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Interaction with host factors exacerbates *Trypanosoma cruzi* cell invasion capacity upon oral infection

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Abstract

Outbreaks of severe acute Chagas' disease acquired by oral infection, leading to death in some cases, have occurred in recent years. Using the mouse model, we investigated the basis of such virulence by analyzing a *Trypanosoma cruzi* isolate, SC, from a patient with severe acute clinical symptoms, who was infected by oral route. It has previously been shown that, upon oral inoculation into mice, *T. cruzi* metacyclic trypomastigotes invade the gastric mucosal epithelium by engaging the stage-specific surface glycoprotein gp82, whereas the surface molecule gp90 functions as a down-modulator of cell invasion. We found that, when orally inoculated into mice, metacyclic forms of the SC isolate, which express high levels of gp90, produced high parasitemias and high mortality, in sharp contrast with the reduced infectivity in vitro. Upon recovery from the mouse stomach 1 h after oral inoculation, the gp90 molecule of the parasites was completely degraded, and their entry into HeLa cells, as well as into Caco-2 cells, was increased. The gp82 molecule was more resistant to digestive action of the gastric juice. Host cell invasion of SC isolate metacyclic trypomastigotes was augmented in the presence of gastric mucin. No alteration in infectivity was observed in *T. cruzi* strains CL and G which were used as references and which express gp90 molecules resistant to degradation by gastric juice. Taken together, our findings suggest that the exacerbation of *T. cruzi* infectivity, such as observed upon interaction of the SC isolate with the mouse stomach components, may be responsible for the severity of acute Chagas' disease that has been reported in outbreaks of oral *T. cruzi* infection.

Keywords: *Trypanosoma cruzi*; Oral infection; Metacyclic trypomastigotes; Host factors; Modulation of infectivity; Cell invasion

1. Introduction

Oral infection by *Trypanosoma cruzi*, the agent of Chagas' disease, was the focus of attention in 2005 when there was an outbreak of acute cases of the disease in the southern State of Santa Catarina, Brazil, as a result of ingestion of sugar cane juice contaminated with the parasite. The source of contamination was attributed to *T. cruzi*-infected triatomine insects that were crushed along with the sugar cane during preparation of the juice. According to health authorities, 26 people were infected and three died. It was also reported in early 2005 that several people were orally infected with *T. cruzi* in the far north State of Amapá, which borders French Guiana. The most recent outbreak of Chagas' disease, with one death, occurred in the northern State of Pará in mid-2006. All infected people had ingested juice made from bacaba, a fruit typical of the region (http://www.promedmail.org/pls/askus/f?p=2400:1001: 428441192295844876 3::NO::F2400_P1001_BACK_PAGE, F2400_P1001_PUB_MAIL_ID:1010,33766; http://portal.saude.gov.br/portal/saude/visualizar_texto. cfm?idtxt=24541). Before these episodes of oral infection, others had been reported. Back in 1986, in the northeast State of Paraíba, after drinking sugar cane juice, many people became ill, 20 were diagnosed as *T. cruzi*-positive and one died (Shikanai-Yasuda et al., 1991). Oral infection with *T. cruzi*, illustrated by these episodes, may be more frequent than previously thought (Pinto Dias, 2006) and, depending on the locality, may constitute an

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important mode of transmission. In the Brazilian Amazon, more than 50% of acute cases of Chagas' disease recorded between 1968 and 2000 were attributable to microepidemics of orally transmitted infection (Coura et al., 2002).

It has been shown in mice that insect-derived metacyclic trypomastigotes inoculated orally invade and replicate in the gastric mucosa, which serves as a unique portal or entry for systemic infection (Hoft, 1996; Hoft et al., 1996). Using metacyclic forms generated in culture, we have found that *T. cruzi* infective capacity by oral route is associated with the ability to interact with gastric mucin and to invade the underlying epithelial cells, through the engagement of the stage-specific surface molecule gp82 (Neira et al., 2003). The gp82 molecule promotes cell invasion by inducing the signal transduction pathways leading to Ca^{2+} mobilization (Ruiz et al., 1998; Neira et al., 2002), which is an essential requirement for parasite internalization (Moreno et al., 1994; Tardieux et al., 1994; Dorta et al., 1995). Metacyclic trypomastigotes of *T. cruzi* strains belonging to highly divergent genetic groups express highly conserved gp82 molecules, with an overall 97.9% identity in amino acid sequence, and 100% identity with regard to the central domain containing the mammalian cell binding site (Yoshida, 2006).

Recent findings from experiments in mice inoculated orally with metacyclic trypomastigotes have indicated that host factors modulate parasite infectivity (Cortez et al., 2006), suggesting the possibility that this is associated with the higher lethality of oral *T. cruzi* infection. In this study, to further clarify the mechanisms of this mode of infection, we performed a series of experiments using, we believe for the first time, a *T. cruzi* isolate from a patient who was infected by oral route.

2. Materials and methods

2.1. Parasites, mammalian cell culture and invasion assays

The *T. cruzi* isolate, designated SC, was derived from a patient at the acute phase of Chagas' disease, who was infected in the state of Santa Catarina, Brasil, in 2005, after ingestion of sugar cane juice. *Trypanosoma cruzi* strains CL, isolated from the insect *Triatoma infestans* (Brener and Chiari, 1963), and G, isolated from an opossum (Yoshida, 1983), were used as references. The parasites were maintained cyclically in mice and in liver infusion tryptose (LIT) medium containing 5% FBS (Cultilab, São Paulo, Brazil). In the case of the CL strain, as its rate of differentiation into metacyclic forms is very low in LIT medium, it was grown for one passage in Grace's medium. Metacyclic forms from cultures at the stationary growth phase were purified by passage through a DEAE-cellulose column (Sigma), as described (Teixeira and Yoshida, 1986). HeLa cells, the human carcinoma-derived epithelial cells and Caco-2 cells, which are derived from a human colonic adenocarcinoma and differentiate into small intestinal-like cells, were grown at 37 °C in Dulbecco's Minimum Essential Medium (DMEM) supplemented with 10% FBS, streptomycin (100 μg/ml; Sigma) and penicillin (100 U/ml; Sigma) in a humidified 5% CO_2 atmosphere. Cell invasion assays were carried out as detailed elsewhere (Yoshida et al., 1989), by seeding the parasites onto each well of 24-well plates containing 13-mm diameter round glass coverslips coated with 1.5×10^5 HeLa cells. After 1 h incubation with parasites, the coverslips with HeLa cells were washed in PBS, fixed in methanol and stained with Giemsa, and the number of intracellular parasites counted. For Caco-2 cells, the following procedure was used: the coverslips were fixed with Bouin, stained with Giemsa and dehydrated with acetone, different acetone/xylol mix, and xylol.

2.2. Oral infection

Four to five week-old female Balb/c mice, bred in our animal facility (CEDEME), were used throughout. All procedures and experiments conformed with the regulations of the institutional Ethical Committee for animal experimentation. Mice were inoculated with *T. cruzi* metacyclic forms by intrafaringeal route (4×10^5 parasites per mouse), using a plastic tube adapted to a 1-ml syringe. Starting on 13 day post-inoculation, parasitemia was monitored twice a week by examining, at the phase contrast microscope, 5 μl peripheral blood samples collected from the tail.

2.3. Recovery of metacyclic forms from the mouse stomach after oral inoculation

Balb/c mice were maintained overnight without food and then, under anesthesia, had their stomach exposed through a cut in the abdomen. Using a thread, the stomach was closed at the pylorus with a knot. After sewing the abdominal cut and following full recovery from anesthesia, the mice received 2×10^8 metacyclic trypomastigotes by intrapharingeal route. One hour later, the abdominal cut was reopened and an additional knot was made at the cardiac sphincter in order to avoid the loss of gastric content. After removal from the abdominal cavity, the stomach was placed in a well of a 6-well plate containing 1 ml of DMEM plus 20% FBS and cut open. The supernatant was collected, avoiding as much as possible of the solid material, and the number of parasites was counted. It was not always possible to obtain clean trypomastigotes and, depending on preparation, they came with more or less gastric components, which appeared as extra bands by Ponceau staining of nitrocellulose membranes containing parasite extracts subjected to SDS–PAGE.

2.4. Immunoblotting

To analyze the expression of metacyclic trypomastigote surface molecules, the parasite soluble extract was loaded onto a 10% SDS–PAGE gel. The total amount of protein in each lane was 7 μg, which corresponded to the extract of 3×10^7 parasites. After blotting the proteins to the nitrocellulose membrane, and staining it with Ponceau-S to check the proper loading of samples and their effective