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Growth behaviour of two *Trypanosoma cruzi* strains in single and mixed infections: *In vitro* and in the intestinal tract of the blood-sucking bug, *Triatoma brasiliensis*

Catarina A.C. Araújo [a], Pedro H. Cabello [b], Ana M. Jansen [a,*]

[a] *Departamento de Protozoologia, Instituto Oswaldo Cruz, FIOCRUZ, Avenida Brasil, 4365 Manguinhos, RJ, Brazil*
[b] *Departamento de Genetica, Instituto Oswaldo Cruz, FIOCRUZ, Avenida Brasil, 4365 Manguinhos, RJ, Brazil*

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Abstract

Competition and cooperation are well-recognized biological phenomena, even among parasites. Co-infection of parasites in a single host leads to several outcomes, one being competition for a limited resource. Here, the behaviour of mixed infection was evaluated using two isolates of *Trypanosoma cruzi*, previously typed as belonging to genotypes TcI and TcII. The growth *in vitro* and in the different compartments of the gut of *Triatoma brasiliensis* was studied. *In vitro* growth showed that MDID/BR/1999/M1 (TcI) has a doubling time of 19.5 h and MIDID/BR/1999/JCPD4 (TcII) of 9.6 h, while the mixed infection group presented a doubling time of 13.9 h. *In vivo*, three groups of infection were done: M1/TcI, JCPD4/TcII and mixed infection (50% of each strain), respectively. All comparisons among the groups were done using the Kruskal–Wallis non-parametric test. The data showed that the *in vitro* culture of mixed populations has a similar pattern to the growth of M1/TcI, apparently suggesting a positively selection for M1/TcI strain, in axenic culture. In the gut of the insects, M1/TcI isolate and mixed infections colonized predominantly the rectal wall and rectal lumen, in contrast to the JCPD4/TcII isolate, which was found mainly colonizing the small intestine. According to the isolates investigated, it could be concluded that the doubling time was not determinant factor for the final composition of a co-infection. Moreover, mixed infections resulted in a homogenous distribution of the parasites, comparing to the isolates studied separately. Apparently, in the gut of the bugs, the simultaneous presence of JCPD4/TcII isolate resulted in an improvement of the number of parasites from M1/TcI.

Keywords: Chagas disease; *Trypanosoma cruzi*; Mixed infections; *Triatoma brasiliensis*

1. Introduction

Chagas disease (American Trypanosomiasis) is a major public health problem in Latin America, affecting 16–18 million people (WHO, 1991; WHO/CTD, 2004). The disease has the haemoflagellate, *Trypanosoma cruzi* (Kinetoplastida: Trypanosomatidae) (Chagas, 1909) as etiologic agent and is transmitted to humans by contamination of the perforated skin with bug faeces, containing the metacyclic stage of the parasite. *Triatoma brasiliensis* (Neiva, 1911) is a native species and the most important vector of Chagas disease in semiarid areas of north-eastern Brazil. This insect colonizes natural and artificial ecotopes, reinvading human dwellings successfully (Silveira and Vinhaes, 1999; Costa et al., 2002). The ability of *T. brasiliensis* to infest household environments remains the primary risk factor for preservation

* Corresponding author. Tel.: +55 21 2598 4324; fax: +55 21 2560 6572.
E-mail address: jansen@ioc.fiocruz.br (A.M. Jansen).

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of the domiciliary transmission of the parasite, since sylvatic bugs, which show high natural infection rates, may set up new domestic dwellings, establishing new *T. cruzi* infections. In addition, *T. brasiliensis* was reported to have the highest percentage of natural infection by *T. cruzi* among the triatomines captured in natural and artificial ecotopes (Costa et al., 2003).

The interactions between *T. cruzi* and the insect hosts continue to be an interesting subject, to be intensely explored. The parasite is extremely heterogenic; both the geographic distribution in its potential hosts and the putative association with the different genetic variants continues to be uncertain (Herrera et al., 2003). *T. cruzi* has been extensively investigated by zymodemes, being subdivided into three main groups: zymodemes Z1, Z2 and Tc/Z3. Currently, *T. cruzi* is characterized as belonging to two main genotypes: TcI and TcII, which have been recognized as associated, in Brazil, to the sylvatic and domestic transmission cycles, respectively.

Despite detection of several developmental stages of *T. cruzi* in the intestinal tract of the triatomines, Chagas (1909) described the epimastigote and trypomastigote as the main forms of the parasite life cycle. Both stages are predominant in the regularly fed insects and the epimastigote form is responsible for parasite multiplication and colonization of the whole intestinal tract of the blood-sucking insect, while trypomastigote forms develop in the rectum and are considered the infectious forms. Considering the high heterogeneity of *T. cruzi*, different genotypes infecting a single host are an observed occurrence in nature (Bosseno et al., 2000; Torres et al., 2004). However, no studies on the evolution of mixed infections are available, although it is to be expected that the development of the parasites will be profoundly influenced inside the blood-sucking insect.

Competition within-host generating selection of parasites is a regularly described phenomenon. Furthermore, the intra-host competition between parasites of different genotypes has been described as a main factor shaping parasite ecology and evolution (Gower and Webster, 2005). Considering host resources, it is expectable that co-infection of different species of parasites will result in resource competition (Vizoso and Ebert, 2005), affecting not only the development, but also the growth, reproduction and survival of the parasite, as well as the state of health of the host (Ebert et al., 2000). In multiclonal populations, natural selection has been described as favouring more virulent strains (Taylor et al., 1998; de Roode et al., 2004, 2005).

Several studies have reported intrinsic characteristics of single *T. cruzi* genotype infections. However, this is a different situation from that which occurs in nature, since hosts are frequently co-infected with more than one parasite strain. The aim of this study was to evaluate the behaviour of mixed infections using two isolates of different *T. cruzi* genotypes (TcI and TcII) in axenic culture and their spatial distribution in the intestinal tract of *T. brasiliensis*, its natural host, following the dynamics of *T. cruzi* growth kinetics in both experimental models.

2. Materials and methods

2.1. Parasites

The TcI strain (MDID/BR/1999/M1) was obtained from a naturally infected *Didelphis albiventris*, in the municipality of Coronel José Dias, while the TcII strain (MDID/BR/1999/JCPD4) was obtained from naturally infected *T. brasiliensis*, in the municipality of João Costa, both regions in north-eastern Brazil, distanced 50 km, in the Piauí State, Brazil (Herrera et al., 2005). Both genotypes circulate in sympatry in this area and were isolated from the natural hosts recently and stored in liquid nitrogen until use. After the isolation of the parasites from the respective hosts, epimastigotes of *T. cruzi* isolates were grown in McNeal, Novy and Nicolle (NNN) medium with liver infusion tryptose (LIT) overlay supplemented with 10% fetal calf serum (Chiari and Camargo, 1984). The parasites were characterized as TcI and TcII by miniexon multiplex PCR characterization, isoenzymes and lectin agglutination (Araújo et al., 2002; Herrera et al., 2005).

2.2. In vitro cultures of T. cruzi genotypes and doubling time calculations

Using the same batch of liver infusion-tryptose (LIT) medium, parasite cultures of M1/TcI, JCPD4/TcII and mixed infections (50% M1 + 50% JCPD4) in an exponential growth phase were grown in triplicate in tissue culture flasks (25 cm^2 surface area), containing 10 ml of LIT medium inoculated with 1×10^5 parasites/ml. The parasites were incubated at 27 °C and counted daily from inoculation onwards using Neubauer haemocytometers, until the death of the flagellates. Before counting, the flasks were always shacked for the detachment of the flagellates. The metacyclic trypomastigote forms were also estimated using Neubauer haemocytometers. The generation number and doubling time were calculated for each different group of infection, between the days 4 and 5 of the growth curve (when at log phase), as follows: generation number = $\log c_f - \log c_i/0.30$ (c_f = final concentration, c_i = initial concentration of parasites counted). Doubling