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Tridimensional ultrastructure and glycolipid pattern studies of *Trypanosoma dionisii*

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ABSTRACT

Trypanosoma (*Schizotrypanum*) *dionisii* is a non-pathogenic bat trypanosome closely related to *Trypanosoma cruzi*, the etiological agent of Chaga's disease. Both kinetoplastids present similar morphological stages and are able to infect mammalian cells in culture. In the present study we examined 3D ultrastructure aspects of the two species by serial sectioning epimastigote and trypomastigote forms, and identified common carbohydrate epitopes expressed in *T. dionisii*, *T. cruzi* and *Leishmania major*. A major difference in 3D morphology was that *T. dionisii* epimastigote forms present larger multivesicular structures, restricted to the parasite posterior region. These structures could be related to *T. cruzi* reservosomes and are also rich in cruzipain, the major cysteine-proteinase of *T. cruzi*. We analyzed the reactivity of two monoclonal antibodies: MEST-1 directed to galactofuranose residues of glycolipids purified from *Paracoccidioides brasiliensis*, and BST-1 directed to glycolipids purified from *T. cruzi* epimastigotes. Both antibodies were reactive with *T. dionisii* epimastigotes by indirect immunofluorescense, but we noted differences in the location and intensity of the epitopes, when compared to *T. cruzi*. In summary, despite similar features in cellular structure and life cycle of *T. dionisii* and *T. cruzi*, we observed a unique morphological characteristic in *T. dionisii* that deserves to be explored.

1. Introduction

Trypanosoma (Schizotrypanum) *dionisii* is a non-pathogenic bat trypanosome closely related to *Trypanosoma cruzi*, the etiological agent of Chaga's disease. In 1978, Baker et al. suggested the phylogenetic proximity between *T. dionisii* and *T. cruzi*, and in 1987, Petry et al. (1987a,b,c) demonstrated that these species share epitopes, defined by monoclonal antibodies (MAbs) anti-epimastigote forms. The phylogenetic proximity between these species was confirmed years later, based on genes of the small subunit ribosomal RNA, and the glycosomal glyceraldehyde phosphate dehydrogenase (Hamilton et al., 2007).

As *T. cruzi*, 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; Wilkins and Baker, 1975; Molyneux, 1991). *In vitro*, metacyclic trypomastigote forms are able to invade and replicate within a large number of mammalian cells. These forms remain in the cytoplasm and transform into amastigotes, the replicative forms. After an intense multiplication phase, amastigotes transform into trypomastigotes and after cellular rupture are released into the medium (Baker et al., 1971; Thorne et al., 1979; Baker and Selden, 1981; Glauert et al., 1982; Molyneux, 1991).

Many *T. cruzi* studies have indicated that parasite and host cell glycoconjugates are involved in the cellular invasion processes (De Arruda et al., 1989; Ming et al., 1993; Ramirez et al., 1993; Giordano et al., 1994; Silva et al., 2006; Cortez et al., 2006a,b; Ferreira et al., 2006). Glycoconjugates containing galactofuranose (Gal*f*) residues have been described in fungi, bacteria, and parasites such as: polysaccharides of *Mycobacterium* sp., *Streptococcus* sp., *Aspergillus* sp; lipopolysaccharides of *Escherichia coli*; glycoproteins of *Crithidia* sp., *Leptomonas samueli* and *T. cruzi*; and glycolipids of *T. cruzi*, *T. dionisii*, *Leishmania* sp., *Histoplasma capsulatum* and *Paracoccidioides brasiliensis* (Alves and Colli, 1975; Mendonça-Previato et al., 1983; Lederkremer and Colli, 1995; Toledo et al., 1995; Branquinha et al., 1999; Pedersen and Turco, 2003; Takahashi et al., 2009; Tefsen et al., 2012)

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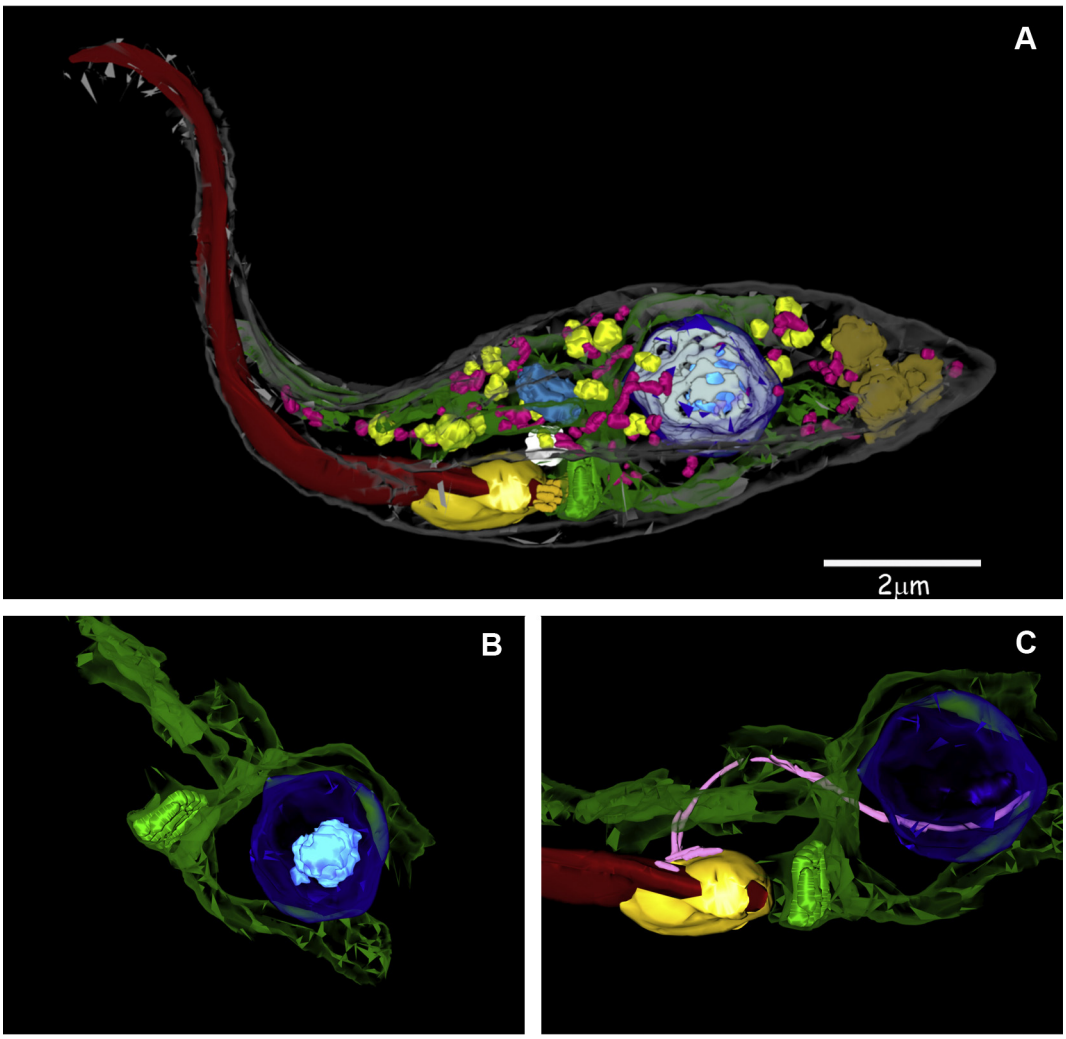

Fig. 1. *T. dionisii* epimastigote G1/S phase (3D reconstruction of micrographs showed at supplementary data, Fig. S1). (A) a general view of organelles distribution. Green: mitochondrion; light green: kinetoplast; dark yellow: flagellar pocket; orange: basal bodies; red: flagellum; royal blue: Golgi; light yellow: electron-dense vesicles; blue: nucleus; light blue: nucleolus; light gray: electron-dense chromatin; brown: reservosomes; dark gray: membrane; pink: non electron-dense vesicles (acidocalsisomes); white: contract vacuole. (B) Detail of the disk shaped kinetoplast (light green) in the middle of the unique mitochondrion (green), the nucleus in blue and nucleolus in light blue. (C) Detail of the cytostome-cytopharynx (light pink) emerging from the flagellar pocket (dark yellow) and contouring the kinetoplast (light green) and the nucleus (blue). Parasites were processed for transmission electron microscopy and the 3D model was generated with Blender® software. Scale bar: 2 μm.

Although the biological role of Gal*f* residues is still unclear, the postulated absence of Gal*f* and galactofuranosidases in mammalian species suggests the intriguing hypothesis that terminal Gal*f* residues play a central role in survival of fungi and parasites, by preventing the action of the host's glycosidases on their glycoconjugates. If this hypothesis is correct, Gal*f* residues are potentially useful as specific target molecules for therapy of parasitic and fungal diseases. But, there is little information about differences of these glycoconjugates containing Gal*f* residues, between pathogenic and non-pathogenic trypanosomatides.

In *T. cruzi* studies, Golgher et al. (1993), showed that Gal*f* residues present in *T. cruzi* glycoconjugates are highly antigenic, since patients with Chagas' disease present anti-Gal*f* antibodies and Bertini et al. (2003a,b) demonstrated that *T. cruzi* trypomastigotes Gal*f*-containing glycoproteins are important for Vero cells invasion. Moreover, Nogueira et al. (2007), demonstrated that Gal*f*-containing GIPCs are important for *T. cruzi* epimastigote forms differentiation, proliferation and interaction with host midgut cells, and Suzuki et al. (2002, 2008) suggested that, in other trypanosomatids, as *Leishmania major*, glycoinositolphospholipids containing Gal*f* residues could be involved in promastigote-macrophage attachment and invasion, using Fab fragments of MAb MEST-1 (which recognizes terminal Gal*f* residue present on GIPL-1 of *L. major* promastigotes), and *p*-nitrophenyl-β-D-galactofuranoside.

Our recent study described similarities on host cell interaction and invasion processes of *T. dionisii* and *T. cruzi*, and also important differences, such as the reduced trans-sialidase activity in *T. dionisii*, which may be one of the factors responsible for intracellular retention of some amastigotes into the parasitophorous vacuoles, preventing their scape into the cytoplasm, and subsequent differentiation to trypomastigotes (infective forms) (Oliveira et al., 2009). We believe that the similarities and differences between these so closely species deserve to be better explored, in order to identify new targets to Chagas' disease vaccines.

The ultrastructure of *T. cruzi* has been extensively described (De Souza, 1984, 1999, 2002, 2008; De Souza et al., 2009a,b; Cunha-e-Silva et al., 2006) and recently several techniques have been developed to analyze it at the three dimensional (3D) level, such as, electron tomography, which provides details about specific organelles (Tocheva et al., 2010).