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Trypanosoma cruzi: Impact of dual-clone infections on parasite biological properties in BALB/c mice

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Abstract

Herein, we have analyzed major biological properties following dual-clone *Trypanosoma cruzi* infections in BALB/c mice. Eight *T. cruzi* clonal stocks, two of each principal genotype, including genotype 19 and 20 (*T. cruzi* I), hybrid genotype 39 (*T. cruzi*) and 32 (*T. cruzi* II) were combined into 24 different dual-clone infections. Special attention was given to characterize biological parameters assayed including: prepatent period, patent period, maximum of parasitemia, day of maximum parasitemia, area under the parasitemia curve, infectivity, mortality, and hemoculture positivity. Our findings clearly demonstrated that features resultant of dual-clone infections of *T. cruzi* clonal stocks did not display either the characteristics of the corresponding monoclonal infections or the theoretical mixture based on the respective monoclonal infections. Significant changes in the expected values were observed in 4.2–79.2% of the mixtures considering the eight biological parameters studied. A lower frequency of significant differences was found for mixtures composed by phylogenetically distant clonal stocks. Altogether, our data support our hypothesis that mixed *T. cruzi* infections have a great impact on the biological properties of the parasite in the host and re-emphasizes the importance of considering the possible occurrence of natural mixed infections in humans and their consequences on the biological aspects of ongoing Chagas' disease.

Index Descriptors and Abbreviations: *Trypanosoma cruzi*; Dual-clone infection; Clonal genotypes; Biological properties; PPP, prepatent period; PP, patent period; MP, maximum parasitemia; DMP, day of maximum parasitemia; PAR, area under the parasitemia curve; INF, infectivity; MORT, mortality; +HEM, hemoculture positivity; MW, Mann–Whitney test; KS, Kolmogorov–Smirnov test; χ^2, chi-square test; SD, number of significant differences; S, significant difference at $P < 0.05$; NS, nonsignificant difference at $P > 0.05$; NR, not recorded due to subpatent parasitemia; I, increased parasitemia; D, decreased parasitemia; *n*, numbers of animals

1. Introduction

Trypanosoma cruzi, the etiological agent of Chagas' disease, is a protozoan parasite that displays extremely heterogeneous biological properties (Andrade, 1976; Toledo et al., 2002), including growth kinetics in cellular and acellular cultures (Dvorak et al., 1980; Laurent et al., 1997; Revollo et al., 1998), tissue tropism (Diego et al., 1998; Melo and Brener, 1978), pathogenicity (Andrade and Magalhães, 1997), ability to multiply and differentiate in the insect vectors (Garcia and Dvorack, 1982; Lana et al., 1998), and susceptibility to chemotherapy (Andrade et al., 1992; Revollo et al., 1998; Toledo et al., 2003; reviewed by Toledo et al., 2004). Recently, its has been considered that the diverse

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spectrum of clinical manifestations of chronic Chagas' disease (Dias, 1992; Rassi and Luquetti, 1992) may be due, at least in part, to genetic diversity of the infecting parasites (reviewed by Macedo et al., 2004).

Tibayrenc and Ayala (1988) have demonstrated that *T. cruzi* organisms undergo predominantly clonal evolution, which should render the parasite genotype relatively stable in space and time. Using this hypothesis, they have identified 43 distinct *T. cruzi* genotypes with some of them named "principal genotypes" including genotypes 19, 20, 39, and 32, which are ubiquitous and very well studied (Tibayrenc and Brenière, 1988). The working hypothesis underlying the *T. cruzi* clonal theory is that the variation of biological and medical properties of this parasite is proportional to their phylogenetic divergence. Recently, several authors have corroborated this hypothesis when working with this set of clonal genotypes (Lana et al., 1998; Laurent et al., 1997; Revollo et al., 1998; Toledo et al., 2002, 2003).

The coexistence of mixed infections in vertebrate and invertebrate hosts had already been demonstrated in both experimental (Deane et al., 1984; Lana et al., 2000; Pinto et al., 1998, 2000) and natural situations (Brenière et al., 1985; Tibayrenc et al., 1985) and this certainly plays an important role in the determining the clinical picture of the disease. Therefore, the principal goal of the present investigation was to analyze the impact of dual-clone *T. cruzi* infections in BALB/c mice. For this purpose, we have analyzed the biological properties of 24 distinct combinations of *T. cruzi* clonal stocks (19, 20, 39, and 32) compared with both the correspondent monoclonal infections and the predicted theoretical patterns of dual-clone infections.

2. Materials and methods

2.1. Parasites

We have included for monoclonal and dual-clone infections eight standard *T. cruzi* clonal stock representatives of the four major clonal genotypes (Tibayrenc, 1998): 19 (Gamba cl1 and OPS21 cl1) and 20 (Cuica cl1 and P209 cl1) both from *T. cruzi* I; the hybrid genotype 39 (Bug2148 cl1 and SO3 cl5) and the genotype 32 (IVV cl4 and MAS cl1) from *T. cruzi* II. These stocks were previously typed with 22 enzyme loci and RAPD (Tibayrenc et al., 1993) and were chosen based on their biological properties (more and less virulent) previously observed in BALB/c mice (Toledo et al., 2002).

2.2. Experimental infections

For monoclonal infections, groups of six female BALB/c mice, 28–30 days old, were intraperitoneally inoculated with 10,000 blood trypomastigotes of a given *T. cruzi* clonal stock. Dual-clone infection was also carried out using groups of six female BALB/c mice, 28–30 days old, intraperitoneally inoculated with two different clones, using 5000 blood trypomastigotes of each clone. The inocula were counted according to Brener (1962). Twenty-four different parasite combinations were analyzed (Table 1).

2.3. Parameters evaluated

Eight biological parameters were considered and included: prepatent period (PPP), patent period (PP), maximum parasitemia (MP), day of maximum parasitemia (DMP), area under the parasitemia curve (PAR), infectivity (INF), mortality (MORT), and hemoculture positivity (+HEM). The reproducibility of the different parameters for monoclonal infections was previously assayed in similar experiments with 5000 and 10,000 parasites.

Parasitemia was daily examined according to Brener (1962), starting at day 4 after inoculation. PPP and DMP for animals with subpatent parasitemia were not recorded. Infectivity (INF) was evaluated by fresh blood examination and/or hemoculture during the first month of infection and expressed as percentage of infected animals. Animals with negative results were eliminated from further analyses. Mortality (MORT) was expressed in cumulative percentage observed during 90 days following infection. Hemoculture was carried out according to the Filardi and Brener (1987), 60 days after inoculation and expressed in percentage of positive hemoculture for each group of animals.

2.4. Statistical analysis

The null hypothesis tested here was that "there was no interference by one clone on the major biological properties of the other one included in the mixture." The data analysis

Table 1
Combinations of representative clones of genotypes 19, 20, 39, and 32 of *T. cruzi* used in BALB/c mice dual-clone infections

Mixture of genotypes	Mixture of clones[a]			
	+ Virulent + Virulent	− Virulent − Virulent	+ Virulent − Virulent	− Virulent + Virulent
19 + 20	Gamba cl1 + P209 cl1	OPS21 cl11 + Cuica cl1	Gamba cl1 + Cuica cl1	OPS21 cl11 + P209 cl1
19 + 39	Gamba cl1 + Bug2148 cl1	OPS21 cl11 + SO3 cl5	Gamba cl1 + SO3 cl5	OPS21 cl11 + Bug2148 cl1
19 + 32	Gamba cl1 + IVV cl4	OPS21 cl11 + MAS cl1	Gamba cl1 + MAS cl1	OPS21 cl11 + IVV cl4
20 + 32	P209 cl1 + IVV cl4	Cuica cl1 + MAS cl1	P209 cl1 + MAS cl1	Cuica cl1 + IVV cl4
20 + 39	P209 cl1 + Bug2148 cl1	Cuica cl1 + SO3 cl5	P209 cl1 + SO3 cl5	Cuica cl1 + Bug2148 cl1
39 + 32	Bug2148 cl1 + IVV cl4	SO3 cl5 + MAS cl1	Bug2148 cl1 + MAS	SO3 cl5 + IVV cl4 cl1

[a] + virulent, clone more virulent; − virulent, clone less virulent. These clones were chosen among 20 representative clones of the four major clonal genotypes.