RT.001A - **GENOME-BASED APPROACHES TO STUDY ANTIGENIC VARIABILITY IN TRYPANOSOMA CRUZI**

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The ability of *Trypanosoma cruzi* to survive in the mammalian host is in part due to the presence of a diverse surface membrane coat. In fact, a remarkable feature of the *T. cruzi* genome is the massive expansion of genes that encode polymorphic surface proteins, which include the trans-sialidase and sialidase-like superfamily (TcS), mucin-associated surface protein (MASP), TcMUC mucins, among others. These T. cruzi genes are often clustered into large haploid and heterogeneous arrays that can be as large as 600 kb and are enriched by retroelements. To gain insights of the level of polymorphism of the largest *T. cruzi* surface protein families, we performed pairwise alignments of all members of each family resulting in a distance matrix that was used to generate multidimensional scaling (MDS) plots. Our data suggest MASP is the most polymorphic *T. cruzi* gene family, followed by TcS. The pattern of dispersion of these two families is very distinct. MASP members display a continuous sequence variation whereas TcS members form eight robust clusters. By mapping characterized TcS proteins in the MDS projection, we were able to identify all of them as members of the four TcS groups previously identified and, more importantly, disclose four new groups. Real-time RT-PCR confirms the expression of genes derived from new groups and immunoblot reveals that they are antigenic. We have also performed B-cell epitope prediction on the MASP gene family and constructed a peptide array with 200 putative epitopes that was screened with sera from acutely *T. cruzi* infected mice. We found that mice IgG and IgM are reactive against several MASP peptides during acute infection. We have also analyzed MASP expression profile in trypomastigotes derived from distinct host cells and from acutely infected mice after sequential passages. Although several MASP genes are co-expressed in the parasite population, the repertoire of expressed genes is distinct in trypomastigotes derived from tissue culture and bloodstream trypomastigotes recovered from sequential passages in mice. We speculate that variations in the large repertoire of potentially antigenic peptides derived from MASP family may favor the parasite escape of immune response during the acute phase of infection.

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RT.001B - **LEISHMANIA (VIANNIA) BRAZILIENSIS TRANSCRIPTOME BY RNA-SEQ: LOOKING FOR GENES RELATED TO VIRULENCE IN TWO DISTINCT ISOLATES**

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Leishmania (Viannia) braziliensis is one of the etiological agents of cutaneous leishmaniasis. This disease presents itself with a broad severity spectrum, from one unique lesion to high destructive mucosal compromising. The mechanisms involved with such variability are not well known. To investigate these mechanisms and to look for target genes involved with *L. (v.) braziliensis* virulence and infectivity we have investigated the transcriptome of two distinct isolates, namely NSL and ET. Both were isolated from human cases but NSL was characterized as more virulent than ET in murine model. Using high-throughput RNA-Seq (454/Roche) we have surveyed the transcriptomes from two life cycle stages, the infective metacyclic and the non infective procyclic. Interesting findings were obtained, such as hundreds of metacyclic specific transcripts in each isolate that may be involved with infectivity/virulence phenomena. Furthermore, transcriptome analysis showed for the first time in *Leishmania* evidence of hetero- and homopolymeric poly-adenylation tracts truncating RNA molecules (rRNAs and mRNAs). It is known that transient internal poly adenylation targets RNA molecules to rapid exonucleolytic degradation. Interestingly, we observed a higher frequency of internal poly(A)-tails truncating ribosomal RNA molecules in metacyclic forms from NSL than from ET. The observed poly(A) truncated coding mRNAs in NSL and ET strains were quite distinct and could eventually explain the differences in virulence of isolates. The internal poly(A)-tailed RNA truncation phenomenon can open new fields on post-transcriptional gene expression regulation in Leishmania and related organisms. It adds further complexity to the paradigm that almost the entire trypanosomatid genome is constitutively transcribed; in that the integrity of messages could play an important role in regulating the pool of transcripts that are effectively accumulated.

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RT.001C - **INVESTIGATING THE FUNCTIONS OF THE STAGE-REGULATED HASPB AND SHERP PROTEINS IN LEISHMANIA METACYCLOGENESIS**

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Leishmania parasites are transmitted to mammalian hosts by the bite of female phlebotomine sand flies. After uptake of intracellular amastigotes during blood-feeding, L. (*Leishmania*) spp. develop within the sand fly midgut to generate mammalian-infective metacyclic promastigotes; this process is termed metacyclogenesis. We have used null and complemented parasite mutants to show that the LmcDNA16 locus (~11.5Kb on chromosome 23 of L. major) is essential for completion of metacyclogenesis in the insect vector (*Sadlova et al. (2010) Cellular Microbiology 12:1765*). This locus contains 2 gene families, coding for the HASP and SHERP proteins, stage-specific molecules that are unrelated to other identified eukaryotic proteins. We are sequentially replacing genes from the locus back into the LmcDNA16 null mutant to investigate their contribution to the metacyclogenesis phenotype. Over-expression of the HASPB and SHERP genes individually suggests that HASPB plays a major role in this process. To investigate further, individual replacement mutants for HASPB and SHERP, designed to support expression of each gene at the correct developmental stage and at wild type level, have been generated and characterised. These have then been used to infect *P. papatasi* female sand flies. The results obtained from these analyses will be discussed in the context of understanding the contribution of the HASP and SHERP proteins to the process of metacyclogenesis *in vivo*.

RT.002A - **IMMUNOBIOLOGY OF LEISHMANIA EXOSOMES**

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Leishmania infection brings about macrophage deactivation, which promotes chronic infection and contributes to disease pathogenesis. Macrophage deactivation appears to involve the export of virulence factors into host cells. We have shown that interferon-γ signaling through the Jak1,2-Stat1 pathway is attenuated in L. donovani infected cells and MAP kinase signaling is similarly deficient with both leading to impaired gene expression. We also identified the leishmania secreted protein elongation factor-1α to be involved in macrophage deactivation and showed EF-1α to be translocated into the cytosol of infected cells. To determine the identity of other leishmania effectors and their mechanisms of secretion, we undertook a global proteomic analysis of leishmania secreted proteins using quantitative mass spectrometry. This identified 358 *bona fide* leishmania secreted proteins, many of which were orthologs of proteins considered to be markers of mammalian exosomes. Subsequently, we demonstrated directly that leishmania release exosomes, and analysis of the exoproteome showed that exosomes account for at least 50% of protein secretion by leishmania. We also found that the cargo profile of leishmania exosomes is influenced by heat shock and low pH, conditions which mimic those experienced by promastigotes after host invasion. Confocal and electron microscopy of leishmania infected cells confirmed the novel finding that leishmania use exosomes to deliver proteins into infected host cells. Likewise, we obtained evidence to show that exosomes deliver leishmania effectors to naïve, uninfected macrophages. In related studies, we demonstrated that wild-type (WT) leishmania exosomes have immunosuppressive properties which, in a cargo-dependent manner, modulate the phenotypes of monocytes, dendritic cells, and T lymphocytes. In contrast to exosomes from WT *L. donovani*, vesicles from HSP100 null *L. donovani* showed a gain-of-function, pro-inflammatory phenotype. Furthermore, proteomic analysis showed that exosomes from WT and HSP100 null leishmania had distinct protein cargo, suggesting that packaging of proteins into exosomes is dependent in part on HSP100. Notably, immunization of mice with WT exosomes from either *L. donovani* or *L. major* exacerbated disease progression after subsequent challenge with homologous organisms, whereas HSP100 null exosomes did not. Moreover, when combined with the Th1 adjuvant CAF01, immunization with both WT and HSP100 null *L. donovani* exosomes reduced parasite loads upon subsequent challenge infection and the HSP100 null exosome/CAF01 combination was the most protective. Taken together, these findings show that exosomes are a major mechanism for protein secretion by leishmania. In addition, the packaging of protein cargo into *L. donovani* exosomes is regulated by HSP100. These vesicles are bio-active and predominantly immunosuppressive. Exosomes appear to function as a mechanism for delivery of effector molecules to host cells to bring about myeloid cell deactivation.

RT.002B - **TRYPANOSOMA CRUZI: TRYPOMASTIGOTES SECRETE VESICLES WITH PROINFLAMMATORY VIRULENCE FACTORS THAT ENHANCE CELL INVASION**

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The surface of Trypanosoma cruzi is heavily coated by glycosylphosphatidylinositol (GPI)-anchored molecules (i.e., mucins, trans-sialidase (TS)/gp85 glycoproteins, and glycoinositolphospholipids-GIPLs) that strongly stimulate the host innate immunity via Toll-like receptor (TLR)-dependent pathways. It remains elusive, nevertheless, how these major plasma membrane glycoconjugates become more readily avalailable to host cognate receptors. One potential mechanism involves the continuous shedding of membrane-bound vesicles by host cell-derived trypomastigotes (Ves). A recent study showed that Ves seem to be enriched of TS/gp85 glycoproteins and other α-galactosyl-containing glycoconjugates. Moreover, pre-treatment of BALB/c mice with Ves, followed by parasite challenge, could significantly exacerbate parasite load and inflammation of the heart, and hasten animal mortality. We provide new insights into the molecular composition of Ves and the mechanism by which they promote host cell invasion and inflammation. Proteomic analysis of Ves by liquid chromatography-tandem mass spectrometry (LC-MS/MS) revealed 110 T. cruzi-specific proteins, of which over half (55%) were trans-sialidase/gp85 glycoproteins, which are well established virulence factors. We also found other virulence factors such as gp63 and mucins, as well as proteins involved in endo-/exocytosis, suggesting an exosome-like biogenesis for Ves. Similar to earlier in vivo observations, Ves induced in vitro high levels of proinflammatory cytokines and nitric oxide by murine BALB/c- and C3He/HeJ-derived macrophages, and considerably increased invasion of host cells. Using TLR2- and TLR4-transfected CHO cells and macrophages derived from TLR2-knockout mice, we clearly demonstrated that both proinflammatory response and host-cell invasion-enhancing activity were mediated by a TLR2-dependent pathway. Finally, both phenomena were abolished when Ves were pre-treated with α-galactosidase, suggesting that Ves virulence factors engage TLR2 and yet unknown host α-galactosyl receptor(s).

RT.002C - **POTENTIAL HOST BIOMARKERS FOR PLASMODIUM VIVAX MORBIDITY: MICROPARTICLES AND CIRCULATING NUCLEIC ACIDS.**

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Microparticles (MPS) - submicrometer vesicles released from cells upon activation or apoptosis - and circulating nucleic acids (CNAs) have been increasingly recognized as powerful diagnostic and prognostic tools for various inflammatory diseases and tumors as their plasma concentrations increase according to severity. Given the marked inflammatory status of *Plasmodium vivax* infection, we investigated the usefulness of MPs and CNAs as biomarkers for malaria morbidity. The results show that: (1) MPs and CNAs levels were significantly increased in plasma from *P. vivax* patients as compared to healthy age-matched malaria-unexposed controls; (2) Platelets-derived MPs and CNAS were associated with acute vivax symptoms, and CNAs levels were strongly associated with vivax morbidity, including a drop in platelet counts, (3) Mps and CNAs levels decrease and reach physiological levels after antimalarial treatment. Together, these data suggest the potential of these inflammatory mediators as sensitive biomarkers for vivax malaria morbidity. Studies are in progress to investigate Mps as source of these CNAS, and if these complexes could bind IgG to amplify the inflammation.
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RT.003A - **IS A PHEROMONE BASED APPROACH TO VISCERAL LEISHMANIASIS CONTROL IN BRAZIL FEASIBLE**

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Lutzomyia longipalpis is a species complex of at least 4 species (or subspecies) that can be identified by their male produced sex pheromones. The most geographically widespread of these species produces a sex pheromone identified as the novel terpene, (*S*)-9-methylgermacrene-B which has a 16 carbon skeleton.
Sex pheromones and other semiochemicals are widely for monitoring and as part of control programs for agricultural insect pests. To answer the question of whether or not a sex pheromone based approach to controlling *L. longipalpis* is feasible we determined first if it was possible to produce sufficient synthetic pheromone in the laboratory at a cost that would make it viable for large scale use in the field. We demonstrated that the sex pheromone could be synthesised in bulk in the laboratory by converting a plant derived intermediate, germacrone, to the insect produced compound in 4 steps.
The next step was to show that the synthetic sex pheromone was as attractive as the natural sex pheromone in the laboratory and to demonstrate that it could be formulated to be attractive in the natural environment. We showed that synthetic (*S*)-9-methylgermacrene-B, attracted females in laboratory experiments and that it could be formulated in dispensers that released the pheromone at a rate similar to that released by a group of aggregating males. Field experiments using experimental chicken sheds showed that we were able to attract both females and males to Centers for Disease Control (CDC) traps (with and without lights) and commercially available sticky traps baited with the pheromone dispensers in the field.
The last step in this feasibility study was to show that the sex pheromone could be used in the "lure-and-kill" approach which is widely used in the agricultural sector. To do this we showed that the pheromone could be used to improve the killing potential of insecticide sprayed experimental chicken sheds.
We found that addition of synthetic pheromone resulted in greater numbers of male and female sand flies being caught and killed at experimental chicken sheds sprayed with insecticide, compared to pheromone-less controls. Furthermore, a ten-fold increase in the amount of sex pheromone released from test sheds increased the number of females attracted and subsequently killed. Treating sheds with insecticide alone resulted in a significant decrease in numbers of males attracted to sheds (compared to pre-spraying levels), and a near significant decrease in numbers of females. However, this effect was reversed through addition of synthetic pheromone at the time of insecticide spraying, leading to an increase in number of flies attracted post-treatment.
From these studies we concluded that synthetic pheromone could play a significant role in more effectively target the sand fly vector of AVL for control. Currently the sex pheromone could be most effectively deployed for sand fly control through combination with existing insecticide spraying regimes or new forms of pesticide application. Development of a standalone pheromone trap remains a possibility for the future, but such devices may require an additional attractive host odour component to be fully effective.
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RT.003B - **A NEW PERSPECTIVE ON THE INTERACTIONS BETWEEN TRIATOMINES AND TRYPANOSOMES**

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Parasite-host interactions represent a complex area of biology, since they involve the development of an organism inside another one, which, in its turn, makes part of an intricate ecological system. Notably, relations between parasites and insects become even more complex, since the environmental temperature they endure can modulate infection dynamics. The infection of *Rhodnius prolixus* with *Trypanosoma cruzi* and *Trypanosoma rangeli* was studied to evaluate factors that could influence parasite virulence. *T. rangeli* is considered

pathogenic to some triatomines, although several authors have not reported virulence. We found two different reaction types in the *T. rangeli* CHOACHI strain. The first one is mainly promoted during parasite passages in culture medium. This population is sensitive to environmental temperature, as shown by the growth of parasites at distinct levels of this parameter, both in culture medium and inside triatomines. These parasites rarely invade salivary glands and produce a severe haemolymph infection phase prolonging intermoult periods, increasing hemolymph volume, lipid load and mortality rates. Insects infected by the virulent reaction type also showed alterations in their behavior, such as altered use of shelters, negative phototaxis and locomotor activity. The second *T. rangeli* reaction type normally attains the metacyclic trypomastigote form in salivary glands and presents low numbers of epimastigotes in the hemolymph. Pathogenic alterations in insects infected by these parasites become slighter, as well as behavioral ones. As both virulence patterns have been described in naturally infected bugs, we suggest that they might have implications on the parasite transmission in nature. To date, *T. cruzi* has not been considered pathogenic to triatomines. We observed that these parasites show increased culture medium growth when exposed to high temperatures (i.e. 30°C). Interestingly, insects infected with *T. cruzi* presented increased mortality rates at this temperature. Additionally, infection by these parasites induced alterations on their locomotor activity, making them less active than control ones. This suggests that *T. cruzi* may also alter the physiology of triatomines, although in a way less apparent than that observed with *T. rangeli* infections. Our results indicate that both parasites can have their virulence modulated depending on several factors, such as life history, i.e., to have a previous passage through a vertebrate, or environmental temperature. More detailed evaluations on insect fitness, as well as the effect of the co-infection by both parasite species, will be necessary to improve our understanding on trypanosome-triatomine interactions.
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RT.003C - **ENVIRONMENTAL CHANGES AND THE IMPACT ON THE LEISHMANIASES TRANSMISSION**

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Currently, besides the biological factors (vector, host and parasite), associated with the context of the transmission of the leishmaniases, environmental and climatic changes, resulting from anthropic actions or by natural events, may be considered as important determining factors in the new epidemiological profiles. Continuous deforestation due to the hydroelectric construction , migrations, the implantation of great agricultural projects, civil wars and military activities are elements that have amplified this scenario, suggesting that the geographical expansion is occurring as a result of the ecological chaos caused by human action, thus making possible, a closer contact with man, wild and/or synanthropic animals and the vectors of human pathogens.
The context of global environmental changes, in which the uncertainties about the nature of their impact on the scale of the local ecosystems, together with the complexities of the new realities of an urban Brazil, imply new challenges in dealing with the process of expansion of the leishmaniases to urban perimeters of medium and large sized cities, as well as in the actions of surveillance and control.
Climatic alterations, together with deforestation and the destruction of natural habitats could reduce the transmission of the leishmaniases, however, such events have resulted in the increase of human infection, as has been observed in relation to the incidence of American Cutaneous Leishmaniasis (ACL), through the change from the wild cycle to the peridomiciliar environment, in areas of South America, particularly in Brazil. Similarly, American Visceral Leishmaniasis (AVL), in Brazil, is not only increasing, but is in a full process of urbanization, clearly a result of disequilibrium in the natural ecosystem.
In the context of ACL, two cycles of transmission stand out by their frequent register in impacted areas, some situations in the domiciliary environment. Lutzomyia (Nyssomyia) whitmani, a vector of Leishmania (Viannia) shawi in Amazonia and Leishmania (Viannia) braziliensis, in the Central-West, North, Northeast, Southeast and South, is the most important vector of ACL in Brazil, adapted to various climates and vegetation covers in association with the majority of the epidemiological circuits of production of the disease, participating in the profile of transmission related to the environmental alterations. A severe form of ACL associated with Leishmania (Leishmania) amazonensis is also encroaching on the anthropic environment with the participation of Lutzomyia (Nyssomyia) flaviscutellata, a vector found in all the geographical regions, with the exception of the South of Brazil, adapted to different types of climate and vegetation.
In relation to AVL, considering that Brazil is facing its expansion and urbanization, the acquisition of knowledge about the determining factors of this new epidemiological profile is imperative. The Brazilian national policies of surveillance and control of AVL find, in the presence of their principal vector, Lutzomyia

(Lutzomyia) longipalpis, in the urban area, one of the greatest challenges for the success of the National Control Program of AVL, clearly highlighting the need for a better understanding of the factors associated with the occupation of new habitats, and for defining the determining factors of this process.
Geo-technologies are important tools in the area of health, and viable studies have been carried out regarding: the analysis of the distribution of patients, variations in the occurrence of epidemics, monitoring of vectors, assessment in real time of emergency or catastrophic situations and the incorporation and aggregation of different variables such as extension, location, time, social-economic characteristics, and environmental, climatic and biological characteristics. Thus, in relation to L. (N.) whitmani, L. (N.) flaviscutellata and L. (L.) longipalpis they will be able to define determining factors of expansion and installation of the cycles of transmission in which these vectors are involved, establishing important correlations between the determining factors of occurrence of the leishmaniases (climatic and environmental) associated with the vectors.
Some studies under development show, from their preliminary results, the relationship between the occurrence of the leishmaniasis and the vegetation (type and state). In relation to ACL associated with L. (N.) whitmani it was possible to verify the various types of vegetation cover associated with the vector, being found most frequently in the biomes of the Amazon, Savannah and Atlantic Forest in association with the areas of the epidemiological circuits with a greater concentration of human cases. And for AVL, populations of the vector L. (L.) longipalpis were analyzed coming from endemic areas in the State of Tocantins, with an ample approach involving environmental and biological factors, decisive in the process of transmission, expansion and urbanization of the disease. The thematic maps showed, over the years, a consistent pattern in space-time evolution in the increasing deforestation, factor that could be reflecting in the number of human cases of AVL.

RT.004A - **VISUAL GENOME-WIDE RNAI SCREENING TO IDENTIFY HUMAN HOST FACTORS REQUIRED FOR TRYPANOSOMA CRUZI INFECTION**

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The protozoan parasite *Trypanosoma cruzi* is the etiologic agent of Chagas disease, currently estimated to affect 8-10 million people in the Americas. Current chemotherapy relies on only two drugs that have limited efficacy and considerable side effects. Therefore, new and more effective drugs are urgently needed. Although some host cellular factors that play a role in *T. cruzi* infection have been uncovered, the molecular requirements for intracellular parasite growth and persistence are still not well understood. To further study these host-parasite interactions and identify human host factors required for *T. cruzi* infection, we performed a genome-wide RNAi screen using cellular microarrays of a printed siRNA library that spanned the entire human genome. The screening was reproduced 6 times and a customized algorithm was used to select as hits those genes whose silencing visually impaired parasite infection. The 162 strongest hits were subjected to a secondary screening and subsequently validated in two different cell lines. Among the fourteen hits confirmed, we recognized some cellular membrane proteins that might function as cell receptors for parasite entry and others that may be related to calcium release triggered by parasites during cell invasion. In addition, two of the hits are related to the TGF-beta signaling pathway, whose inhibition is already known to diminish levels of *T. cruzi* infection. This study represents a significant step toward unveiling the key molecular requirements for host cell invasion and revealing new potential targets for antiparasitic therapy.

RT.004B - **TDR TARGETS: A CHEMOGENOMICS RESOURCE FOR NEGLECTED DISEASES**

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The TDR Targets Database (http://tdrtargets.org) is an online resource that seeks to facilitate the prioritization of molecular targets for drug development, allowing users to numerically weight the evidence available for each gene. The database associates gene information from human pathogens with genomic and functional information from various sources. In order to expand the coverage of chemical information associated with targets from complete genomes, we integrated various chemical datasets, associating them with protein targets based on manual curation of the literature. Compounds were obtained from DrugBank, PubChem and Starlite (ChEMBL) databases. A cheminformatics pipeline was developed in-house to calculate physicochemical properties and identifiers for each molecule, used to facilitate searches and cross-linking to other databases. Using this pipeline, we have now integrated 825,814 compounds. Bioactive compounds obtained from the Starlite/ChEMBL database (439,984) have information on their activity (IC50s, MICs, etc.) and their targets. Using the OrthoMCL database of orthologous proteins, we have identified 4,338 pathogen proteins that share the same ortholog group with at least one target in ChEMBL. These proteins could be transitively linked to 173,416 compounds. Using these data we are starting to define rules to propose new associations between compounds and targets. As a first step, we are analyzing groups of orthologs that contain pathogen genes without any chemical information, but whose non-pathogen members carry information on compounds that have significant inhibitory activity. This procedure could be useful to identify known compounds that could be tested on pathogens. Additional chemical leads can then be identified by chemical similarity. Preliminary results, taking into account only four assays (measurement of EC50, IC50, MIC and Ki), allowed us to identify new chemical leads for 17 ortholog groups that carry essential genetic phenotypes.

RT.04C - **IN SILICO APPROACHES FOR THERAPEUTIC INNOVATION**

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The process of drug design has the computational tools needed to increase the speed and decrease the costs involved in development of new products related to therapeutic innovation. The use of computers in this area has taken significant proportions, particularly in the area of computer-based molecular design, with several studies of structure prediction and molecular properties, for example. The term "in silico" follows the trend of the terms "in vitro" and "in vivo" and refers to the silicon chip present in computers, being widely used in medicinal chemistry literature to describe the use of computational methods in molecular modeling, especially with regard to systems of biological interest. The importance of "in silico" approach has been perceived by the scientific community around the world and the pharmaceutical industry ("big pharma"). It is estimated that the use of these methods can reduce costs and development time of a new drug by 50% [Geldenhuys et. al. 2006; McGee, 2005]. This often occurs because the number of molecules that need to be synthesized and tested experimentally becomes drastically reduced due to the high predictability and reliability of computational methods ("in silico"), thereby shortening the development time for a new drug. Virtual screening or molecular docking methods, for example, are widely used in this field. The molecular docking determines if there is favorable interaction energy between two molecules (ligand and biological target) in order to elucidate the molecular mechanisms responsible for the pharmacological potency of these ligands or potential drugs. The docking procedure seeks for the position and orientation that maximizes these intermolecular interactions. Thus, the ligand and the target (typically a protein) form a complex by structural complementarity and energetic stabilization. One of the main objectives of the "in silico" studies is to predict the intensity and specificity with which small and medium-sized molecules, usually called ligands (drugs, potentially) bind to the active site of a biological receptor, typically a biomacromolecule (drug target), thus altering its biochemical cycle through modulation of its biological response. This presentation will address some aspects of these “in silico” approaches, particularly related with anti-parasitic drugs.

RT.005A - **HOST SERINE PEPTIDASES AND TOLL-LIKE RECEPTOR 4 IN THE INTERACTION OF LEISHMANIA MAJOR WITH PHAGOCYTES**

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The sucessful establishment of Leishmania infections relies on the parasite's ability to survive in the inhospitable environment of professional phagocytes and to evade the host's responses. While *Leishmania* amastigotes reside in macrophages, the majority of freshly inoculated metacyclics undergo a transient passage through neutrophils, before infecting macrophages. Recently, *L. major* ecotin-like inhibitor of serine peptidases 2 (ISP2) was implicated as a virulence factor for modulating the activity of host serine peptidases during the interaction with macrophages. *L. major* has three ecotin-like genes: *ISP*1, expressed in promastigotes and metacyclics, *ISP*2, expressed in the three life stages and *ISP*3, whose expression was not detected so far. ISP2 inactivates S1A family peptidases such as trypsin, neutrophil elastase and cathepsin G. *L. major* lines lacking *ISP*2 and *ISP*3 (Δ*isp*2/*isp*3) are internalized more efficiently by macrophages due to up-regulation of phagocytosis. Increased uptake was mediated by the concerted triggering of the complement type 3 receptor (CR3), Toll-like receptor 4 (TLR4) and the activity of neutrophil elastase (NE), present at the surface of macrophages. Parasites lacking *ISP*2/*ISP*3 were partially eliminated from macrophages within 24h after phagocytosis, and the remaining parasites exhibited delayed growth. Parasite survival required inhibiton of NE and neutralization of TLR4, but was dissociated from CR3. Superoxide scavengers or catalase prevented parasite death, suggesting that ISPs play a role in parasite oxidant resistance in macrophages. Increased phagocytosis of Δ*isp*2/*isp*3 was reverted to WT levels in RAW cells expressing dominant-negative serine/threonine kinase R (PKR). Neutralisation of TLR4, CD11b or the inhibiton of NE prevented increased uptake of Δ*isp*2/*isp*3 by RAW, while it had no effect in parasite phagocytosis by PKR-DN-RAW, suggesting that PKR acts downstream of the TLR4-NE pathway. Live imaging of *L. major* phagocytosis by RAW showed that WT parasites are rapidly surrounded by host cell lysosomes while Δ*isp*2/*isp*3 are temporarily encircled by lysosomes, but move away from them a few minutes after internalisation. The interaction of metacyclics with murine bone marrow-isolated neutrophils was also evaluated. Parasites were found with the cell body inside neutrophils and the flagella protuding outward from the host cell. Multiple Δ*isp*2/*isp*3 were observed in a single neutrophil, in contrast to a maximum of three WT parasites per neutrophil. Motile parasites were relased from neutrophils within 12h and, in contrast to the observed in macrophages, Δ*isp*2/*isp*3 survived the passage through neutrophils and exited at higher numbers. Neutrophils isolated from NE knock-out mice were used to address the influence of this peptidase in *L. major* uptake/release. While WT *L. major* were released at similar numbers from NE-KO or C57B6 neutrophils, the release of Δ*isp*2/*isp*3 from NE-KO neutrophils was reduced. These observations suggest that NE contributes to the increased uptake and/or the release of Δ*isp*2/*isp*3 by neutrophils. Neutrophil-derived parasites were cultivated for 7 days and their infectivity to macrophages was subsequently evaluated. Neutrophil-derived WT *L. major* were more infective to macrophages, as compared to parasites that had not been in contact with neutrophils and this phenotype was maintained after at least three passages in culture. However, neutrophil-derived Δ*isp*2/*isp*3 were as infective as neutrophil-derived WT parasites, suggesting that the lack of inhibiton of neutrophilic serine peptidases influences the outcome of parasite infectivity to macrophages later on. Those results favour the hypothesis that the inhibiton of host SPs, in particular of NE by ISPs contributes to the parasite's ability to survive in professional phagocytes.

Supported by:The Wellocme Trust, FAPERj, CNPq

RT.005B - **AS SWEET AS IT CAN GET: INFLUENCE OF SIALIC ACID IN TRYPANOSOMA CRUZI HOST INTERACTION**

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A number of studies have demonstrated the importance of differential sialylation for $CD8^+$ T cells with respect to their development, activation via the TCR, and cytotoxic responses. Here we provide insights into the impact of surface sialylation of $CD8^+$ T cells on the response to *Trypanosoma cruzi*. Infection with *T. cruzi* is of particular interest in this context, as the parasite releases into the host plasma large amounts of enzymatically active and inactive proteins belonging to the *trans*-sialidase (TS) family. Our results demonstrate that upon activation by protozoal infection cytotoxic $CD8^+$ T lymphocytes are desialylated, exposing beta-galactose residues, enhancing their effector activity. Subsequently, *T. cruzi* uses its TS enzyme to resialylate bared terminal beta-galactose on $CD8^+$ T cell surface, attenuating antigen-specific $CD8^+$ T cell response and increasing mouse mortality. Our findings also indicated that CD43 (a highly sialylated mucin expressed on leukocyte surface) is a target receptor for TS on the $CD8^+$ T cell surface. Accordingly, CD43 deficient mice are more resistant to parasite infection coinciding with an increased $CD8^+$ T cell mediated cytotoxic response. In addition, we found that sialic acid transferred by alpha-2,3-sialiltrasferase-IV (ST3Gal-IV) play a important role during *T. cruzi* infection. Mice lacking ST3Gal-IV infected with *T. cruzi* had a significant reduction of parasitemia and mortality. Consistent with these observations, antigen-specific $CD8^+$ T cells isolated from infected ST3Gal-IV KO mice showed an increased and antecipated cytotoxic activity. Together these observations show that sialylation of $CD8^+$ T cells by TS could be the mechanism that delays $CD8^+$ T cell responses during *T. cruzi* infection. These results open a new perspective for the role of sialic acid in the development of adaptive immunity during infection by *T. cruzi*.
Supported by:CNPq, FAPERJ, The National Institute for Vaccine Technology (INCTV-CNPq),

RT.005C - **NEUTROPHILS EFFEROCYTOSIS INDUCES A M2B PHENOTYPE IN MACROPHAGES**

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Clearance of apoptotic exudate neutrophils (efferocytosis) induces either pro- or anti-inflammatory responses in mouse macrophages depending on host genetic background. In this study, we investigated whether neutrophil efferocytosis induces a stable macrophage phenotype that could be recalled by late restimulation with LPS. Bone marrow-derived macrophages previously stimulated by pro but not anti-inflammatory neutrophil efferocytosis expressed a regulatory/M2b phenotype characterized by low IL-12 and high IL-10 production following restimulation, increased expression of LIGHT/TNFsuperfamily 14, Th2-biasedTcell responses, and permissive replication of Leishmania major. Induction of regulatory/M2b macrophages required neutrophil elastase activity and was partially dependent on TLR4 signaling. These results suggested that macrophage differentiation to a regulatory phenotype plays a role in resolution of inflammation but could contribute to increased humoral Ab responses and parasite persistence in the infected host.
Supported by:CNPq

RT006A - **LEISHMANIASIS: THE WHO PERSPECTIVE**

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Leishmaniasis shares all characteristics of a neglected tropical disease and, in addition, the lack of social and political recognition according to its disease burden. Underlying reasons are the gap in a coherent concept on how to control it from case management to vector control, the lack of updated epidemiological information, the need to define specific control programmes according to geographical areas due to the different epidemiological cycles, and the absence of a strategic plan shared by governments and stakeholders.
In the current presentation are presented the steps forward to change the paradigma of leishmaniasis as a neglected tropical disease, more specifically (i) the Resolution "Control of Leishmaniases" approved by the World Health Assembly (WHA 2007/60.13), (ii) the Expert Committee meeting and the publication of the Technical report series 949, (iii) the document "Leishmaniais, the country profile", (iv) the role of control programmes in the Indian subcontinent, East Africa, the Middle East and Maghreb, Caucasian and Central Asian countries, and America, (v) the inclusion of all medicines for Leishmaniasis in the Essential
Drug List, and (vi) the negotiations with the pharmaceutical industry to reduce the drug prices or to reach specific donations.
The contribution of AECID and the collaboration of Sanofi-aventis and WHO mad eit possible to launch a comprehensive programme that opens new perspectives for Leishmaniasis control.

RT006B - **CHAGAS DISEASE IN THE AMAZON REGION – RISKS AND PERSPECTIVES**

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Chagas disease in the Panamazon can be considered a sylvatic enzootic of wild reservoir hosts from six orders: Marsupialia, Chiroptera, Rodentia, Edentates (Xenartra), Carnivora and Primates of 33 species found infected with Trypanosoma cruzi. Over 25 species of wild triatomines vectors in nine genera, most of them infected with T. cruzi, were identified on that region. Invasion of houses by adventitious vectors infected with T. cruzi is frequent in the Amazon region, and focal preadaptation and adaptation of native triatomines to human dwellings has been reported from several areas of different countries, mainly in Brazil, Venezuela and Colombia.
Hundreds of acute cases of human Chagas disease have been reported from the Amazon region mainly in the Brazilian Amazon. The first acute cases were reported by Floch and Tasqué (1941) and Floch and Comain (1948) in French Guiana.
Chagas disease in the Brazilian Amazon
In 1969 Shaw et al. described the first acute cases of Chagas disease in the Brazilian Amazon. Since then about five hundred acute cases have been recorded, most of them in the states of Pará, Amapá and Amazon, reported as consequence of microepidemics, isolated cases or described through serological surveys.
Since Carlos Chagas (1924) has confirmed as Trypanosoma cruzi, the parasites isolated from Saimiri sciureus by Aben-Athar, many reservoirs of the parasite were described in the Amazonian region. On the other hand, at least 16 species of triatomines, 10 of which infected with T. cruzi, were identified on that region.
A national serological survey about the prevalence of Chagasic infection, accomplished by SUCAM (National Health Foundation), from 1975 to 1980, indicated a prevalence of 1.88% in the State of Amazonas, with a concentration of 6.3-6.8% in areas of Rio Negro. In 1977, six autochtonous cases sera positive for infection by T. cruzi were described in the same region.From 1991 on, we have accomplished various serological surveys and sectional studies in the district of Barcelos and among the riverside population of Rio Negro and its affluents. These studies have revealed a high serological prevalence of chagasic infection, PCR positiveness and isolation of T. cruzi, particularly among workers of piaçava's gathering and their families, as well as among sylvatic reservoirs and vectors of the region.
Risks and perspectives of Chagas disease in the Amazon Region
The risks of Chagas disease in the Amazonian region are related to deforestation and to the possible adaptation of sylvatic triatomines to human dwellings; or, yet, to the transposition of the

domestic cycle from endemic areas to the Amazon region, due to migration of infected people and/or to the carriage of vectors and domestic reservoirs from the endemic areas to that region. Under some circumstances, Amazonian sylvatic triatomines can invade houses, contaminate foodstuffs, or attack forest workers. Some populations of T. maculata, P. geniculatus, P. herreri, and R. stali are adapted to artificial ecotopes in a few areas. Prevalence of human T. cruzi infection may be estimated as 1-2%, but reaches about 5% in some places. The identification of ~ 300 acute cases associated with family outbreaks (probably inked to the ingestion of contaminated food) has changed the traditional view of the Amazon as a region free of human Chagas disease. The number of acute cases without any relation with those outbreaks (over 197) suggests that vector-borne transmission via direct contact between humans and sylvatic triatomines is more important than compulsory case reporting indicates. Autochthonous cases of dilated myocardiopathy with fatal evolution have been documented, and severe acute cases are reported with increasing frequency. Most of these cases are associated with infection by T. cruzi 1, which circulates widely both in the Amazon and in other endemic regions.

RT.006C - **ECOLOGICAL GENETICS OF PHLEBOTOMINES: VECTOR INCRIMINATION AND LEISHMANIASIS CONTROL**

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Leishmaniasis is often a zoonotic disease, and the landscape epidemiology can be distinctive for each transmission cycle involving a parasitic *Leishmania* species and one or a few species of reservoir hosts and phlebotomine sandfly vectors. For many decades this helped to encourage a zoological slant to leishmaniasis research, with this bias being accentuated by the search for molecular markers to identify species of microscopically similar parasites and beige-coloured sandflies. Research on leishmaniasis epidemiology has moved on, becoming less focused on eco-epidemiology and more concerned with modelling, certainly for zoonotic visceral leishmaniasis caused by *Leishmania infantum* in the Mediterranean region, Asia and the Americas (Quinnell, R.J. and Courtenay, O. 2009. Transmission, reservoir hosts and control of zoonotic visceral leishmaniasis. *Parasitology* 136:1915-1934. Ready, P.D. 2010. Leishmaniasis emergence in Europe. *Eurosurveillance* 15: pii = 19505). Ecological genetics has been developed for both parasites and vectors, but the focus is too often on the distribution of neutral markers between species, rather than assessing the frequencies of alleles and phenotypes known to affect infectivity, susceptibility and immunological responses, and searching for their distributions within and between populations. The current presentation will use recent research on the vectors of *L. infantum* in the Mediterranean region and the Americas to illustrate how ecological genetics could inform different approaches to leishmaniasis control as well as to vector incrimination. Examples will include testing for adaptive selection of sandfly salivary peptides in relation to vaccination strategies. Epidemiological modelling and applied ecological genetics are only possible in areas where the causative agents of leishmaniasis are known and can be routinely identified to species using standardized biochemical or molecular tools. This has long been recognized in the Americas, unlike in parts of the Old World where there has been a tendency to assume that one parasite species predominates locally (Parvizi, P., Mazloumi-Gavgani, A.S., Davies, C.R., Courtenay, O. and Ready, P.D. 2008. Two *Leishmania* species circulating in the Kaleybar focus of 'infantile visceral leishmaniasis', northwest Iran: implications for deltamethrin dog collar intervention. *Transactions of the Royal Society of Tropical Medicine & Hygiene 102, 891-897*). Brazilian teams are well aware of the diversity of the leishmaniasis transmission cycles that can be found in relatively small areas of their country, where the complexity of parasite-vector-host interactions could restrict the use of applied ecological genetics. One of the challenges is to assess what it means to describe *Lutzomyia longipalpis* as a "permissive vector". What proportions of the wild populations of this widespread neotropical sandfly are susceptible to different phenotypic strains of *L. infantum*?

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RT.007A - **HEAT SHOCK PROTEIN GENE EXPRESSION AND REGULATION IN TRYPANOSOMA CRUZI**

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The heat shock proteins (HSPs) are a group of chaperone proteins that have their gene expression increased in response to heat shock and other stressing agents. HSPs are grouped in families according to their size or functional characterization. Some HSP families have received more attention because of their important function in the cell. Among these is the HSP70 and the chaperonin (HSP60 and HSP10), and HSP100 families. In Trypanosoma cruzi, the causative agent of Chagas' disease, and other trypanosomatids, gene regulation is exerted mainly at the post-transcriptional level by modulation of mRNA stability and translation. We have investigated the gene expression patterns and regulatory mechanisms in this organism. The HSP70 mRNA's half-life increases after heat shock, and the stabilization is dependent on protein synthesis. In a cell-free RNA decay assay, a U-rich region in the 3' untranslated region (UTR) is a target for degradation, which is reduced when in the presence of protein extracts from heat shocked cells. In a transfected reporter gene assay, both the 5'- and 3'-UTRs confer temperature-dependent regulation. Both UTRs must be present to increase mRNA stability at 37°C, indicating that the 5'- and 3'-UTRs act cooperatively to stabilize HSP70 mRNA during heat shock. We have also shown that HSP104 is induced upon heat shock, and the levels of the corresponding mRNA are increased. In addition, a 3D structure of T. cruzi HSP104 is being generated through molecular modelling. The 3D model is being constructed using ClpB of Thermus thermophilus as a template. Finally, we are currently investigating how HSP10 gene expression is regulated in this organism and also how it is coordinated with HSP60 gene expression, since both protein products are part of the same macromolecular structure. The relevance and conservation of the HSP gene expression and regulation in T. cruzi will be discussed.
Supported by:CNPq and FAPERJ.

RT.007B - **TRYPANOSOMA CRUZI GENOMICS: FOCUS ON GPI BIOSYNTHESIS AND STAGE SPECIFIC GENE EXPRESSION**

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During its life cycle, T. cruzi undergoes major morphological and biochemical adaptations that are dependent on stringent regulatory mechanism of stage-specific gene expression. The interactions that occur with its different hosts are mediated by a variety of surface glycosylphosphatidylinositol (GPI) anchored molecules. Therefore, parasite genome sequences involved in the control of stage-specific gene expression and the biosynthetic pathways of GPI anchors are attractive targets for new therapies for Chagas disease. Using the T. cruzi genome database, we identified all T. cruzi genes involved in GPI biosynthesis and protein attachment, as well as the gene encoding inositolphosphorylceramide synthase, a unique parasite enzyme involved in lipid remodeling of the GPI anchor. After transforming yeast conditional mutants, we showed that the T. cruzi DPM1, GPI10, and GPI12 genes encode functional enzymes in yeast. To further investigate the role of T. cruzi GPI anchored proteins, we disrupted the GPI8 gene, which encodes the catalytic subunit of the enzyme complex responsible for GPI-protein attachment. To identify genome sequences involved in the control of stage-specific gene-expression, we developed a transfection vector containing the firefly and Renilla luciferase reporter genes, in which sequences derived from 3'UTR of stage-specific mRNAs can be inserted and the expression of the reporter can be evaluated in all stages of parasite life cycle. We showed that sequences derived from the alpha tubulin gene, which is up-regulated in epimastigotes, and amastin, which is up-regulated in amastigotes, resulted in increased luciferase activity and mRNA levels in epimastigotes and amastigotes, respectively. We also showed that the spliced leader sequence and poly-A tail were inserted in the predicted sites of the reporter mRNA and that deletions in the alpha tubulin 3'UTR resulted in decreased luciferase expression because it affects poly-adenylation. In contrast to tubulin and amastin 3'UTR, the presence of the 3'UTR from a trans-sialidase and MASP genes, whose expression is higher in trypomastigotes, resulted in increased luciferase activity in trypomastigotes without a corresponding increase in luciferase mRNA levels.
Supported by:CNPq, FAPEMIG, CAPES, HHMI

RT.007C - **TRYPANOSOMA CRUZI REGULOME: A GROWING DATABASE AND FRAMEWORK FOR GENE EXPRESSION REGULATION DATA MINING**

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Trypanosoma cruzi is an interesting model for studying gene expression control, due to several characteristics as lack of transcriptional gene-specific regulation, complex life cycle, interaction with distinct hosts, etc. We and others researchers are conducting several high-throughput characterization studies of diverse levels of molecular biology information, including distinct approaches from genomics, transcriptomics, proteomics, ribonomics and interatomics fields. In this regard, we have used RNA-Seq and LC-MS/MS to analyze the transcriptome, ribonome and proteome modulation in many *T. cruzi* biological systems, as life cycle (four major forms), differentiation (metacyclogenesis, epimastigogenesis etc) and infection processes, environmental responses (mainly stress), knock-outs, drug response (ergosterol inhibition), mRNA decay (actinomycin treatment) and protein-mRNA interaction, among others. These results are interesting per se, enabling a better view of the individuallized processes, but much more can be extracted if we are able to organize these datasets in a specialized database, and integrate them in a higher view of increased complexity. This is not an easy task, and we are currently implementing a specific framework suitable for more complex analysis. However, although the amount of disponible data is increasing rapidly, we still lack enough information, reinforcing the need for further research. Here, we will present a brief overview of the different datasets we have generated, the general description of the database and integration framework, as well as some initial results from simple scale data integration.
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