measurements will be necessary to confirm this. If Ln^{3+} ions competitively block Ca^{2+} ion uptake in this system, the presence of millimolar concentrations of Ca^{2+} in culture medium would explain the ineffectiveness of low concentrations of Ln^{3+} ions.

Inhibition of lymphocyte activation in vivo could contribute to the purported anti-inflammatory properties of the lanthanides. This suggestion is supported by the observation that several steroidal and non-steroidal anti-inflammatory drugs inhibit lymphocyte proliferation in vivo [30, 31]. Gold chloride shares this property [32], an interesting finding in light of the antiarthritic properties of gold drugs. More than one author has speculated that lanthanides might find therapeutic use in arthritis [5, 6, 10, 11].

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Absence of immunosuppression in DBA/2 mice vaccinated with *Trypanosoma cruzi* treated with actinomycin-D

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Summary. The subcutaneous (s.c.) vaccination of DBA/2 mice with 4 weekly doses of 3×10^7 living metacyclic forms of *T. cruzi*, Y strain, obtained from culture in axenic medium and treated for 24 h with actinomycin-D (50 μg/10^7 parasites), a drug that promotes an irreversible blockade of the parasite replication, do not induce any detectable degree of humoral and cellular immunosuppression as assessed by a) the production of anti-SRBC antibodies, b) the permanence of delayed cutaneous reaction to *T. cruzi* antigen, to PPD and DNCB and c) the degree of blastogenic transformation of spleen lymphocytes in the presence of the specific antigen.

Key words. T. cruzi; immunology; *T. cruzi* vaccination; DBA/2; actinomycin-D; immunosuppression.

Acute infection of mice with *T. cruzi* usually induces a state of immunosuppression, varying in duration and depending on the biological characteristics of the parasite, the dose and route of its inoculation and the genetic constitution of the host [2, 4, 8–11]. Several hypotheses have been formulated to try to explain the mechanism of this suppression [1, 5–7].

A potent immunogen is obtained by treatment of viable metacyclic culture or blood forms of *T. cruzi*, Y strain, with an adequate dose of actinomycin-D, a drug that

blocks DNA synthesis, rendering the parasites irreversibly incapable of in vitro and in vivo multiplication [8]. In this publication we show that the vaccination of DBA/2 mice with metacyclic *T. cruzi* treated with actinomycin-D does not induce detectable immunosuppression of humoral and cellular responses to different immunogens, as assessed by tests performed 15 and 30 days after the last dose of vaccination.

Material and methods

Animals. DBA/2 female mice, 4–6 weeks old, weighing 18 ± 20 g were used in all experiments.

Parasites. Viable trypanosome metacyclic forms of *T. cruzi*, Y strain, were obtained from epimastigotes cultured in LIT-agar-blood medium at 28 °C during 2 weeks. Cultured parasites were purified by chromatography on DEAE-cellulose (Sigma Chemical Co. USA) equilibrated with phosphate-saline-glucose, pH 8.0, ionic strength 0.185.

The parasites were then treated with actinomycin-D (Sigma Chemical Co. USA) (50 μg/10^7 parasites) in RPMI medium (GIBCO Laboratories, Grande Island, USA) for 24 h at 28 °C. The parasites were washed twice before use.

Vaccination schedule. Mice were injected s.c. with 4 doses of 3×10^7 actinomycin-treated motile metacyclic parasites at 7-day intervals. Unvaccinated animals were used as control.

Plaque-forming-cells (PFC). Determinations of the number of splenic cells forming direct hemolytic plaques [3] were carried out 4 days after i.p. injection of 10^7 SRBC.

Cutaneous hypersensitivity to PPD (purified protein derivative). Three weeks after an i.p. injection of 10^7 viable BCG, 5 animals, from vaccinated and control groups, were ear-tested by intradermal injection with 10 μg of PPD. The ear thickness was determined 48 h later with a dial micrometer (Mitutoyo, Japan).

Contact hypersensitivity to DNFB. The vaccinated animals were sensitized to DNFB by epicutaneous application of 20 μl of 0.5% 2,4-dinitrofluorbenzene (Sigma Chemical Co. USA) solution to abdominal skin. Reactions were elicited 15 days later by application of 20 μl of 0.1% DNFB in the ear. The ear swelling was read 48 h later with a dial micrometer.

Delayed cutaneous reaction to T. cruzi. 10 mice vaccinated 30 days before with actinomycin-treated *T. cruzi* were subjected to the i.d. footpad test with 50 μg of a sonicated extract from *T. cruzi*, Y strain, epimastigotes. The footpad swelling were read 48 h later.

Blastogenic transformation of lymphocytes. Mice vaccinated 30 days before with actinomycin-treated *T. cruzi* were inoculated i.p. with 100 μg of viable BCG. 21 days later the animals were killed and their spleen cells cultivated in RPMI medium supplemented with 10 μg/ml gentamycin (Schering, Rio de Janeiro), 3 mM L-glutamine (Sigma Chemical Co. USA), AB inactivated normal serum and 0.05 mM 2-mercaptoethanol (Eastman Kodak Co. USA). To each well of a microtiter plate were added 4×10^5 spleen cells and either 10 μg/ml phytohemagglutinin-P (PHA, Sigma Chemical Co. USA), or 20 μg/ml PPD or 50 μg/ml *T. cruzi* antigen, in 0.1 ml RPMI medium. Control wells received spleen cell suspension only. Cells were cultivated at 37 °C for 72 h. 20 h before cell harvesting, each well received 1 μCi of tritiated thymidine (^{3}H)TDR, sp. act. 6.7 Ci/mM (New England Nuclear Co., USA). The amount of ^{3}H-thymidine incorporated by lymphocyte DNA was measured in a liquid scintillation counter.

Statistics. The statistical analysis was carried out using Student's t-test for independent small samples.

Results

The data presented in tables 1 and 2 show the results of immunological studies carried out in DBA/2 mice 30 days after s.c. vaccination with 4 weekly doses of 3×10^7 of mobile metacyclic actinomycin-D-treated *T. cruzi*, Y strain.

The number of hemolytic plaques against SRBC formed by 10^6 spleen lymphocytes were found to be statistically similar in both vaccinated and unvaccinated animals.

Table 1. Animals vaccinated with *T. cruzi* treated with actinomycin-D and submitted to: A) immunization with SRBC 4 days before tests for PFC; B) intracutaneous tests with *T. cruzi* antigen; reaction evaluated by footpad swelling (48 h); C) immunization with viable BCG, evaluated by ear swelling test; D) sensitization with 0.5% DNFB, challenged 48 h later.

Groups (5 animals)	Plaque forming cells (10^6 spleen cells)	Footpad swelling (48 h) 50 μg *T. cruzi* antigen (×0.01 mm)	Ear swelling (48 h) (×0.01 mm)	
			10 μg PPD	20 μl DNFB 0.1%
A	2487 ± 420	–	–	—
	2518 ± 447[a]	–	–	–
B	–	21 ± 0.20	–	–
	–	2 ± 0.05[a]	–	–
C	–	–	6.45 ± 0.97	–
	–	–	5.13 ± 1.25[a]	–
D	–	–	–	14.66 ± 1.66
	–	–	–	12.67 ± 1.17[a]
p(t)[b]	NS[c]	< 0.01	NS	NS

[a] Unvaccinated control animals. [b] Probability, Student's t-test. [c] No significance.

Table 2. Spleen cells ^{3}H-thymidine incorporation in the presence of 50 μg/ml *T. cruzi* antigen, 20 μg PPD and 10 μg PHA: 1) animals vaccinated with *T. cruzi* treated with actinomycin-D; 2) normal unvaccinated animals (control); 3) vaccinated and sensitized with BCG 20 days before test; 4) sensitized with BCG only (control).

Groups (5 animals)	^{3}H-thymidine incorporation (cpm ± SD) × 10^{-3}		
	T. cruzi antigen (50 μg/ml)	PHA (10 μg/ml)	PPD (20 μg/ml)
1	7.82 ± 0.93	15.05 ± 1.50	–
2	0.92 ± 0.25	16.53 ± 1.02	–
3	–	–	3.27 ± 0.72
4	–	–	3.83 ± 0.64
P(t)[a]	< 0.001	NS[b]	NS

[a] Probability, Student's t-test. [b] No significance.

Similar results were consistently demonstrated in several lots of mice. The vaccinated mice displayed normal delayed (48 h) footpad reactions to 50 μg *T. cruzi* antigen. Vaccinated animals immunized with BCG or DNFB, and later challenged, showed normal specific ear swelling reactions to intradermal tests with 10 μg PPD and to epicutaneous tests with 0.1% DNFB solution. At the same time, these animals gave normal in vitro responses to *T. cruzi* antigen, to PPD and PHA as assessed by ^{3}H-thymidine incorporation.

Discussion

The results of the experiments presented here showed clearly that the metacyclic culture forms of *T. cruzi*, Y strain, previously treated with an adequate dose of actinomycin-D (50 μg/10^7 parasites) and injected s.c. at 7-day intervals (3×10^7 non-replicating parasites), do not induce humoral or cellular immunosuppression in adult DBA/2 mice.

Experiments carried out in our laboratory have repeatedly shown that these metacyclic forms of *T. cruzi*, Y strain, which have become incapable of multiplication by the intercalating action of actinomycin-D on the parasite DNA, represent an excellent immunogen, capable of inducing a vigorous stimulation of the immune response followed by a long lasting state of immunostimulation.

Several reasons can be invoked to explain the favorable results obtained in the vaccination with our immunogen: 1) There was no multiplication of the parasites inside the antigen-presenting cells (macrophages, dendritic cells); this made possible the presentation of *T. cruzi* antigens to T-lymphocytes under conditions much more efficient than those found in macrophages heavily loaded with replicating parasites. 2) The parasites were unable to enter nonphagocytic cells, thus the disturbing effects of a high degree of tissue parasitism were avoided. 3) The actinomycin-D-treated *T. cruzi* keep their mRNA with an apparently normal function for a relatively long period of time (about 15 days in vitro). 4) As shown here, the actinomycin-D-treated parasites can no longer induce immunosuppression, even after repeated s.c. inoculations of large numbers.

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Lysosomal mutations increase susceptibility to anaesthetics

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Summary. The anaesthetic responses of homozygous mutant mice were compared with those of their normal heterozygous littermates. The two recessive mutations studied were beige (*bg*) and reduced pigmentation (*rp*). Homozygosity for either significantly increased the sleeping time of both sexes after treatment with pentobarbital, tribromoethanol or the steroid anaesthetic alphaxalone.
Key words. Pentobarbital; tribromoethanol; alphaxalone; sleeping time; beige mutation; reduced pigmentation gene; mice.