

RESISTANCE OF MICE IMMUNIZED WITH KILLED CULTURE TRYPOMASTIGOTES AGAINST INFECTION BY INSECT-DERIVED TRYPOMASTIGOTES OF TRYPANOSOMA CRUZI

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SUMMARY

Mice immunized with heat or merthiolate-killed culture trypomastigotes of the non-virulent G strain were resistant to the challenge by insect-derived trypomastigotes of the CL strain of *Trypanosoma cruzi*. No parasitemia was detected, by direct microscopic examination of blood samples, in 90% of immunized mice while all control animals developed a high parasitemia. Trypsinization before heat-inactivation, or fixation with paraformaldehyde, apparently reduced the immunogenicity of the G strain trypomastigotes. Mice immunized with trypomastigotes treated by either of these procedures were not protected against infection by virulent *T. cruzi*. Analysis of the ¹³¹I-labeled surface proteins of G strain trypomastigotes inactivated by the various methods suggests that these components are involved in eliciting protective immunity against *T. cruzi* infection.

INTRODUCTION

Infection by *Trypanosoma cruzi*, the agent of Chagas' disease, is initiated when the metacyclic trypomastigotes contained in the feces of triatomid vectors penetrate into the vertebrate host. In the acute phase of the disease the parasites multiply intracellularly as amastigotes and circulate in the blood as trypomastigotes. In the chronic phase a steady balance is established between host and parasite during which parasitemia is hardly detectable. The resistance acquired by chronically infected animals appears to be mediated by cellular as well as by humoral immune response (BRENER²).

Aiming at the control of Chagas' disease through vaccination, many attempts have been made to immunize animals against experimental *T. cruzi* infection. Negative results or partial protection with reduced parasitemia have been reported (BRENER & CAMARGO⁴). Immunogens tested included attenuated forms of the parasite (BASOMBRIO & BESUSCHIO¹), organisms inactivated by irradiation (HANSON

et al.⁷; OKANLA et al.¹³), killed parasites (KIERSZENBAUM & BUDZCO⁸; NEAL & JOHNSON¹²) or sub-cellular fraction (LEON et al.¹⁰). In most experiments blood trypomastigotes, and more extensively epimastigotes, have been used.

In the present study we compared the effectiveness of culture trypomastigotes of *T. cruzi*, killed by different methods, in the immunization of mice against challenge by trypomastigotes from the insect vector. In addition, we also examined the effect of the various inactivation procedures on the surface proteins of culture trypomastigotes.

MATERIAL AND METHODS

Two strains of *T. cruzi* were used: strain CL, isolated by BRENER & CHIARI³ and strain G, isolated by Mena Barreto from an opossum captured in the Amazon region. The parasites were maintained in mice and in triatomid vectors *Triatoma infestans*. Cultures

were established by transferring blood from infected mice to liver infusion tryptose medium (CAMARGO⁵) and the parasites were maintained in this medium for 5 to 7 serial subcultures.

Trypomastigotes obtained from *T. infestans* were used to infect mice. Triatomids with 2 month-old infections were fed on mice and 30-60 minutes later the drops of clean transparent liquid (urine) eliminated by the insects, and containing almost pure (> 90%) metacyclic trypomastigotes, were pooled and used to inoculate mice. When large numbers of trypomastigotes were required, the parasites were harvested from the culture medium, washed 2 times in phosphate buffered saline containing 5.4% of glucose (PSG), pH 8.0, passed through a DEAE-cellulose column and eluted with PSG. The eluate contained only trypomastigotes, as determined by microscopic examination.

For immunization of mice we used DEAE-cellulose-purified culture trypomastigotes of the G strain, killed by various treatments. We selected procedures that did not alter the morphology of the parasites, as determined by light microscopy: a) heating at 50°C for 10 min; b) treatment with 0.1 mg/ml of merthiolate for 10 min at room temperature, followed by 2 washings with PSG; c) fixation with 2% para-formaldehyde for 30 min at room temperature, followed by 3 washings with PSG; d) treatment with 1 mg/ml of trypsin for 30 min at 37°C, in PSG, followed by 3 washings with PSG and heating at 50°C for 10 min. Groups of 10 albino male mice received, by intraperitoneal route, 6 weekly doses of 10⁷ killed metacyclic trypomastigotes of the G strains. Ten days after the last immunizing dose, the vaccinated mice, as well as age and sex matched controls, were challenged with 2 x 10³ virulent trypomastigotes of the CL strain derived from insect vec-

tors. The course of infection was followed for 30 days after inoculation of infective forms by examining 5 µl blood samples under a phase microscope.

Trypomastigotes were labeled with ¹³¹I by the Iodo-Gen method (MARKWELL & FOX¹¹), as described by CAMARGO et al.⁶ Iodinated parasites were disrupted with 0.5% Nonidet P-40 in the presence of the following protease inhibitors: leupeptin (25 µg/ml), antipain (25 µg/ml), aprotinin (2 trypsin inhibiting units/ml). The supernatant obtained by centrifugation at 12,000 x g for 10 min was diluted in 0.08M Tris buffer, pH 6.8, containing 2% SDS, 10% glycerol, 10% mercaptoethanol and 6M urea, and analysed by sodium dodecyl sulphate poly-acrylamide gel electrophoresis (LAEMMLI⁹) in slab gels, using 3% and 7% gels for the stacking and running gels respectively. After fixation in a mixture of 25% methanol and 7% acetic acid solution, the gel was exposed to an X-ray film. Proteins with molecular weights ranging from 14,400 to 94,000 were used as markers.

RESULTS

The efficiency of G strain trypomastigotes killed by different methods in affording protective immunity to *T. cruzi* infection was determined. As shown in Table I, only mice immunized with heat or merthiolate-killed trypomastigotes, but not with trypomastigotes treated with para-formaldehyde or with trypsin before heating, were resistant to *T. cruzi* challenge. In mice immunized with heat or merthiolate-inactivated organisms the parasitemia was either undetectable (90% of cases) or was transient and greatly reduced (10% of cases), while all control animals developed a high parasitemia (Fig. 1).

T A B L E I

Resistance of mice immunized with killed culture trypomastigotes against challenge with virulent forms of *T. cruzi* (*)

Immunization with G strain trypomastigotes killed by	No. of mice with negative parasitemia/No. challenged	% of protected mice
Heating	18/20	90
Trypsin + Heating	1/9	11
Merthiolate	18/20	90
Para-formaldehyde	2/20	10
Non-immunized control	0/30	0

(*) Mice were inoculated intraperitoneally with 6 weekly doses of killed G strain trypomastigotes from culture and challenged with 2 x 10³ CL strain trypomastigotes from *T. infestans*. The course of infection was followed for 30 days by examining 5 µl-blood samples under a contrast phase microscope

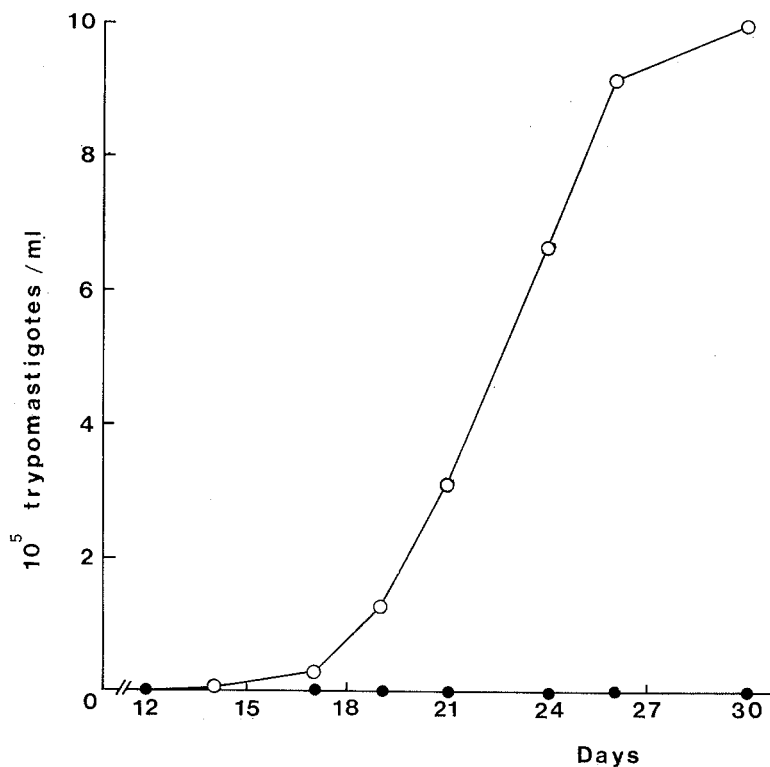


Fig. 1 — Time-course of *T. cruzi* infection in mice inoculated with 2×10^8 metacyclic trypomastigotes of CL strain derived from *T. infestans*. Non-immunized mice (○-○), mice immunized with heat or merthiolate-killed G strain trypomastigotes from culture (●-●). The values correspond to average parasitemia of 10 animals

Analysis of ^{131}I -labeled surface proteins of G strain metacyclic trypomastigotes killed by different procedures revealed that the 3 major bands, with apparent molecular weights between 70,00 and 100,00, present in non-treated trypomastigotes (Fig. 2, lane 1), were preserved in either heat or merthiolate-inactivated parasites (Fig. 2, lanes 2 and 4, respectively). However, after para-formaldehyde treatment, the 85,000 molecular weight component was barely detectable (Fig. 2, lane 5). And trypsinization extensively digested the 3 major surface proteins (Fig. 2, lane 3).

DISCUSSION

In this paper we report that mice immunized with heat or merthiolate-killed trypomastigotes resisted effectively to the challenge with metacyclic trypomastigotes of *T. cruzi* derived from the insect vector. Parasitemia was negative in 90% of immunized mice and barely detectable in the remaining 10%, while control animals developed a high parasitemia. However, protection was not complete since the presence

of parasites in mice with apparently negative parasitemia could indirectly be demonstrated through hemoculture. Immunization with trypomastigotes treated with para-formaldehyde or with trypsin before heating did not protect the animals.

It is interesting to note that the same ^{131}I -labeled surface proteins found in non-treated trypomastigotes were also detected in heat or merthiolate-inactivated trypomastigotes, whereas treatment with trypsin or para-formaldehyde significantly altered the trypomastigote surface protein profile. Taken together with the immunization experiments, these results suggest that the surface proteins of trypomastigotes may be involved in inducing protective immunity against *T. cruzi* infection. SNARY¹⁴ reported that *T. cruzi* glycoproteins confer partial protection against metacyclic trypomastigote challenge. Another indication of the immunogenic properties of trypomastigote surface proteins is provided by the observation that heat-killed trypomastigotes of *T. cruzi* CL strain, which differs from the G strain in its surface protein make-up (YOSHIDA¹⁵), are not

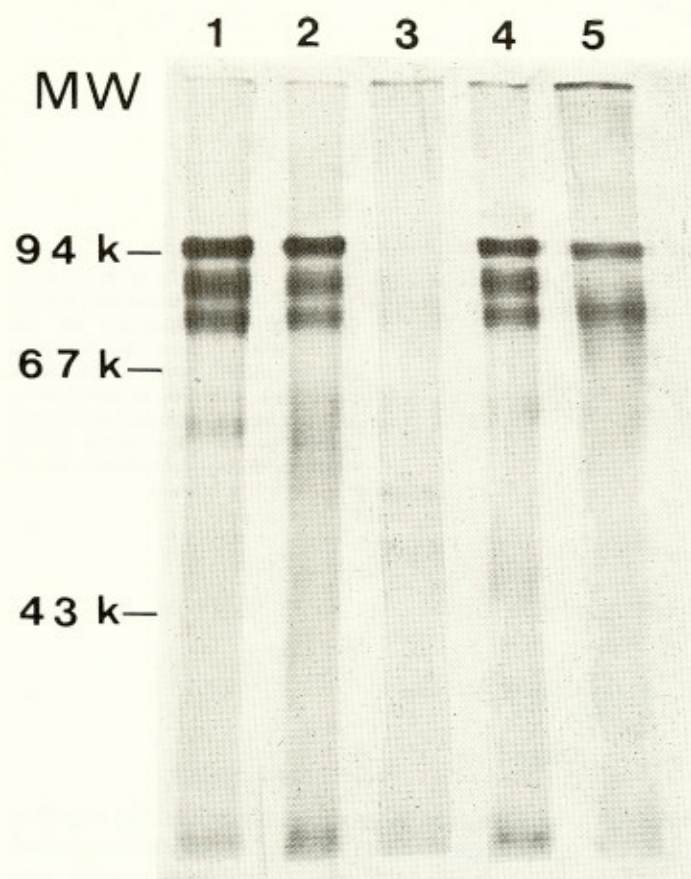


Fig. 2 — Sodium dodecyl sulphate polyacrylamide gel electrophoresis of ^{125}I -labeled extracts of culture trypomastigotes of *T. cruzi* G strain. Before surface labeling the parasites were subjected to heating (lane 2), trypsin treatment (lane 3), merthiolate (lane 4) and para-formaldehyde (lane 5). Lane 1 corresponds to the ^{125}I -labeled extracts of untreated trypomastigotes

as effective as the G strain in eliciting protective immune response.

The present study on the immunization of mice against *T. cruzi* suggests that a systematic search for an appropriate strain and immunization procedure may eventually lead to a vaccine which confers full protection.

RESUMO

Resistência de camundongos imunizados com tripomastigotas de cultura mortos contra infecção por tripomastigotas de *Trypanosoma cruzi* provenientes do inseto

Camundongos imunizados com tripomastigotas de cultura da cepa G, mortos pelo calor ou mertiolato, mostraram-se resistentes à infecção por tripomastigotas da cepa CL de *T. cruzi* provenientes do inseto vetor. Em 90% dos camundongos imunizados não foi detectada parasitemia patente ao exame microscópi-

co enquanto todos os animais-controle desenvolveram alta parasitemia. Tripsinização seguida de aquecimento, ou fixação com para-formaldeído, aparentemente reduziram a imunogenicidade de tripomastigotas da cepa G, visto que camundongos imunizados com tripomastigotas tratadas por qualquer destes métodos não foram protegidos contra infecção por *T. cruzi*. A análise de proteínas de superfície de tripomastigotas da cepa G, inativados por diferentes métodos, marcados com ^{125}I , sugere que esses componentes de superfície estão envolvidos na indução da imunidade protetora contra *T. cruzi*.

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