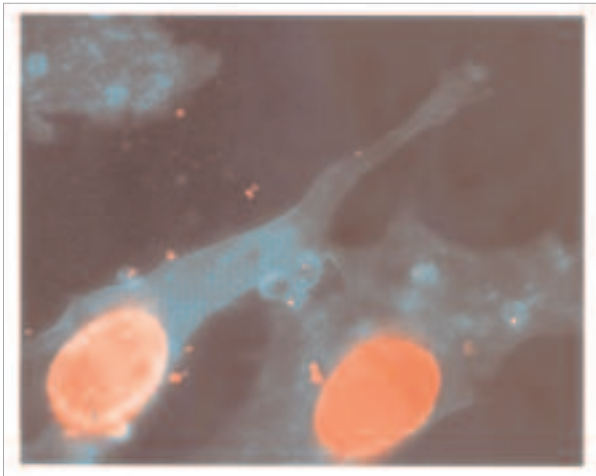


MOLECULAR STUDIES ON *Trypanosoma cruzi* AND ITS INTERACTION WITH CELLS AND FACTORS FROM THE HOST *IN VITRO* AND *IN VIVO*

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Trypanosoma cruzi amastigotes recruit lipid raft marker GM1 during host cell invasion

Trypanosoma cruzi, the etiological agent of Chagas' disease, is a highly relevant microorganism from both medical and biological points of view. Although vectorial transmission is mostly under control in the Southern Cone, *T. cruzi* transmission still prevails in Latin America where 15-16 million people are infected. Outbreaks of acute cases of Chagas' disease by oral infection have been reported in different areas of Brazil. Our group has extensively studied host cell invasion by trypomastigotes and amastigotes, an essential step in the establishment of infection in man and other vertebrate hosts. Oral infection has become the focus of our studies in the last years. The mechanisms of *in vitro* and *in vivo* *T. cruzi* infection are beginning to be unveiled. Parasite molecules involved in host cell interactions are being analyzed regarding their expression in different developmental forms of *T. cruzi* strains, and also regarding their genomic organization and structure.

We intend to study the interaction of *T. cruzi* with host cells and factors both *in vitro* and *in vivo*. The project involves the analysis of molecules previously identified by our group as well as the identification and characterization of new molecular and cellular components. Among our objectives is the identification of host cell receptors for parasite surface proteins, and the study of the intracellular traffic of *T. cruzi* infective forms. We plan to use host cells transfected with different genes as well as co-infection models with other trypanosomes and invasive bacteria.

Concerning the metacyclic surface antigens, we intend to identify transcribed and translated genes by hybridizations with DNA microarrays and partial sequencing of proteins with mass spectroscopy, respectively. Recombination mechanisms responsible for the generation of variants in these multigenic families will be studied in parasites transfected with artificial and specially designed *T. cruzi* chromosomes. These studies will bring new information on the mechanisms controlling gene expression in this parasite and on the evolution of multigenic families.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Host cell invasion by *T. cruzi* is a complex process in which various host as well as parasite components interact, triggering the activation of signaling cascades and calcium mobilization in both cells. Our studies with metacyclic trypomastigotes from different strains have elucidated the mechanisms of parasite entry into target cells.

Outbreaks of severe acute Chagas' disease, acquired by oral route, have been frequently notified in Brazil. We have investigated in the murine model the molecular basis of oral *T. cruzi* infection using an isolate derived from a patient with severe clinical manifestations, upon ingestion of contaminated sugar cane juice. The role of metacyclic surface glycoproteins in oral infection was established.

We found that recombinant GP82 protein is able to inhibit the development of murine melanoma cells. When injected with GP82 in the tumor area, the mice that had received tumorigenic cells developed melanoma at significantly lower pace and survived longer, as compared to control.

Studies on *T. cruzi*-host interactions revealed unique features of amastigote expression that appeared to be host-dependent. In the double-infection model using *Vero cells* harboring the bacterium *C. burnetii* we observed strain and infective form dependences in both invasion and trafficking properties. Studies aiming at the identification of membrane components that might be involved in host-parasite interactions revealed for the first time that host cell cholesterol and membrane lipid raft components are involved in both trypomastigote and amastigote invasion of mammalian cells. This work was honored with the cover of *International Journal Parasitology*.

Analysis of the expression of gp82 and gp90 genes in the digestive tract of the insect vector *R. prolixus* showed that the stabilization of these mRNAs is linked with their translation. We identified gp82 alleles lacking the motifs for adhesion of the parasite to mammalian cells.

Computer models were used to estimate the variation generation capacity of surface gene families. We found that genes relevant to host-parasite interactions exhibited high volatility (anti-robust pattern), which may be related to the capacity of the parasite to evade the host immune system. Measures of genetic robustness may detect variations between potential drug targets at the protein level. The simulations showed that nuclear genes tend to be relatively more robust against random, multiple-point mutations than surface protein genes.

MAIN PUBLICATIONS

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