MC.01 - **AFTER 100 YEARS HOW WELL DO WE KNOW LEISHMANIA (VIANNIA) BRAZILIENSIS VIANNA 1911?**

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In 1911, only 2 years after graduating from Rio de Janeiro's Faculty of Medicine, Gaspar Vianna, gave the name *Leishmania braziliensis* to a parasite that he had found in a patient with disseminated lesions. He did this because he considered that both the pathology and parasite morphology were different from those associated with typical cutaneous leishmaniasis. Prof Leônidas Deane commenting on Vianna's naming of the parasite said "He erroneously based his decision on morphological characters that he considered to be peculiar to our tegumentary leishmaniasis. However the species created by Vianna is accepted today by the majority of parasitologist but for other reasons". It is ironical that Ross similarly created the genus *Leishmania* for reasons that today would not be valid. Vianna's patient resided in São João deAlém Paraiba, a small village situated in the Paraiba river valley in Minas Gerais. This then is the type area for the species but unfortunately no isolates exist from this locality. Different isolates have been used as the standards for this species but so far none have been designated as a neotype. So we really do not know what parasite Vianna was dealing with. For many years it was considered that *L. braziliensis* was the only parasite causing cutaneous leishmaniasis and mucocutaneous leishmaniasis throughout the Americas. As more strains were isolated from the different clinical forms of leishmaniasis it was evident that this was not the case and the name *L. braziliensis* was used for leishmanial parasites from areas where the mucosal form of the disease is endemic. During the 1970' and 80's the species was characterized both biologically and biochemically and in 1987 it became the type species of the subgenus (*Viannia*), a clade of leishmanial parasites that only exists in tropical America. *L. (V.)braziliensis* has the greatest geographical distribution of any of the American Leishmania species being found in 16 countries. Parasites identified as *L.(V.)braziliensis* have been found in 25 species of sand flies in the Americas as well as a variety of small ground loving mammals. In Brazil alone, at least 6 sand fly species have been incriminated as vectors and 5 species of small mammals as reservoirs. These numbers are bound to increase as better field and laboratory methods are applied to epidemiologies in different biomes. Combinations of different vectors and reservoirs result in many different eco-epidemiological cycles. Within these cycles different selective pressures are at work and molecular tools are now untangling this network of genetically different parasites that we call *L. (V.)braziliensis*. Clinical data indicates that in some regions, such as Bolivia, that mucosal involvement is commoner. But is this due to the parasite or the genetic background of the population or both? RAPD was one of the first molecular tools to give us an insight into the population structure of this parasite, indicating that different genotypes existed in different geographical regions. Could some be more pathogenic than others? In Bahia one genotype was associated with mucosal lesions and another with disseminated lesions. Microsatellite studies in Peru and Bolivia have indicated that there are high levels of interbreeding suggesting that the life cycle may alternate between clonal and sexual propagation. Finding *L(V.)braziliensis* hybrids with such species as *L(V.)panamensis*, *L(V.)guyanensis* and *L(V.)peruviana* is in keeping with this sexuality. The realization that *L(V.)braziliensis* is a complex of genetically different parasites raises many academic and practical questions such as - do they respond differently to treatment, how feasible are control measures and is it justifiable to keep using a single name given the high level of heterogeneity? In 100 years we have come a long way in understanding Vianna's parasite. Its genome has recently been sequenced and this with the data generated from other research areas means that we now have very many more questions than answers.

Supported by:CNPq FAPESP

MC.02 - **TRANSCRIPTOME ANALYSIS BY RNA-SEQ: INSIGHTS INTO LEISHMANIA BIOLOGY**

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The availability of the genome sequence from several *Leishmania* species has revolutionized our ability to investigate the biology and pathophysiology of these parasites. The advent of new high throughput sequencing (HTS) technologies now offers new opportunities to elucidate regulation of gene expression during the parasite lifecycle. We have used high throughput RNA sequencing (RNA-seq) techniques to analyze the transcriptomes of *Leishmania major* Friedlin V1 (LmjF), *L. donovani* (LdoS), *L. amazonensis* (LamP), *L. braziliensis* (LbrM), and *L. tarentolae* (LtaP), which represent all three forms (cutaneous, visceral and mucocutaneous) of leishmaniasis. Several approaches were utilized to construct different RNA-seq libraries; including random, not-so-random (NSR) or oligo(dT)-priming for first strand cDNA synthesis (to map the 3' end of mRNAs), and Splice Leader (SL) priming for second strand cDNA synthesis (to map the 5' end of mRNAs). The latter was particularly effective, since it could be performed on total RNA (including that isolated from infected macrophages and animal lesions) and provides data indicating both SL site location and abundance of each mRNA species. In LmjF, we have mapped the SL and polyA sites for >98% and 85% of the mRNAs, respectively, and identified changes in transcription abundance and SL site location between the procyclic, metacyclic and amastigote stages. In LdoS, we have mapped SL sites for >99% and polyA sites from ~80% of protein coding genes and shown that >1000 mRNAs change abundance by more than 2-fold during axenic promastigotes-to-amastigote differentiation. In addition, several hundred genes showed changes in SL site location during differentiation. By precisely defining the 5' and 3' ends of mRNAs, these results have enabled us to refine the structural annotation of these genomes, resulting in re-annotation of several hundred protein-coding sequences (CDSs) and RNA genes. The studies in LamP, LbrM and LtaP were focused on changes in mRNA abundance due to specific gene deletions or changes in culture conditions. The results from these experiments have helped us elucidate fundamental aspects of *Leishmania* biology, such as transcription initiation and termination. Comparison of SL site location and mRNA abundance between the five *Leishmania* species are currently underway.
Supported by:NIAID

MC.03 - **THE CROSSING OF ENDOTHELIAL BARRIERS BY PLASMODIUM SPOROZOITES**

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The sporozoite, the malaria parasite stage injected by the mosquito, invades and multiplies within host hepatocytes. How sporozoites cross the liver sinusoidal barrier to reach hepatocytes has been much studied over the years, and the established 'gateway model' is that sporozoites translocate exclusively through Kupffer cells, the resident macrophages in the liver. Here, we show by intravital imaging in rodents that sporozoites use multiple, including Kupffer cell-independent pathways to cross the barrier. Most crucially, sporozoites use their ability to wound the membrane of and traverse host cells both to resist destruction by Kupffer cells while gliding in the sinusoids and to cross the barrier through endothelial and Kupffer cells. Therefore the host cell traversal ability of the *Plasmodium* sporozoite plays a dual role in the liver in crossing cell barriers and resisting phagocytosis, and is thus an appealing target for halting sporozoite progression at multiple steps of its journey in the host.
Supported by:ANR

MC.04 - **SUBVERSION AND MODULATION OF HOST IMMUNITY BY LEISHMANIA AMAZONENSIS PARASITES**

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Autophagy is a highly conserved cellular process for targeting aged proteins or organelles for lysosomal degradation, and is also involved in the generation of innate and adaptive immune responses. While autophagy can contribute to infection control, some intracellular pathogens can subvert or use host autophagy-related pathways for their advantage. We have previously reported that priming of macrophages with IFN-gamma promotes the growth of L. amazonensis amastigotes in an arginase-independent manner. We hypothesize that this enhanced parasite growth and its related immunopathogenesis are linked to induction and/or alterations in the host autophagic pathways. Here we show that cells deficient in the autophagy-related genes (Atg) are less permissive for parasite infection than their wild-type controls, and that the IFN-gamma-mediated growth enhancement is virtually abolished in the Atg-deficient cells. Western blot, quantitative RT-PCR, and immunoelectron microscopy studies reveal molecular details concerning autophagy induction in IFN-gamma-treated and infected cells. This study provides a better understanding of immunopathogenesis associated with L. amazonensis infection. It calls for additional studies for the underlying mechanism(s), by which the parasite exploits the host autophagy machinery for its long-term survival and disease pathogenesis.
Supported by:NIH/NIAID

MC.05 - **KINASES INVOLVED IN STRESS RESPONSES OF TRYPANOSOMES**

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Trypanosomes undergo several morphological transformations to adapt to each host environment. The signals that induce these processes and the mechanisms by which the parasites achieve each new biochemical status are still poorly understood. In eukaryotic cells, sensing the environment results in the control of translation by affecting specific protein kinases. One group of signaling kinases phosphorylates the translation initiation factor 2 (eIF2α). This phosphorylation decreases the recycling of eIF2, a factor required for finding the AUG codon, promoting translation of genes involved in responses to the different stresses. We have generated an antibody that recognizes specifically the phosphorylated form of the unusual eIF2α of *Trypanosoma cruzi.* We found that starvation of epimastigotes causes eIF2α phosphorylation and decrease in protein synthesis and polysomes. The same starvation causes differentiation of epimastigotes into infective metacyclic forms. Overexpression of eiF2α mutated in the phosphorylation site prevents the formation of metacyclic forms, indicating that its phosphorylation occurs during nutrient stress induction of differentiation. Another group of signaling enzymes that is modulated by environmental changes are kinases named target of rapamycin (TOR). They are inhibited when nutrients are limiting, causing translation arrest and induction of cell division. Trypanosomes contain four kinases with characteristics of TOR. One of them, TOR-like 1 was characterized in *Trypanosoma brucei.* It is located in cytosolic granules that relocalize upon osmotic stress. RNAi of TbTOR-like1 causes grow arrest, potentiated by elevated osmotic conditions. Knock down cells accumulate polyphosphates, required for osmotic resistance of the parasite, differently from other *T. brucei* TORs. Proteomic analysis revealed that RNAi of TbTOR-like 1 have increased levels of mitochondria metabolism. ThusTOR-like 1 signaling controls the energy balance responses due to variations in osmotic conditions. These two sets of kinases are thus, key players for trypanosome adaptation to the environmental changes.
Supported by:FAPESP, CNPq

MC,06 - **FREE RADICALS AND RESISTANCE TO INTRACELLULAR PARASITES**

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The role of nitric oxide in resistance to intracellular parasites has been extensively studied. However, the involvement of reactive oxygen species is not completely understood. In this work, we used mice that lack the NADPH-dependent phagocyte oxidase (phox-/-) to address the role of reactive oxygen species during infection with Leishmania amazonensis and Trypanosoma cruzi . Phagocytes from these mice lack the ability to produce superoxide. We found that, in this model, control of parasite growth is independent from reactive oxygen species from phox. However, during infection with L. amazonensis, we found a larger inflammatory infiltrate at early time points of infection. Interestingly, at later time points, phox-/- mice healed lesions more efficiently than wild-type (wt) controls. During T. cruzi infection, albeit the similar parasitemia and organ parasitism, phox-/- mice showed higher mortality than wt. This mortality may be due to the higher systemic levels of nitric oxide and consequent lower blood pressure. Our hypothesis is that, in the presence of phox, nitric oxide reacts with superoxide forming peroxinitrite. The latter kills parasites, but does not have an effect in blood pressure. Hence, we have uncovered two effects of reactive oxygen species during parasitic infections which are not directly involved with killing of intracellular parasites.
Supported by:CAPES, CNPq, FAPEMIG

MC.07 - **AUTOPHAGY IN TRYPANOSOMATIDS**

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Autophagy is a catabolic process involving the degradation and recycling of a cell's own constituents, through the action of lysosomal enzymes. Analysis of the Leishmania major and *Trypanosoma brucei* genomes reveals that these trypanosomatids contain many of the ATG genes demonstrated to be involved in autophagy in yeast and mammalian cells. These include the small ubiquitin-like modifiers ATG8 and ATG12 and we have used GFP-ATG8 and GFP-ATG12 fusion proteins as molecular markers for monitoring the pathway in *L. major* and *T. brucei*. In Leishmania, autophagosomes are most prevalent during differentiation of the multiplicative procyclic promastigotes to metacyclic promastigotes and from metacyclic promastigotes to amastigotes, suggesting an important role for autophagy in these remodelling processes. Live cell imaging experiments with GFP-ATG8 and fluorescent markers for various organelles, including glycosomes and the mitochondrion, have been used to monitor autophagosome cargo during differentiation. In addition, Leishmania mutants defective in autophagy have been used to investigate the importance of peptidases and autophagy in life-cycle progression in Leishmania. We have also generated an RNAi compatible *T. brucei* cell line expressing GFP-ATG8, which has allowed us to track the formation of autophagosomes and to investigate the function of ATG genes in African trypanosomes.

MC.08 - **THE BRAZILIAN CHAGAS DISEASE SEROPREVALENCE NATIONAL SURVEY (2001-2008)**

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A survey of the seroprevalence of Chagas disease was conducted in a sample of Brazilian individuals up to five years of age in all rural areas of Brazil, with the exception of Rio de Janeiro State. The rationale was to investigate new cases of Chagas disease to verify the effect of the control of the vector attained in recent years.

The initial sample size was of 150,549 children living in 2,201 municipalities of Brazil (40.2% of the municipalities). Blood (two drops) was collected on Whatman 1 filter paper from 104,954 children and screened in a single laboratory (in Goiania) by two techniques: indirect immunofluorescence and an enzyme linked immunoassay. For quality control, 10% of all samples and all those tested positive or indetermined were tested again with both techniques, in addition to a western blot assay (TESA blot, bioMerieux ®), in another laboratory (in Sao Paulo).

All children with confirmed positive results (n=104, 0.1%) were visited again for venous collection of their blood, as well as their mothers and siblings. The infection was confirmed in only 32 of these children (0.03%). From them, 20 (0.025%) mothers were also infected, characterizing congenital transmission. In eleven of them, the mothers were not infected, suggesting vectorial transmission. In one case the mother died and 13 cases were not localized. In 41 cases only the mother was confirmed as infected, these children had their blood drawn when they were less than six months of age and on the re-test did not have antibodies against T. cruzi anymore; they were classified as passive transference of maternal antibodies. In another 18 children, no antibodies were found in the mother or the child. The presumably 11 children infected by vector transmission were distributed mainly in the Northeast region of Brazil (States of Piaui, Ceara, Rio Grande do Norte Paraiba, and Alagoas), North (Amazonas State) and South (Parana State). Most of these states had not reported prior infestation by the vector Triatoma infestans.

As a sub-product of this survey, 20 cases of vertical transmission were detected however, the unexpected result was their geographical distribution. More than half of them (n=12) were found in a single State (Rio Grande do Sul) where only 4,529 samples were collected (4.3% of the total number of samples). In this region of Brazil, the predominant group of T. cruzi is TcVI, which differs from the group found in Central Brazil (TcII). Although mechanisms underlying maternal transmission of the parasite are not clearly understood, factors related to the parasite and to the genetic background of the host have been implied. These findings clearly indicate the importance of the parasite as directly responsible for this mechanism of transmission. The low number of vertical transmission cases in Brazil has been highlighted previously (around 1%), but without a clear explanation. This seems to be different in this particular region of Brazil, which shares many geographical similarities with the neighbor countries (Argentina, Uruguay), in which the frequency of congenital transmission is higher (around 5%).

Our results show the success of the vector control campaigns through regular and systematic insecticide spraying of rural houses, performed from 1975 through 1996. Brazil was certified by the South Cone Initiative for the virtual elimination of Triatoma infestans in 2006. In addition, this is the first description of geographical differences in congenital transmission probably related to the group of *T. cruzi*.