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Detection of Antigens with Affinity for Host Cell Membrane Polypeptides in Culture Supernatants of *Trypanosoma cruzi*

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Parasite antigens which bind to host cell molecules of approximately 32 and 34 kilodaltons (kDa) were identified in supernatant fluids obtained from axenic cultures of *Trypanosoma cruzi*. These parasite components were first detected in culture supernatants obtained after 2 weeks in culture. Immunoblot analysis of culture supernatants exhibiting binding activity revealed the presence of several parasite antigens ranging in molecular mass from approximately 26 to 290 kDa. Gel filtration (Sephacryl S-300) analysis of culture supernatants revealed four major peaks, but only the highest-molecular-mass peak (containing several parasite antigens ranging from 27 to 250 kDa) possessed binding activity for the host cell molecules.

The adsorption of *Trypanosoma cruzi* antigens to mammalian host cells in vitro is a well-documented phenomenon. Ribeiro Dos Santos and Hudson (14) detected the binding of *T. cruzi* antigens (derived from Y-strain amastigotes) to a variety of different mammalian cell lines by using indirect immunofluorescence. Similarly, Williams and co-workers (18) demonstrated the adsorption of numerous radioactively labeled polypeptides of *T. cruzi* (derived from Y-strain epimastigotes) to S2 cells (mouse muscle tumor) in vitro. Many investigators have demonstrated that both infected and noninfected cells possess *T. cruzi* antigens on their surfaces following the rupture of infected cells in vitro (1, 4, 12). In addition, Boschetti et al. (5) have reported the specific binding of a ^{125}I-labeled surface protein (83 kilodaltons [kDa]) of *T. cruzi* (derived from EP-isolate trypomastigotes) to Vero cells in in vitro culture. This 83-kDa glycoprotein was isolated from highly adherent activated parasites but was not found in extracts prepared from trypomastigotes which had been recently removed from cultures of Vero cells.

In previous studies, we identified host cell membrane molecules (32 and 34 kDa) which bind antigens of *T. cruzi* as well as live parasites in a highly specific manner (8). In the present study, we report that antigens released by *T. cruzi* (Brazil and Tulahuen strains) during axenic culture also demonstrate a selective affinity for two host cell membrane molecules of approximately 32 and 34 kDa. In addition, we have used immunochemical techniques to partially characterize parasite molecules which are released during in vitro culture.

MATERIALS AND METHODS

Cell cultures. PSC3H murine fibroblasts, SVB6KH murine fibroblasts, and P388D1 murine macrophages were grown in RPMI 1640 (GIBCO Laboratories, Grand Island, N.Y.) adjusted to pH 7.2 with 25 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid) and supplemented with 10% fetal bovine serum, gentamicin sulfate (50 μg/ml), penicillin G (100 U/ml), and streptomycin (100 μg/ml) (complete RPMI 1640). Cells were grown in 75-cm^2 flasks (Corning Glass Works, Corning, N.Y.) and were maintained at 37°C in a 5% CO_2 atmosphere at 70 to 80% relative humidity.

Parasite cultures. Parasites of Brazil and Tulahuen strains of *T. cruzi* were grown in an undefined liver infusion tryptose (LIT) medium (13). For timed studies, five 25-cm^2 T flasks (Corning) containing 10 ml of LIT medium were inoculated with approximately 10^7 fibroblast-derived trypomastigotes (FDTs). After 1 week of culture at 27°C, the contents of each 25-cm^2 flask were transferred to separate 1-liter Erlenmeyer flasks containing 100 ml of LIT medium. At weekly intervals, the contents of one flask were centrifuged at 1,300 × *g* for 30 min at 4°C. A 2-ml sample of culture supernatant was passed through a 0.22-μm-pore-size Acrodisc filter (Gelman Sciences Inc., Ann Arbor, Mich.) and was frozen immediately at −70°C for later electrophoretic and immunoblot analysis. The remaining culture supernatant was filtered through a sterile 0.22-μm-pore-size filter and stored at 4°C for later use in binding assays.

PSC3H fibroblasts infected with blood-form stages of the Brazil strain were also maintained in order to provide a source of FDTs for the study (17).

Parasite-specific antisera. Antigen extracts were prepared by solubilizing purified parasites in 50 mM Tris hydrochloride buffer (pH 8.0) containing 10 mM 3-[(3-cholamidopropyl)-dimethyl-ammonio]-1-propanosulfonate (CHAPSO) (Pierce Biochemical Co., Rockford, Ill.) as previously described (8). New Zealand White rabbits were immunized with a primary injection of trypomastigote antigen extract or culture-form antigen extract in an equal volume of Freund incomplete adjuvant (2 mg of total antigen). The primary immunization consisted of 10 subcutaneous 100-μl injections along the back of each animal. The rabbits were boosted intravenously with 1 mg of total antigen in Dulbecco phosphate-buffered saline (DPBS) 5 to 6 weeks after the primary immunization. After 7 to 10 days, the rabbits were bled by venipuncture and the serum was tested by immunoblot analysis. Each animal was boosted and bled periodically in order to obtain large quantities of parasite-specific antiserum. Control serum was also obtained from each rabbit prior to immunization.

Plasma membrane isolation. Plasma membranes were isolated from murine cells according to a previously described adaptation (8) of the polycationic bead technique of Jacobson (10) and Cohen et al. (7). Affi-Gel 731 beads (Bio-Rad Laboratories, Richmond, Calif.) were used as a polycationic solid support.

Membrane proteins were eluted from the beads by adding an equal volume of sample buffer (0.08 M Tris hydrochloride [pH 6.8], 0.1 M dithiothreitol, 2.0% sodium dodecyl sulfate

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[SDS], 10% glycerol) to the bead preparation and then incubating them for 10 min in a 75°C water bath with intermittent vortexing at high speed. The bead preparation was then placed on ice and sonicated at 20 kHz with 10 5-s pulses. The supernatant fluid containing solubilized membrane proteins was subjected to SDS-polyacrylamide gel electrophoresis (PAGE). Tracking dye (0.2% bromophenol blue) was added to the sample prior to electrophoresis.

Electrophoresis and protein blotting. Samples of conditioned LIT medium and selected samples of Sephacryl S-300 fractionated conditioned LIT medium were solubilized in an equal volume of sample buffer (0.8 M Tris hydrochloride [pH 6.8], 2.0% SDS, 0.1 M dithiothreitol, 10% glycerol, 0.2% bromophenol blue) by boiling for 2 min at 100°C. Prior to electrophoresis, selected fractions of conditioned LIT medium were concentrated by ultrafiltration in a Filtron Omegacell concentration unit (3,000-M_w cutoff; Pharmacia, Piscataway, N.J.). Protein concentrations were determined by the Bradford technique (6).

Host cell plasma membrane preparations were solubilized in sample buffer as described previously, and samples were applied directly to the wells of the stacking gel.

Electrophoresis of all samples was performed in polyacrylamide slab gels containing 0.1% SDS, with a 5% (wt/vol) polyacrylamide stacking gel and a 12.5% (wt/vol) polyacrylamide resolving gel, by the method of Laemmli (11). The following molecular weight markers were used: myosin, β-galactosidase, phosphorylase *b*, bovine serum albumin, ovalbumin, carbonic anhydrase, and soybean trypsin inhibitor (Bio-Rad). Separated proteins were transferred electrophoretically to 8-mm strips of nitrocellulose (0.45-μm pore size; Bio-Rad). Electrophoretic transfer was performed at 30 V overnight at 4°C in a Trans-blot cell (Bio-Rad) in the presence of 25 mM Tris (pH 8.3), 192 mM glycine, and 20% methanol. The efficiency of transfer was determined by staining selected strips containing antigen and strips containing molecular weight markers in 0.1% (wt/vol) amido black in 40% (vol/vol) methanol–2% (vol/vol) acetic acid. The strips were destained in 70% (vol/vol) methanol–7% (vol/vol) acetic acid.

Detection of parasite antigens by immunoblot analysis. Nitrocellulose strips containing conditioned LIT medium, fresh LIT medium, or selected Sephacryl S-300 fractions of conditioned LIT medium resolved by electrophoresis were analyzed by an indirect enzyme-labeled antibody assay. The strips were rinsed in DPBS following the electrophoretic transfer and incubated in DPBS containing 3% bovine serum albumin, 1% ovalbumin, 3% normal goat serum, and 0.1% Tween 20 (all from Sigma Chemical Co., St. Louis, Mo.) for 2 h as a blocking step. Rabbit anti-FDT antiserum, rabbit anti-culture forms (CF) antiserum, and normal rabbit serum (diluted 1:50 in DPBS containing 10% fetal bovine serum) were the primary antisera for the assay. Horseradish peroxidase-labeled goat anti-rabbit total immunoglobulin (Fisher Scientific Co., Pittsburgh, Pa.) diluted 1:1,000 in DPBS was used as a second antibody. The strips were incubated first in primary antibody and then in second antibody for 1 h with three 10-min rinses in DPBS containing 0.1% Tween 20 following each incubation. The strips were developed in 120 ml of substrate solution (0.34 mM 4-chloro-1-naphthol dissolved in 20 ml of cold methanol and diluted in 100 ml of Tris-saline buffer (pH 7.4) activated by the addition of 60 μl of 30% H_2O_2.

Binding assay. Following electrophoretic transfer, nitrocellulose strips containing host cell membrane proteins were rinsed briefly in DPBS and incubated for 2 h in the blocking solution described above. All incubations were performed in a 25-well acrylic incubation tray (Bio-Rad) placed in a high-humidity water bath set at 37°C. Nitrocellulose strips containing resolved membrane proteins were used as solid affinity supports to assay for the binding of parasite antigens. The strips were exposed to 10 ml of the appropriate test solution (conditioned LIT medium, weekly samples of conditioned LIT medium, Sephacryl S-300 fractions of conditioned LIT medium, or fresh LIT medium) for 4 h. The strips were then washed three times, for a total of 30 min in DPBS containing 0.1% Tween 20. Specific binding of parasite components was detected by using the enzyme-labeled antibody assay described above.

Gel filtration. Medium from in vitro cultures of *T. cruzi* in the stationary phase of growth (conditioned LIT medium) was cleared of intact parasites and parasite debris by centrifugation at 1,300 × *g* for 30 min at 4°C and passage through a sterile 0.22-μm-pore-size Acrodisc filter (Gelman). A Sephacryl S-300 (Pharmacia) column was prepared according to the instructions of the manufacturer and equilibrated in Tris-saline buffer (40 mM Trizma HCl, 9.7 mM Trizma base, 0.2 M NaCl [all from Sigma]), pH 7.4. Conditioned LIT medium (3 ml) was applied to the column (1.5 by 50 cm), and the sample was eluted in Tris-saline buffer (pH 7.4). A_{280} was monitored with an 8300 Uvicord II (UKB), and results were graphically recorded with a series 5000 Recordall (Fisher).

RESULTS

Specific molecular interactions between parasite antigens and murine cell membrane components. Parasite-derived material present in conditioned LIT medium (Brazil and Tulahuen strains) exhibited a specific binding interaction with membrane polypeptides purified from PSC3H murine fibroblasts (Fig. 1a, lanes 2 and 3), SVB6KH murine fibroblasts (Fig. 1b, lanes 2 and 3), and P388D1 murine macrophages (Fig. 1c, lanes 2 and 3). The reactive host cell membrane polypeptides had relative mobilities corresponding to molecular masses of approximately 32 and 34 kDa. No reactivity was observed with the 32- and 34-kDa polypeptides when the strips were incubated in fresh LIT medium (Fig. 1, lanes 1). A polypeptide of approximately 60 kDa was also visible on all strips in Fig. 1a and b, including those for the fresh medium controls, and is most likely a cross-reactive host cell polypeptide which was detected by the rabbit anti-FDT antisera in this assay.

When binding assays were performed with conditioned LIT medium obtained from axenic cultures of *T. cruzi* (Brazil and Tulahuen strains) ranging from 1 to 5 weeks in age, binding activity was detected by week 2 of culture and persisted through week 5 of culture (Fig. 2), at which time the cultures were discontinued.

Immunoblot analysis of weekly samples of conditioned LIT medium. Weekly samples of conditioned LIT medium (Brazil strain) were subjected to SDS-PAGE and analyzed by immunoblotting. Antigens with relative mobilities corresponding to molecular masses of approximately 65, 53, and 27 kDA were detected in conditioned LIT medium by week 1 of axenic culture by the rabbit anti-FDT antiserum (Fig. 3a, lane 2). In week 2 of culture, the rabbit anti-FDT antiserum detected additional antigens of 175, 80, 50, and 26 kDa (Fig. 3a, lane 3). A component of approximately 220 kDa was detected in week 4 culture supernatant (Fig. 3a, lane 5) and a component of approximately 290 kDa was detected in week 5 culture supernatant (Fig. 3a, lane 6) by the rabbit anti-FDT antiserum.

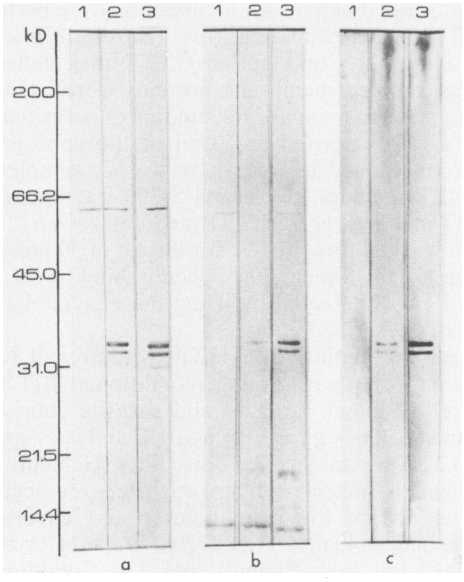

FIG. 1. Binding of components of conditioned LIT medium to host cell membrane polypeptides. Nitrocellulose strips contained PSC3H fibroblast membrane proteins (a), SVB6KH fibroblast membrane proteins (b), or P388D1 macrophage membrane proteins (c). Lanes 1, Fresh LIT medium; lanes 2, conditioned LIT medium (Brazil strain); lanes 3, conditioned LIT medium (Tulahuen strain). Antigen binding was detected by enzyme-labeled assay with rabbit anti-FDT as a primary antiserum.

The rabbit anti-CF antisera reacted with components of 175, 65, 53, 50, 43, 40, and 37 kDa in the week 1 culture supernatant (Fig. 3b, lane 2). Additional antigens of 90, 80, 27, and 26 kDa were detected in week 2 (Fig. 3b, lane 3), and

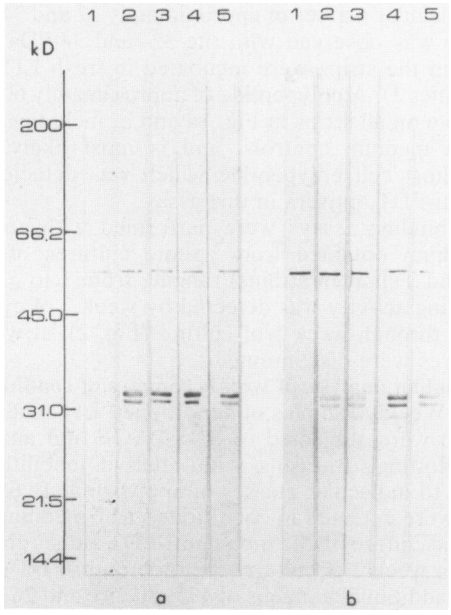

FIG. 2. Analysis of binding activity in weekly samples of conditioned LIT medium. Nitrocellulose strips contained PSC3H fibroblast membrane proteins resolved by SDS-PAGE. (a) Brazil-conditioned LIT medium; (b) Tulahuen-conditioned LIT medium. Lanes 1 through 5, Samples from weeks 1 through 5, respectively.

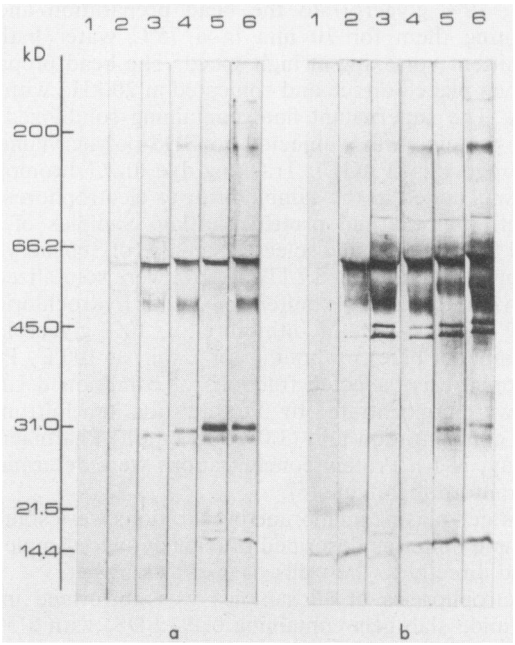

FIG. 3. Immunoblot analysis of weekly samples of conditioned LIT medium (Brazil strain). Samples were subjected to SDS-PAGE and electroblotted onto 8-mm nitrocellulose strips. Parasite antigen was detected by an enzyme-linked antibody assay utilizing rabbit anti-FDT antiserum (a) or rabbit anti-CF antiserum (b). Lanes 1, Fresh LIT medium; lanes 2 through 6, conditioned-medium samples from weeks 1 through 5, respectively.

in week 5 of culture, an antigen of approximately 290 kDa was detected by the CF-specific antiserum (Fig. 3b, lane 6). No reactivity was observed with components ranging in molecular mass from 26 to 250 kDa when the samples were exposed to normal rabbit serum (data not shown) or when fresh LIT medium was incubated with rabbit anti-FDT antiserum or rabbit anti-CF antiserum (Fig. 3, lanes 1), indicating that the antigens detected in the assay were of parasitic origin.

Sephacryl separation of conditioned LIT medium and analysis of binding activity. Samples of conditioned LIT medium were separated by gel filtration in an effort to determine which specific parasite components present in the medium were responsible for binding activity. The separation of conditioned LIT medium (Brazil strain) through a Sephacryl S-300 column resulted in four major peaks as determined by A_{280} (Fig. 4). Individual fractions constituting each peak were then combined and tested for binding activity with nitrocellulose strips containing PSC3H fibroblast membrane proteins. The results of this analysis showed that only peak 1 (the peak with the largest M_w) possessed detectable binding affinity for the 32- and 34-kDa host cell membrane polypeptides (Fig. 5). The peak 1 sample (Brazil strain) was concentrated by ultrafiltration, subjected to SDS-PAGE, and analyzed by immunoblotting. Antigens with relative mobilities corresponding to molecular masses of approximately 80, 68, and 27 kDa were detected in the peak 1 sample by both the rabbit anti-FDT and rabbit anti-CF antisera (Fig. 6, lanes 1 and 3). The rabbit anti-CF antiserum reacted with additional components of approximately 40 and 37 kDa that were not recognized by the FDT-specific antiserum (Fig. 6, lane 3). In addition, a series of high-molecular-mass antigens ranging from approximately 80 to 250 kDa were recognized

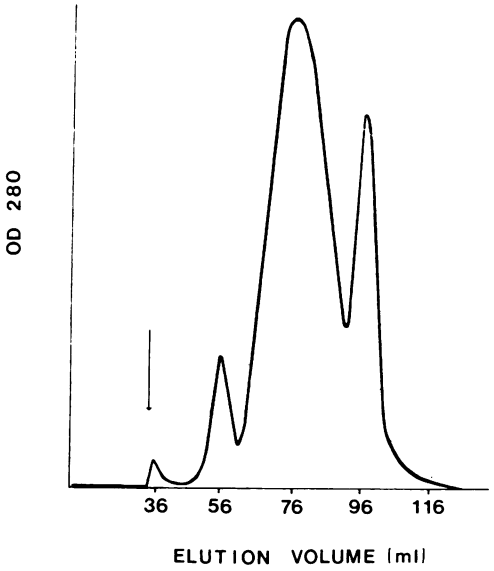

FIG. 4. Gel filtration analysis of conditioned LIT medium (Brazil strain) on Sephacryl S-300. The eluent used was Tris-saline buffer (40 mM Trizma HCl, 9.7 mM Trizma base, and 0.2 M NaCl [pH 7.4]). Arrow indicates the elution position of blue dextran (2,000 kDa).

only by the rabbit anti-FDT antiserum (Fig. 6, lane 1). No reactivity was observed when blots were probed with normal rabbit serum (Fig. 6, lane 2).

DISCUSSION

The release of antigens by *T. cruzi* into culture medium has been documented by several investigators. Rimoldi et al.

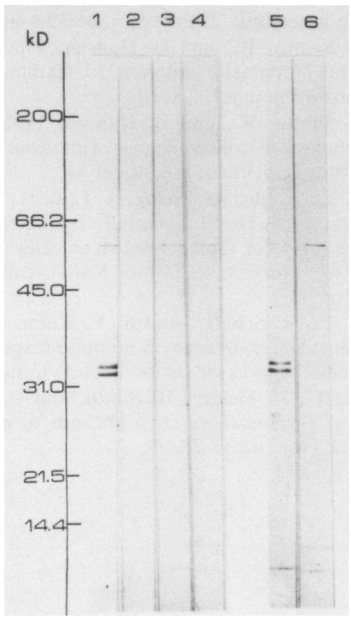

FIG. 5. Analysis of binding activity in Sephacryl S-300 fractions of conditioned LIT medium. Nitrocellulose strips contain PSC3H membrane proteins resolved by SDS-PAGE. Lanes 1 through 4, Strips incubated with peaks 1 through 4, respectively; lane 5, strip incubated with nonfractionated conditioned LIT medium (Brazil strain); lane 6, strip incubated with fresh LIT medium. Antigen binding was detected by an enzyme-linked antibody assay (see text).

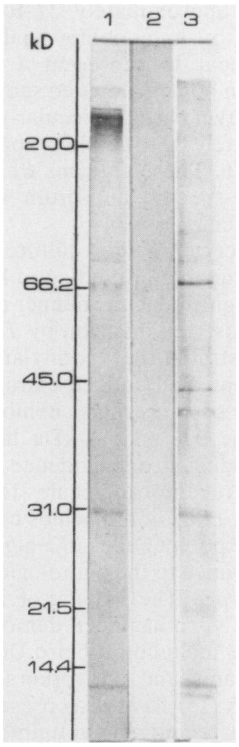

FIG. 6. Immunoblot analysis of the peak 1 fraction of conditioned LIT medium (Brazil strain). Samples were subjected to SDS-PAGE and electroblotted onto 8-mm nitrocellulose strips. Parasite antigen was detected by an enzyme-linked antibody assay utilizing rabbit anti-FDT antiserum (lane 1), normal rabbit serum (lane 2), and rabbit anti-CF antiserum (lane 3).

(16) described the release of molecules ranging in molecular mass from 87 to 155 kDa by trypomastigote stages of *T. cruzi* within 4 h of in vitro culture. The shed antigens were shown to accelerate the intrinsic decay of the alternative and classical pathways of C3 convertases. In addition, Andrews and co-workers (2) have documented the release of *T. cruzi* surface glycoproteins during in vitro culture, and Martin et al. (12) have shown that a complex polysaccharide is released into axenic culture media during log-phase growth of epimastigote stages. Gottlieb (9), Araujo (3), and Martin et al. (12) have provided evidence that antigens are also released by *T. cruzi* into the blood of infected hosts. The release of antigens by *T. cruzi* may, therefore, be a normal aspect of the process of growth and differentiation.

In this study, we have demonstrated that during axenic culture, culture forms of both the Brazil and Tulahuen strains of *T. cruzi* release molecules which possess binding activity for host cell molecules of approximately 32 and 34 kDa. This highly specific molecular interaction was observed with three different murine cell lines. Binding assays performed with conditioned LIT medium obtained from axenic cultures of *T. cruzi* (Brazil and Tulahuen strains) ranging in age from 1 to 5 weeks demonstrated that parasite antigens with an affinity for host cell molecules are detected in media by week 2 of culture. In culture, these parasite antigens appear in a high-molecular-weight aggregate which can be separated from other components present in the medium by gel filtration (Sephacryl S-300). It is only the high-molecular-weight fraction (peak 1) which retains binding activity for host cell membrane polypeptides of 32 and 34 kDa.

Several parasite antigens, including major components

which range from approximately 27 to 250 kDa, can be detected in peak 1 by immunoblot analysis. As expected, these antigens appear to represent a subset of parasite antigens identified in nonfractionated samples of conditioned LIT medium. However, high-molecular-mass antigens ranging from approximately 80 to 250 kDa appear to be enhanced in the peak 1 sample. These antigens were detected only by the trypomastigote-specific antiserum and therefore may represent stage-specific antigens.

The current observation that culture forms of *T. cruzi* release molecules capable of binding to host cell membrane polypeptides in a highly specific manner may have relevance to the process of host cell invasion by *T. cruzi*. In previous studies, we demonstrated that biotinylated antigen extracts of *T. cruzi*, nonconjugated antigen extracts of *T. cruzi*, and live infective stages of *T. cruzi* also exhibit a specific binding interaction with the 32- and 34-kDa host cell membrane polypeptides, providing strong evidence that these polypeptides may serve as receptor molecules for *T. cruzi* (8).

The release by *T. cruzi* of molecules capable of binding to host cell membrane polypeptides in a highly specific manner may also have relevance to the pathological consequences of infection with *T. cruzi*. The binding of *T. cruzi* antigens to mammalian cells in vitro has been demonstrated by a number of investigators, including Ribeiro Dos Santos and Hudson (14) and Williams and co-workers (18). Ribeiro Dos Santos and Hudson (15) have also presented evidence that the binding of parasite antigens to uninfected host cells can target them for destruction by antibody-dependent, complement-mediated lysis and by cytotoxic cells of the immune system of the host. Araujo (4), Abrahamsohn and Kloetzel (1), and Martin et al. (12) have demonstrated that both infected and noninfected cells possess parasite antigens on their surfaces following the rupture of infected cells in in vitro cultures. Araujo (4) further demonstrated that cells possessing parasite antigen were susceptible to destruction by lymphocytes (T enriched) obtained from chronically infected mice.

The results of the present study demonstrate that *T. cruzi* releases molecules with an affinity for specific host cell membrane polypeptides during axenic culture. Ongoing investigations are directed toward the further isolation and characterization of the specific parasite antigen(s) involved in this apparent receptor-ligand interaction.

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LITERATURE CITED

1. **Abrahamsohn, I. A., and J. K. Kloetzel.** 1980. Presence of *Trypanosoma cruzi* antigen on the surface of both infected and uninfected cells in tissue culture. Parasitology **80**:147–152.
2. **Andrews, N. W., K.-S. Hong, E. S. Robbins, and V. Nussenzweig.** 1987. Stage-specific surface antigens expressed during the morphogenesis of vertebrate forms of *Trypanosoma cruzi*. Exp. Parasitol. **64**:474–484.
3. **Araujo, F. G.** 1982. Detection of circulating antigens of *Trypanosoma cruzi* by enzyme immunoassay. Ann. Trop. Med. Parasitol. **76**:25–36.
4. **Araujo, F. G.** 1985. *Trypanosoma cruzi*: expression of antigens on the membrane surface of parasitized cells. J. Immunol. **135**:4149–4154.
5. **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.
6. **Bradford, M. M.** 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72**:248–254.
7. **Cohen, C. M., D. Kalish, B. S. Jacobson, and D. Branton.** 1977. Membrane isolation on polylysine-coated beads. Plasma membrane from HeLa cells. J. Cell Biol. **75**:119–134.
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. **Gottlieb, M.** 1977. A carbohydrate-containing antigen from *Trypanosoma cruzi* and its detection in the circulation of infected mice. J. Immunol. **119**:465–470.
10. **Jacobson, B. S.** 1980. Improved method for isolation of plasma membrane on cationic beads. Membranes from *Dictyostelium discoideum*. Biochim. Biophys. Acta **600**:769–780.
11. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
12. **Martin, U. O., D. Afchain, M. Loyens, C. Maidana, and A. Capron.** 1989. *Trypanosoma cruzi*: circulating polysaccharide factors excreted in vitro and in vivo. Medicina (B. Aires) **49**:33–36.
13. **Powell, M. R., and R. E. Kuhn.** 1987. Measurement of cytolytic antibody in experimental Chagas' disease using a terminal radiolabeling procedure. J. Parasitol. **66**:399–406.
14. **Ribeiro Dos Santos, R., and L. Hudson.** 1980. *Trypanosoma cruzi*: binding of parasite antigens to mammalian cell membranes. Parasite Immunol. **2**:1–10.
15. **Ribeiro Dos Santos, R., and L. Hudson.** 1980. *Trypanosoma cruzi*: immunological consequences of parasite modification of host cells. Clin. Exp. Immunol. **40**:36–41.
16. **Rimoldi, M. T., A. Sher, S. Heiny, A. Lituchy, C. H. Hammer, and K. Joiner.** 1988. Developmentally regulated expression by *Trypanosoma cruzi* of molecules that accelerate the decay of complement C3 convertases. Proc. Natl. Acad. Sci. USA **85**: 193–197.
17. **Tarleton, R. L., R. Schafer, and R. E. Kuhn.** 1983. Effects of extracts of *Trypanosoma cruzi* in immune responses: induction of a nonspecific suppressor factor. Infect. Immun. **41**:978–986.
18. **Williams, G. T., L. Fielder, H. Smith, and L. Hudson.** 1985. Adsorption of *Trypanosoma cruzi* proteins to mammalian cells in vitro. Acta Trop. **42**:33–38.