Integral Approach to Evaluation of the Pathogenic Activity of *Trypanosoma Cruzi* Clones as Exemplified by the Mexican Strain

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Comparative histopathological study and analysis of parasite load in different muscle groups were carried out in BALB/c mice during the acute phase of Chagas disease. Activities of C104 clone of *T. cruzi* strain TPAP/MX/2002/Albarrada and the parental strain were compared. Panoramic 2D-microscopy imaging of sample surface was used and quantitative analysis of parasitism and pathologic damage was performed. The infection rates in various muscle groups were as follows: myocardium=abdominal muscles=lumbar muscles=femoral muscles←diaphragm for the clone and myocardium¬abdominal muscles=lumbar muscles=femoral muscles→ diaphragm for the parental strain.

Key Words: *Trypanosoma cruzi; muscle tissue tropism; myocardium; diaphragm; myositis*

Chagas disease caused by *Trypanosoma cruzi* (*T. cruzi*) flagellar protozoon is a serious public health problem in America. In mammalian host, the disease evolves through three periods: acute, intermediate, and chronic [1]. During the acute phase, the bloodstream forms invade the host tissues and are converted into intracellular proliferating forms (amastigotes). Myocyte parasitism, myositis, degeneration, and necrosis of myofibers were observed during the infection [1].

T. cruzi strains are multiclonal populations. The distribution of the parasites in the host tissues is determined by specific features of the clones constituting the infective strain. According to the polyclonal theory, uneven distribution of the parasites in tissues is determined by specific features of the clones constituting the integral strain [2]. Hence, some clone can be tropic to a certain or several tissue types and infect them to the same degree. The host by its reaction modifies the infective characteristics of the parasite and these host-parasite relations presumably explain different virulence of the same clone in different hosts. Similar logics, however, may be applied also for different tissues within the same host, so that their differential infection/damage can be expected even with a single clone. However, parasite distribution in different groups of muscles and their invasion in the same host have been never evaluated quantitatively.

We compared parasitism and histopathological changes in the heart and four groups of skeletal muscles during the acute phase of *T. cruzi* infection in mice.

MATERIALS AND METHODS

T. cruzi strain TPAP/MX/2002/Albarrada was isolated from *Triatoma pallidipenis* (Stål, 1868) recollected in

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Colima, Mexico. Four strains were isolated from this strain by micromanipulation [3].

Amplification of a part of miniexon nontranscribed intergenic site of *T. cruzi* DNA was carried out using 3 oligonucleotides: common *T. cruzi* primer TCC (5'-CCCCCCTCCCAGGCCACACTG-3') and specific for genetic groups I and II isolated from the hypervariable site: TC1 (5'-GTGTCCGCCACCTCCTTCGGGCC-3') and TC2 (5'-CCTGCAGGCACACGTGTGTGTG-3') [4]. PCR mixture contained 1 mM $MgCl_2$, 0.2 mM each dNTP, 2.5 U Tag platinum, 50 ng DNA, and 200 ng each primer. The following thermal profile was used: 1 min at 94°C; 27 cycles - 30 sec at 94°C, 30 sec at 55°C, and 30 sec at 72°C; and the final step - 10 min at 72°C. Silvio and CL Brener strains served as the control for genetic groups TcI and VI, respectively. Amplification products were analyzed in 1.5% agarose gel stained with ethidium bromide.

BALB/c mice were infected with bloodstream trypomastigotes (10^5/mouse) at the age of 8 weeks. Tissue specimens were collected on day 24 after infection at the peak of parasitemia; the animals were sacrificed with lethal dose of sodium phenobarbital. Sections of the myocardium, abdominal, lumbar, femoral, and diaphragmatic muscles were fixed in 10% formalin in buffer solution, embedded in paraffin, and stained with hematoxylin and eosin.

Digital imaging of the entire area of each specimen was realized by bloodstream trypomastigotes (at ×20) obtained by a digital photocamera (Carl Zeiss). The percentage of sites with intact muscle fibers and visual fields (at ×20) with mononuclear infiltration were evaluated and the amastigote accumulations were counted. Interactive Measurement Tools and Automeasures Plus modulus (Axiovision 4.8.1 software) were used.

Statistical analysis was carried out using Friedman's test (NPAIR1WAY procedure for Statistical Analysis System; SAS, 1989). Multiple paired comparisons were carried out using Dunn's test with macro% Dunn for SAS, suggested by P. Juneau [5]. The differences were considered significant at $P<0.05$.

All experiments were carried out in accordance with the Guide for the Care and Use of Laboratory Animals, NIH [6].

RESULTS

During genetic characterization of clone C104, a characteristic 350 b.p. product was obtained indicating that this clone belongs to TcI like the majority of Mexican strains [7]. Our results indicated that muscle tissue tropism of clone C104 differed from that of the parental strain (Fig. 1), which could be attributed to the polyclonal structure of Albarrada strain, where different clones contribute to histotropism. All infected mice developed acute myositis (Table 1). No mononuclear infiltration was found in the muscles of intact animals (about 99% intact muscle tissues).

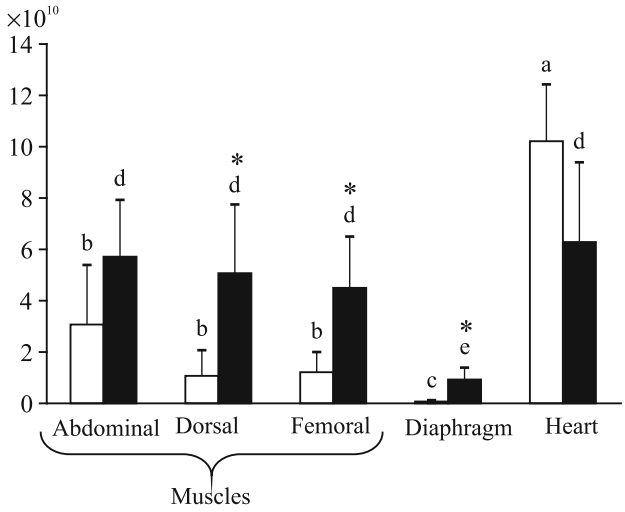

Fig. 1. Tissue parasitism (amastigote accumulation per mm³ tissue) in the heart and muscles of mice infected with parental Albarrada strain (light bars) and clone C104 (dark bars). *$p<0.05$ in comparison with parental strain (Friedman's test). The groups without significant differences were denoted as *a*, *b*, and *c* for C104 and *d* and *e* for the parental strain. Dunn's test for multiple comparisons showed the following values: $p=0.0283$ and variability coefficient 119.06 for clone C104; $p=0.0112$, variability coefficient 151.02 for the parental strain.

It should be noted that the diaphragm was significantly less parasitized and damaged than other striated muscles and the heart. It is intriguing, because *T. cruzi* was known to propagate in the host via the bloodstream [1] and the blood supply to the diaphragm was more intense than to other striated muscles [8]. Presumably, the parasite propagation through the bloodstream in this case was not a limiting factor. By the way, *T. cruzi* was capable of infecting a wide spectrum of mammalian cells *in vitro* [9], due to the presence of membrane proteins mediating the parasite invasion on these cells. On the other hand, the internal metabolic conditions of the host cell were extremely significant for the intracellular stage of the parasite development cycle.

Oxidative glycolytic metabolism is different in different muscle fibers, subdivided into fast-twitch glycolytic (FG), fast-twitch oxidative-glycolytic (FOG), and slow-twitch oxidative (SO). The FOG and SO fibers are characterized by high activities of the aerobic metabolism enzymes [10]. On the other hand, *T. cruzi* is highly sensitive to oxidative stress [11], and hence, muscles with high levels of SO and/or FOG fibers should be less suitable for parasite survival and, consequently, would be less affected during infection. Aerobic metabolism with low phosphorilase activity and low resistance to anaerobic conditions predominates

TABLE 1. Histopathological Parameters of Infected Muscles

Tissue/organ	A		B	
	percentage of visual field (at ×40) per sample with mononuclear infiltration, %		percentage of intact muscle fibers, %	
	clone C104	parental strain	clone C104	parental strain
Abdominal muscles	94.50 a	84.10 b	90.03 b	93.24 b
Diaphragm	53.00 b	35.32 c	96.30 a	93.24 b
Dorsal muscles	89.00 b	74.25 b	79.74 c	88.20 b
Femoral muscles	100.00 a	96.45 a	81.25 c	86.45 b
Heart	90.00 a	100.00 a	86.76 b	81.23 c
Mean P values (coefficient of variation)	0.0005 (27.19)	0.0041 (38.5)	0.0001 (98.44)	0.007 (9.42)

Note. No significant differences in the means denoted by the same letters (a, b, or c) for each variable (columns A and B). *n*=11.

in the diaphragm of small animals — mice [12], while other muscles are characterized by different proportion of muscle fibers [13].

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