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SPC001 - ORAL INFECTION BY TRYPANOSOMA CRUZI

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Oral infection constitutes the most important mode of T. cruzi transmission in some geographical regions and has been responsible in recent years for frequent outbreaks of acute Chagas disease in Brazil, Venezuela and Colombia. Juices made from sugar cane, guava or açaí fruit, contaminated with T. cruzi metacyclic trypomastigote (MT) forms have been incriminated as source of infection. In the murine model of oral T. cruzi infection, the parasites have been found to invade the gastric mucosal epithelium. Mucin-like molecules protect MT from destruction in the gastric milieu. The evidences are that gp82, a MT-specific surface molecule highly conserved among genetically divergent parasite strains and resistant to degradation by pepsin at acidic pH, binds specifically to gastric mucin (GM), the main macromolecular component of the mucus that protects the stomach mucosa. Upon migrating through the mucus layer, the parasites reach the underlying target cells, which are invaded in a gp82-mediated manner, as inferred from cell invasion experiments using human epithelial cells.
We have found that different T. cruzi strains vary considerably in their ability to infect by the oral route. Strains (CL, Y82) that express high levels of gp82 and low levels of gp90, a MT-specific surface molecule that functions as a negative regulator of host cell invasion, are the most effective in invading the gastric epithelial cells. Four days post oral infection with these parasites, large number of amastigote nests were detected in the histological preparations of the stomach. This implied that they efficiently traversed the mucus layer to reach the target cells. Accordingly, in assays using GM-coated transwell filters, to mimic MT migration through the mucus layer, efficient migration was confirmed. Strains (Y30, 569, 588) deficient in gp82 and expressing gp30, a surface molecule that shares ~58% sequence identity with gp82 and is also involved in cell invasion, were poorly infective when given orally into mice. Fewer amastigote nests were found in the stomach of mice infected with gp82-deficient strains than in mice that received gp82-expressing strains. The ability of these strains to enter target cells in vitro was comparable but the efficiency of gp82-deficient MT in migrating through the GM-coated transwell filter was lower. As the GM-binding capacity of gp30 is lower than that of gp82, the gastric mucus possibly acts a barrier for gp82-deficient strains. Some strains (SC, 573, 587), expressing both gp82 and gp30 in addition to high levels of gp90, exhibited reduced cell invasion capacity in vitro but differential ability to invade gastric epithelial cells in vivo. Strains (SC, 573) that expressed a gp90 isoform susceptible to pepsin degradation in vivo successfully invaded and proliferated in the gastric epithelium, whereas the in vivo cell invasion capacity of strain 587 expressing pepsin-resistant gp90 was very low.
Attempts to interfere in the course of oral infection by highly infective T. cruzi strains (CL, Y82) pointed out that blocking MT traversal through the mucus can reduce parasite burden in the stomach. Upon oral administration of a synthetic peptide (p7), corresponding to the GM-binding site of gp82, shortly before MT inoculation, the number of amastigote nests in the mouse stomach was significantly lower than in mice that received a control peptide (p7*) with the same composition as p7 but with a scramble sequence. Assays with transwell filters coated with GM mixed with peptide p7 showed a reduced MT migration as compared to filters coated with GM alone or with GM plus control peptide p7*. Taken together, our observations highlight the importance of MT surface molecules gp82 and gp90 as promoter and negative modulator of infection not only in vitro but also in vivo. **Supported by:**FAPESP, CNPq.
Keywords: Trypanosoma cruzi; oral infection; metacyclic trypomastigotes

CO001 - INTRACELLULAR IRON ACQUISITION AND ROS GENERATION ARE CRITICAL REGULATORS OF LEISHMANIA AMAZONENSIS VIRULENCE

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Iron is an essential element but is toxic when in excess. For this reason, cellular iron uptake and storage has to be tightly regulated. Until recently, very little was known about the mechanisms by which Leishmania parasites acquire iron and the iron-containing essential porphyrin heme. Studies from our laboratory showed that the availability of iron in the culture medium regulates the expression of a number of genes in L. amazonensis, including genes encoding the ferric iron reductase LFR1, the ferrous iron transporter LIT1, and the heme transporter LHR1. These membrane proteins mediate iron and heme acquisition by the parasites, and are required for virulence in Leishmania amazonensis (1-4). Surprisingly, we found that exposure to iron-depleted medium triggers the differentiation of L. amazonensis promastigotes into the infective intracellular stage, amastigotes, independently of the classical temperature and pH differentiation signals. This iron-dependent differentiation process requires expression of the iron transporter LIT1 and involves generation of reactive oxygen species (ROS) (5). Our recent results suggest that the L. amazonensis mitochondrial iron transporter MIT1 and mitochondrial superoxide dismutase FeSOD-A play an important role in the amastigote differentiation process, by promoting iron-dependent generation of the signaling molecule H2O2. We also found that L. amazonensis has the ability to modulate iron availability inside its host cell, by interfering with expression of the macrophage iron exporter ferroportin (6). Collectively, these recent findings are revealing the critical role that iron acquisition and utilization plays in the virulence of Leishmania.

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Keywords: Leishmania; iron; transporter

CO002 - CELL MORPHOGENESIS, SHAPE AND PATHOGENICITY IN KINETOPLASTID PARASITES

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This talk will review current knowledge in how shape and form in kinetoplastid parasites is defined by the internal cytoskeleton. I will cover published and unpublished material from our laboratory, together with some insights from collaborative projects with colleagues. I will focus on how the parasite cytoskeleton – both microtubule and various filament systems – are assembled and coordinated with membrane systems to define cell shape. Then, how modern post-genomic approaches are providing insights to hundreds of molecular components of these cytoskeletal systems leading to an understanding of assembly and inheritance of multicomponent complexes during cell division. Finally, I will deal with genomic comparisons across Trypanosoma and Leishmania, coupled with modulation of individual gene expression via RNAi. Conclusions here allow us to suggest mechanisms by which the various shapes of trypanosomes and Leishmania kinetoplastid parasites are altered within the life cycle, and in evolution, such that they are fitted for their different ecological niches in the vector and host. The trypanosome shape and form is, in the main, defined by an internal microtubule based cytoskeleton. However, a defining feature of these organisms is the existence of a flagellum whose nature varies somewhat between cell types. Epimastigotes, trypomastigotes and amastigotes are distinguished by the substructure of their flagella, the length of its attachment to the cell body and other functional elaborations. Evolutionary cell biology and comparisons with other model organisms highlights conserved features and specialisations of kinetoplastid flagella. Our studies of the 3D structure of flagella and basal bodies has provided insights to how the structure is defined as either a 9+2 or 9+0 microtubule organelle involved in the biology of movement, shape, sensing, proliferation and pathogenicity. The basal body complex – defining a "master organiser" region of the cell – orchestrates a variety of cellular microtubules and filament systems essential for inheritance of cell shape and completion of cytokinesis, in addition to regulating kinetoplast position and segregation. Critically, it also defines perhaps one of the most important pathogenicity attributes – the flagellar pocket. Using Cellular 3D tomography we have been able to map the fine structure of the basal bodies, their duplication and segregation in the cell cycle. Also, we have addressed how the very divergent amastigote flagellum is formed during the promastigote to amastigote differentiation in Leishmania. Finally, using proteomics and gene tagging technologies we have determined the spatial position of over 200 proteins of the flagellum, basal body and flagellum attachment zone. Such tagging and inducible expression systems have facilitated new insights to how the various components of the cytoskeleton are assembled and morphed during the cell cycle to produce the overall shape of a kinetoplastid parasite cell type.

CO003 - THE BIOLOGY OF MATING IN *LEISHMANIA*

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Sexual reproduction is evident in different parasitic protozoa, including *Leishmania*, which until recently was considered to be exclusively asexual. We have previously shown that genetic exchange in *Leishmania* can occur during their growth in the sand fly vector and experimentally demonstrated intra- and inter-species mating that in each case generated full genomic hybrids. The inter-species mating between *L. major* and *L. infantum* generated progeny clones that displayed variable skin and viscera tissue tropisms in mouse models of cutaneous and visceral leishmaniasis. Whole genome sequencing is being be carried out to identify the parasite genes involved in the tissue specific tropisms. We also generated hybrids between L. major from the Middle East and *L. amazonensis* from Brazil. Despite their genetic distance and distinct karyotypes, hybrid progeny were obtained at frequencies similar to those reported for L. major intra-species crosses, indicating that genetic distance is not a barrier to mating. In more recent studies, we assessed the fertility of F1 L. major hybrids by backcross and outcross mating attempts. Several hybrids were generated, though at significantly lower frequencies than those

involving parental crosses, indicating that the F1 progeny have reduced mating competency, but they are not sterile. Whole genome sequencing of the backcross lines provide clear evidence for recombination events and a Mendelian pattern of inheritance in *Leishmania*. Lastly, we have studied the self-mating competency in *Leishmania*. Our results indicate that self-mating in *Leishmania* is possible and can result in alteration in DNA content and produce phenotypic variation. Since opportunities for outcrossing in the sand fly vector are extremely rare, self-mating may be the more important reproductive strategy contributing to the remarkable diversity in *Leishmania*.

Keywords: Leishmania; genetic exchange; sand flies

CC001 - INNATE RESISTANCE AGAINST TOXOPLASMA GONDII: AN EVOLUTIONARY TALE OF MICE, CATS, AND MEN

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Recent studies have revealed remarkable species specificity of the Toll-like receptors (TLRs) TLR11 and TLR12 and the immunity-related GTPase (IRG) proteins that are essential elements for detection and immune control of Toxoplasma gondii in mice, but not in humans. The biological and evolutionary implications of

these findings for the T. gondii host-pathogen relationship and for human disease will be discussed.

Keywords: Toxoplasma gondii; toll-like receptors; co-evolution