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Trypanosoma cruzi: Infectivity modulation of a clone after passages through different hosts

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Abstract

Although *Trypanosoma cruzi* virulence can be modified through passages in vivo or long-term in vitro culture, the mechanisms involved are poorly understood. Here we report modifications in the infectivity of a *T. cruzi* clone after passages in different hosts without detectable changes in parasite genetic patterns. A clone was obtained from a *T. cruzi* IIe isolate and showed to be less virulent than the original isolate ($p < 0.05$). This clone was enzymatically similar to the original isolate as shown by multilocus enzyme electrophoresis. Infection of this clone was compared by successive passages in mice and guinea pigs. The mouse-passaged subline became more virulent for both host species compared to the guinea pig-passaged subline ($p < 0.05$). The clone line displayed similar random amplified polymorphic DNA patterns before and after passages in different hosts suggesting that alterations in virulence could be a result of a differential expression of virulence factors.

Index Descriptors and Abbreviations: *Trypanosoma cruzi*; Clones; Selection; Infectivity; DNA, deoxyribonucleic acid; RNA, ribonucleic acid; RAPD, random amplified polymorphic DNA; MLEE, multilocus enzyme electrophoresis; LIT, liver infusion tryptose medium; LIT-HSP, LIT plus fetal bovine serum, hemin, and penicillin–streptomycin; CTA1, clone line from TolAc1 isolate; TAE, Tris-acetate EDTA; GPI, glucose-6-phosphate isomerase

1. Introduction

Trypanosoma cruzi, the etiological agent of Chagas' disease, consists of distinct parasite progenies, which circulate among humans, insect vectors, and mammalian reservoirs. In the process of geographic dispersion, *T. cruzi* has developed a great diversity of populations, which show distinct biological characteristics (Murta and Romanha, 1999). It is well established that *T. cruzi* is extremely heterogeneous in terms of host specificity, pathogenicity, virulence (Toledo et al., 2002), and molecular markers, such as isoenzyme and kinetoplastic-DNA restriction patterns (Barnabé et al., 2000; Morel et al., 1980).

Polymorphisms in ribosomal RNA genes, mini-exon genes, and microsatellite fingerprinting indicate the presence of at least two principal genetic lineages, *T. cruzi* I and *T. cruzi* II (Souto et al., 1996). Tibayrenc et al. (1986) proposed a complex multiclonal structure for *T. cruzi* and postulated that parasite propagation occurs with little or no genetic exchange (Tibayrenc and Ayala, 1987). According to this model, every clone represents a lineage that reproduces by binary fission and remains unchanged for many generations until mutation or eventual horizontal genetic exchange occur. However, recent experimental studies have reported genetic exchange among parasites of the *T. cruzi* I lineage under defined experimental conditions (Gaunt et al., 2003).

Studies based on cloned and non-cloned *T. cruzi* populations reinforce the heterogeneity hypothesis and the theory that populations are composed of subpopulations with different characteristics (Andrade, 1999; Dusanic et al., 1994). Thus, the characteristic behavior of one isolate would be the result of the interaction of several clones. The coexistence of multiple parasite strains in vertebrate and invertebrate hosts has been reported (Lana et al., 2000; Pinto et al., 1998). The time when the sample is taken and the isolation method can

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act as selective factors (Deane et al., 1984). Successive passages in mice or axenic culture medium can completely eliminate one or more strains present in the original population. It has also been reported that maintenance of *T. cruzi* in culture affects gene and antigenic expression of metacyclic trypomastigotes (Contreras et al., 1998). All these mentioned factors could account for the virulence increase in *T. cruzi* populations after in vivo passages or the attenuation process after long-term in vitro culture.

The main goal of this study was to determine if a particular *T. cruzi* clone could modify its virulence when submitted to different stress situations. For this purpose, we decided to analyze the genetic and biological characteristics of a single cell derived clone after successive passages in different host species. In this case, the heterogeneity of the original isolate was eliminated by cloning the initial inoculum. Furthermore, we assessed the virulence of this clone in mice compared to the original isolate.

2. Materials and methods

2.1. Parasites

The *T. cruzi* parasites used in this work derived from the *T. cruzi* isolate TolAc1 (original isolate), which comes from a *Triatoma infestans* collected in an endemic zone for Chagas disease, in the province of Chaco, Argentina. Diosque et al. (2003) classified this isolate as belonging to *T. cruzi* IIe lineage, according to multilocus enzyme electrophoresis analysis (MLEE) for 15 enzymatic loci.

Parasites were maintained at 29 °C in axenic cultures of Liver Infusion Tryptose Medium (LIT) supplemented with 10% fetal bovine serum, 1% hemin, and 100 U/ml of penicillin–streptomycin (LIT-HSP). In general, these cultures produce >95% epimastigote forms of the parasite. For enrichment of the cultures in infective trypomastigote forms, filtrates of triatomine gut homogenate were added (1%) to the cultures (Isola et al., 1986). Complement-resistant forms were selected by incubation in fresh human serum.

2.2. Cloning procedure

To derive a clone line from a single parasite, serial dilutions of the original isolate culture (TolAc1) were seeded into microplate wells and examined under an inverted microscope to confirmed well containing single parasites. Single cells were transferred into glass hemolysis tubes containing 2 ml of LIT-HSP and cultured at 29 °C. The clone used in the present study, CTA1, was derived by this procedure.

2.3. Genetic characterization of CTA1 clone

CTA1 clone and the original isolate were examined at 15 enzymatic loci by MLEE. The MLEE analysis was undertaken according to Ben Abderrazak et al. (1993) with slight modifications. We also developed random amplified polymorphic DNA assays (RAPD) to evaluate the genetic characteristics of the clone after passages through different hosts. DNA was extracted with phenol:chloroform method and used to carry out the RAPD technique. The amplification reactions were performed according to Brisse et al. (1998) using two random primers, A10 (5′GTGATCGCAT3′) and R16 (5′CTCTGCGCGT3′) (Operon Technologies). RAPD products were analyzed by electrophoresis in 1.6% agarose gels in TAE buffer (Tris-acetate 40 mM, EDTA 1 mM), stained with ethidium bromide and visualized by ultraviolet light.

2.4. Animal infections and parasitemia curves

One- or four-week-old male Balb/c mice and one- to three-month-old guinea pigs (*Cavia porcellus*) were used. Animal care guidelines of the Health Sciences Faculty of the National University of Salta were strictly followed. The propagation assays involved submitting CTA1 to successive mouse to mouse and guinea pig to guinea pig passages. The animals were inoculated by intraperitoneal route either with 1×10^3–1×10^4 *T. cruzi* bloodstreams forms per animal or 1×10^5–1×10^7 metacyclic trypomastigotes as specified. Blood was collected in heparinized glass capillary pipette by sectioning the tail tip from mice and by footpad puncture in guinea pigs. Ten microliters of blood was placed between slide and coverslip and the number of parasites per 100 fields was recorded under a microscope ($400\times$).

2.5. Statistical analysis

The statistical significance of differences between parasitemia curves was calculated with the 2-tailed Wilcoxon signed rank test and the Mann–Whitney *U*-test. For measurement of variability within groups of animals, the standard error of the mean (SEM) was used. These tests and calculations were incorporated in the Graph Pad Prism Software (Graph Pad Software Inc., USA). Values are expressed as means ± SD from a minimum of three separate experiments.

3. Results

3.1. Genetic characterization of CTA1 clone

The phylogenetic identity of CTA1 clone obtained from the isolate TolAc1 was evaluated by MLEE. The CTA1 clone displayed the same *T. cruzi* IIe profile as compared to the original isolate for the 15 enzymatic loci examined with the MLEE technique (data not shown).

The genetic variability of CTA1 clone and TolAc1 isolate was examined by RAPD after passage in mice or guinea pigs. No differences were observed between the clone and the original isolate for the two primers used. The data presented in Fig. 1 indicates no differences between the culture-derived clone and the clone maintained in different