INFECTION AND IMMUNITY, Apr. 1982, p. 342-350
0019-9567/82/040342-09$02.00/0

Vol. 36, No. 1

Side Effects of Immunization with Live Attenuated *Trypanosoma cruzi* in Mice and Rabbits

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Received 8 September 1981/Accepted 8 December 1981

Immunity against lethal, bloodstream forms of *Trypanosoma cruzi* was achieved in mice by preinoculation of $\geq 10^5$ culture epimastigotes of an attenuated *T. cruzi* strain (TCC). The risks of TCC inoculation in terms of pathogenicity or eventual increase in virulence of TCC progeny were evaluated. No pathogenic parasites could be selected from TCC progeny by either mouse, triatome, or culture passages. Immunizing doses of live TCC did not induce in adult mice alterations resembling chronic Chagas' disease, as judged by patterns of mortality, tissue damage, autoantibodies, or parasite recovery. On the basis of the same criteria, however, a remarkable similarity could be established between the disease caused in mice by inoculation of low numbers (10^2) of pathogenic trypomastigotes and human chronic Chagas' disease. Although patent parasitemias were never revealed in fresh blood mounts obtained from TCC-inoculated mice, a few hemocultures and xenodiagnoses gave positive results, particularly soon after inoculations at birth. The parasites recovered by either method remained in the attenuated, epimastigote stage. In rabbits, no local lesions, fever, weight loss, or histopathological alterations were detected after subcutaneous inoculation of 10^7 TCC organisms, although one fifth of the animals yielded positive hemocultures of epimastigotes. The contrasting host response to cultured epimastigotes as compared with blood trypomastigotes indicates that, in experimental Chagas' disease, immunoprotection is not necessarily associated with immunopathology.

The antigenic variation expressed by most trypanosomes stands as a major obstacle frustrating attempts at vaccination. This would not seem to be the case with American trypanosomiasis (Chagas' disease), since the inducing agent, *Trypanosoma cruzi*, has not been shown to express variable antigenic types. Periodic peaks of parasitemia indicating escape from immunity have not been observed. The inoculation of epimastigotes from attenuated culture strains of *T. cruzi* induces in mice solid and lasting resistance against lethal trypomastigotes (15, 17).

The possibility of vaccinating against *T. cruzi* would thus seem tenable on the grounds of stability and strength of the antigens involved in protection. The impediments, however, involve rather the safety of immunogens and the possible untoward effects of induced immunity. After inoculation of live, attenuated *T. cruzi* cultures in mice, parasites have been occasionally recovered (2, 11), raising doubts as to whether the protection afforded by such inocula against lethal homologous parasites is mediated by sterile immunity, premunition, or even by the induction of chronic Chagas' disease. Moreover, even the inoculation of subcellular *T. cruzi* immunogens may induce lesions akin to those of chronic Chagas' disease [18; E. L. Segura, P. Cabeza Meckert, M. Esteva, R. Gelpi, A. R. Campanini, E. Subias, and R. P. Laguens, Medicina (Buenos Aires) **40**:807, 1981], thus suggesting that immunization may carry the hazard of unwanted immunopathology.

The studies reported here were undertaken to analyze the risk of pathogenicity which follows the inoculation of an immunizing culture strain of *T. cruzi* (TCC) and to determine whether the immunoprotection obtained is associated with immunopathology. For this purpose, experiments were carried out in both mice and rabbits to determine whether (i) parasites could be recovered from TCC-inoculated (immunized) animals, analyzing the pattern and conditions of recovery; (ii) the recovered parasites were pathogenic; (iii) under selective conditions, pathogenic derivates could be isolated from TCC sublines; and (iv) TCC-inoculated animals had any feature in common with those suffering from chronic Chagas' disease, on the basis of

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clinical, parasitological, serological, and histopathological observations.

MATERIALS AND METHODS

Animals. Outbred Swiss and inbred BALB mice of both sexes were used. Swiss mice were originally obtained from Carlos Malbrán Institute of Microbiology, Buenos Aires, and BALB mice were obtained from Jackson Laboratory, Bar Harbor, Maine. Both strains have been bred in our laboratory for several years. For specific purposes (see Results), mice of other strains were included. The ages and weights of the mice used varied with the experimental protocols (see Results). Adult Swiss mice were heavier (average weight, 32 g at 2 months) than BALB mice (average weight, 27 g at 2 months).

Female, 30-day-old Norfolk rabbits, weighing 880 g each (average), were used to test the effects of parasites. These animals were bred in our laboratory by parent animals obtained from a commercial supplier.

Parasites: mouse passage strain. The Tulahuén strain of *T. cruzi* was used throughout. It was passaged every 10 to 12 days in 1-month-old Swiss mice by intraperitoneal inoculation of blood containing 10^5 trypomastigotes. These inocula produced high parasitemia and were 100% lethal within 20 days.

Attenuated culture strain (TCC). An in vitro derivate of the Tulahuén strain was obtained from the Department of Microbiology, University of Buenos Aires. After initial mouse-to-mouse passages, which produced a strain of considerable virulence, a substrain was derived in vitro. This line has been maintained in a biphasic medium at 29°C for at least 8 years. During this period, variations in virulence were detected (16) which were consistent with a progressive loss of in vivo infectivity along with adaptation to in vitro growth. During this investigation, the parasites were subcultured weekly in glass bottles with biphasic liver-heart infusion medium (1), the agar layer containing 20% rabbit blood. A total of 100 IU of penicillin and 100 μg of streptomycin were added per ml of medium. Giemsa-stained smears showed that over 99% of the parasites were slender epimastigotes. Stout and spheric forms increased in older cultures. No trypomastigotes were observed.

Parasites used for inoculation were harvested from 7- to 14-day-old cultures which were in late logarithmic to early stationary phases of growth curves. The culture fluid was filtered through two layers of sterile gauze and shaken gently to minimize clumps and obtain a uniform dispersion of single parasites. After determining the concentration of a sample in a Neubauer chamber, parasites were diluted to the desired concentration in fresh culture medium, and 0.2 ml was injected intraperitoneally.

Fresh blood mounts. Blood obtained by sectioning the tail tip in mice or by heart puncture in rabbits was collected in a 0.01-ml capillary pipette and placed between a slide and a cover slip (20 by 20 mm; fresh blood mount). Mobile parasites were looked for in at least 100 0.32-mm-diameter microscope fields.

Xenodiagnosis. Groups of 10 second- or third-instar *Triatoma infestans* which had been fasted for a month were allowed to feed on each pentobarbital sodium-anesthesized mouse for 45 min in the dark. This feeding was repeated on two occasions that were 20 days apart. At 10 and 30 days after the last meal, fresh mounts of the pooled bug feces were diluted in culture medium and examined under a microscope for 15 min or until parasites were found.

Hemoculture. Blood collected under sterile conditions was seeded (0.2 ml) into Kahn tubes containing 1 ml of biphasic medium (see above). One milliliter of fresh medium was added every 20 days, and mobile parasites were searched for periodically under an inverted microscope until day 60. Two to five tubes were used per mouse.

Histopathology. Tissue samples from autopsied animals were fixed in 10% formaldehyde, and histological, hematoxylin–eosin-stained sections were studied. The lesions found (see Results) were scored blindly as severe, moderate, slight, or absent by one or two observers.

Serology. Animals were bled by retroorbital or heart puncture. After separation, the sera were used immediately or kept frozen at −20°C. Indirect immunofluorescence over tissue sections was carried out by using the technique of Coons and Kaplan (3). Cryostat sections of mouse skeletal muscle and myocardium adhered to glass slides were incubated with 1:10 test serum dilutions for 30 min at 37°C. The section was then rinsed with a 0.9% NaCl solution and incubated with an anti-mouse gamma globulin serum (Cappel Laboratories, Downington, Pa.) for 30 min at 37°C. After rinsing and mounting, the slides were observed under a fluorescence microscope.

RESULTS

Stability of the attenuated phenotype in TCC. Inoculation of up to 10^7 TCC organisms in newborn mice was apparently innocuous, no lethality with patent parasitemia having been observed in over 460 mice. To detect a possible shift toward more virulent progeny in different TCC sublines, a standard, short-term inoculation test was used; newborn (12- to 48-h-old) Swiss mice received 10^6 TCC organisms intraperitoneally. The lack of mortality and of parasitemia detectable in fresh blood mounts at different intervals within a 60-day period was taken as evidence for the attenuated behavior of the parasites.

Modified media. Since an increase in the infectivity of other Tulahuén sublines has been reported (16) after subculture in a medium containing either human or sheep blood in the agar base, TCC was cycled in such media. The inoculation tests of these sublines were negative (Table 1).

Mouse passage sublines. Hemocultures allowed the occasional recovery of epimastigotes from TCC-inoculated mice (see below). Parasites recovered after 15 and 370 days in vivo were submitted to inoculation tests, which were negative (Table 1). Serial blind passages from mouse to mouse were performed by inoculating 10^6 TCC organisms intraperitoneally in newborn mice. A week later, a homogenate was prepared from the blood, spleens, livers, lungs, hearts,

TABLE 1. Attenuation of various *T. cruzi* sublines: inoculation tests

Strain	Subline	TCC dose[a]	Positive parasitemia/no of determinations[b]	No. of mice dead at day 60[c]/total inoculated
TBT[d]	Mouse passage	1×10^3	6/7	7/7
TBT	63-Day culture, biphasic medium	1×10^6	3/5	0/5
TCC	Culture, biphasic medium	1×10^6	0/6	0/6
TCC	Culture, biphasic medium	1×10^7	0/11	0/11
TCC	First culture passage, human blood	1×10^6	0/63	0/18
TCC	Fifth culture passage, human blood	1×10^6	0/78	0/24
TCC	First culture passage, sheep blood	1×10^6	0/32	0/8
TCC	Fifth culture passage, sheep blood	1×10^6	0/54	0/17
TCC	First culture passage, goat blood	1×10^6	0/28	0/6
TCC	Fifth culture passage, goat blood	1×10^6	0/21	0/5
TCC	First mouse passage, hemoculture at day 15	1×10^6	0/200	0/32
TCC	First mouse passage, hemoculture at day 370	1×10^6	0/55	0/32
TCC	First to fourth blind mouse passages	ND[e]	0/30	0/28
TCC	Fifth blind mouse passage	ND	0/32	0/22
TCC	Triatome passage[f]	4×10^2	0/25	0/5
TCC	Triatome passage[g]	2×10^4	0/30	0/10

[a] Number of parasites inoculated intraperitoneally into newborn mice.
[b] Determinations were made on fresh blood mounts, 100 fields.
[c] The number of mice weaned was slightly less (overall, 5.78%) than the number born. This happened with either non-inoculated, sham-inoculated, or TCC-inoculated newborns. Therefore, litters were checked daily, and parasites were searched for in fresh blood mounts of the dead mice and their siblings. If found, death was attributed to trypanosomiasis. If not, lost mice were discounted from the total.
[d] TBT, Tulahuén blood trypomastigotes.
[e] ND, Not determined.
[f] Infected feces from triatomes fed with cultures (6).
[g] Infected feces from triatomes fed on suckling mice, inoculated at birth.

and muscles of four mice and reinoculated intraperitoneally into other newborn mice. This procedure was repeated five consecutive times. No mortality, parasitemia (Table 1), or histopathological lesions were detected in any passage.

Triatome passage sublines. Insects were infected with TCC by two procedures. In the first procedure, they were allowed to feed directly on TCC cultures across a latex membrane (6). In the second procedure, they fed on suckling mice which had recently been inoculated with TCC. Although the feces of these bugs contained numerous epimastigotes, the inoculation tests of the triatome-cycled parasites were negative (Table 1). No histopathological lesions (see below) were detected at day 60 in the inoculated mice. Other trials which failed to reveal an increase in the virulence of TCC included culture at 37°C, admixture of human blood to the inoculum, and inoculation into several strains of mice (C3H/Ep, C57BL/Ks, BALB × DBA, Swiss × AKR, BALB × C57BL/Ks, and BALB × AKR).

Long-term search for chronic pathology in mice immunized with live TCC. Preinoculation with TCC protected mice against lethal inocula of Tulahuén blood trypomastigotes. After determining the range of useful immunizing TCC doses, an investigation was undertaken to determine whether these doses might produce chronic pathogenic effects at different times postinoculation.

Titration of immunizing doses of live TCC. Groups of 1-month-old mice received single immunizing intraperitoneal inoculations of either culture medium alone or TCC in 10-fold-increasing doses, from 10 to 10^7. All animals were challenged intraperitoneally 44 days later with 5×10^4 Tulahuén blood trypomastigotes. It was observed that, whereas small TCC doses had no protective effect, higher doses were 100% protective (Table 2). Since 10^5 TCC organisms was a dose inducing full protection in both BALB and Swiss mice, further studies on the risk of immunization were performed in mice inoculated with doses exceeding 10^5 TCC organisms.

Life-span, appearances, and spleen and body weights of TCC-inoculated mice. Newborn or adult Swiss mice received intraperitoneal inocu-

TABLE 2. Titration of cultured *T. cruzi* (TCC strain) doses which immunize against blood trypomastigotes

Mouse strain	TCC dose[a]	No. dead at day 60/total (%)	
Swiss	10^7	0/3	(0)
Swiss	10^6	0/6	(0)
Swiss	10^5	0/6	(0)
Swiss	10^4	0/6	(0)
Swiss	10^3	2/6	(33)
Swiss	10^2	3/6	(50)
Swiss	10	3/4	(75)
Swiss	Medium alone	5/10	(50)
BALB	10^7	0/4	(0)
BALB	10^6	0/6	(0)
BALB	10^5	0/6	(0)
BALB	10^4	1/6	(17)
BALB	10^3	6/6	(100)
BALB	10^2	6/6	(100)
BALB	10	6/6	(100)
BALB	Medium alone	8/8	(100)

[a] Number of parasites preinoculated intraperitoneally into adult mice challenged later with 5×10^4 trypomastigotes.

lations of either medium alone or TCC and were kept under observation for their entire life-span (28 months). No differences in survival were detected between TCC-inoculated and normal animals (Fig. 1). No weight loss or general signs of disease were observed. There was a temporary increase (×1.60) in average spleen weight, which peaked at 2 weeks and returned to normal values at 4 weeks post-inoculation. In comparison, trypomastigotes in sublethal doses of as low as 10 parasites produced acute signs of infection (dyspnea, hunched position, inactivity, furry hair), weight loss, and lasting splenomegaly (average spleen weight, ×2.45).

Histopathology. After inoculating mice with 10^6 TCC organisms intraperitoneally, animals were sacrificed at different times for autopsy and histological studies. Because the tissue lesions (Fig. 2 and 3) induced by blood trypomastigotes of the parental Tulahuén strain were most often found in the skeletal muscle and heart (8), these tissues were selected for study. Samples were routinely taken from the quadriceps muscle, colon, and myocardium.

After TCC inoculation into 1-month-old mice, 9 animals were examined after 1 month, 15 after 5 months, and 11 after 10 months. Only 2 of these 35 mice presented slight focal mononuclear infiltrates in their skeletal muscles. In comparison, 17 of 19 mice inoculated with Tulahuén blood trypomastigotes had moderate to intense lesions (myositis and myocarditis [Fig. 4 through 6]).

Some lesions were found, however, after TCC inoculation at birth; 10 BALB mice which received 10^6 TCC organisms intraperitoneally at birth were sacrificed at day 14. Slight or moderate myositis with no amastigote nests was found in five mice (Fig. 7). No lesions suggestive of Chagas' disease were observed in the hearts, smooth muscles, livers, spleens, lymph nodes, lungs, kidneys, or skins of these mice.

Antibodies against muscle. Among 28 mice which had received 10^6 TCC organisms intraperitoneally, immunofluorescence studies failed to reveal significant increases in antinuclear factors and antibodies against red, white, or smooth muscle (Table 3). In comparison, parallel determinations in mice inoculated with Tula-

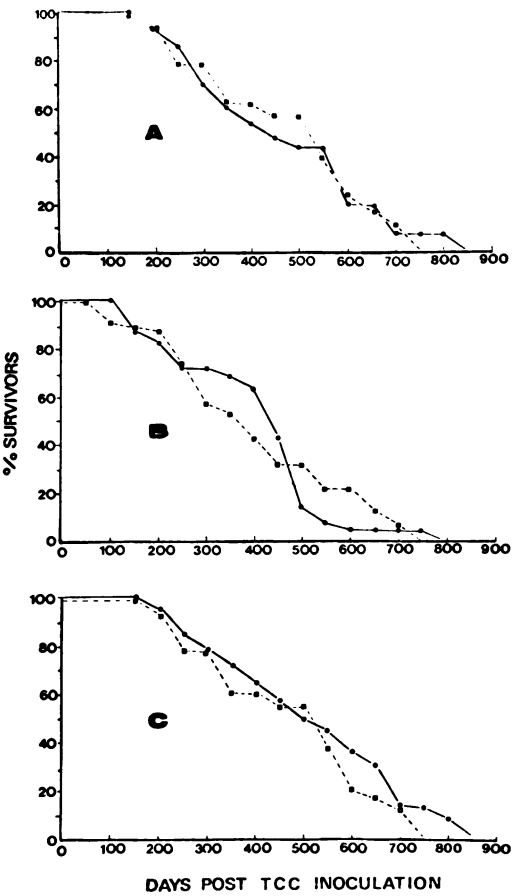

FIG. 1. Survival of mice as a function of time. Symbols: ●—●, mice inoculated with *T. cruzi* (TCC strain); ■- - -■, controls inoculated with culture medium. (A) Swiss mice (70 days old) were inoculated with 2×10^6 TCC organisms (experimentals, $n = 35$; controls, $n = 18$); (B) BALB mice (48 h old) were inoculated with 2×10^6 TCC organisms (experimentals, $n = 39$; controls, $n = 30$); (C) Swiss mice (48 h old) were inoculated with 2.7×10^6 TCC organisms (experimentals, $n = 52$; controls, $n = 18$).

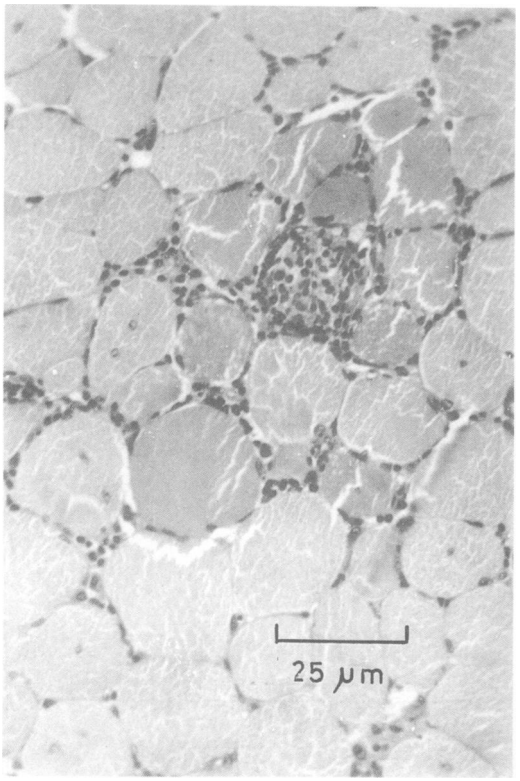

FIG. 2. Hematoxylin–eosin-stained transversal section of skeletal muscle from a Swiss mouse chronically infected with *T. cruzi* (Tulahuén blood trypomastigotes). A heavy interstitial lymphomonocytic infiltrate and fibers with different degrees of eosinophilia are present.

huén blood trypomastigotes revealed an increase in antibodies against smooth muscle ($P < 0.02$) and appearance of antibodies against red muscle ($P < 0.01$).

Detection and recovery of parasites. Trypanosomes were searched for in blood at various times after TCC inoculation, using direct parasitoscopy, xenodiagnosis, and hemoculture. In over 530 determinations, the direct observation of fresh blood mounts never revealed any parasites. The variables studied included age at inoculation (from 2 to 488 days), mouse strain (eight different strains), and age of the culture (5 to 32 days).

Xenodiagnoses allowed the regular detection of parasites only in mice inoculated with TCC at birth and tested before day 30 (Fig. 8). Determinations made later or in mice inoculated at ages 1 (Fig. 8) and 7 (0 of 10; data not shown) months only exceptionally detected parasites. In comparison, xenodiagnoses performed in mice chronically infected with 10^2 Tulahuén blood trypomastigotes were 80% (12 of 15) positive at 5.5 months post-inoculation.

Hemocultures performed in mice inoculated with TCC at birth allowed the regular recovery of parasites during the first 20 days. Thereafter, recovery was variable but more frequent (overall rate 16%) than recovery with xenodiagnosis (Fig. 9).

Inoculation of rabbits. Five 30-day-old rabbits received subcutaneous dorsal inoculations of 10^7 TCC organisms. Periodical records were kept of average body weights on days 2, 7, 14, 21, 27, 42, 49, and 56; rectal temperatures on days −3, −1, 0, 1, 2, 5, 11, 12, 35, and 56; aspect of inoculation sites on days 0, 1, 2, 5, 9, 14, 21, 27, 42, 49, and 56; fresh blood mounts on days 10, 21, 30, and 60; hemoculture on days 10 and 60; and autopsies with histological studies at day 60 (up to 17 sections of heart, skeletal muscle, colon, and lung were studied). Except for two of four positive hemocultures taken in one rabbit, no alterations were observed in either inoculated or control animals. Average body weight pro-

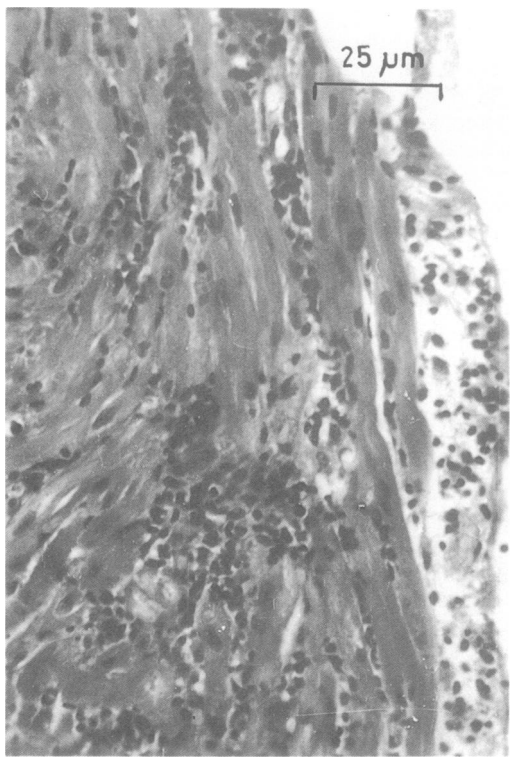

FIG. 3. Hematoxylin–eosin-stained section of heart tissue (atrium) from a Swiss mouse chronically infected with *T. cruzi* (Tulahuén blood trypomastigotes). A significant interstitial lymphomonocytic infiltrate can be observed, which also involves epicardial tissue (right edge).

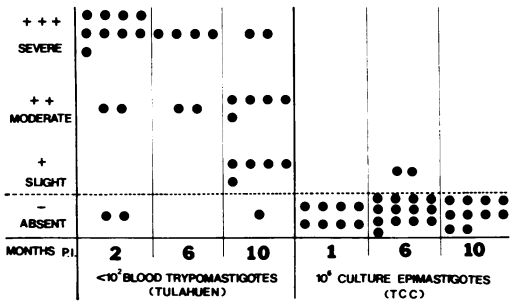

FIG. 4. Evaluation of tissue damage found in skeletal muscles of Swiss mice inoculated with *T. cruzi*. Lesions consisted of myositis (interstitial mononuclear infiltrates and edema), followed by fiber degeneration. One sample was missing.

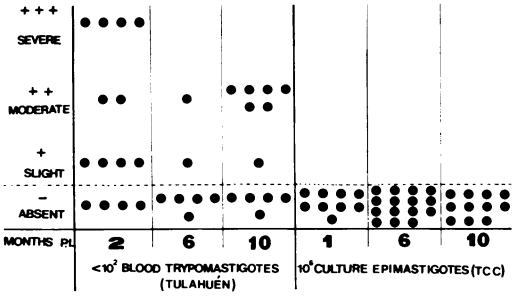

FIG. 6. Evaluation of tissue damage found in smooth muscles (colons) of Swiss mice inoculated with *T. cruzi*. Lesions consisted of mononuclear infiltrates.

gressed normally from 880 g when inoculated to 1,820 g when autopsied. The sites of inoculation, except for the needle puncture, were unrecognizable by inspection or palpation.

DISCUSSION

The potential risk of using live vaccines against *T. cruzi* infection has mostly been evaluated in terms of infectivity of the immunizing parasites. Since under favorable experimental conditions, parasites of all culture strains tested (2, 14, 16) have been recovered from immunized animals, the concept prevails that attenuated *T. cruzi* cultures are unsafe as immunogens.

On similar grounds, however, organisms not regarded as pathogens are demonstrably infective. These include vaccines widely used in humans, such as BCG, smallpox, polio, and yellow fever. With the use of each of these vaccines, recovery of the live organism from vaccinated individuals has been demonstrated (4, 5, 9, 10). It seemed, therefore, that beyond the mere recovery of parasites, other safety criteria were worth analyzing in the TCC subline. These included pathogenicity of culture, mouse or triatome-cycled parasites, possible immunopathological effects, and late consequences of TCC inoculation.

One set of experiments was designed to determine whether the attenuated behavior was a circumstantial or a permanent phenotype of the TCC subline. A circumstantial attenuation occurs when a virulent strain, such as Tulahuén, enters the epimastigote stage upon in vitro cultivation. A usually lethal Tulahuén dose became unable to kill mice after a single in vitro passage (Table 1). Should this be the basis for TCC attenuation, it might soon reverse as a favorable environment (triatome gut, mouse blood) selects the differentiated, trypomastigote stage. Yet all

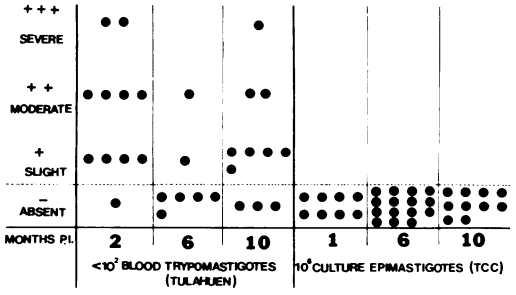

FIG. 5. Evaluation of tissue damage found in myocardia of Swiss mice inoculated with *T. cruzi*. Lesions consisted of intense perivascular or moderate interstitial mononuclear infiltrates, either focal or diffuse. Two samples were missing.

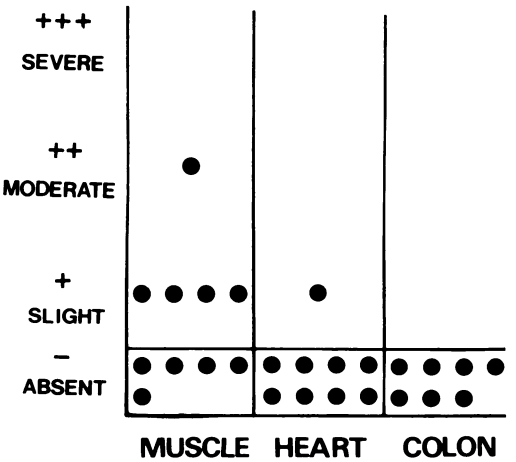

FIG. 7. Evaluation of tissue damage found among 10, 14-day-old BALB mice inoculated with 10^6 cultured *T. cruzi* organisms (TCC strain) at birth. Tissues and lesions were as described in the legends to Fig. 4, 5, and 6. Four samples were missing.

TABLE 3. Anti-muscle autoantibodies detected by immunofluorescence in Swiss mice inoculated with culture (TCC) or blood strains of *T. cruzi*

Inoculum[a]	No. of mice	% Positive increase in:			
		Antibodies against:			
		Skeletal muscle		Smooth muscle	Antinuclear factors
		Red	White		
TCC epimastigotes	28	16	7	0	3
Blood trypomastigotes	11	64[b]	0	36[c]	18
Normal controls[d]	17	18	6	0	6

[a] Mice were 1 to 3 months old when inoculated. Dose was 10^6 TCC organisms and ranged from 1×10^2 to 2×10^5 trypomastigotes. The following times elapsed between parasite inoculation and serum sampling. TCC: 1 month (10 mice), 5 months (8 mice), and 7.5 months (5 mice); trypomastigotes: 4.5 months (3 mice), 6 months (3 mice), 11 months (1 mouse), and 12 months (4 mice).

[b] $P < 0.01$ by the chi-square test.

[c] $P < 0.02$ by the chi-square test.

[d] 9 months old.

attempts to generate or select such stages from TCC were unsuccessful. Either culture-triatome or culture-mouse-triatome recycling yielded only epimastigote-containing triatome stools, not demonstrably infective or pathogenic for newborn mice. Mouse passage yielded only occasionally positive hemocultures of attenuated epimastigotes. Serial mouse-to-mouse passage was unsuccessful, suggesting that the capacity of TCC epimastigotes to differentiate into trypomastigotes may be lost or blocked as a consequence of prolonged adaptation to in vitro culture.

The behavior of TCC in virulence tests contrasts with that found in Tul Oh (16), a *T. cruzi* subline related to TCC ancestry. Since this subline was found to be virulent for mice after cultivation in human blood-containing medium, it would so far be premature to attribute a permanent or mutational character to the attenuation found in TCC.

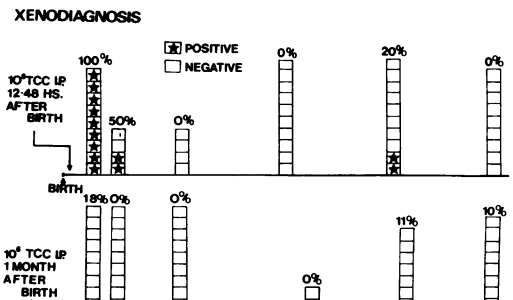

FIG. 8. Xenodiagnoses performed in mice inoculated with 10^6 cultured *T. cruzi* organisms (TCC strain). Each square represents a mouse. The left edge of each column indicates, on the month scale, the time post-inoculation when the first triatome application was performed.

The resistance to reinfection elicited by injection of attenuated *T. cruzi* cultures has been attributed to either persisting infection (7, 15), also called premunition, or to sterile immunity (12). The presence of trypanosomes does not seem to be a requirement for resistance to reinfection, as shown by Morrow et al. (13) in nifurtimox-cured mice challenged 4 months post-immunization. The pattern of TCC recovery after immunizing inoculation revealed a few cases of persistent infection lasting for at least a year. In most mice, however, such evidence was negative. Proof that these animals became sterilized was difficult to obtain, since negative hemocultures and xenodiagnoses provided no solid evidence that they were free of parasites; there are limits of detection, and a repository of noncirculating parasites may remain in some tissues. In spite of these considerations, the pattern of TCC recovery, restricted to mice inoculated at birth and examined within a month, supported the theory that after TCC inoculation, cured infections could prevail in a majority of mice.

Chronic pathology, similar in several respects to human Chagas' disease, can be observed in various animal models after inoculation of sublethal *T. cruzi* doses. The recovery of parasites from TCC-inoculated mice and rabbits prompted the search for similar pathology in these animals. This was approached through clinical, serological, and histopathological determinations. Comparative studies were performed between TCC- and Tulahuén blood trypomastigote-inoculated Swiss mice for periods exceeding a year; rabbits were controlled periodically for a 2-month period. Immunizing TCC doses were innocuous for 1-month-old mice, as judged by several criteria which simultaneously re-

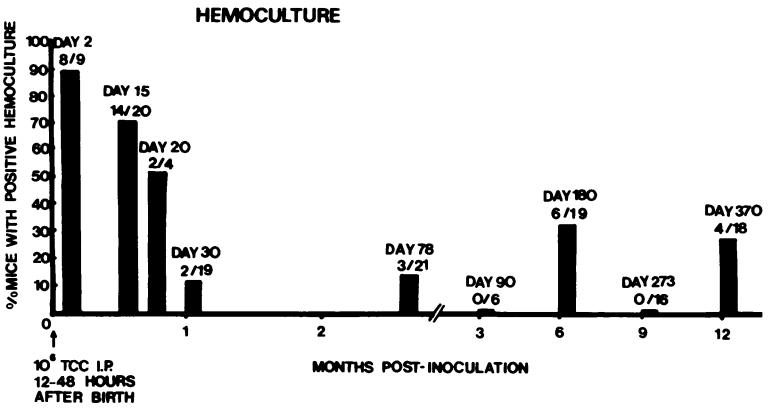

FIG. 9. Percentage of positive hemocultures plotted against time after inoculation of 10^6 cultured *T. cruzi* organisms (TCC strain) into newborn mice.

vealed chronic pathology in trypomastigote-inoculated mice. No indication of disease was found in rabbits, even though one of five yielded positive TCC hemocultures. In newborn mice, however, the inoculation of 10^6 TCC organisms led to findings consistent with an acute, self-limiting disease. The transient peak of parasitemia, detectable by hemoculture in most mice, was associated with slight, reactive muscle infiltrates, detectable in half of the animals at day 14. These findings are consistent with the alterations found by Pizzi (14) in a detailed, although shorter, study of mice inoculated with attenuated *T. cruzi* cultures. The tissue alterations he described consisted of acute inflammation and restoration of normality within a month. It is clear from our results that the alterations found after TCC inoculation do not bear any resemblence to those associated with Chagas' disease.

The pattern of antibodies against muscle did not distinguish between the sera of TCC-immunized and normal mice. The fact that positive sera correlated with tissue lesions after inoculation of trypomastigotes and not with parasite immunity after TCC inoculation may help in interpreting the still debated origin of autoantibodies in Chagas' disease.

Since immunization of mice and rabbits with subcellular *T. cruzi* material may produce tissue lesions in the heart and skeletal muscle [18; Segura et al., Medicina (Buenos Aires) **40**:807, 1981], questions arise as to why such an effect is lacking in mice immunized with live, attenuated parasites. The explanation may be related to the amount and dynamics of distribution of the parasite material injected. The former method involves the injection of repeated doses with a rather high (1,000 μg per mouse) protein content. Immunization with live parasites, on the other hand, seems to require a mild infectious process. This difference may offer a basis for the induction of either predominantly pathogenic or protective responses.

Because findings about the immunopathology of chronic Chagas' disease point to possible untoward effects of vaccination, the results presented here are encouraging, since they constitute direct evidence that strong protective immunity can be obtained without immunopathology.

ACKNOWLEDGMENTS

We are grateful to C. D. Pasqualini for unrelenting support, E. D. de Isola for providing the original seed of the TCC subline, and to A. Bellouard, B. Basombrío, and M. B. Casanova for their technical assistance.

This investigation was supported by grants from the Consejo Nacional de Investigaciones Científicas y Técnicas.

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