MC001 - A GLYCOCONJUGATE VACCINE ELICITS B AND T CELL-DEPENDENT PROTECTION AGAINST EXPERIMENTAL TRYPANOSOMA CRUZI INFECTION

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Chagas disease (ChD) is a devastating neglected tropical illness caused by the protozoan parasite Trypanosoma cruzi. It is estimated that 8-11 million people are chronically infected in Latin America, representing a substantial social and economic burden. Owing to the extensive global migration of asymptomatic, chronically infected individuals from endemic regions, ChD now affects thousands of people in nonendemic regions like North America and Europe. The two available drugs for ChD chemotherapy have limited efficacy in the chronic phase of the disease and are rather toxic. Moreover, there is no preventive or therapeutic vaccine for human ChD, despite numerous experimental efforts targeting almost entirely parasite proteins. Highly abundant T. cruzi trypomastigote surface glycoconjugates, such as glycosylphosphatidylinositol (GPI)-anchored mucin glycoproteins (tGPI-mucins), contain highly immunogenic terminal α-galactopyranosyl residues (α-Gal), which are not expressed in human cells. Accordingly, very high levels of trypanolytic, protective anti-α-Gal antibodies are observed in both acute and chronic stages of human ChD. Although glycoconjugates are the major surface antigens of the parasite, they remain completely unexplored as potential vaccine candidates for ChD. Here, we developed a glycan-based vaccine that confers full protection against T. cruzi infection in an α1,3-galactosyltransferase (α1,3GalT)-knockout (KO) mouse model (C57BL/6 background), which closely mimics the human humoral response against the parasite. Animals were vaccinated with a neoglycoprotein, containing the synthetic Galα1,3Galβ1,4GlcNAc (Galα3LN) trisaccharide, the major parasite epitope for the immunodominant trypanolytic anti-α-Gal antibodies, covalently linked to bovine serum albumin (BSA) as carrier protein. In contrast to control animals, all α1,3GalT-KO mice vaccinated with Galα3LN-BSA showed long-lasting, high titers of lytic, protective IgG anti-α-Gal antibodies that conferred full protection against consecutive lethal parasite challenges. In addition, Galα3LN-BSA-immunized mice showed much lower parasitemia, and very low parasite load in the heart, as determined by qRT-PCR, when compared to control BSA-vaccinated animals. Purified murine anti-α-Gal IgG (IgG1>IgG2b>IgG3>IgG2a) Abs agglutinated and killed trypomastigote forms in a complement-independent manner and, when transferred to naïve mice, could partially protect the animals against lethal parasite challenge.
In vivo depletion experiments indicated that the antibody-mediated protection was dependent on CD4+ helper T cells. Taken together, our results demonstrate that a glycan-based vaccine targeting highly immunogenic T. cruzi-specific glycan structures could be an effective approach to prevent or treat Chagas disease. Support: NIH/NIAID grant # 1R21AI115451-01 (to ICA and KM); NIH/NIHMD Grant # 2G12MD007592 (to BBRC/UTEP). CNPq grant # 470737/2013-1 (to AFM). ICA is a Special Visiting Researcher scholar from the Science Without Borders Program, CNPq, Brazil.
Keywords: Chagas disease; vaccine; glycoconjugates

MC002 - "INNATE IMMUNE RESPONSE IN THE CONTROL AND PATHOLOGY OF LEISHMANIA BRAZILIENSIS INFECTION"

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The studies in the pathogenesis of Leishmania braziliensis infection have been mainly focused in the adaptative immune response as IFN-γ production by CD4+ T cells is the main cytokine that activate macrophage for leishmania killing. However, although T cells are important to control parasite proliferation and dissemination, Th1 cell activation is not able to prevent development of disease, and both CD4+ and CD8+ T cells have been associated with pathology. As leishmania survives in macrophages and these are the major cells responsible for leishmania killing it is important to understand the role of this cell in the pathogenesis of leishmaniasis. Additionally while most of the studies in cutaneous leishmaniasis (CL) evaluate

patients in the late phase of the disease when the ulcer is already present, we have been able to identify patients in the very early phase of disease before ulcer appearance called early CL (ECL). Moreover we have followed a cohort of household contacts (HC) of CL patients which allow to study individuals exposure to L.braziliensis but who do not develop disease, subclinical (SC) L.braziliensis infection, and subjects that after exposure are not able to control parasite growth and develop CL. The expression of toll like receptors (TLR), monocyte subsets, the respiratory burst and cells expressing cytokine were performed by flow cytometer and chemokines and cytokines were also measured in supernatants. Gene expression was performed by microarray analysis. A cohort study enrolled 308 HC of CL patients without history or evidence of previous CL. HC were enrolled in 2010 and immunologic studies were performed every two years including characterization of cytokine and chemokine profile, source of cells expressing cytokines, ability of monocytes to kill leishmania and the leishmania skin test (LST).
The frequency of intermediate monocytes were enhanced in ECL and CL and ex vivo expression of TLR2 and TLR4 was higher in CL than in cells from HS. L.braziliensis infection enhanced TLR expression as well as expression of TNF and the respiratory burst. Intermediate monocytes were the main cells expressing TLR and secreting TNF. The frequency of cells expressing reactive oxygen species (ROS) was higher than cells express NO. ROS production was higher in CL than in SC L.braziliensis infection but monocytes from SC L.braziliensis infection killed more leishmania than cells from CL. Monocytes from ECL and CL produce high amount of TNF, IL-1β, CXCL9 and CXCL10 and gene expression of MMP9 was enhanced in biopsies of ECL and CL. Secretion of MMP9 was dependent of TNF. This group of experiments emphasized the role of monocyte from ECL and CL in the inflammatory response in CL and pointed out for the role of IL-β, TNF and MMP9 in ulcers development in CL patients.
Of the 308 HC enrolled in the cohort study exposure to L.braziliensis infection based on a positive LST or production of IFN-γ was documented in 134 individuals. There was a weak association between the LST and the ability of cells to produce IFN-γ. While a positive LST was not associated with protection, the production of IFN-γ in the absence of LST was associated with protection. NK cells where the major source of IFN-γ and both monocytes and macrophages from HC who had SC infection had a greater ability to kill leishmania than cells from CL patients. The production of CXCL9 was higher in HC exposure to L.braziliensis who progress to CL than in subjects who remain with SC infection. These data show that in an endemic area of L.braziliensis ability to control the infection and prevent development of CL is associated with production of IFN-γ by NK cells and greater ability of monocyte to kill leishmania. Alternatively production of CXCL9 was association with progression from injection to disease. **Supported by:**Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq); National Institutes of Health
Keywords: Leishmania ; braziliensis ; infection

MC003 - FUNCTIONAL GENOMICS OF DRUG RESISTANCE IN LEISHMANIA

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Resistance in Leishmania is often due to gene copy number variations (CNVs) and to point mutations. Next generation sequencing (NGS) of Leishmania cells selected for resistance to anti-leishmania drugs has allowed the detection of a plethora of resistance mechanisms. These include chromosome aneuploidy, gene amplification or deletion of specific loci and point mutations. As CNVs is an important mechanism of resistance we developed a sensitive method, termed Cos-Seq, for the identification of both resistance and drug-target genes in Leishmania using cosmid-based complementation coupled to NGS. This approach was first validated using the well-studied model-drug methotrexate and then applied to the five major antileishmanials, antimony, miltefosine, paromomycine, amphotericin B and pentamidine. This technique led to the isolation of an unprecedented number of known and new drug target and resistance genes to antileishmanials. Functional validation of several of these loci highlighted known and novel drug targets, and resistance genes for the six drugs that were studied. Several genes/loci were also shown to contribute to resistance to more than one antileishmanials. This novel method will expedite the discovery of drug targets and resistance mechanisms.**Supported by:**CIHR
Keywords: Leishmania; drug resistance; genomics

MC004 - PARASITE PERSISTENCE MECHANISMS IN TRYPANOSOMA CRUZI INFECTION AND CHAGAS DISEASE

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Although Trypanosoma cruzi infection is generally controlled by host immune responses, it is rarely cleared, resulting in a chronic infection and the development of cardiac pathology in many infected subjects. The ability of T. cruzi to persist long-term despite otherwise effective host immune control mechanisms appears to be due to a number of factors, including the rather silent nature of the host cell infection process and the deflection of host immune responses toward a set of highly variable and constantly changing parasite surface antigens. The data supporting these conclusions and the implications of these finding for vaccine development will be discussed. Also to be discussed are new methods for the study and disruption of these immune evasion mechanisms.
Keywords: Persistence; evasion; vaccines

MC005 - NEGLECTED PATHWAYS HIDDEN BEHIND GLYCOLYSIS IN BLOODSTREAM TRYPANOSOMES

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The bloodstream forms of Trypanosoma brucei, the causative agent of sleeping sickness, rely solely on glycolysis for ATP production. It is generally accepted that pyruvate is the only end-product excreted from glucose metabolism, with virtually no production of succinate and acetate, the main end-products excreted from glycolysis by all the other trypanosomatid adaptative forms, including the procyclic insect form of T. brucei. We have revisited the central metabolism of the bloodstream form trypanosomes by using a combination of reverse genetics (RNAi and knockout) and NMR spectrometry analyses of end-products excreted from metabolism of the main carbon sources consumed by the parasite (glucose and threonine). Although, acetate and succinate are minor end-products of glucose metabolism (~5% and ~2% of excreted end-products from glucose breakdown, respectively), their production are essential for viability of the parasite. These data highlight that the central metabolism of the bloodstream form trypanosomes contains unexpected essential pathways, although minor in terms of metabolic flux compared to the glycolytic rate, which could be targeted for the development of trypanocidal drugs. **Supported by:**CNRS, Université de Bordeaux
Keywords: Trypanosoma, brucei; glucose and threonine metabolism; new pathways

MC006 - *TRYPANOSOMA CRUZI* EXTRACELLULAR AMASTIGOTES AND THE HOST CELL: RECENT FINDINGS ON HOST ACTIN CYTOSKELETON AND PARASITE MICROVESICULES

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When invading non-professional phagocytic HeLa cells, *T. cruzi* extracellular amastigotes (EA) subvert host cell actin filaments in a phagocytosis like process cells. During this process, EAs induce an actin-rich cup like at the cell surface and we show now that the parasites release microvesicles within this synaptic structure. Along with actin, other microfilament modulators are recruited to the cups. Recently, we have examined the contribution of host GTPases signaling pathways (Cdc42/N-WASP, Rac1/WAVE2 and RhoA), ERM plasma membrane-actin filament linker proteins (Ezrin, Radixin and Moesin) and parasite microvesicules. Host cell invasion by EAs was inhibited in HeLa cells stably depleted for Cdc42/N-WASP and Rac1/WAVE2 pathways, but not RhoA. Using GFP-tagged constructions, we observed recruitment and colocalization of these proteins with actin at EA invasion sites, both in fixed and live cells.

Regarding ERM proteins, depletion of Ezrin and Radixin but not Moesin reduced EA invasion in HeLa cells. GFP-tagged wild type ERMs were recruited and colocalized to actin at EA invasion sites but a closed conformation (inactive) Ezrin mutant did not. By time-lapse confocal microscopy, we observed that ERM depleted cells presented delayed and reduced actin recruitment when compared to the control groups. We showed that EA are able to release vesicular structures (100-200 nm) coated with Ssp-4 not only inside the phagocytic cup but also when adhered to poly-L-lysine coated coverslips or to cells. We observed that HeLa cell invasion by G strain EAs was inhibited if cells were preincubated with vesicles derived from Y strain EA supernatant (less infective strain). Alternatively, EA invasion from Y strain was enhanced only when cells were pre incubated with the soluble protein fraction from G strain EA supernatant. These findings unveil new details on this unique parasite - host cell interaction.
Supported by:FAPESP, CNPq, Capes
Keywords: Trypanosoma cruzi; extracellular amastigotes; host cell invasion

MC007 - STUDIES OF THE LEISHMANIA DSRNA VIRUS LRV1 IN ANIMAL MODELS AND HUMAN DISEASE

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Leishmania species in South America often bear the dsRNA Leishmania virus 1 (LRV1). Like most Totiviruses, LRV1 virus is neither shed nor infectious, and thus may be viewed as a persistent endobiont. L. guyanensis LRV1 has been associated with hypervirulence and increased metastasis, the latter being a hallmark of the more severe forms of leishmaniasis (Ives et al. Science 2011). Intriguingly, while many Leishmania have lost the RNA interference (RNAi) pathway, Viannia have retained it (Lye et al PLoS Pathogens 2010). In many host species RNAi plays key roles with viruses, and we are studying its roles in Leishmania biology and virology. Lbr and Lguy LRV1-infected parasites showed high levels of viral siRNAs, from 0.4-1.5% of total siRNAs, whose structure and size mirrored normal cellular siRNAs (Atayde et al. Mol Micro 2013). While thus resistant to 'endogenous' RNAi activity, we proceeded to test the ability of an LRV1 stem-loop transgene to target LRV1. Analysis of LRV1 capsid levels by FACS and viral RNA by RT-PCR showed complete loss LRV1, accompanied by massive overproduction of LRV1-siRNAs. We speculate this could overcome the ability of the virus to resist 'natural' levels of RNAi activity. Notably, macrophage cytokine responses showed similar LRV1-dependent changes in L. braziliensis as observed in L. guyanensis. This extends LRV1-dependent hypervirulence responses for the first time to L. braziliensis, and establishes RNAi as a useful tool for generating isogenic LRV1-deficient lines.

An important question involves potential links of LRV1 with Leishmania pathogenicity in human infections, where disease manifestations differ greatly from those seen in murine models. While several recent reports showed significant correlations of LRV1 with mucocutaneous disease in humans, consistent with the increased metastasis seen in murine models, other reports did not observe this. The basis for this discordant results is unknown and under study. Recently we showed that the presence of LRV1 was associated with increased relapse and/or treatment failures in human L. braziliensis-infected patients treated with pentavalent antimonials in Peru and Bolivia (Aduai et al, J. Infectious Diseases 2015). Similarly, LRV1 was similarly associated with increased pathology and relapses in L. guyanensis patients treated with pentamidine in French Guyana (Bourreau et al, J. Infectious Diseases 2015). The mechanism of LRV1-mediated treatment failures is currently unknown. Potentially, alterations in host immune or metabolic responses may decrease drug efficacy; alternatively (and not exclusively), increased parasite numbers could overcome the often marginal efficacy of existing anti-leishmanial agents. Regardless, the association of LRV1 with clinical drug treatment failure could serve to guide more effective treatment of tegumentary disease caused by L. braziliensis.

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Keywords: Drug treatment failures; chemotherapy; virulence factors

MC008 - TRYPANOSOMA CRUZI PHENOTYPIC DIVERSITY: A ROLE FOR MUCIN-LIKE MOLECULES

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Due to its predominant clonal proliferation, *Trypanosoma cruzi* exhibits a highly-structured population, composed of multiple strains displaying considerable genetic drift. This genetic variability, in turn has a major impact at the phenotypic level, when parameters such as antigenic profile, virulence, growth rate, pathogenicity, tissue tropism, and sensitivity to anti-chagasic drugs are considered. Therefore, one of the main objectives of Chagas Disease research remains to elucidate the molecular and cellular basis underlying *T. cruzi* phenotypic variation. The surface coat of *T. cruzi* is covered in different glycoconjugates which contribute to parasite protection and to the establishment of a persistent infection. Mucins are major components of this coat. These are glycoproteins attached to the parasite membrane through a glycosylphosphatidylinositol anchor in which the oligosaccharide chains are *O*-glycosidically linked to threonine or serine residues via NAcGlc units. *TcSMUG L* comprises a group of genes coding for small *T. cruzi* mucins anchored to and secreted from the surface of replicative, insect-dwelling developmental forms (i.e. epimastigotes). Here, we show that the N-terminal peptide of TcSMUG L mucins promotes adhesion of epimastigotes to the posterior midgut epithelial cells of the triatomine vector *Rhodnius prolixus*, a key step for metacyclogenesis (i.e. conversion of epimastigotes to infective, metacyclic trypomastigotes). In addition, we show results obtained from transgenic lines over-expressing TcSMUG L products that further support this idea, and that indicate that these molecules play also a key role during metacyclogenesis *in vitro*. Interestingly, and in spite of showing a high degree of conservation across paralogues and orthologues from different isolates, *TcSMUG L* expression at both mRNA and protein levels is quite variable among *T. cruzi* strains. Here, we also show that differences in *TcSMUG L* expression correlate with bias in threonine codon utilization between strains, which correlates with changes in the levels of adenosine-to-inosine editing of tRNAs, and particularly of threonine decoding tRNAs, suggesting tRNA editing as a forcible step in controlling expression of *TcSMUG L* (and likely other *T. cruzi* genes) while driving codon adaptation. Moreover, *in vivo* manipulation of tRNA editing deaminase (ADAT) complex expression leads to increases in the levels of tRNA adenosine-to-inosine editing, and significantly impacts the expression of TcSMUG L proteins but not *TcSMUG L* transcripts. These variations are also observed upon ectopic expression of synthetic *TcSMUG L* constructs displaying contrasting threonine codon bias in isogenic parasite background. Overall, our data indicate that TcSMUG L mucins are key determinants of the infectivity of *T. cruzi* towards the insect population and that, due to inter-strain differences on their expression, these molecules might also contribute to the parasite phenotypic variability. Regarding the latter phenomenon, our data support the existence of a novel control mechanism likely operating at the level of *TcSMUG L* mRNA translation elongation. These observations open a novel perspective on regulation of gene expression in T. cruzi (and trypanosomatids in general) which still relies extensively on post-transcriptional events, yet our findings depart from the more widely accepted mechanism of regulation at the level of mRNA abundance. **Supported by:**ANPCyT (Argentina) and Fundacion Bunge y Born (Argentina).

Keywords: Trypanosoma cruzi; mucin; codon adaptation

MC009 - TO INFINITY AND BEYOND: ON THE DIVERSITY AND DISTRIBUTION OF *TOXOPLASMA GONDII*.

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Toxoplasma gondii is an extraordinary parasite. It has successfully colonized every conceivable ecological niche from the Arctic tundra to the rainforests of Brazil and from the desserts of Australia to the marine ecosystems of North America. One might suppose that such widespread distribution might have provided a strong selective pressure leading to to diversification of the parasite, yet population genetic studies show a pattern where a restricted number of genotypes dominate most regions. These studies highlight the greater diversity among parasite isolates in South America, raising questions about the origins and evolutionary history of the parasite. Such genotyping studies have given a relatively clear picture of the global population structure of Toxoplasma but it is interesting to consider how stable current biogeographical patterns of strain distribution will be under future conditions of land use, ecosystem and climate change. This question depends heavily on the ecology of toxoplasmosis, on the way in which strains flow through different hosts and the way in which this impacts on opportunities for transmission and recombination. Epidemiological studies suggest that Toxoplasma can exploit may different routes of transmission, for example in aquatic mammals it is suggested that infections are acquired from oocysts in runoff water, while in sheep and mice there is evidence that vertical transmission plays a role. These studies indicate that transmission networks may vary across ecosystems providing a mechanism for partitioning of strains. To unravel these networks requires the development and application of molecular probes to track the transmission of Toxoplasma isolates. Here the conservative nature of the T.gondii genome presents a considerable challenge, as relatively intensive genotyping is required to discriminate among strains. The challenges of identifying transmission networks require us to develop analytical approaches that will operate a local 'ecosystem' level. We discuss the application of whole genome sequencing and the development of SNP based multilocus probes to support molecular epidemiology studies. Furthermore we advocate a need for intensive 'sympatric' studies to analyse the flow of toxoplasma genotypes in the environment. These issues are discussed in the light of recent advances in the field and in relation to our own studies Uganda and Mexico We consider the challenges of estimating levels of allelic variation and recombination and developing strategies to monitor genotype:phenotype relationships in the field.

Keywords: Toxoplasma; biodiversity; ecology

MC010 - IDENTIFICATION OF VIRULENCE FACTORS REQUIRED FOR VISCERAL LEISHMANIASIS

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A central question in Leishmania research is why most species cause cutaneous infections but others cause fatal visceral disease. Interestingly, L. donovani causes both visceral and cutaneous leishmaniasis in Sri Lanka. To investigate this phenomenon, L. donovani clinical isolates were obtained from a cutaneous leishmaniasis (CL-SL) and from a visceral leishmaniasis (VL-SL) patient in Sri Lanka. The CL-SL isolate was severely attenuated in visceral infection in BALB/c mice compared to the VL-SL isolate which was highly virulent. These observations reveal that the different pathologies in Sri Lanka are due to different strains of L. donovani. In addition to these clinical isolates, we have also serially passaged the CL-SL through the spleen of mice for over one year to obtain a more virulent strain capable of high visceral infection termed IV-SL. These 3 different L. donovani parasites (CL-SL, VL-SL and IV-SL) represent excellent strains for investigating the genetic basis for visceral disease. Comparative analysis of these 3 genomes from L. donovani will be presented. In order to determine the function of genes potentially involved in the visceral disease phenotype, we have also developed a novel way to study the genome of L. donovani which involves using the Crisper-Cas9 gene editing system. The use of this system to study gene function in Leishmani will also be presented. **Supported by:**Canadian Institutes of Health Research
Keywords: Visceral disease; virulence; genome editing

MC011 - CELLULAR AND MOLECULAR BIOLOGY OF *LEISHMANIA* SPP. TELOMERES: INSIGHTS ABOUT THE EVOLUTIVON OF TELOMERE MAINTENANCE

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Telomeres, the nucleoprotein structures that protect chromosome ends from degradation and fusion, have been studied in different eukaryotes due to their role in maintain genome stability. Here we are going to present the state of the art about Leishmania telomeres biology. Leishmania telomeres are composed by the conserved TTAGGG repeated sequence which is maintained by the action of telomerase. The composition of Leishmania telomeric chromatin revealed the existence of few proteins that were able to bind the G-rich telomeric strand among which is RPA-1. In Leishmania, RPA-1 structurally differs from their eukaryote counterpart even in the mode of interaction with the telomeric DNA. A deep in silico analysis showed no reliable evidence of a trypanosomatid putative homologue to any of the G-rich telomere binding proteins described so far, although most of them are considered telomeric RPAs. In contrast, orthologs to double-stranded telomeric factors were characterized in trypanosomes and Leishmania. In addition, the two main components of parasite telomerase ribonucleoprotein complex, the reverse transcriptase TERT and the RNA (LeishTER) present many parasite specific features although share conserved structural domains and motifs with other eukaryotes. LeishTER and TERT probably represent the minimal Leishmania telomerase ribonucleoprotein (RNP) complex since they co-immunoprecipitate and colocalize in a cell cycle-dependent manner. Recently, we were able to show that the chaperone Hsp83 (ortholog of Hsp90) is also part of this complex. The regulation of these interactions as well as enzyme biogenesis and composition are probably regulated during parasite development since we detected clear differences in telomere length among parasite life stages. Thus, we are currently trying to understand the associations between the Leishmania telomerase RNP components and the telomeric chromatin as well as the role played by TERRA expression from Leishmania subtelomeres. **Supported by:**FAPESP
Keywords: Leishmania; telomeres; telomerase

MC012 - LEISHMANIA'S EXOSOMES: A NEW VIRULENCE FACTOR TO CONSIDER.

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Exosomes have been involved in immune and tumor cell communications as well as in host-pathogen interactions. Amongst the pathogens shown to actively secrete exosomes are Leishmania spp., a group of parasitic protozoans of public health importance worldwide. Despite several studies describing the secretion of exosomes by these parasites in vitro, observation of their formation and release in vivo has remained a major challenge. In this study, we demonstrate exosome secretion by Leishmania in a biological context and provide evidence that these vesicles influence the progression of experimental leishmaniasis. We show that Leishmania constitutively secretes authentic exosomes in vivo within the lumen of the sand fly midgut through a mechanism homologous to the mammalian exosome pathway. Through egestion experiments, we demonstrate that Leishmania exosomes are part of the sand fly inoculum, therefore suggesting that these vesicles are co-egested with the parasite during the insect's blood meal exerting their own influence on the host infectious process. Indeed, the co-inoculation of mice footpads with Leishmania plus midgut-isolated or in vitro-isolated Leishmania exosomes results in a significant increase in footpad swelling compared to the inoculation of parasites alone. Taking advantage of the strong similarity between in vivo- and in vitro-isolated Leishmania exosomes, we use in vitro preparations to study the immunological process behind lesion exacerbation. Notably, co-injection of Leishmania with exosomes produced exacerbated lesions through overinduction of inflammatory cytokines, in particular of IL-17a. Our data indicate that Leishmania exosomes are an integral part of the parasite's infectious life cycle and propose to add these vesicles to the repertoire of virulence factors associated to vector-transmitted infections. To our best knowledge, this is the first direct demonstration of exosome secretion by eukaryotic cells in vivo. **Supported by:**Canadian Institute of Health Research
Keywords: Leishmania; exosome; virulence factor