Surface Antigens of Metacyclic Trypomastigotes of *Trypanosoma cruzi*

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The surface antigen makeup of metacyclic trypomastigote forms of strain G of *Trypanosoma cruzi*, which produce a subpatent infection in mice, differed from those of the virulent strains Y and CL. A 100,000-molecular-weight protein, barely detectable on the Y or CL cell surface, appeared as the main surface antigen of the G metacyclic trypomastigotes. In addition, the G metacyclic forms differed from those of the virulent strains in their susceptibility to complement-mediated immunolysis.

*Trypanosoma cruzi*, the causative agent of Chagas’ disease, is a protozoan parasite with a complex life cycle. In vertebrate hosts *T. cruzi* is found as intracellular amastigotes and bloodstream trypomastigotes, whereas epimastigotes multiply in the digestive tract of insect vectors. Epimastigotes can further differentiate to form metacyclic trypomastigotes which are infective to mammalian hosts.

Clinical manifestations of infection by *T. cruzi* vary widely, but the cause of such diversity is unclear. Since the variability of the clinical picture of Chagas’ disease may reflect infection by distinct *T. cruzi* strains (2), attempts have been made to characterize strains of *T. cruzi*, particularly by analyzing the cell surface components (1, 12, 14). However, by using either amastigotes, blood trypomastigotes, or culture epimastigotes, the presence of common, rather than strain-specific, surface antigens has been reported.

The present work is aimed at characterizing the surface components of metacyclic trypomastigotes, which so far have been poorly studied, and at verifying the possibility of distinguishing strains of *T. cruzi* through the determination of the surface antigen makeup at this developmental stage.

Three strains of *T. cruzi* were used: Y, isolated from an acute case of Chagas’ disease (13); CL, derived from the vector *Triatoma infestans* (3); and G (obtained from E. P. Camargo and originally from Mena Barreto), isolated from an opossum in the Amazon region. The parasites were maintained through cyclic passages in outbred albino mice and in the triatomid *Dipetalogaster maximus*. Short-time cultures were established by seeding blood trypomastigotes from infected mice into liver infusion-tryptose medium (4). Parasites were maintained in this medium for four to five passages. To obtain pure trypomastigotes, cultures of *T. cruzi* in liver infusion-tryptose medium were harvested, washed twice in phosphate-buffered saline (pH 8.0) containing 5.4% glucose, and then passed through a DEAE-cellulose column, using the same buffer for the eluent. The eluate contained 99 to 100% metacyclic trypomastigotes, whereas epimastigotes were retained on the top of the column.

Immune sera were prepared by injecting rabbits with four doses of purified metacyclic trypomastigotes of strain Y or G killed either by heating at 50°C for 10 min or by treatment with merthiolate (0.1 mg/ml) for 10 min. Anti-CL serum was produced in rabbits by giving two intravenous injections of $10^8$ live culture forms at 15-day intervals. Human immune sera were from Hospital das Clínicas, São Paulo, Brazil.

Surface labeling of the parasites with $^{131}$I was carried out by the Iodo-Gen method (11), as described by Carmago et al. (5). Metacyclic trypomastigotes iodinated by this procedure retained full infectivity to mice. The iodinated parasites were treated with Nonidet P-40 at a final concentration of 0.5% in the presence of the following protease inhibitors: leupeptin (25 μg/ml), antipain (25 μg/ml), and phenylmethylsulfonyl fluoride (Sigma Chemical Co.) (1 mM). The supernatant obtained by centrifugation at 8,000 × g for 10 min was used as parasite extract. Labeled extracts were immunoprecipitated with various immune sera, according to the procedure of Kessler (6) as previously described (16). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (10) was performed in slab gels, using 3% and 5 to 12% gradient gels for the stacking and running gels, respectively. After fixation in 25% methanol–7% acetic acid solution, the gel was dried and exposed to an X-ray.
film (Sakura Film Co.). Proteins with molecular weights ranging from 14,000 to 94,000 (Pharmacia Fine Chemicals) were used as markers.

I observed a considerable difference in the patterns of $^{131}$I-labeled extracts of metacyclic trypomastigotes of strains Y, CL, and G (Fig. 1). Two surface components with apparent molecular weights of 70,000 and 85,000 were shared by the three strains of T. cruzi. In addition to these, a protein with an apparent molecular weight of 60,000 appeared as a strong band in the CL extracts, whereas in the G extract a 100,000-molecular-weight band of high intensity was detected. Upon reaction of the parasite extracts with the various immune sera, sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the immunoprecipitates revealed a remarkable difference in the G surface antigens, as compared with those of strain Y or CL (Fig. 2). The most prominent band of the G extract, i.e., the 100,000-molecular-weight protein, was also the main component immunoprecipitated with the rabbit anti-G serum (Fig. 2A, lane b). This antigen was also recognized as a weak band by the anti-CL serum and as a strong band by one of the sera from chronically infected patients (Fig. 2A, lanes d and e, respectively). In contrast, the 100,000-molecular-weight component could not be detected on the Y or CL cell surfaces by the same rabbit anti-G serum (data not shown) or by the chagasic human serum (Fig. 2B, lanes c and g), which strongly react with G metacyclic forms. Furthermore, the immunoprecipitation patterns of Y and CL extracts with anti-Y and anti-CL rabbit sera (Fig. 2B, lanes a, b, e, and f) were very similar to each other and differed from those of strain G (Fig. 2A, lanes c and d).

The surface antigen profile of Y and CL metacyclic trypomastigotes in this study differs from that reported by Nogueira et al. (12), which could in part be ascribed to the fact that I labeled the parasites with $^{131}$I instead of $^{125}$I, using the...
forms differed significantly in mice from the mechanisms.

reaction reported sera metacyclic trypomastigotes tryptose derived from cyclic preparations method. Iodo-Gen antigens with ml under number of motile Antiserum" Anti-G II was determined It is ND, Not c b ml. lysed of 107 parasites were lysed only by sera of T. cruzi strainsa CL 0 parasitemia 100 parasitemia taken or 106 pure metacyclic trypomastigotes derived from liver infusion-

trypomastigote medium.

Susceptibility to complement-mediated immunolysis was another distinctive feature of T. cruzi G, as compared with strains Y and CL. Whereas Y and CL metacyclic forms were resistant to in vitro lysis by antisera against the metacyclic stage, G metacyclic trypomastigotes were completely lysed by the same sera in a complement-dependent reaction (Table 1). In the case of blood trypomastigotes, it has been reported that the parasites are lysed only by sera from animals with ongoing infection (7–9). Lysis of G metacyclic forms, however, occurred with sera from animals immunized with dead organisms.

It is interesting to note that the course of infection in mice inoculated with G metacyclic forms differed significantly from that produced by strains Y and CL. Animals inoculated with

Iodo-Gen method. Moreover, it should be pointed out that whereas previous analysis of T. cruzi surface antigens (12) has been performed with trypomastigotes either mixed with epimastigotes or contaminated with complement components, I have determined surface antigens of pure metacyclic preparations free from extraneous proteins, as judged by iodinated patterns of metacyclic forms obtained in macromolecule-free medium (15), which were comparable to those of trypomastigotes derived from liver infusion-

Trypomastigotes, at a final concentration of 10^7 cells per ml, were incubated with 0.2 ml of fresh normal or immune serum in a total volume of 0.25 ml. After 60 min at 37°C, samples were taken and the percent lysis was determined by counting the number of motile trypomastigotes in a hemacytometer under a phase-contrast microscope.

Antisera were obtained by immunizing rabbits with heat-killed (I) or merthiolate-killed (II) pure metacyclic trypomastigotes of strain Y or G.

d ND, Not done.

10^5 purified metacyclic trypomastigotes of strain Y or CL developed high parasitemia (Fig. 3), and some mortality was also observed. In contrast, G metacyclic forms repeatedly failed to produce patent parasitemia in mice, even after the injection of 10^6 or more organisms (Table 2), and infection could be only indirectly detected by either xenodiagnosis or hemoculture.

Taken together, the present findings suggest that determination of surface antigens and susceptibility to complement-mediated immunolysis of metacyclic trypomastigotes may be a valuable tool to distinguish virulent and nonvirulent strains of T. cruzi.

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LITERATURE CITED


