# INTERACTION BETWEEN *Trypanosoma cruzi* AND HOST: LIGANDS, RECEPTORS AND DETERMINANTS OF INTRACELLULAR DEVELOPMENT

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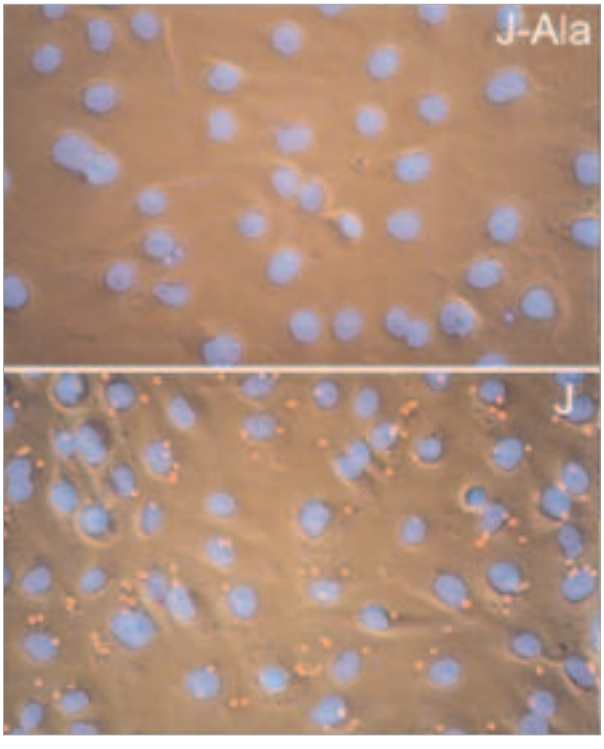


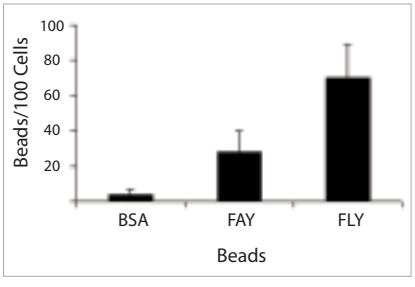

*FLY binds to epithelial cell. Tissue-cultured cells were incubated with =(J). FLY-coated beads (red) or (J_Ala) FLA-coated beads (control peptide, = small blue spots). After washing, the cells were fixed with methanol and the nuclei were stained with Hoechst (blue)*

Our Laboratory has dedicated in characterizing a family of proteins (TC-85) involved in the adhesion of the parasite to epithelial cells and to laminin. The objectives of our project are: (i) to verify if the adhesion domain of the TC85 family, selected *in vitro* for epithelial culture cells and components of the extracellular matrix, correspond to *in vivo* using phage display technique; (ii) to compare *in vitro*, with established lines, the results of adhesions of members of the TC85 family and their subfamilies in primary cultures of cardiomyocytes, neurons and Schwann cells, in order to verify if there is some correspondence between members of the TC85 family and different receptors of these different cell types; (iii) to identify, as a complementary procedure, molecules of the parasite which bind specifically to each component of the extracellular matrix. The same methodology will be used to identify, in the different cell types, the receptors for members of the TC85 family; (iv) to determine if the individuals of the *T. cruzi* population – intracellular trypomastigotes and amastigotes, express proteins of one or two principal subfamilies of TC85. For this, monoclonal (or monospecific policlonal) antibodies, aimed at exclusive epitopes of each subfamily, will be obtained and the parasites analyzed by immunofluorescence or high resolution microscopy; (v) to verify if members of the TC-85 family, or their peptides, induce changes in the host cell, such as increase in the synthesis of the extracellular matrix components or increase tissues and macrophages adhesion/endocytosis in culture cells; (vi) o study the transport of glucose and the expression of its transporter (mRNA and protein) and energetic metabolism throughout the intracellular differentiation of *T. cruzi* in the mammal. This data, compared to that referring to transport of proline, will make possible to establish the profile of the preferential sources of carbon in each stage. If this task proves successful, we will try to clone the transporters of proline and other aminoacids.

## SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Chagas' disease is a chronic, debilitating and incapacitating illness, caused by the protozoan parasite *Trypanosoma cruzi* when infective trypomastigotes invade host cells. The main focus of our work is the identification and characterization of molecules involved in the interaction between the parasite and the host cell, as well as the transport of amino acids (proline, glutamate, aspartate) by the parasite, trying to understand the carbon and energy sources for the intracellular survival of the parasite.

Previously, we had described a glycoprotein family from *T. cruzi* involved in the adhesion step to the host cell (TC85). Recombinant proteins encoded by two genes of the family bind to laminin and to cytokeratin 18 and the binding sites were mapped. FLY, one of the conserved domains of the family is the cytokeratin binding site. FLY promotes dephosphorylation and reorganization of CK18 in epithelial cells and activation of the ERK1/2 signaling cascade culminating in an increase of approximately 9-fold in the number of parasites/cell. Inhibition of ERK1/2 phosphorylation blocks by 57% the host cell infection by *T. cruzi*. The possibility that FLY foracting as a pathogenic factor *in vivo* was then verified. Phages expressing the FLY domain bind specifically to the endothelium of organs affected in patients with Chagas´disease. Interestingly, FLY Balb/c mice, primed intraperitoneally with a single dose of FLY and infected with blood trypomastigotes, presented higher parasitism and mortality rates, with a decrease in IFN-gamma and NO production by spleen cells. The presence of FLY-containing molecules in "Membrane Vesicles", continuously shed from the surface of the parasite, may be the delivery system employed by *T. cruzi* to prime the host cell for infection. Membrane vesicles- treated animals develop severe heart pathology, with intense inflammatory response and higher mortality probably due to the increase of IL-4 and IL-10 synthesis.




*Quantification of peptides binding to cells*

## MAIN PUBLICATIONS

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