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Evolution of infection in mice inoculated by the oral route with different developmental forms of *Trypanosoma cruzi* I and II

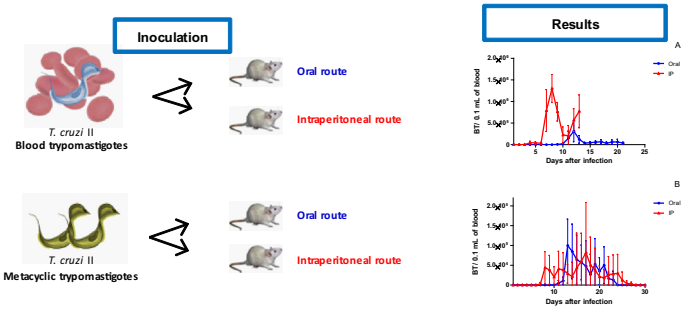
Greicy Brisa Malaquias Dias [a], Ana Paula Gruendling [a], Silvana Marques Araújo [a,b], Mônica Lúcia Gomes [a,b], Max Jean de Ornelas Toledo [a,b,*,1]

[a] Post-Graduate Program in Health Sciences at the State University of Maringá (UEM), Av. Colombo 5790, Bloco 126, CEP 87020-900 Maringá, Paraná, Brazil
[b] Department of Basic Health Sciences at the UEM, Av. Colombo 5790, Bloco I-90, CEP 87020-900 Maringá, Paraná, Brazil

HIGHLIGHTS

- The development of *Trypanosoma cruzi* infection in mice was induced by the oral route.
- It varied significantly according to the genetics and number of parasites inoculated.
- The inoculum volume and developmental stages also influenced the course of infection.
- Mice inoculated with metacyclic trypomastigotes showed more histopathological changes.

GRAPHICAL ABSTRACT



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ABSTRACT

Oral infection has become the most important transmission mechanism of Chagas disease in Brazil. For this study, the development of *Trypanosoma cruzi* infection in mice, induced by the oral and intraperitoneal (IP) routes, was compared. Four groups of Swiss mice were used to evaluate the influence of parasite genetics, number of parasites, inoculation volume and developmental stages on the development of the orally induced infection: 1 – blood trypomastigotes (BT) via oral; 2 – BT via IP; 3 – culture metacyclic trypomastigotes (MT) via oral; and 4 – culture MT via IP. Animals inoculated orally showed levels of parasitemia, as well as infectivity and mortality rates, lower than animals inoculated via IP, regardless of DTU (discrete typing unit) and inoculum. Animals infected with TcII showed higher levels of these parameters than did animals infected with TcI. The larger volume of inoculum showed a greater capacity to cause an infection when administered via the oral route. BT infection was more virulent than culture MT infection for both routes (oral and IP). However, mice inoculated orally with BT showed lower levels than via IP, while mice inoculated orally with culture MT showed similar levels of infection to those inoculated via IP. Mice inoculated with culture MT showed more histopathological changes than those inoculated with BT, regardless of the inoculation route. These results indicate that this alternative experimental model is useful for evaluating infection by *T. cruzi* isolates with subpatent parasitemia and low virulence, such as those belonging to the TcI and TcIV DTUs, which are prevalent in outbreaks of orally transmitted Chagas disease.

Abbreviations: AUC, area under the parasitemia curve; BT, blood trypomastigotes; ChD, Chagas disease; d.i., day after inoculation; DMP, day of maximum parasitemia; DTUs, discrete typing units; FBE, fresh blood examination; HC, hemoculture; IP, intraperitoneal; LIT, liver infusion tryptose; MPP, maximum peak of parasitemia; DMOR, mean day of mortality; PP, mean patent period; PPP, mean pre-patent period; TP, mean total parasitemia; MT, culture metacyclic trypomastigotes; %MOR, percentage of cumulative mortality; %INF, percentage of infected mice; PCR, polymerase chain reaction; UEM, State University of Maringá; T, Student's *t* test.

* Corresponding author. Address: Departamento de Ciências Básicas da Saúde, Universidade Estadual de Maringá, Av. Colombo 5790, Bloco I-90, Zona 7, CEP: 87020-900 Maringá, Paraná, Brazil. Fax: +55 21 44 3011 4860.

E-mail addresses: gremalaquias@hotmail.com (G.B.M. Dias), aninha_gru@bol.com.br (A.P. Gruendling), smaraujo@uem.br (S.M. Araújo), mlgomes@uem.br (M.L. Gomes), mjotoledo@uem.br (M.J.O. Toledo).

[1] Present address: Laboratório de Biologia de Tripanosomatídeos, Instituto Oswaldo Cruz/FIOCRUZ, Av. Brasil, 4365 – Manguinhos, Rio de Janeiro, RJ CEP: 21040-900, Brazil.

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1. Introduction

Chagas disease (ChD) or American trypanosomiasis is present in 21 countries, and despite advances in control, it is still being actively transmitted in many Latin American countries and constitutes an important public-health problem (PAHO, 2006). Studies indicate an incidence of approximately 200,000 cases per year; this is the third most common parasitic infection in the world, after malaria and schistosomiasis (World Health Organization, 2005).

In 2006, the Brazilian Ministry of Health received the International Certification of Elimination of Chagas Disease Transmission by *Triatoma infestans*, conferred by the Pan American Health Organization. Although vector transmission is controlled, experimental evidence and field observations demonstrate continued oral transmission of the etiological agent *Trypanosoma cruzi* to humans and several species of mammals. Various types of food and carriers of the flagellate have been implicated in this transmission route, usually via infected triatomines related to human cases in the immediate vicinity of the event (de Noya et al., 2010; Dias, 2006). Oral infection has become the most important transmission mechanism of ChD in Brazil, since vector transmission and *T. cruzi* transmission through blood supplies have been controlled (Coura, 2006). During the period of October 2005–2010, 756 cases of acute ChD were reported in Brazil (Brazilian Ministry of Health, 2012). Importantly, 703 (93.0%) of these cases occurred in the Amazonian states, where oral transmission is prominent.

Orally acquired human infection is not a recent epidemiological occurrence for ChD. The first well-documented incidence of oral transmission occurred in the southern state of Rio Grande do Sul, and was attributed, among other sources, to food contaminated by opossum urine (Nery-Guimarães et al., 1968). Concurrent outbreaks of acute ChD have been reported in different regions of Latin America, most likely due to transmission by alternative routes (Coura, 2006).

Several outbreaks attributed to oral infection have been recorded in different regions of Brazil, including Catolé da Rocha, Paraíba (Shikanai-Yasuda et al., 1991), Macaúbas, Bahia (Dias et al., 2008) and Redenção, Ceará (Roque et al., 2008) in the Northeast. Oral outbreaks have also been recorded in regions that were previously not considered endemic for ChD, such as the Amazonian states of Pará, Amapá and Maranhão in the North (Valente et al., 2009; Pinto et al., 2008), and in the municipality of Navegantes, Santa Catarina in the South (Steindel et al., 2008). In all these outbreaks, some cases involved severe acute forms of human illness and deaths, and were attributed to local contamination of fruit juices in the North/Northeast outbreaks and sugar-cane juice in the South.

Different biological, biochemical, genetic and molecular characterization studies have demonstrated the great heterogeneity of *T. cruzi* species (Macedo and Segatto, 2010). Today, their strains are grouped into six discrete typing units (DTUs), termed TcI–TcVI (Zingales et al., 2009). Several of these DTUs have been found in human cases associated with oral-transmission outbreaks, with TcI, TcIII and TcIV predominating in northern Brazil (Monteiro et al., 2012; Marcili et al., 2009; Valente et al., 2009) and TcII predominating in the south, where mixed infections with TcI and TcVI have recently been detected as well (Andrade et al., 2011; Steindel et al., 2008). Although the oral route is the most common form of parasite transmission between animals in nature, most experimental studies are performed with mice inoculated intraperitoneally, and few studies on the development of experimental *T. cruzi* infection via this route exist (Camandaroba et al., 2002; Deane et al., 1963).

Therefore, we aimed to standardize the conditions for the study of experimental oral infection in mice, with IP inoculation as the comparative parameter, analyzing the influence of the genetic strain, developmental form, quantity and volume of parasites inoculated.

2. Materials and methods

2.1. Mice

Male Swiss mice aged 21–28 days old from the Central Animal Facility of the State University of Maringá (Universidade Estadual de Maringá – UEM) were used for this study. Authorization for the use of animals for experimentation was given by the Ethics Committee on Animal Use in Experiments of UEM (process No. 113/09).

2.2. T. cruzi strains and developmental forms of the parasite

In all experiments, the Y strain of *T. cruzi*, and in comparative studies to evaluate the influence of parasite DTU on the development of oral infection, the Colombian strain were used as reference strains for the DTUs *T. cruzi* II (TcII) and *T. cruzi* I (TcI), respectively (Zingales et al., 2009).

2.2.1. Blood trypomastigotes (BT)

For experiments with TcI and TcII, trypomastigotes obtained from the blood of previously infected Swiss mice were utilized. Samples were collected through the retro-orbital plexus vein with the anticoagulant heparin. The blood was centrifuged at 1500 *g* to separate its components, and the buffy coat containing trypomastigotes was removed, resuspended in LIT (liver infusion tryptose) culture medium, counted by the Brener (1962) technique, and adjusted to a concentration of 1×10^4 and 5×10^4 BT/0.1 mL. For the remaining experiments with BT, blood was centrifuged at 1200 rpm to separate the blood components, and the supernatant containing the parasites was removed. After addition of LIT medium, the trypomastigotes were counted by the Brener (1962) technique. For the experiments to evaluate the influence of volume, the inocula were adjusted to 1 x 10^4 and 5×10^4 BT in 0.1 and 1.0 mL; and for the experiment with different developmental forms, the inocula were adjusted to 5×10^4 BT/1.0 mL.

2.2.2. Culture-derived metacyclic trypomastigotes (MT)

Trypomastigotes were obtained from acellular culture in LIT medium during the stationary phase. Parasites present in the culture were counted in a Neubauer chamber, and the inocula were adjusted to 2×10^6 culture MT/1.0 mL.

2.3. Experimental groups

2.3.1. Influence of parasite DTU and inoculum volume

Groups of 20 mice which received two different inoculations of blood trypomastigotes (BT) were inoculated with either the TcI or TcII strains. These groups were divided into four subgroups of five animals inoculated with: (a) 5×10^4 BT via the oral route; (b) 5×10^4 BT via the intraperitoneal route (IP); (c) 1×10^4 BT via oral; and (d) 1×10^4 BT via IP. For the volume test, a group of 20 mice inoculated with 5×10^4 BT of the Y strain, divided into four subgroups of five animals, were inoculated with volumes of: (a) 1.0 mL via oral; (b) 1.0 mL via IP; (c) 0.1 mL via oral; and (d) 0.1 mL via IP.

2.3.2. Influence of different developmental forms

A total of 80 mice were used, divided into two groups: (a) 40 animals inoculated via oral divided into two subgroups, 20 with