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# Polymorphism of the 35- and 50-Kilodalton Surface Glycoconjugates of *Trypanosoma cruzi* Metacyclic Trypomastigotes

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**In this study, we examined the immunochemical properties of the 35- and 50-kDa (35/50-kDa) surface glycoconjugates expressed on the surface of metacyclic trypomastigotes of *Trypanosoma cruzi* using three different monoclonal antibodies directed to this component. The 35/50-kDa surface antigen was expressed by metacyclic trypomastigotes of 11 different strains of *T. cruzi* and displayed a significant degree of molecular polymorphism. Results of immunoblotting and complement-mediated lysis were consistent with such diversity. Different monoclonal antibodies reacted with distinct epitopes on the 35/50-kDa antigen, which is resistant to proteases and behaves as an amphiphilic component.**

There is increasing evidence that the entry of *Trypanosoma cruzi* trypomastigote forms into mammalian cells involves energy-dependent attachment (23) followed by parasite internalization (4, 21, 23). The attachment and internalization steps seem to depend on the expression of specific ligands on the parasite surface which interact with corresponding receptors at the host cell membrane. Parasite ligands, particularly proteins and glycoproteins, have been identified, and their putative role in the invasion process has been inferred from inhibition studies with monoclonal antibodies (MAbs) (1, 22, 29, 30) or through direct binding assays (4, 7, 8).

We have previously reported on a stage-specific 90-kDa glycoprotein and doublet glycoconjugates of 35 and 50 kDa, which are expressed on the surface of metacyclic trypomastigotes (2, 25, 29) and are identified by MAbs 1G7 and 10D8, both capable of neutralizing the parasite infectivity in vivo and partially inhibiting the invasion of host cells in vitro (2, 29, 30). For the 35- and 50-kDa (35/50-kDa) antigen, recent observations indicate that it is a cell adhesion molecule which, in its purified form, can significantly reduce, in a dose-dependent manner, the entry of metacyclic trypomastigotes into mammalian cells in vitro (19).

Mortara et al. (16) have shown that the metacyclic trypomastigote 90-kDa surface antigen is ubiquitous among different *T. cruzi* strains and displays some structural polymorphism. In this study, we investigated whether the 35/50-kDa antigen of metacyclic trypomastigotes also has such a wide distribution among *T. cruzi* isolates, and we also attempted to further characterize the glycoconjugates by using three monoclonal antibodies. We observed that the glycoconjugates, which are protease-resistant amphiphilic molecules of the parasite membrane, are expressed as a polymorphic component in different *T. cruzi* strains.

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## MATERIALS AND METHODS

**Parasites.** The 11 strains of *T. cruzi* used in this study have been previously described (16). They were isolated at different geographical locations from a variety of hosts: F (9), CL (5), Tulahuen (17), Costalimai (13), Dm28, Dm30, and Guafitas (donated by Victor Contreras, Universidade de Carabobo, Valencia, Venezuela), G (28), MD (provided by Maria Deane from Instituto Oswaldo Cruz, Rio de Janeiro, Brazil), M226 (14), and Y (24). Parasites were maintained alternately in mice and in axenic cultures. Epimastigotes were grown in liver infusion-tryptose medium (6), and metacyclic trypomastigotes were purified from aged liver infusion-tryptose cultures by passage through DEAE-cellulose columns (16).

**Production of MAbs to *T. cruzi* metacyclic trypomastigotes.** MAbs 10D8 and 2B10 were prepared as previously described (30). MAb 3F5 was obtained as follows. BALB/c mice were immunized with G-strain metacyclic trypomastigote cytoskeletons, isolated as insoluble pellets after parasite lysis at 0°C in appropriate buffer solutions containing 1% Nonidet P-40 (15), denatured with 0.1% sodium dodecyl sulfate (SDS), and emulsified with Freund's complete adjuvant (Sigma Chemical Co., St. Louis, Mo.). Spleen cells from immunized mice were fused with SP2/0-Ag14 plasmacytoma cells, and culture supernatants were screened by indirect immunofluorescence on formaldehyde-fixed parasites or cytoskeletons. Positive hybrids were cloned twice by the limited dilution procedure. Immunoglobulin (Ig) and light-chain isotyping was done by an MAb-based enzyme-linked immunosorbent assay (Immunoselect; GIBCO/BRL). MAb 1G7 has been described elsewhere (2, 16, 25).

**Lysis and agglutination assays.** For the complement-mediated lysis assay, 10 μl of a suspension of freshly isolated metacyclic forms ($10^8$/ml) was incubated with 40 μl of heat-inactivated (56°C, 30 min) normal mouse serum or ascitic fluid from a mouse bearing a hybridoma producing MAb to *T. cruzi*. After the mixture was incubated for 10 min at room temperature, 50 μl of normal human serum was added as source of complement, and the parasites were further incubated at 37°C for 30 min. The percent lysis was determined by counting under a phase-contrast microscope

the relative numbers of live motile metacyclic forms versus lysed parasites.

Agglutination was assayed by a similar protocol but omitting complement and using serial dilutions of the MAbs. Agglutinates were visualized under a phase-contrast microscope.

**SDS-PAGE and immunoblotting.** *T. cruzi* extracts were prepared by dissolving $10^6$ parasites per μl in sample buffer for SDS-polyacrylamide gel electrophoresis (PAGE) containing 90 mM Tris-HCl (pH 6.8), 10% SDS, 10% β-mercaptoethanol, 5% glycerol, and 0.5% bromophenol blue. Extracts were subsequently boiled for 2 min and subjected to electrophoresis (11) in 6 to 16% polyacrylamide linear gradient microslab gels (12). Electrophoresed components and molecular weight markers (low-molecular-weight range; Pharmacia Fine Chemicals, Piscataway, N.J.) transferred to nitrocellulose (0.45-μm pore size; Millipore) or nylon (Hy-Bond; Amersham) membranes (26) were visualized by staining with Ponceau S; membranes were subsequently soaked in 5% defatted powdered milk in phosphate-buffered saline (PBS) for at least 1 h. Nitrocellulose sheets or strips were subsequently incubated with culture supernatants of different hybrids, and bound immunoglobulins were visualized after incubation with anti-mouse Ig conjugated to peroxidase (Sigma) and reaction with diaminobenzidine (0.2 mg/ml) and $H_2O_2$ (5 μl of a 30% solution in 30 ml of PBS).

**Periodate oxidation of carbohydrate residues.** Antigens immobilized onto nitrocellulose were subjected to periodate oxidation as described previously (27). Briefly, after SDS-PAGE, transfer to nitrocellulose, and Ponceau S staining and destaining, strips were washed with 50 mM sodium acetate (pH 4.5) for 10 min and then incubated for 1 h at room temperature in the dark with 10 mM sodium periodate (Sigma) in the same buffer. Control strips were incubated without periodate. The strips were subsequently washed with PBS and incubated with 50 mM sodium borohydride (Sigma) in PBS for 30 min. Excess borohydride was removed with PBS, and strips were processed for immunoblotting as described above.

**Surface labeling of metacyclic forms with galactose oxidase and sodium boro[$^3$H]hydride.** Metacyclic trypomastigotes (G strain) were washed in RPMI 1640 medium and resuspended to a density of $10^8$ parasites in 100 μl of medium. They were treated for 45 min at room temperature with 1 U of galactose oxidase (Sigma) and then washed three times in medium and resuspended in 150 μl of medium containing 750 μCi of $NaB^3H_4$ (16 Ci/mmol; Research Products International Corp., Mt. Prospect, Ill.). After another 45-min incubation at room temperature, the parasites were washed three more times in medium, lysed with 1% Nonidet P-40 in the presence of protease inhibitors (16), and processed for immunoprecipitation as described previously (25). The immunoprecipitates were dissolved in boiling SDS-PAGE sample buffer and electrophoresed as described above. The gels were stained, destained, and treated with AMPLIFY (Amersham) before being dried and exposed to X-ray films at −70°C in the presence of an intensifying screen.

**Protease treatment of live parasites and parasite extracts.** Live metacyclic trypomastigotes (G strain) were incubated with increasing concentrations of trypsin (Sigma) or pronase (Boehringer GmbH, Mannheim, Germany). After 60 min at 37°C, parasites were washed three times in the presence of either 100 μg of soybean trypsin inhibitor per ml or 1 mM phenylmethylsulfonyl fluoride before lysis in SDS-PAGE sample buffer and immunoblotting analysis.

Metacyclic trypomastigote extracts (also from the G strain) prepared in SDS-PAGE sample buffer were incubated in the presence of increasing papain (BDH, Poole, England) concentrations for 15 min at room temperature. After this period, samples were subjected to SDS-PAGE and immunoblotting.

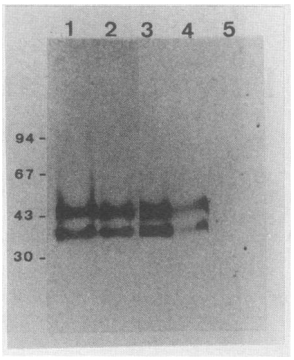


FIG. 1. Metacyclic trypomastigote surface antigen recognized by MAbs 10D8, 2B10, and 3F5. *T. cruzi* trypomastigotes (G strain) from axenic cultures were labeled by the galactose oxidase and sodium boro[$^3$H]hydride procedure, and parasite lysates (lane 1) were immunoprecipitated with MAbs 10D8 (lane 2), 2B10 (lane 3), and 3F5 (lane 4) and normal mouse serum (lane 5). For MAb 3F5, rabbit Ig anti-mouse IgG (10 μg/ml) was used before *Staphylococcus aureus* incubation. Numbers on left show sizes in kilodaltons.

**Phase separation of hydrophobic components in Triton X-114.** Metacyclic trypomastigotes from the G strain were lysed at 0°C in the presence of 1% Triton X-114 and protease inhibitors (3). Amphiphilic and hydrophilic components were separated by centrifugation (500 × *g*, 1 min) after the temperature was raised to 37°C for 1 min or 1 h (3). Both phases were collected and subjected to SDS-PAGE and immunoblotting as described above.

**Purification of 35/50-kDa glycoconjugates.** The purification of the 35/50-kDa glycoconjugates was based on the method of Previato et al. (18). Briefly, individual preparations of $3 \times 10^9$ to $5 \times 10^9$ purified G-strain epimastigotes or metacyclic trypomastigotes were washed in PBS and pelleted. The pellets were resuspended in 5 ml of distilled water, mixed with an equal volume of phenol, heated at 80°C for 15 min, and then centrifuged at 6,000 × *g* for 20 min. The aqueous phase was collected, dialyzed against distilled water, and lyophilized. The dried preparation was resuspended in 400 μl of 10 mM Tris-HCl (pH 7.5) containing 10 mM $MgCl_2$ and treated with DNase and RNase (100 μg/ml) at 37°C for 3 h. The digested material was extracted with phenol and dialyzed against PBS. The final preparation, devoid of nucleic acid as judged by lack of staining with ethidium bromide, was subjected to SDS-PAGE to be stained with Schiff's reagent. The amount of glycoconjugate in the purified sample was estimated by a colorimetric method for carbohydrates (10).

## RESULTS

**Reactivity of MAbs directed to the 35/50-kDa surface glycoconjugates of *T. cruzi* metacyclic trypomastigotes.** In the same manner as the previously described MAb 10D8 (30), MAbs 3F5 (IgA) and 2B10 (IgG3) recognized doublet molecules of 35 and 50 kDa after surface labeling of metacyclic trypomastigotes (G strain) with galactose oxidase-sodium boro[$^3$H]hydride (Fig. 1).

The three MAbs displayed different reactivities with meta-

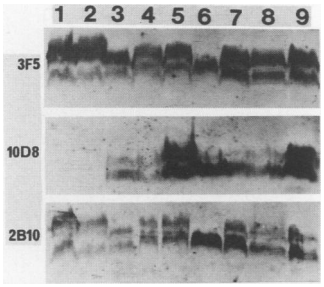


FIG. 2. Reactivity of MAbs 3F5, 10D8, and 2B10 with the 35/50-kDa glycoconjugates of different *T. cruzi* strains. Metacyclic trypomastigote extracts of *T. cruzi* strains were processed for immunoblotting as described in Materials and Methods. Only the 35/50-kDa regions of the immunoblots, processed in parallel, are shown. The *T. cruzi* strains are as follows. Lanes: 1, Y; 2, CL; 3, Tulahuen; 4, F; 5, G; 6, MD; 7, M226; 8, Costalimai; 9, Guafitas.

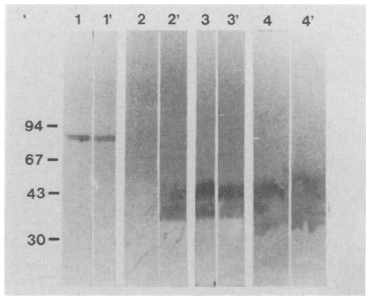


FIG. 3. Effect of periodate treatment on the reactivity of MAbs directed to the 35/50-kDa *T. cruzi* glycoconjugates. Cell extracts (G strain in lanes 1, 2, and 3 and CL strain in lane 4) were blotted onto nitrocellulose membranes after SDS-PAGE and treated with sodium periodate. Both untreated (lanes 1', 2', 3', and 4') and treated (lanes 1, 2, 3, and 4) nitrocellulose strips were incubated with MAb 1G7, which reacts with the 90-kDa surface glycoprotein (lanes 1 and 1', as a control; see reference 30), 2B10 (lanes 2 and 2'), and 3F5 (lanes 3 and 3' and 4 and 4'). Molecular sizes in kilodaltons are shown on the left.

cyclic trypomastigotes of different *T. cruzi* strains. On immunoblots (with nitrocellulose or nylon membranes), 10D8 gave no reaction with the Y or CL strain, whereas 3F5 and 2B10 detected the 35/50-kDa antigen in all strains examined (Fig. 2). MAb 3F5 gave a stronger reaction with some strains (e.g., Y, CL, Tulahuen, and Guafitas) when compared with 2B10. The glycoconjugate bands of the Y and CL strains are broader than those of the remaining antigens (Fig. 2). In the MD strain, all MAbs detected only the 35-kDa component (Fig. 2). A 35/50-kDa doublet band with mobility on SDS-PAGE identical to that of G-strain glycoconjugates was detected by MAb 10D8 also in the Dm28 and Dm30 strains (data not shown). MAbs 10D8, 2B10, and 3F5 also reacted with epimastigotes of the 11 strains examined, and the pattern of reactivity on immunoblots was comparable to that of metacyclic trypomastigotes (data not shown).

**Complement-mediated lysis and agglutination of *T. cruzi* metacyclic trypomastigotes with MAbs directed to 35/50-kDa surface glycoconjugates.** MAb 10D8 was shown to strongly agglutinate metacyclic trypomastigotes of all *T. cruzi* strains examined (Table 1), showing that the glycoconjugates are expressed on the surface of all isolates. However, when metacyclic trypomastigotes were subjected to complement-mediated lysis in the presence of MAb 10D8, different susceptibilities were observed among strains (Table 1). Metacyclic trypomastigotes of six strains (Y, CL, Tulahuen, F, M226, and Costalimai) were extensively destroyed, whereas the other five isolates (G, MD, Dm28, Dm30, and Guafitas) were resistant to 10D8 complement-mediated lysis. In addition, we observed that MAbs 3F5 and 2B10 were also highly agglutinating for the Y, CL, Tulahuen, and G strains and lysed metacyclic forms of these strains in the presence of complement (data not shown). The lytic effect toward the G strain shown by MAbs 3F5 and 2B10 is in contrast to the result obtained with MAb 10D8.

TABLE 1. Agglutination and complement-mediated lytic activity of MAb 10D8 directed to the 35/50-kDa surface glycoconjugates from *T. cruzi* metacyclic trypomastigotes of different strains

| *T. cruzi* strain | Agglutination titer[a] (10D8) | % Lysis[b] | |
|---|---|---|---|
| | | 10D8 | NMS |
| Y | 64 | 98 | 2 |
| CL | 128 | 96 | 0 |
| Tulahuen | 128 | 93 | 2 |
| F | 64 | 96 | 0 |
| G | 256 | 0 | 0 |
| MD | 128 | 24 | 3 |
| M226 | 256 | 100 | 8 |
| Costalimai | 256 | 100 | 0 |
| Dm28 | 256 | 11 | 0 |
| Dm30 | 256 | 0 | 0 |
| Guafitas | 256 | 0 | 0 |

[a] The reciprocal of the highest dilution of ascitic fluid in which rosettes containing more than 10 metacyclic trypomastigotes was defined as the agglutination titer. The agglutination titer in normal mouse serum was negative for all strains.

[b] Percent lysis was scored by counting at least 200 parasites. NMS, normal mouse serum.

**Effect of periodate oxidation on reactivities of MAbs 10D8, 2B10, and 3F5.** Immunoblotting and complement-mediated lysis assays, showing distinct reactivities of the three MAbs with individual strains, suggested that they reacted with different epitopes. When the periodate oxidation test (27) was performed on immunoblots of parasite extracts, it was observed that the epitope recognized by MAb 3F5 was resistant to the periodate treatment, in contrast to the epitopes reactive with MAbs 2B10 and 10D8, which were completely abolished (Fig. 3) (30). These observations suggest that, unlike the binding sites of MAbs 10D8 and 2B10, the 3F5 epitope may not be a carbohydrate.

**Resistance to proteases and amphiphilic nature of the 35/50-kDa surface glycoconjugates.** To proceed one step further in the elucidation of the chemical nature of the 35/50-kDa antigen, we did the following experiments. Intact metacyclic trypomastigotes (G strain) were subjected to protease treatments. Trypsin or pronase had no effect on the electrophoretic mobility of the antigen and did not impair the binding of MAb 3F5, even at relatively high concentrations (Fig. 4). Furthermore, the intensity of the glycoconjugate bands was essentially the same for the protease-treated parasites as for the control parasites (Fig. 4), indicating that the protease treatment did not cause substantial release of the glycoconjugate from the parasite surface. In control samples, the 90-kDa surface glycoprotein was significantly degraded (data not shown). Similar results were obtained when parasite extracts prepared in SDS-PAGE sample buffer were digested with papain. In these experiments, neither the electrophoretic mobility nor the 3F5-binding capacity of the glycocon-

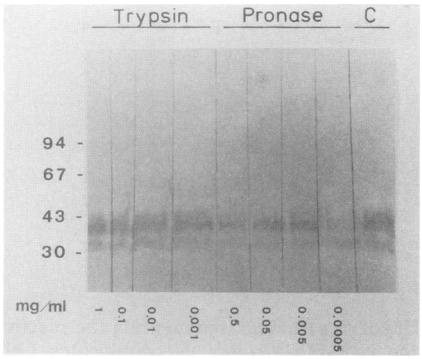


FIG. 4. Effect of protease treatment of live *T. cruzi* metacyclic trypomastigotes on the mobility of the 35/50-kDa glycoconjugates. A total of $10^7$ parasites (G strain) were incubated for 1 h at 37°C with variable trypsin and pronase concentrations (indicated at the bottom), washed in the presence of excess protease inhibitors, dissolved in SDS-PAGE sample buffer, and processed for immunoblotting with MAb 3F5. Lane C, control untreated sample. Molecular sizes in kilodaltons are shown on the left. Note that the protease treatments do not affect the mobility or the intensity of the glycoconjugate bands.

jugate immobilized on nitrocellulose was altered, in comparison with the 90-kDa antigen, which was extensively degraded (Fig. 5).

The hydrophilic or amphiphilic properties of the glycoconjugate were examined by the Triton X-114 phase separation test (3). When parasite lysates prepared with Triton X-114 were subjected to phase separation, the 35/50-kDa glycoconjugates were found exclusively in the detergent phase (Fig. 6). The incubation of parasite lysates at 37°C for 1 h did not affect the partition of either parasite antigen (Fig. 6). Figure 6 also shows that a fraction of the 35/50-kDa antigen remains associated with the cytoskeletal fraction after detergent extraction, which is consistent with the isolation of MAb 3F5 with these preparations as immunogens.

**Distinct mobilities on SDS-PAGE of 35/50-kDa glycoconjugates isolated from epimastigotes and metacyclic trypomastigotes.** Glycoconjugates purified from epimastigotes and

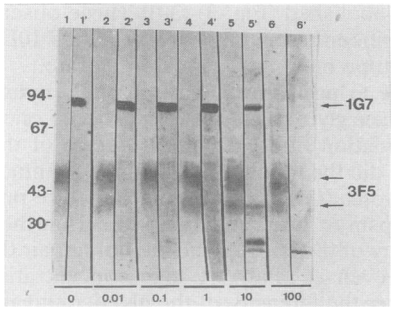


FIG. 5. Effect of papain digestion of metacyclic trypomastigote extracts on the mobility of the 35/50-kDa glycoconjugates. A total of $10^7$ metacyclic trypomastigotes (G strain) were lysed in SDS-PAGE sample buffer and incubated with increasing papain concentrations (indicated at the bottom). Nitrocellulose strips were cut in half and incubated with MAb 3F5 (1, 2, 3, 4, 5, and 6) or MAb 1G7 (1′, 2′, 3′, 4′, 5′, and 6′). Note that the mobility of the 35/50-kDa glycoconjugate is not affected, whereas the degradation of the 90-kDa antigen is clearly visible. Molecular sizes in kilodaltons are shown on the left.

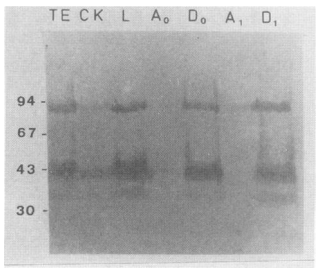


FIG. 6. Behavior of the 35/50-kDa glycoconjugates in Triton X-114 solutions. G-strain metacyclic trypomastigotes were lysed in 1% Triton X-114 solutions, subjected to phase separations by the method of Bordier (3), and analyzed by immunoblotting with a mixture of MAbs 3F5 and 1G7. Lanes: TE, total cell extract; CK, cytoskeletal fraction, insoluble in Triton X-114 at 0°C; L, lysate, soluble in TX-114 at 0°C; $A_0$ and $D_0$, aqueous and detergent phases, respectively, isolated immediately after cell lysis; $A_1$ and $D_1$, aqueous and detergent phases, respectively isolated after incubation of lysates for 1 h at 37°C. Note that both antigens partition exclusively in the detergent phases. Numbers on left show sizes in kilodaltons.

metacyclic forms of the G strain were subjected to SDS-PAGE. Staining of the gel with Schiff's reagent revealed doublet bands of approximately 35 and 50 kDa in the epimastigote preparation that migrated slightly faster than the metacyclic trypomastigote component (Fig. 7).

## DISCUSSION

In this study, we further characterized the 35/50-kDa surface glycoconjugates of *T. cruzi* metacyclic trypomastigotes. By using three different MAbs, 3F5, 2B10, and 10D8, we found that the 35/50-kDa antigen is expressed at the parasite surface as a polymorphic component by all 11 *T. cruzi* strains examined (Fig. 2 and Table 1). In one strain, MD, only the 35-kDa band was detected by any of the three MAbs (Fig. 2). Of note is the fact that MAb 10D8, in contrast to MAbs 3F5 and 2B10, failed to detect the glycoconjugates in the Y and CL strains on immunoblots, although it reacted with live metacyclic forms (Table 1). We also observed that formaldehyde fixation precludes the binding of MAb 10D8 to Y- and CL- but not to G-strain parasites (data not shown). It appears, therefore, that the 10D8 epitope expressed by these two *T. cruzi* strains is rendered nonreactive when the antigen is immobilized on nitrocellulose or nylon membranes or chemically modified by formaldehyde fixation. Since the

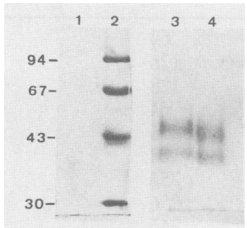


FIG. 7. Migration in SDS-PAGE of the 35/50-kDa glycoconjugates purified from *T. cruzi* epimastigotes or metacyclic trypomastigotes. Glycoconjugates purified from metacyclic trypomastigotes (lanes 1 and 3) or epimastigotes (lane 4) of the G strain were subjected to SDS-PAGE, and the gel was stained with Coomassie brilliant blue (lanes 1 and 2) or with Schiff's reagent (lanes 3 and 4). Lane 2 contains molecular size markers, indicated (in kilodaltons) on the left.

35/50-kDa glycoconjugates are detected by MAb 10D8 in other strains, this is suggestive of an epitope polymorphism. Thus, it is possible that in Y- and CL-strain glycoconjugates, MAb 10D8 recognizes an epitope related to but distinct from that expressed by other strains.

Complement-mediated lysis assay with MAb 10D8 revealed that metacyclic trypomastigotes of six *T. cruzi* strains are extensively lysed, whereas those of the other five strains are minimally or not lysed (Table 1). It is possible that this also reflects a discrete epitope polymorphism. However, as whole parasites are implicated in the lysis assay, the failure of MAb 10D8 in triggering the lytic cascade in some strains could be due to inhibitory effects of neighboring molecules at the cell surface.

The distinct reactivity of MAbs 3F5, 2B10, and 10D8 with metacyclic trypomastigotes on immunoblots (Fig. 2), in addition to the fact that, unlike MAbs 2B10 and 10D8, the binding of 3F5 is not blocked by periodate oxidation (Fig. 3), indicates that these MAbs recognize different epitopes on the glycoconjugates. While MAbs 2B10 and 10D8 appear to react with carbohydrate epitopes, the nature of the 3F5 epitope has been more elusive. One interesting possibility is that the 3F5 epitope could be a peptide segment, although we had no indications that a polypeptide was part of the 35/50-kDa antigen. The glycoconjugate is resistant to a variety of protease treatments (Fig. 4 and 5) and to radiolabeling by $^{125}I$ or [$^{35}S$]methionine (30). Very recently, however, chemical analysis of the purified antigen by Michael Ferguson at the University of Dundee, Dundee, Scotland, has revealed that the glycoconjugate does contain a peptide portion (23a). Another feature of the 35/50-kDa antigen is its amphiphilicity. When subjected to phase separation in Triton X-114, it partitions exclusively in the detergent phase (Fig. 6). Recent experiments have shown that in vitro-incorporated fatty acids can be released by exogenous phosphatidylinisitol phospholipases C, indicating that the 35/50-kDa glycoconjugate is inserted into the *T. cruzi* membrane through a glycophosphatidylinositol anchor (23a).

MAbs 10D8, 2B10, and 3F5 reacted with both epimastigotes and metacyclic trypomastigotes of all 11 *T. cruzi* strains in a similar way, either on immunoblots or by agglutination assay. However, the epimastigote antigen may differ significantly from that of the metacyclic trypomastigotes. For instance, the 35/50-kDa glycoconjugates purified from epimastigotes migrated slightly faster than metacyclic trypomastigote antigen in SDS-PAGE (Fig. 7). Ruiz et al. (19) have found differences in the antigenic properties of the 35/50-kDa glycoconjugates from the two developmental stages. The epimastigote antigen is not recognized by sera from mice immunized with heat-killed metacyclic forms. Furthermore, the capacity of the epimastigote glycoconjugates to adhere to host cells is significantly lower than that displayed by the metacyclic trypomastigote antigen (19). In this regard, it is interesting that Sacks and Silva (20) have described for *Leishmania major* a developmentally regulated glycolipid of infective promastigotes which appears to be a modified form of the noninfective-stage glycolipid. In *T. cruzi*, we could envisage an alteration of the epimastigote 35/50-kDa glycoconjugate during differentiation to metacyclic trypomastigotes, leading to acquisition of cell-binding capacity.

Finally, it is of relevance that surface glycoconjugates of 35 and 50 kDa, which presumably participate in the process of host cell invasion by metacyclic trypomastigotes, are a ubiquitous component among *T. cruzi* strains isolated from a variety of hosts in widely separated geographical regions in South America.

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## REFERENCES

1. **Alves, M. J. M., G. Abuin, V. Y. Kuwajima, and W. Colli.** 1986. Partial inhibition of trypomastigote entry into cultured mammalian cells by monoclonal antibodies against a surface glycoprotein of *Trypanosoma cruzi*. Mol. Biochem. Parasitol. **21**:75–82.
2. **Araguth, M. F., M. M. Rodrigues, and N. Yoshida.** 1988. *Trypanosoma cruzi* metacyclic trypomastigotes: neutralization by the stage-specific monoclonal antibody 1G7 and immunogenicity of 90 kD surface antigen. Parasite Immunol. **10**:707–712.
3. **Bordier, C.** 1981. Phase separation of integral membrane proteins in Triton X-114 solutions. J. Biol. Chem. **256**:1604–1607.
4. **Boschetti, M. A., M. M. Piras, D. Henriquez, and R. Piras.** 1987. The interaction of a *Trypanosoma cruzi* surface protein with Vero cells and its relationship with parasite adhesion. Mol. Biochem. Parasitol. **24**:175–184.
5. **Brener, Z., and E. Chiari.** 1963. Variações morfológicas observadas em diferentes amostras de *Trypanosoma cruzi*. Rev. Inst. Med. Trop. Sao Paulo **5**:220–224.
6. **Camargo, E. P.** 1964. Growth and differentiation in *Trypanosoma cruzi*: origin of metacyclic trypomastigotes in liquid media. Rev. Inst. Med. Trop. Sao Paulo **6**:93–100.
7. **Davis, C. D., and R. E. Kuhn.** 1990. Detection of antigens with affinity for host cell membrane polypeptides in culture supernatants of *Trypanosoma cruzi*. Infect. Immun. **58**:1812–1816.
8. **Davis, C. D., and R. E. Kuhn.** 1990. Selective binding of *Trypanosoma cruzi* to host cell membrane polypeptides. Infect. Immun. **58**:1–6.
9. **Deane, M. P., and J. Kloetzel.** 1974. Lack of protection against *Trypanosoma cruzi* by multiple doses of *T. lewisi* culture forms. A discussion on some strains of "lewisi." Exp. Parasitol. **35**:406–410.
10. **Dubois, M., K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith.** 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. **28**:350–356.
11. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
12. **Matsudaira, P. T., and D. Burgess.** 1978. SDS microslab linear gradient polyacrylamide gel electrophoresis. Anal. Biochem. **109**:396–399.
13. **Mello, D. A., and M. M. Borges.** 1981. Primeiro encontro do *Triatoma costalimai* naturalmente infectado pelo *Trypanosoma cruzi*: estudos de aspectos biológicos da amostra isolada. Mem. Inst. Oswaldo Cruz Rio J. **76**:61–69.
14. **Mello, D. A., M. M. Borges, and L. H. Chiarini.** 1980. Crescimento e diferenciação *in vitro* de cepas do *Trypanosoma cruzi* isoladas de animais silvestres. Rev. Saude Publica **14**:569–581.
15. **Mortara, R. A.** 1989. Studies on trypanosomatid actin. I. Immunochemical and biochemical identification. J. Protozool. **36**:8–13.
16. **Mortara, R. A., M. F. Araguth, and N. Yoshida.** 1988. Reactivity of stage-specific monoclonal antibody 1G7 with metacyclic trypomastigotes of *Trypanosoma cruzi* strains: lytic property and antigen polymorphism. Parasite Immunol. **10**:369–378.
17. **Pizzi, T. P., M. D. Rubio, R. Prager, and R. C. Silva.** 1952. Acción de la cortisona en la infeccion experimental por *Trypanosoma cruzi*. Bol. Chil. Parasitol. **7**:22–24.
18. **Previato, J. O., A. F. Andrade, M. C. Pessolani, and L. Mendonça-Previato.** 1985. Incorporation of sialic acid into *Trypanosoma cruzi* macromolecules: a proposal for a new metabolic route. Mol. Biochem. Parasitol. **16**:85–96.

19. **Ruiz, R. C., J. C. Gonzalez, V. L. Rigoni, and N. Yoshida.** The 35/50 kDa surface antigen of *Trypanosoma cruzi* metacyclic trypomastigotes, an adhesion molecule involved in host cell invasion. Parasite Immunol., in press.
20. **Sacks, D. L., and R. P. Silva.** 1987. The generation of infective stage of *Leishmania major* promastigotes is associated with the cell surface expression and release of a developmentally regulated glycolipid. J. Immunol. **139:**3099–3106.
21. **Schenkman, S., N. W. Andrews, V. Nussenzweig, and E. S. Robbins.** 1988. *Trypanosoma cruzi* invade a mammalian epithelial cell in a polarized manner. Cell **55:**157–165.
22. **Schenkman, S., C. Diaz, and V. Nussenzweig.** 1991. Attachment of *Trypanosoma cruzi* trypomastigotes to receptors at restricted cell surface domains. Exp. Parasitol. **72:**76–86.
23. **Schenkman, S., E. S. Robbins, and V. Nussenzweig.** 1991. Attachment of *Trypanosoma cruzi* to mammalian cells requires parasite energy, and invasion can be independent of the target cell cytoskeleton. Infect. Immun. **59:**645–654.
23a. **Schenkman, S., et al.** Unpublished data.
24. **Silva, L. H. P., and V. Nussenzweig.** 1953. Sobre uma cepa de *Trypanosoma cruzi* altamente virulenta para o camundongo branco. Folia Clin. Biol. **20:**191–203.
25. **Teixeira, M. M. G., and N. Yoshida.** 1986. Stage-specific surface antigens of metacyclic trypomastigotes of *Trypanosoma cruzi* identified by monoclonal antibodies. Mol. Biochem. Parasitol. **18:**271–282.
26. **Towbin, H., T. Staehelin, and J. Gordon.** 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA **76:**4350–4354.
27. **Woodward, M. P., W. W. Young, and R. A. Bloodgood.** 1985. Detection of monoclonal antibodies specific for carbohydrate epitopes using periodate oxidation. J. Immunol. Methods **78:** 143–153.
28. **Yoshida, N.** 1983. Surface antigens of metacyclic trypomastigotes of *Trypanosoma cruzi*. Infect. Immun. **40:**836–839.
29. **Yoshida, N., S. A. Blanco, M. F. Araguth, M. Russo, and J. Gonzalez.** 1990. The stage-specific 90-kilodalton surface antigen of metacyclic trypomastigotes of *Trypanosoma cruzi*. Mol. Biochem. Parasitol. **39:**39–46.
30. **Yoshida, N., R. A. Mortara, M. F. Araguth, J. C. Gonzalez, and M. Russo.** 1989. Metacyclic neutralizing effect of monoclonal antibody 10D8 directed to the 35- and 50-kilodalton surface glycoconjugates of *Trypanosoma cruzi*. Infect. Immun. **57:** 1663–1667.