



Trypanosoma cruzi: Immunoglobulin isotype profiles during the acute phase of canine experimental infection with metacyclic or blood trypomastigotes

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ABSTRACT

A detailed investigation has been carried out about the serological profiles of groups of dogs experimentally infected with metacyclic (MT) or blood (BT) trypomastigotes of Berenice-78 *Trypanosoma cruzi* strain. Peripheral blood was collected from infected dogs and uninfected controls, weekly during 35 days following the acute phase of infection, and immunoglobulin profiles were determined by ELISA. Dogs infected with BT exhibited unaltered levels of IgG2, increases in IgM, IgE, IgA, IgG and IgG1. In contrast, dogs infected with MT presented unaltered levels of IgE and IgG1 and an increase in IgM, IgA, IgG and IgG2 levels. Compared with the MT group, animals infected with BT showed significant increases in IgM on days 7, 14 and 28, in IgA on days 7, 14 and 21, in IgE on days 7 and 14, in IgG on days 14 and 28, and in IgG1 on days 7, 14 and 21. Parasitemia levels of the infected animals were measured over the same time period. No correlations were found between the immunoglobulin profiles and the parasitemia levels. The results demonstrated that the inoculum source (BT or MT) influence the immunoglobulin isotype profile that may drive distinct outcome of acute canine Chagas disease.

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1. Introduction

The etiological agent of Chagas disease is the flagellate protozoan *Trypanosoma cruzi*. In regions that are endemic for the disease, the main mechanism of infection is by contamination with metacyclic trypomastigotes (MT) eliminated by triatomine vectors (Dias, 2006). Blood transfusion represents the second most important infection mechanism, especially in urban areas where vectors are not present (Sanchez-Guillen et al., 2002). Whilst MT and epimastigotes are commonly found in triatomine vectors, amastigotes and blood trypomastigotes (BT) represent, respectively, the main proliferative intracellular and bloodstream forms found in the vertebrate hosts (Andrade and Andrews, 2005). Although both MT and BT forms of *T. cruzi* are fully functional with respect to parasite–host interaction and/or target cell invasion (Meirelles et al.,

1982; Ramirez et al., 1993), the two forms differ regarding their surface molecules (Yoshida, 2006).

Trypanosoma cruzi infections are associated with immunological and immunopathological reactions that may result from non-specific polyclonal activation (Montes et al., 2006) or suppressive effects (Abrahamsohn and Coffman, 1995). In addition, immune control of the infection involves the participation of CD4⁺ and CD8⁺ T cells (Sher and Coffman, 1992; Tarleton et al., 1992). Together with cellular immune responses, anti-parasite humoral responses also play a major role in the control of parasitemia and in resistance to *T. cruzi* infection (Krettli and Brener, 1976).

Anti-*Trypanosoma cruzi* IgG antibodies are produced at the beginning of the acute phase of the disease (Lana et al., 1991; Carneiro et al., 2007) and react mainly with surface molecules of the infective trypomastigote forms. However, most epitopes shared by trypomastigote and epimastigote forms belong to internal antigens (Umezawa et al., 1996), a fact that may explain why epimastigote antigens do not result in high IgG reactivity with acute phase sera. The major antibody isotypes involved in the elimination of blood forms of the parasite and in decreasing mortality rates are IgG1 and IgG2 (Brodszyn et al., 1989; Cordeiro et al., 2001).

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Dogs may be employed as standardized experimental models, reflecting the natural history of evolution of human Chagas disease, since these animals develop acute and chronic phases of the infection with morphological and clinical characteristics that are very similar to those observed in man (Andrade and Andrade, 1980; Lana et al., 1992). Bahia et al. (2002) studied parasitemia and histopathological aspects and demonstrated that the source of the inoculum remarkably influence the evolution of the infection in dogs.

In order to gain an insight into the effect of the inoculum source on the humoral immune response in infected animals, we have performed a detailed comparative analysis of the anti-*T. cruzi* immunoglobulin profiles during the acute phase of infection in experimental dogs that had been inoculated with BT or MT forms of *T. cruzi*.

2. Materials and methods

Details of the study were presented to and approved by the Ethical Committee of the Universidade Federal de Ouro Preto, Minas Gerais, Brazil. All procedures described were carried out in compliance with current Brazilian criteria relating to Experimental Biology and Medicine as described in the guidelines issued by the Colégio Brasileiro de Experimentação Animal (2006). All of the animals employed in this study were maintained in the central animal facility of the Universidade Federal de Ouro Preto, Minas Gerais, Brazil.

2.1. Parasites, dogs and study design

MT and BT forms of the Berenice-78 (Be-78)¹ *T. cruzi* strain (Lana and Chiari, 1986) were isolated, respectively, from nymphs of *Triatoma infestans* and female Swiss Webster mice that had been previously infected in our laboratory.

Before the beginning of the study, 12 dogs born in the kennel of the Universidade Federal de Ouro Preto, MG, Brazil, belonging to two different litters, were treated with anti-helminthics and immunized against more common canine infectious pathogens. Animals were maintained in quarantine for 16 weeks receiving drinking water and a balanced commercial feed *ad libitum*.

When the animals completed 120 days of age, groups of 4 dogs were inoculated intraperitoneally with either MT or BT forms of Be-78 *T. cruzi* strain (2000/kg body weight), while the remaining 4 dogs were maintained uninfected as control group. Each group of 4 dogs was maintained in isolated place until the beginning of the experiment.

2.2. Parasitemia evaluation

Parasitemia in the infected animals was evaluated since the 10th day after infection until day 35. The number of parasites in fresh blood collected by vein-puncture of the ear veins was determined under the optical microscope according to Brener (1962). Briefly this methodology consists in examination of 5 µL of infected blood on the microscope slide covered by 22 × 22 mm slide. Parasites present in 50 microscope fields were counted and multiplied by the microscope factor (calculated for each objective). The parasitemia curves represent the mean value of blood trypomastigotes/0.1 mL of blood from all infected dogs of each group at each time point.

2.3. Parasite antigen

Soluble antigen from epimastigotes of *T. cruzi* Y strain were obtained from an axenic culture of the protozoan at exponential

growth phase in liver infusion tryptose (LIT) medium. Parasites were washed three times by centrifugation (800g; 10 min) in phosphate buffer saline (pH 7.2), followed by three ultrasound cycles (each of 1 min at 40 W) with a Sonifier Cell Disruptor® (Branson Sonic Power Co., Danbury, CT, USA) cooled in an ice bath. The sonicated material was centrifuged (18,500 rpm; 1.5 h; 4 °C) and the supernatant transferred to dialysis tubes and dialyzed against phosphate buffer saline for 36 h, with four phosphate buffer saline changes every 6 h. Finally, the remaining material was filtered under aseptic conditions through a 0.22 µm filter and an aliquot was taken to determine protein concentration by the method of Lowry et al. (1951). The bulk of the sample was adjusted to a protein concentration of 1000 µg/mL and stored in small aliquots at –70 °C prior to use.

2.4. Determination of immunoglobulin profiles by enzyme linked immunosorbent assay

Peripheral blood (5 mL) was collected from the brachial cephalic veins of the experimental dogs prior to inoculation (on day 0) and weekly thereafter (on days 7, 14, 21, 28 and 35), and placed in vials in the absence of anticoagulant. Serum samples were stored at –70 °C and enzyme linked immunosorbent assays (ELISA) were carried out using a modified version of the Voller et al. (1976) method. Polystyrene microtiter plates (MaxiSorp™ surface, Nalge Nunc Int., Rochester, NY, USA) were coated with 200 µL of a solution antigenic (Final concentration = 0.5 µg/well) from epimastigotes of *T. cruzi* in carbonate buffer (pH 9.6) and incubated overnight at 4 °C. After incubation, the plates were washed four times with phosphate buffered saline (PBS) containing 0.05% Tween 20, and blocked for 45 min at 37 °C with 100 µL of foetal bovine serum (5%) in PBS per well. Plates were then incubated at 37 °C for 45 min with 100 µL of serum sample diluted 1:80, the wells were washed three times and incubated at 37 °C for 45 min with specific monoclonal peroxidase-conjugated antibodies (Bethyl Laboratories Inc., Montgomery, TX, USA) previously diluted as follows: goat anti-dog IgG1 (anti-heavy chain specific), 1:16,000; IgM (anti-µ chain specific), 1:500; IgA (anti-α chain specific), 1:500; IgE (anti-ε chain specific), 1:1000, or sheep anti-dog IgG and IgG2 (both anti-heavy chain specific), 1:16,000. Following four further washes (as above), reaction was initiated by the addition of 100 µL of 0.1 M citrate buffer (pH 5.0) containing 0.03% *o*-phenylenediamine and 0.012% H₂O₂, followed by incubation at 37 °C for 10 min. The reaction was stopped by the addition of 32 µL of 2.5 M H₂SO₄ and the absorbance value (OD_{492 nm}) measured with an ELISA plate reader (Molecular Devices, E Max, USA).

2.5. Statistical analysis

Statistical analyses were performed with the aid of the Prism 4.0 software package (Prism Software, Irvine, CA, USA). Data collected for the BT and MT groups at identical times after infection were compared using the Mann–Whitney test. Repeated measurement analyses for the longitudinal study were performed using the Friedman test followed by Dunn's multiple comparison test. Spearman's rank tests were employed in order to investigate correlations between immunoglobulin levels and parasitemia curves. In all cases, differences were considered statistically significant when the probabilities of equality (*p*-values) were ≤ 0.05.

3. Results

3.1. Immunological parameters

The kinetic profiles of the anti-*T. cruzi* isotypes IgM, IgA, IgE, IgG, IgG1 and IgG2 were determined by ELISA assay over a period of 35

¹ Abbreviations used: Be-78, Berenice-78; BT, blood trypomastigotes; C, uninfected control group; IFI, indirect immunofluorescence; LIT, liver infusion tryptose; MT, metacyclic trypomastigotes; PBS, phosphate buffered saline.

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