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Trypanosoma cruzi: mechanisms of cell-invasion and intracellular survival

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Trypanosoma cruzi is strikingly non-selective in its choice for host cells in vitro, invading fibroblasts, epithelial, endothelial and other cell types with high efficiency. However, in the mammalian host, T. cruzi predominantly infects myocardial cells, macrophages and cells of the autonomic nervous system (1, 2). Since cells in vivo are mostly organized into tissues and/or attached to extracellular matrices, it is conceivable that in this situation they can only be infected through restricted cellular domains. To determine whether T. cruzi entry could be influenced by cell polarity we studied the infection of cultured Madin-Darby canine kidney (MDCK) epithelial cells. When confluent, these cells are polarized with their plasma membrane separated by tight junctions into two domains, apical and basolateral. Both metacyclic forms derived from cultured epimastigotes and tissue-culture-derived trypomastigotes entered confluent MDCK cells preferentially through their basolateral domains. Sparsely plated MDCK cells were better infected, and

scanning electron microscopy showed that 92% of the parasites entered at the edges of cells. These observations may be explained by postulating that the basolateral domain and the edges of epithelial cells contain the receptors for metacyclic and bloodstream trypomastigotes, or that this phenomenon is a consequence of the increased membrane activity occurring at cell edges (3).

Trypomastigotes invade cells through the formation of tight endocytic vacuoles. Lysosome fusion with these vacuoles does not result in destruction of the parasites, which shortly after are found free in the cytoplasm of the host cell (4, 5).

During this period the trypomastigotes change into amastigotes, expressing a stage-specific major surface glycoprotein, Ssp-4 (6). Amastigotes, when taken up by macrophages, also escape from the phagosome and multiply in the cytoplasm (7-9). These observations indicated that T. cruzi should be capable of inducing the disruption of the phagosome membrane. Using a hemolytic assay we found that amastigotes and trypomastigotes secrete into the culture medium heat-labile, trypsin sensitive molecules which lyse erythrocytes from various animal species. The activity of this hemolysin is maximal at pH 5.5 and undetectable at neutral pH, suggesting that it functions in acidic intracellular compartments. Production of the hemolysin is abolished in the absence of glucose, or by addition of

puromycin or the metabolic inhibitors sodium azide and 2-deoxyglucose. Sieving experiments with erythrocyte ghosts indicated that lesions larger than 10 nm in diameter were formed on the target membranes (10).

Using a especially formulated weak base which contains dinitrophenol groups easily detectable by antibodies (DAMP-11), we could show that indeed the T. cruzi-containing vacuoles are acidic. Further evidence for the possible involvement of the T. cruzi hemolysin in disruption of these phagosomes was obtained in experiments in which infected cells were incubated with the weak bases ammonium chloride and chloroquine. These agents disrupt the pH gradient of cells, raising the pH of the normally acidic intracellular compartments. Using transmission electron microscopy we observed an inhibition of between 67% and 95% in the number of parasites which had escaped from the vacuoles, after 2 hrs in the presence of these drugs (Ley et al, in preparation).

This work was supported by the MacArthur Foundation, the UNDP/WHO Special Program for Research and Training in Tropical Diseases and CNPq.

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