elusive. Our group

has re-visited this event by employing a combination of fluorescent and electron microscopy methodologies, which allowed us to make 3D reconstructions of infected tsetse tissues. We found that, although pockets of trypanosomes can be seen within PM layers, there is no evidence to support degradation of this tissue. Instead, we discovered that trypanosomes invade the ES via an early infection of the proventriculus (PV - point of PM production), a process that also accounts for the formation of “parasite pockets”, which are then carried along the entire length of the gut as the PM gets synthetized. We propose that midgut establishment does not occur in the anterior midgut, rather the PV, and that early PV establishment is crucial to establish a gut infection. Furthermore, we suggest a new role for the PM in that it acts as a “sticky trap” that envelops parasites, which are subsequently excreted when the PM reaches the hindgut. **Supported by:** Wellcome Trust
Keywords: African trypanosomes; tsetse fly; vector-parasite interactions