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INFECTION WITH DIFFERENT *TRYPANOSOMA CRUZI* POPULATIONS IN RATS: MYOCARDITIS, CARDIAC SYMPATHETIC DENERVATION, AND INVOLVEMENT OF DIGESTIVE ORGANS

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Abstract. We tested four isolates of *Trypanosoma cruzi* to assess parasite virulence and ability to cause myocarditis, cardiac sympathetic denervation, and histopathologic alterations in organs of the digestive system. The susceptibility of rats depends on the population of *T. cruzi*, with the ABC strain and the CL-Brener clone killing all animals, regardless of the parasitemic pattern. All tested *T. cruzi* populations caused acute myocarditis, but failed to induce histologic alterations in the intestine. The Cl-Brener and ABC isolates caused esophageal myositis. The myocarditis caused by the ABC, CL-Brener, and Y isolates was severe, but resolution started at the end of the acute phase. In contrast, the Col 1.7G2 clone induced mild and sustained myocarditis. Our results also showed that *T. cruzi* populations able to induce severe acute myocarditis caused marked sympathetic denervation, but recovery of normal cardiac histology and innervation occurred. The sustained myocarditis induced by Col 1.7 G2 clone failed to cause sustained denervation.

INTRODUCTION

Chagas' disease (American trypanosomiasis), a long-lived disease caused by the hemoflagellate protozoan *Trypanosoma cruzi*, affects about 18 million people in Central and South America,[1] and it is becoming a health concern even in the United States.[2] Patent parasitemia (trypomastigotes) and proliferation of amastigotes in several tissues characterize the acute phase. The parasites then become rare in the blood and tissues, with most patients progressing to an asymptomatic form of the chronic phase of the disease. However, about 20–30% of the infected people progress to a symptomatic chronic phase with cardiac and/or digestive involvement.[3–5] Cardiomyopathy is the most common clinical manifestation of the chronic phase, with high mortality caused by congestive heart failure or arrhythmia. The digestive involvement may have epidemiologic implications since megacolon and megaesophagus have not been associated with chronic Chagas' disease in Colombia, Venezuela, Central America, and Mexico.[5] Studies of the hearts of patients who died of chagasic cardiomyopathy showed neuronal depopulation in the parasympathetic cardiac ganglia.[6] In patients with megaesophagus and megacolon, the reduction of the number of neuronal cell bodies in the myenteric plexus is also very impressive.[3,7] In an experimental model of Chagas' disease in rats, instead of counting neuron cell bodies, the autonomic innervation of the heart was assessed by histochemical and biochemical methods. Severe or complete disappearance of both sympathetic[8,9] and parasympathetic[10] nerve terminals was demonstrated to occur at the end of the acute phase. Afterwards, there is a gradual recovery of both kinds of autonomic innervation. At least the sympathetic denervation is independent of neuronal death or damage in cervical ganglia,[11] with the target being the cardiac nerve terminals.[12] These works showed that the rat is a suitable model for the study of *T. cruzi*-induced acute myocarditis and mechanisms involved in autonomic denervation. However, in all our previous studies, the *T. cruzi* infection was provided by inoculating 27–29-day old rats with a rather elevated inoculum (3×10^5 trypomastigotes) and only one strain of *T. cruzi*, the Y strain, was tested. This paper aims at a better understanding of the rat as a model for Chagas' disease by testing lower inocula and four different isolates of *T. cruzi*. Besides assessing the parasite virulence and ability to induce parasitism and inflammatory processes in the heart and organs of the digestive system, an important goal was to investigate the capability to induce sympathetic denervation in the heart.

MATERIALS AND METHODS

Parasites. Four isolates of *T. cruzi* were used: 1) the Col 1.7G2 clone derived from the Colombian strain[13] isolated from a Colombian patient with cardiomyopathy; 2) the CL-Brener clone recently isolated by Z. Brener and M. E. S. Pereira (FIOCRUZ at Belo Horizonte, Minas Gerais, Brazil) from the blood of a mouse chronically infected with the CL strain,[14] which was originally isolated from *Triatoma infestans*; 3) the ABC strain[14] obtained from a Brazilian patient with megacolon and cardiomyopathy; and 4) the Y strain[15] isolated from a Brazilian patient with Chagas' disease.

Infection and groups. Holtzman rats (27–29 days old) were divided into four groups, with each one being infected with one *T. cruzi* population. Each rat was inoculated intraperitoneally with 0.1 ml of mouse blood containing 10,000 trypomastigotes/50 g of body weight. Male rats were used as follows: 42 rats were infected with the Col 1.7G2 clone, 47 with the CL-Brener clone, and 27 with the ABC strain. In view of the high mortality induced by the CL-Brener clone and ABC strain, two additional groups of male rats were infected with 1,000 trypomastigotes/50 g (29 rats infected with the ABC strain and 32 rats with the CL-Brener clone). The Y strain-infected group was composed of 79 female rats. Previous studies showed no difference between male and female rats regarding the acute myocarditis and atrial denervation induced by the Y strain.[8,9] Fifty-four rats of the same sex and age were used as controls for histopathologic and sympathetic innervation studies.

The parasitemia was estimated in 5 μl of peripheral blood as described elsewhere[16] on alternate days from day 3 post-

infection until the end of the parasitemic period, as determined by the absence of circulating parasites for three consecutive alternate days. Mortality was assessed daily and the mortality rate was calculated after exclusion of the animals killed before the end of the patent parasitemia period.

Control and infected animals killed were under ether anesthesia at different periods of infection. Care was taken to kill the animals around the middle and end of the acute phase according to the parasitemic curves. Studies on the chronic phase were restricted to 120 days post-inoculation, except for the Col 1.7G2-infected group, in which the chronic phase was studied at days 74, 100, and 220 post-infection.

Histologic and histoquantitative methods. The following organ fragments were obtained from control and infected animals: the base of ventricles, the left auricular appendage, and fragments of inferior one-third of the esophagus, jejunum, and rectum. In the Y strain-infected group, the ventricles were not used. After fixation in 4% phosphate-buffered paraformaldehyde for 24 hr, the tissues were routinely processed for embedding in Paraplast (Polysciences,Warrington, PA). Seven-micron-thick sections were stained with hematoxylin and eosin.

The cardiac parasitism and inflammatory processes were estimated at 70-μm intervals to avoid recounting the same pseudocyst. The volumetric proportion of amastigote nests and inflammation were assessed with a Zeiss (Öberkohen, Germany) Kpl integrating eyepiece with 100 hits at a final magnification of 400×. For each cardiac region (right ventricle, left ventricle, ventricular septum, and left auricular appendage), the histologic structures coinciding with each hit were counted until a total of 5,000 hits was obtained. The histologic structures were classed as amastigotes nests in cardiomyocytes, cardiomyocytes without nests, normal stroma plus blood vessels, inflammatory processes, and artifacts. The percentages were statistically analyzed with SigmaStat (St. Louis, MO) software.

Histochemical study of the sympathetic innervation. For studying the noradrenergic innervation, the right auricular appendages were sectioned at 30 μm in a cryostat at −30°C. The sections were subjected to a glyoxylic acid-induced fluorescence method[17] for catecholamines, as routinely used in our laboratory.[12] The density of the fluorescent nerve terminals and preterminals was estimated in at least four sections of each atrial fragment at a magnification of 80× under a Leitz (Wetzlar, Germany) Orthoplan microscope with an HBO 100 mercury lamp. In each microscopic field, denervation, when present, was classed as discrete, moderate, severe, or complete. The final score was given after analysis made by at least three of the investigators, with two of them having examined all fragments.

RESULTS

Parasitemic curves and mortality. The analysis of all parasitemic curves allowed the identification of three parasitemic patterns (low, moderate, and high), with the aim of facilitating the comparison of different *T. cruzi* populations. Low parasitemias were characterized by individual values less than 600 trypomastigotes/5 μl of blood. Moderate parasitemias had individual values greater than 600 with peaks less than 5,000 trypomastigotes/5 μl. In high parasitemias, peak values were greater than 5,000 trypomastigotes/5 μl. The proportion of animals displaying each parasitemic pattern and its mortality rate are shown in Table 1.

TABLE 1
Incidence of the parasitemic patterns in rats and their mortality rate for each *Trypanosoma cruzi* population

T. cruzi Population (no.)	Parasitemic pattern (%)			Mortality rate per pattern (%)		
	Low	Moderate	High	Low	Moderate	High
Y strain (72)	33	43	24	8	27	100
ABC strain (27)	32*	60*	8*	100	100	100
ABC strain 1,000 (23)	65	35	0	80	62.5	NA
CL-Brener (41)	12	36.5	51.5	60	53	90
CL-Brener 1,000 (25)	40	60	0	25	100	NA
Col 1.7G2 (33)	70	30	0	10	31	NA

* Presumed values (all animals died before the end of the acute phase).
NA = not applicable.

The parasitemic patterns and the absolute mortality rate for each *T. cruzi* population are shown in Figure 1. The Col 1.7G2 clone caused the longest period of patent parasitemia and the Y strain the shortest one. After the inoculation of 10,000 trypomastigotes, low parasitemia characterized the infection with the Col 1.7G2 clone. In the infection induced by all other *T. cruzi* populations, moderate plus high parasitemia predominated. However, in animals infected with 1,000 trypomastigotes/50 g of body weight (ABC and CL-Brener), high parasitemias could not be detected. The inoculation of 10,000 trypomastigotes of the ABC strain caused the death of all animals in the period of ascending parasitemia regardless of its pattern. In the other groups, the animals died mainly after the period of patent parasitemia (Y strain), at its end (Col 1.7G2), or in the descending phase (CL-Brener). No animal with high parasitemia survived the acute phase. A few (6%) CL-Brener-infected animals survived the acute phase but died before day 100 post-inoculation. The inoculum of 1,000 trypomastigotes of both the ABC strain and the CL-Brener clone failed to induce high parasitemia, but the mortality was still elevated.

Tissue parasitism and inflammatory process in the heart. Myocardial parasitism and inflammation were always present in the heart during the acute phase, regardless of the *T. cruzi* population (Table 2). At the middle of the acute phase, the numbers of amastigote nests in cardiomyocytes were significantly lower in rats infected with the Col 1.7G2 clone compared with the values obtained in animals inoculated with 10 times less trypomastigotes of the ABC strain or CL-Brener clone. At the end of the acute phase, the nests became rare or virtually absent in all experimental infections. However, an amastigote nest in cardiomyocytes could still be found in an animal killed 220 days after the inoculation with the Col 1.7G2 clone (Figure 2C) The myocardial inflammation was significantly lower in the Col 1.7G2-infected animals during the acute phase. However, in contrast to the other infections, the inflammatory processes remained significantly different from those of the controls during the chronic phase in all cardiac regions of all Col 1.7G2-infected animals. At day 220 post-infection with this clone, a sustained inflammation was still clearly present (Figure 2).

Despite the higher inflammatory scores induced by both the ABC and CL-Brener isolates (inoculum = 1,000), the

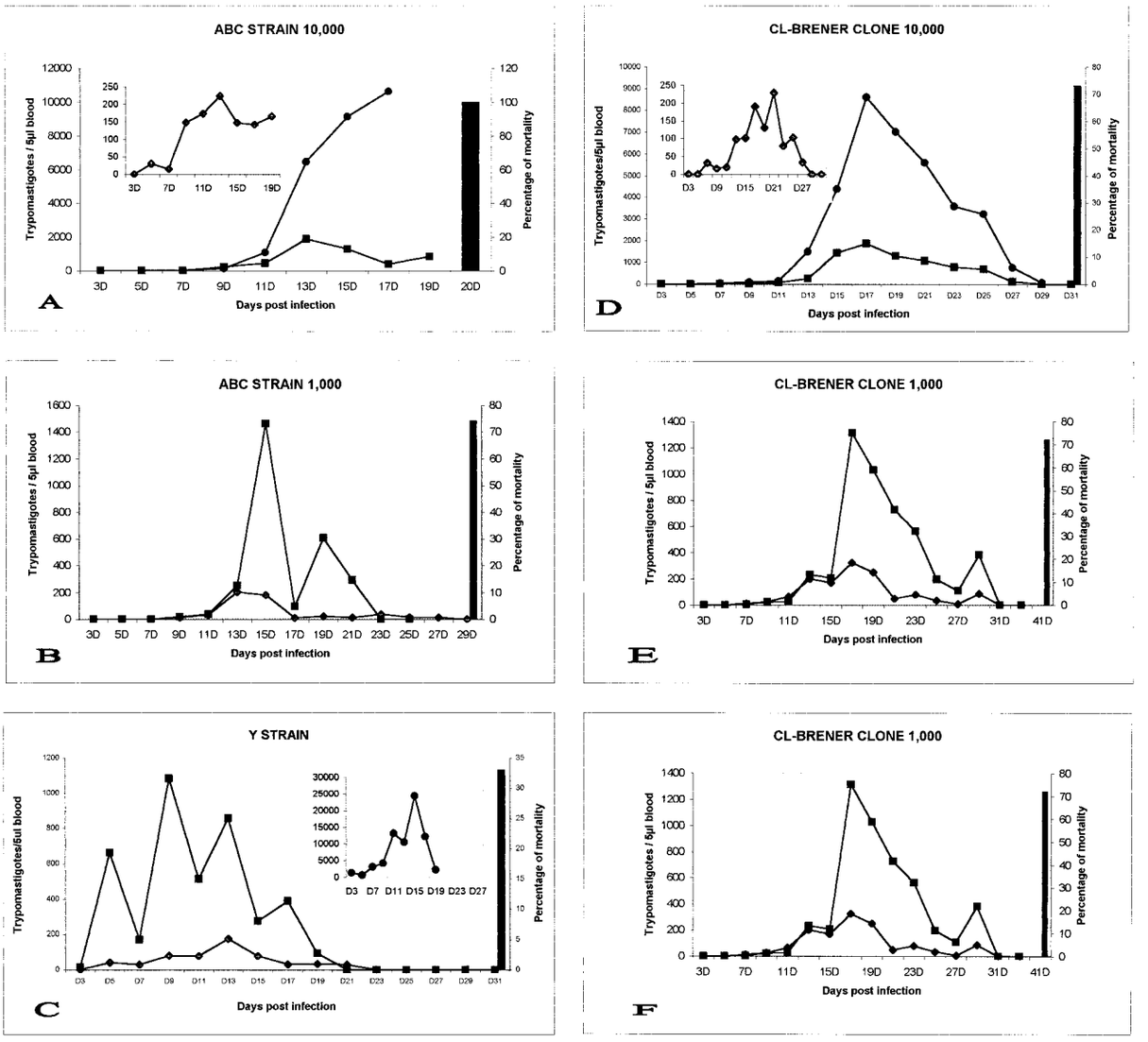


FIGURE 1. Parasitemic patterns in rats inoculated with different *Trypanosoma cruzi* populations (A–F). ♦ = low; ■ = moderate; ● = high. The solid bars show total mortality for each population. In A and D insets show low values; in C inset shows high values.

inflammation became significantly less intense at end of the acute phase and the cardiac histology was similar to that of control rats in the surviving animals killed at day 120 post-infection. However, sparse groups of infiltrating cells could still be observed in the ventricles. By comparing the ABC with the CL-Brener infection (inoculation of 1,000 trypomastigotes), the parasitism and inflammation were significantly higher in all ventricular regions of ABC-infected animals at the middle of the acute infection.

The histologic analysis showed a preferential location of the acute inflammatory process at the peripheral (epicardic) one-third of the ventricular free walls. In the ventricular septum, the inflammation concentrated at the endocardic myocardium facing the right ventricle cavity, that is, in the right septal wall. Even in the ABC infection with 10,000 trypomastigotes, the distribution of the amastigote nests depicted such ventricular gradient, despite the clearly high parasitism, except in the right ventricle of some animals in which severe inflammation and parasitism occurred all over the free wall thickness. The inoculation of 10,000 trypomastigotes of the ABC strain caused a devastating effect in the heart. After rupture of the numerous amastigotes nests (Figure 3A), intense inflammatory process (Figure 3B) parallel the severe damage of cardiomyocyte (vacuolation, reduction in the amount of myofibrils, and alteration of their striation pattern), and large distances among the cardiomyocytes (edema) were particularly evident in the right ventricle. In spite of the tendency for higher inflammatory values in the right ventricular free wall during the acute phase induced by all tested *T. cruzi* populations, significant differences were observed only for day 18 of the ABC strain infection (Table 2).

Parasitism and inflammation were quantitatively determined only in the auricular appendage of Y strain-infected animals with the aim of supporting the innervation studies. The parasitism values (mean ± SD) obtained at days 15 and

TABLE 2
Amastigote nests and inflammation in the right (RV) and left ventricles (LV), ventricular septum (VS), and left atria (LA) of rats

Strain/clone (no.)	Parameters	Day of infection	% of nests and inflammation (mean ± SD)			
			RV	LV	VS	LA
Col 1.7G2 inoculum = 10,000	nests					
(5)		20	0.008 ± 0.01	0.016 ± 0.036	0.012 ± 0.011	0.08 ± 0.12
(5)		30	0.076 ± 0.085	0.06 ± 0.082	0.016 ± 0.026	0.04 ± 0.047
(3)		42	0.00	0.00	0.00	0.00
	inflammation					
(5)		20	12.84 ± 4.5[a]	11.22 ± 8.20[a,b]	10.80 ± 2.51[a]	7.95 ± 4.06[a]
(5)		30	14.11 ± 6.45[a,b]	10.41 ± 5.07[a,b]	9.56 ± 4.21[a,b]	7.20 ± 2.91[a]
(3)		42	17.61 ± 1.52[b]	14.22 ± 2.99[b]	9.82 ± 0.62[a,b]	7.14 ± 2.02[a]
(6)		74	10.90 ± 2.5[e]	9.37 ± 4.51[a]	9.66 ± 5.88[a,b]	9.39 ± 3.74[a]
(4)		100	9.43 ± 2.90[a]	7.14 ± 1.7[a]	7.015 ± 2.07[b]	7.73 ± 1.40[a]
CL-Brener inoculum = 10,000	nests					
(6)		17	1.10 ± 0.96	0.27 ± 0.17	0.29 ± 0.15	0.48 ± 0.46
(8)		25	1.99 ± 4.62	1.23 ± 2.32	0.79 ± 1.54	0.35 ± 0.77
(9)		30	0.011 ± 0.027	0.028 ± 0.05	0.002 ± 0.006	0.004 ± 0.013
	inflammation					
(6)		17	24.8 ± 4.06[a]	20.18 ± 4.61[a]	22.53 ± 5.17[a]	22.25 ± 4.41[a]
(8)		25	19.53 ± 7.69[a,b]	17.28 ± 3.47[a]	16.19 ± 4.53[b]	17.38 ± 5.24[a]
(9)		30	14.84 ± 3.72[b]	15.93 ± 6.94[b]	14.69 ± 7.94[c]	9.95 ± 3.29[b]
CL-Brener inoculum = 1,000	nests					
(7)		16	1.08 ± 0.90	0.64 ± 0.70	0.29 ± 0.36	0.50 ± 0.27
(7)		30	0.00	0.00	0.00	0.00
	inflammation					
(7)		16	21.93 ± 3.22[a]	19.62 ± 3.77[a]	17.80 ± 3.58[a]	20.67 ± 7.30[a]
(7)		30	11.37 ± 3.61[b]	10.30 ± 2.20[b]	9.62 ± 8.38[b]	9.22 ± 2.46[b]
(5)		120	2.06 ± 0.86[c]	1.98 ± 0.53[c]	1.90 ± 0.59[c]	1.60 ± 0.13[c]
ABC inoculum = 1,000	nests					
(6)		18	3.60 ± 1.56	4.43 ± 1.92	2.60 ± 1.26	0.56 ± 0.11*
(5)		25	0.80 ± 1.79	1.60 ± 3.68	1.20 ± 2.68	0.00
	inflammation					
(6)		18	31.92 ± 0.30[a,*]	21.55 ± 0.26[a]	21.36 ± 0.3[a]	29.93 ± 0.30[a]
(5)		25	16.22 ± 2.94[b]	14.06 ± 0.70[b]	15.07 ± 3.62[b]	14.26 ± 0.17[b]
(5)		120	1.98 ± 0.50[c]	1.99 ± 0.33[c]	1.87 ± 0.12[c]	1.72 ± 0.20[c]
Inflammation in control rats						
(11)		16–18	2.57 ± 1.19	1.83 ± 0.37	1.77 ± 0.40	2.12 ± 0.63
(10)		25–30	3.60 ± 2.32	3.29 ± 1.41	3.05 ± 1.22	2.71 ± 1.29
(4)		42–73	3.28 ± 1.07	2.19 ± 0.47	2.17 ± 0.45	2.47 ± 0.45
(4)		100–120	1.64 ± 0.33	1.18 ± 0.30	1.40 ± 0.64	1.98 ± 0.68

[a,b,c] Inflammation values in each cardiac region with the same letters are statistically similar. SD = standard deviation.
* Value is different from others in the same horizontal line.

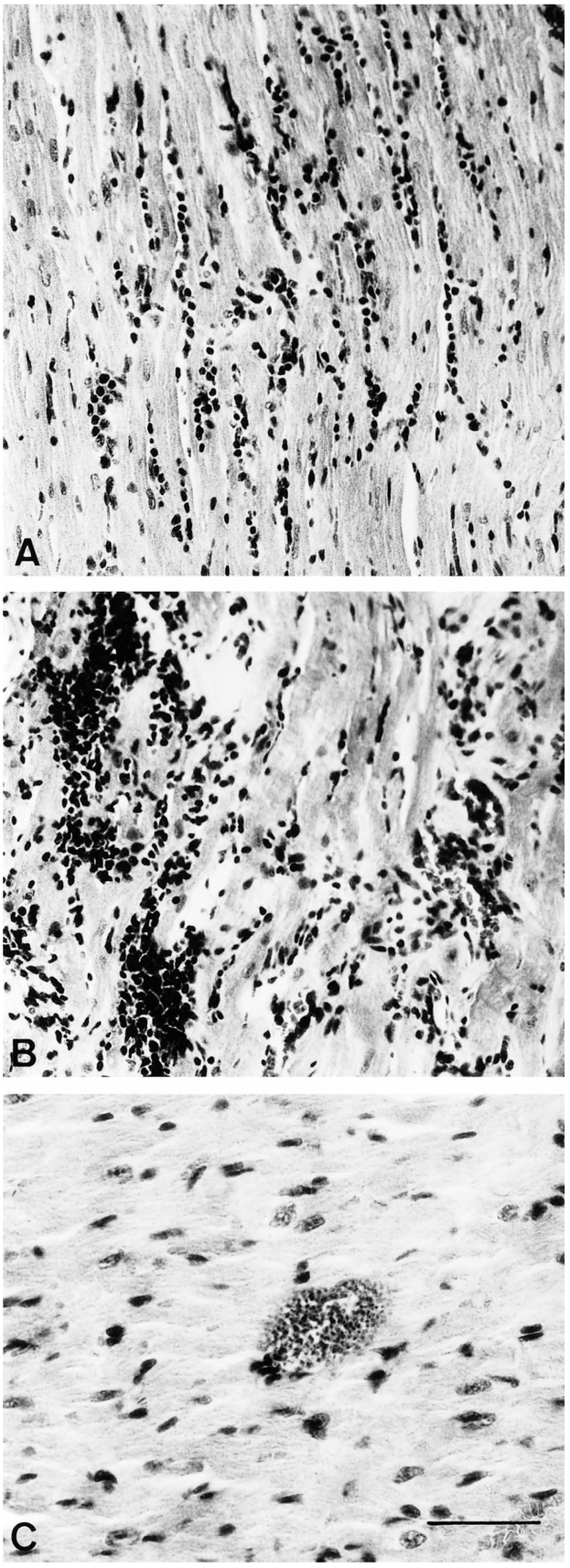
20 post infection were 0.76 ± 0.70 and 0.84 ± 0.96, respectively (n = 9 at both periods). The values for inflammation at days 15 (6.59 ± 2.17) and 20 (5.78 ± 3.01) post-infection differed from control values (0.46 ± 0.23, n = 5). At day 30 of infection, the inflammatory process had already been largely resolved (n = 16), although sparse and small groups of inflammatory cells were found.

Parasitism and inflammation in digestive organs. All *T. cruzi* populations failed to cause detectable parasitism and inflammatory process in the intestine (jejunum and rectum) at the middle and end of the acute phase as well as at day 100 or 120 post-inoculation with 10,000 trypomastigotes. The esophagus also remained unaffected during the infection with the Col1.7G2 clone or Y strain. However, some amastigote nests were seen in the esophageal muscle layer of all ABC-strain-infected animals killed at day 20.

The inoculation of 1,000 trypomastigotes of either the CLl-Brener clone or the ABC-strain caused discrete to severe inflammatory processes in the esophageal striated muscle layer in all infected animals at end of the acute phase. However inflammation was already present at the middle of the acute phase in the ABC-infected animals. At day 120 post-inoculation, no alteration was found in esophagi of the surviving CL-Brener or ABC-infected animals.

Cardiac noradrenergic innervation. All *T. cruzi* populations were able to cause a reduction in the amount of noradrenergic nerve terminals in the auricular appendages during the acute phase of the infection (Figure 4), as summarized in Table 3. In parallel with the histologic findings, the Col 1.7G2 clone caused the mildest denervation, with most animals showing discrete to moderate reduction of the fluorescent nerve terminals. With all other infections, severe or virtually complete denervation prevailed at the end of the acute infection.

In the Y strain-infected group there was correlation between the degree of denervation and the pattern of parasitemia in rats killed at days 20 and 30 post-inoculation. All animals with low parasitemias showed no or discrete denervation. Those with moderate and high parasitemias developed severe or complete denervation. However, the inflammation scores were very variable among the animals, with no correlation between the degree of denervation and the intensity of the inflammation. In all other groups, no corre-



lation could be made between parasitemia, inflammation, and denervation.

The noradrenergic innervation of atrial blood vessels was usually more resistant to the infection than the myocardial one. For example, some animals with moderate and severe myocardial denervation showed well-preserved vascular innervation in all infected groups, especially at the middle of the acute phase. Recovery of both myocardial and vascular innervation was generally observed in all infected animals that survived the acute phase, although myocardial areas with signs of denervation could still be found in animals at days 100–120 post-infection.

DISCUSSION

Before weaning, rats are very sensitive to *T. cruzi* infection but juvenile (approximately 30 days old) and adult rats have been considered to be resistant to *T. cruzi.*[18] Because of this, inocula varying from 5×10^3 to 1×10^6 parasites/rat or per gram of body weight[19–21] have been used to infect adult and juvenile rats. Despite the use of the rat as experimental model of Chagas' disease in many studies, comparative studies involving different *T. cruzi* populations and low inoculum values have not been done. Such comparative studies are rare even in the best studied species, the mouse. Our paper clearly shows that the susceptibility of rats depends on the population of *T. cruzi.* The ABC strain and CL-Brener clone killed all or almost all animals during the acute phase induced by the inoculation of 1×10^4 trypomastigotes, regardless of the parasitemic pattern. Even with a lower inoculum (1×10^3), both populations killed more than 70% of the infected rats.

The four *T. cruzi* populations tested in the present study were able to produce myocardial parasitism and inflammation but failed to induce appreciable alteration in intestinal smooth muscle. Regarding the esophagus, only the CL-Brener and ABC isolates induced consistent inflammatory processes restricted to the esophageal striated muscle layer. These results showed low or absence of tropism for smooth muscle either in the intestines or esophageal *muscularis mucosae.* Interestingly, the very sensitive BALB/c mice developed persistent inflammatory processes in the intestinal smooth muscle layer after inoculation of 50 trypomastigotes of the Col1.7G2 clone. This intestinal inflammatory process persisted for three months and became even more intense, and analysis using the polymerase chain reaction (PCR) confirmed the presence of the parasite in all animals.[22]

An important new finding disclosed by our histologic and histoquantitative analyses of the rat heart concerns the ability of *T. cruzi* populations to cause sustained or chronic myocarditis. The Col1.7G2 clone induced the longest acute phase with the lowest parasitemic values, the lowest mortality rate, and mild and sustained myocarditis. This sustained myocar-

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FIGURE 2. Different aspects of the chronic myocarditis observed in rats infected with the Col 1.7G2 clone of *Trypanosoma cruzi* and killed at day 220 post-inoculation **A,** diffuse and moderate; **B,** intense and focal; **C,** amastigote nest in an area without any inflammatory process. Bar = 100 μm.

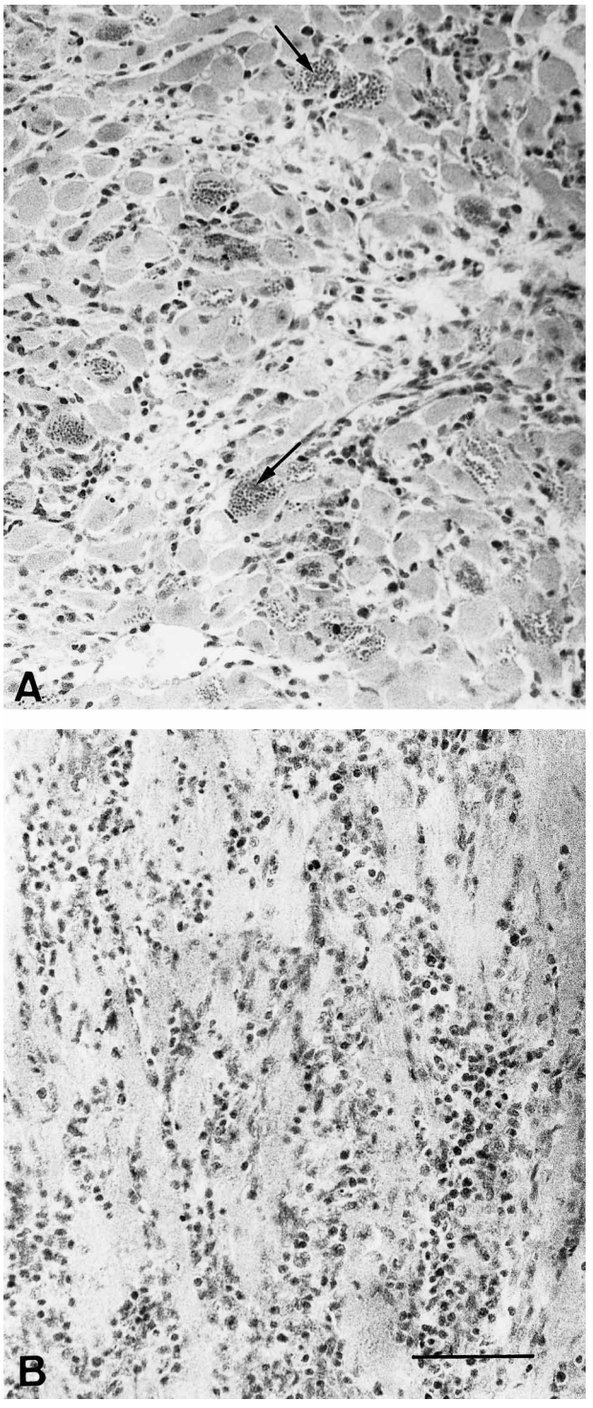

FIGURE 3. Aspects of the acute myocarditis in ABC-strain-infected rats killed at day 20 post-inoculation. **A,** numerous amastigote nests (**arrows**) and a diffuse inflammatory process. **B,** the intense infiltrate that follows the rupture of the nests. Bars = 100 μm.

ditis characterized all cardiac regions of all Col1.7G2-infected rats at least until day 220-post infection. The myocarditis values peaked at the end of the acute phase in the ventricle free walls, but remained about the same in the septum and auricular appendage at all periods of infection. In contrast, the Y and ABC strains and the CL-Brener clone induced a shorter acute phase in which the myocarditis was more intense at its middle, becoming significantly reduced at its end and virtually absent at day 120 of infection. The sustained inflammatory processes induced by the Col1.7G2 clone seems to involve persistent amastigote proliferation since rare nests were found until day 220. However, very rare amastigote nests were also found in ventricles of Y strain-infected rats at day 120 post-inoculation with 3×10^5 trypomastigotes in absence of persistent myocarditis.[9] Therefore, other factors might be involved in the ability of different *T. cruzi* population to induce sustained or chronic myocarditis. Judging by our results, a *T. cruzi* population able to induce an intense and fast host response results in a shorter acute phase (patent parasitemia) with complete recovery of the normal cardiac histology in the surviving animals.

The presence of parasites in the hearts of humans with Chagas' disease (chronic fibrosing myocarditis) was demonstrated by PCR,[23] reinforcing the notion that persistence of parasites is able to sustain the fibrosing myocarditis.[3,24] A question that cannot be tested in humans concerns the notion that chronic chagasic cardiomyopathy originates in the long-lasting indeterminate phase of the disease. Judging by our results in rats, the possibility of a sustained and mild myocarditis persisting for years until the onset of progressive fibrosis cannot be ruled out. *Trypanosoma cruzi* exists in nature as highly variable populations.[25–27] It is possible that in humans, some *T. cruzi* populations can behave like clone Col1.7G2 does in rats. In humans, the right ventricle is affected more than the left one in chronic Chagas' heart disease. In the murine model of the disease, inflammation and parasitism are also more severe in the right ventricle of the heart.[28] Our results in rats confirmed the tendency for greater involvement of the right ventricle, although this was statistically proved only for ABC strain-induced infection.

In mice, virulence and pathogenic capability vary for different *T. cruzi* populations.[25] However, no study has demonstrated the autonomic denervation induced by different *T. cruzi* populations, regardless of the host. We have confirmed that inoculation of 3×10^5 trypomastigotes of the Y strain in juvenile rats induces high parasitemia and intense acute myocarditis, as first demonstrated.[8] Such an inoculum, in parallel with the myocarditis, caused severe to complete cardiac sympathetic denervation assessed either by biochemical,[8] histochemical,[9] or ultrastructural[12] methods. The inoculation of 1×10^4 trypomastigotes caused different parasitemic patterns and the animals with low parasitemias may have had the cardiac sympathetic innervation preserved. However, the infections induced by the ABC strain and CL-Brener clone (inoculum = 1,000) induced severe acute myocarditis and sympathetic denervation, even in animals with low parasitemias. On the other hand, the Col 1.7G2 clone, characterized by low parasitemias, caused discrete (if any) denervation despite the sustained character of the myocarditis. Thus, some conclusions can be made based on our results involving parasitemia, myocarditis, and denervation. First, parasitemia might not reflect what is going on in the heart and other organs. Second, a sustained and mild myocarditis seems to be unable to cause histochemically detectable denervation. Third, the ability to rapidly halt the acute phase and control the myocarditis, as occurs in the Y, ABC,

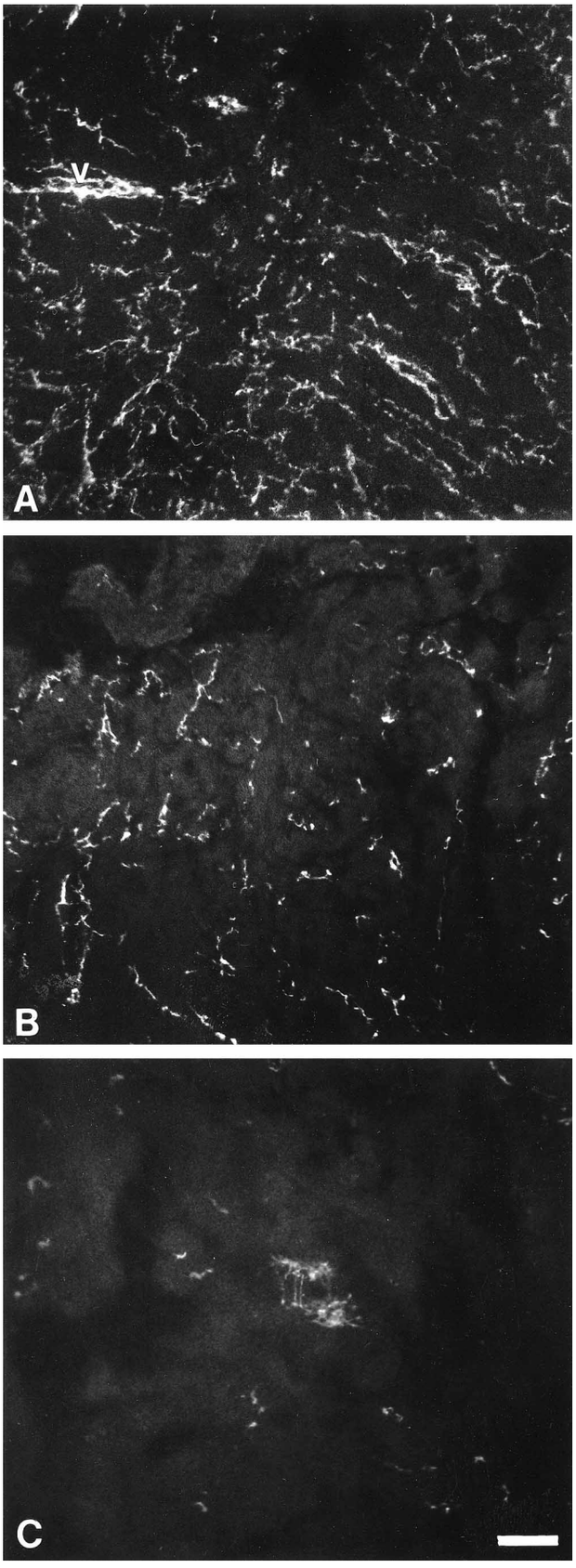

TABLE 3

Effect of different *Trypanasoma cruzi* populations on the myocardial sympathetic innervation in rats tested at the auricular appendage

Strain/clone period of infection (no.)	Degree of denervation				
	None	Discrete	Moderate	Severe	Complete
Col 1.7G2—10,000					
22 d (7)	0	4	2	1	0
30 d (5)	2	0	2	1	0
42 d (7)	3	3	1	0	0
74 d (6)	6	0	0	0	0
106 d (4)	4	0	0	0	0
220 d (4)	4	0	0	0	0
CL-Brener—10,000					
17 d (6)	0	0	4	2	0
25 d (8)	0	2	1	2	3
30 d (9)	1	0	2	5	1
CL-Brener—1,000					
16 d (7)	0	2	2	3	0
30 d (7)	1	0	2	4	0
120 d (6)	3*	2*	1	0	0
ABC—10,000					
20 d (9)	0	0	0	3	6
ABC—1,000					
18 d (6)	0	0	1	3	2
25 d (5)	0	0	1	2	2
120 d (5)	4	1*	0	0	0
Y—10,000					
15 d (4)	1	0	3	0	0
20 d (11)	0	3	0	1	7
30 d (16)	3	2	4	1	6
97 d (14)	8	1	4	1	0
uninfected rats (40)	40				

* Some animals exhibited areas with moderate denervation.
d = day.

and CL-Brener infections, might be crucial for the denervation process.

Different murine models of Chagas' disease have demonstrated the participation of cytokines and αβ T cells in parasite clearance and consequently in suspension of the acute phase.[29] On the other hand, γδ T cells have been implicated in mechanism leading to tissue damage and death.[30] In the myocardium, immunostaining has showed a predominance of $CD8^+$ T lymphocytes with few $CD4^+$ lymphocytes and macrophages.[31] Moreover, there is evidence for the participation of activated macrophages in the control of *T. cruzi* infection through the production of nitric oxide,[32–34] as occurs in infections with other intracellular parasites. The expression of tumor necrosis factor-α, interleukin-1β, and the inducible nitric oxide synthase (NOS2) is increased in the heart of infected mice. Immunocytochemical analysis has localized NOS2 in inflammatory cells, cardiomyocytes, and endothelial cells.[28] The few studies in rats have substantiated the participation of cytokines and inducible NOS2 in the

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FIGURE 4. Noradrenergic innervation of the auricular appendages. **A, v**ascular (**v**) and myocardial fluorescent nerve terminals in a control rat. **B,** moderate denervation in a Col 1.7G2 clone-infected rat killed at day 42 post-inoculation. **C,** virtual complete myocardial denervation in a CL-Brener clone-infected rat at day 25 of infection. In the center, two blood vessels show reduced innervation. Bar = 100 μm.

pathophysiology of acute chagasic myocarditis[21] or favor a major participation of $CD8^+$ cells.[34] Therefore, the mechanism for clearance of parasite could damage nerve terminals. Results obtained in rats depleted of radiosensitive cells favor a major participation of macrophages in such damage.[36] In the human chagasic chronic cardiomyopathy, there is faster or more severe autonomic denervation in comparison with other dilated cardiomyopathies, and sustained and severe chagasic myocarditis is probably involved.[37] There is *in vitro* evidence for murine macrophage-induced lesions of sympathetic neurons[38] and in rats, nitric oxide can be involved in neuronal death induced by *T. cruzi* infection.[39] The role played by nitric oxide in the denervation process, as well as the characterization of the infiltrating cells induced by different *T. cruzi* populations, is currently being investigated.

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