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Unique behavior of *Trypanosoma dionisii* interacting with mammalian cells: Invasion, intracellular growth, and nuclear localization

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ABSTRACT

The phylogenetic proximity between *Trypanosoma cruzi* and *Trypanosoma* (Schizotrypanum) *dionisii* suggests that these parasites might explore similar strategies to complete their life cycles. *T. cruzi* is the etiological agent of the life threatening Chagas' disease, whereas *T. dionisii* is a bat trypanosome and probably not capable of infecting humans. Here we sought to compare mammalian cell invasion and intracellular traffic of both trypanosomes and determine the differences and similarities in this process. The results presented demonstrate that *T. dionisii* is highly infective *in vitro*, particularly when the infection process occurs without serum and that the invasion is similarly affected by agents known to interfere with *T. cruzi* invasion process. Our results indicate that the formation of lysosomal-enriched compartments is part of a cell-invasion mechanism retained by related trypanosomatids, and that residence and further escape from a lysosomal compartment may be a common requisite for successful infection. During intracellular growth, parasites share a few epitopes with *T. cruzi* amastigotes and trypomastigotes. Unexpectedly, in heavily infected cells, amastigotes and trypomastigotes were found inside the host cell nucleus. These findings suggest that *T. dionisii* although sharing some features in host cell invasion with *T. cruzi*, has unique behaviors that deserve to be further explored.

1. Introduction

Trypanosoma (Schizotrypanum) *dionisii* is a non-pathogenic bat trypanosome related to *Trypanosoma cruzi*, the etiological agent of Chagas' disease that according to the World Health Organization (WHO, 2002) affects 18 million of people in Latin America. There are approximately 300,000 new infections annually. *T. (S) cruzi*-like bat trypanosomes have a cosmopolitan distribution and have been reported to be host-restricted (Branquinha et al., 1999; Marinkelle, 1976). During its life cycle, *T. dionisii* alternates different developmental forms between hosts: epimastigotes and metacyclic trypomastigotes in the invertebrate host, and bloodstream trypomastigotes and amastigotes in the mammalian host (Baker et al., 1971; Molyneux, 1991; Wilkins and Baker, 1975). *In vitro*, metacyclic trypomastigote (MT) forms are able to invade and replicate within a large number of mammalian cells. These forms remain in the cytoplasm and transform into amastigotes (replicative forms). After an intense multiplication phase, amastigotes transform into trypomastigotes and are released in the proximity after cellular rupture (Baker et al., 1971; Baker and Selden, 1981; Glauert et al., 1982; Molyneux, 1991; Thorne et al., 1979).

A number of *T. cruzi* studies with mammalian cells culture have indicated that parasite and host cell glycoconjugates are involved in the cellular invasion process (Giordano et al., 1994; Ming et al., 1993; Ramirez et al., 1993; Silva et al., 2006), and that *T. cruzi* entry in non-phagocytic cells involves lysosomal recruitment to the region of parasite/cell adhesion resulting in parasitophorous vacuoles formation (De Souza, 2005; Tardieux et al., 1992). In addition, pre-treatment of host cells with cytoskeleton modifying agents like cytochalasin D, nocodazole or the PI3 kinase inhibitor wortmannin may also influence *T. cruzi* mammalian cell invasion (Andrade and Andrews, 2004; Fernandes et al., 2006; Mortara, 1991; Procópio et al., 1998; Schenkman et al., 1991b; Woolsey et al., 2003; Woolsey and Burleigh, 2004).

In 1978, Baker et al. referred to the phylogenetic proximity between *T. dionisii* and *T. cruzi* (Baker et al., 1978) and, in 1987 Petry et al. (1987a,b,c) suggested that *T. dionisii* and *T. cruzi* share antigenic components and epitopes defined by monoclonal antibodies anti-epimastigote forms (Petry et al., 1987a,b,c). In spite of early studies on the interaction of *T. dionisii* trypomastigotes with mammalian cells in culture (Baker et al., 1971, 1972b; Baker and Green, 1973; Glauert et al., 1982) the detailed mechanisms of host cell invasion

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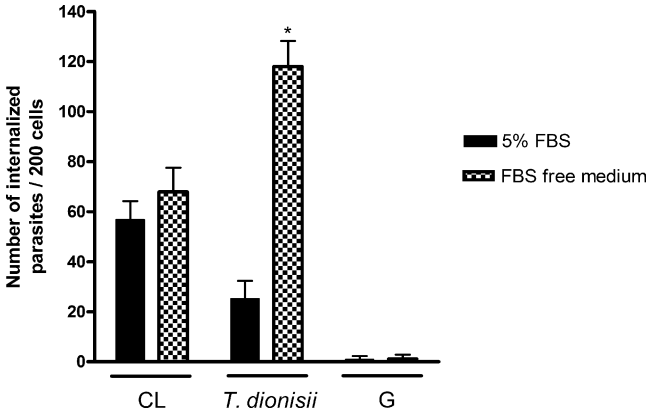

Fig. 1. *T. dionisii* invades host cells in comparable levels to *T. cruzi* CL strain. COS-7 cells were submitted to invasion assays in the presence (black bars) and absence (hatched bars) of FBS. Differences between *T. dionisii*, *T. cruzi* CL and G strain are significant ($p < 0.01$). Values are the mean of three independent experiments, carried out in triplicate cover slips. (*) Indicates significant difference between number of internalized parasites (*T. dionisii*) in the presence or absence of FBS ($p < 0.01$).

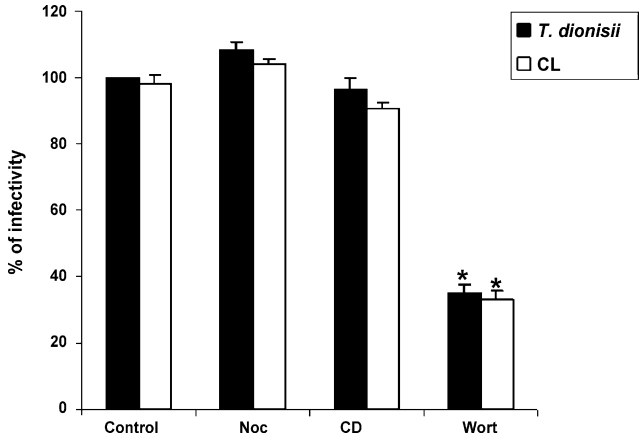

Fig. 2. Pre-treatment of host cells with wortmannin decreases the invasion indexes of both *T. cruzi* (CL strain) and *T. dionisii*. COS-7 cells were pre-treated with the indicated drug (NOC: nocodazole; CD: cytochalasin D; WORT: wortmannin) and then incubated with *T. cruzi* CL stain and *T. dionisii* metacyclic trypomastigotes, at 20:1 parasites:cell ratio. (*) Indicates significant difference in the % of infectivity between treated cells and the respective control ($p < 0.01$).

by this parasite have not yet been analyzed from the perspective of the more recent findings reported for *T. cruzi*.

In the present study we analyzed aspects of mammalian cell entry, intracellular traffic and compared to the closely related and well described *T. cruzi* cell invasion model. We identified similarities between invasion strategies of both species, as well as contrasting differences that deserve to be further investigated.

2. Materials and methods

2.1. Parasites and mammalian cells

T. dionisii epimastigotes (TCC 495, isolated from bat *Carollia perspecillata*, Rondônia, Brazil, and kindly provided by Dr. Marta M. G.

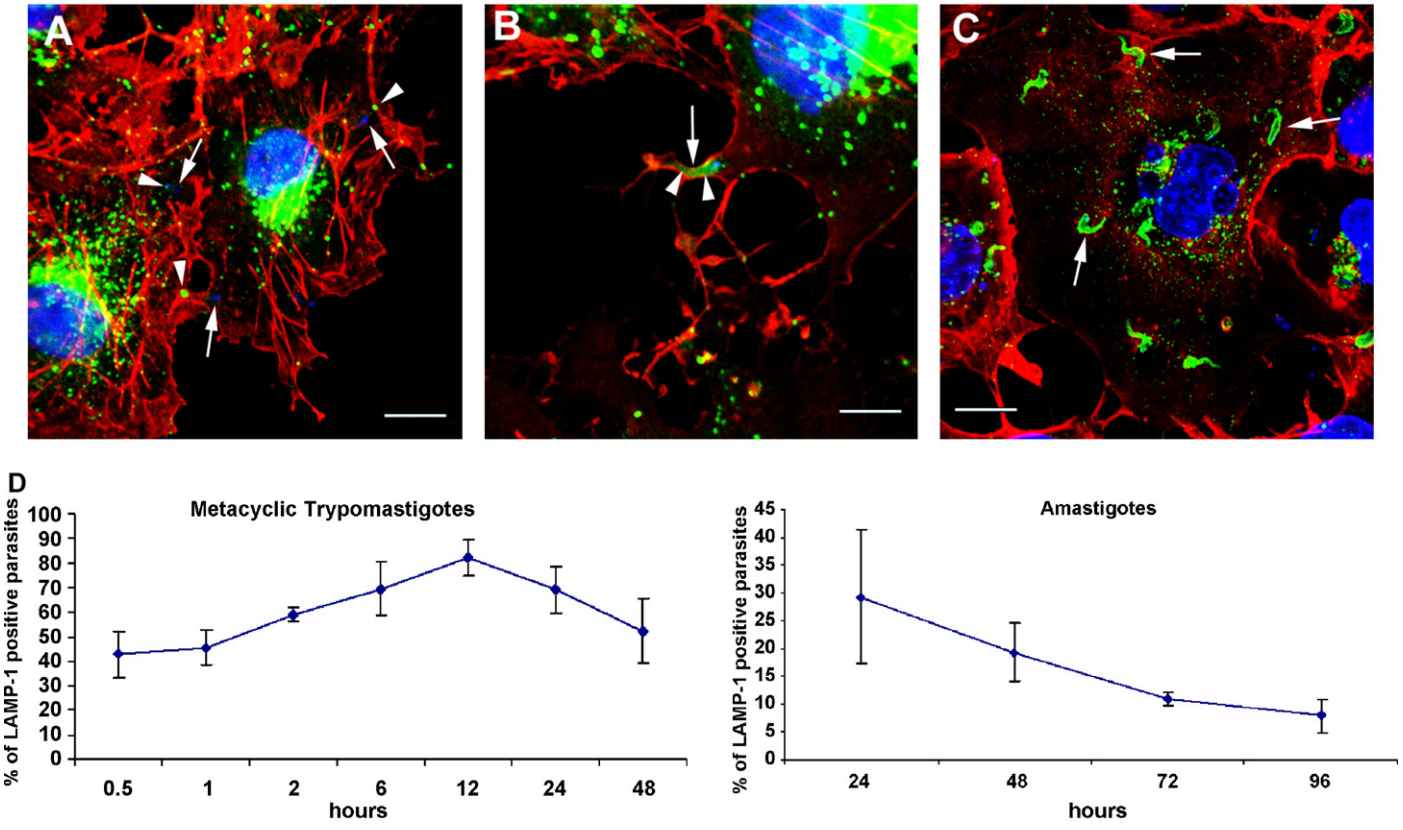

Fig. 3. *T. dionisii* metacyclic trypomastigotes recruit lysosomes during invasion and may be found in LAMP-1 positive vacuoles in COS-7 cells. (A) 15 min of infection: lysosomes (arrowheads) close to internalized parasites (arrows). (B) 30 min of infection: parasite inside tight LAMP-1 positive vacuole (arrowheads), resembling an actin-rich pseudopodium (arrow). (C) 12 h of infection: most parasites reside inside LAMP-1 positive vacuoles (arrows). Actin labeled in red, nuclei and kinetoplasts in blue (DAPI) and monoclonal antibody anti-LAMP-1 in green. Single optical sections acquired by confocal microscopy. Magnification bars: A, C: 10 μm; B: 5 μm. (D) Kinetics of formation of LAMP-1 positive parasitophorous vacuoles during *T. dionisii* invasion. COS-7 cells were infected with metacyclic trypomastigote forms and fixed at different time points. Error bars represent the standard error, within an average of 100 scored parasites at each time point, in two independent experiments. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)