Analysis of *Trypanosoma cruzi* Antigens Bound by Specific Antibodies and by Antibodies to Related Trypanosomatids

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Received 6 December 1985/Accepted 1 April 1986

Antigens of the epimastigote stage of *Trypanosoma cruzi* were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis under reducing conditions and examined for their ability to bind antibodies in sera from humans infected with this organism or infected with one or both of the related protozoa *Leishmania braziliensis* and *Leishmania donovani* by protein blot analysis and enzyme-linked immunosorbent assay. Most of the antigens were bound by antibodies against each one of the organisms. A group of antigens with M_r s between 31,000 and 21,000 were bound by antibodies against *T. cruzi* only. These antigens were isolated and used in an enzyme-linked immunosorbent assay for the differential diagnosis of Chagas' disease, with excellent results. All sera from individuals proven to be infected with *T. cruzi* reacted with the antigens, whereas none of the sera from individuals proven to be infected with *L. braziliensis* or *L. donovani* reacted with the antigens, even when tested at a low dilution. Biochemical characterization of the isolated antigens revealed the presence of protein and carbohydrate. The reactivity of the isolated antigens with antibodies was completely abolished by pronase and partially abolished by sodium periodate. Protein blot analysis of sera from mice immunized with the antigens revealed a major large band with an M_r between 31,000 and 21,000 and a minor band with an M_r of 45,000, suggesting sharing of epitopes between antigens of different M_r s. These sera did not agglutinate or lyse live epimastigotes. Indirect immunofluorescent antibody tests with live and dead epimastigotes revealed that antibodies in the sera only bound to Formalin-killed organisms.

The flagellated trypanosomatids *Trypanosoma cruzi*, *Leishmania braziliensis*, and *Leishmania donovani*, causative agents of Chagas' disease and cutaneous and visceral leishmaniasis, respectively, are endemic in many countries in South America, and their geographic distribution overlaps in many areas. This, and the fact that the organisms share antigenic determinants, as demonstrated by significant cross-reactions in serological tests (10, 11), has had adverse consequences in the accurate diagnosis of the resulting infections, particularly of Chagas' disease (9). Although serological cross-reactions can be resolved in individual cases by the use of different assays and procedures (10), they can be confusing and misleading in serological surveys and epidemiological studies (15). Because of this, the World Health Organization (22) has assigned high priority to research work on antigens of specific parasites. This has considerably stimulated research on the identification and isolation of *T. cruzi* antigens that offer attractive possibilities for use in serological assays for the detection of *T. cruzi*-specific antibodies (12, 17, 18).

In this study, the protein blotting technique was used to examine the antigens of the epimastigote stage of *T. cruzi* that are recognized by antibodies in serum from individuals infected with *T. cruzi*, *L. braziliensis*, or *L. donovani*. Specific antigens were isolated, partially characterized biochemically, and used in an enzyme-linked immunosorbent assay (ELISA) for antibody detection.

MATERIALS AND METHODS

***T. cruzi*.** *T. cruzi* epimastigotes were obtained from cultures in exponential growth phase in brain heart infusion medium as previously described (2). The organisms were pelleted by centrifugation at $1,000 \times g$ for 20 min and washed twice by centrifugation in Dulbecco phosphate-buffered saline (PBS) containing 2% glucose. The final pellet was

solubilized in sample buffer (10 mM Tris hydrochloride [pH 6.8], 0.15 M NaCl, 2% sodium dodecyl sulfate, 1 mM phenylmethylsulfonyl fluoride) at the ratio of 5×10^8 organisms per ml of buffer. Solubilization was for 1 h at room temperature. Thereafter, the solubilized preparation was centrifuged at $10,000 \times g$ for 2 min in a Beckman microfuge to sediment coarse particles. The supernatant was used immediately, as described below.

Polyacrylamide gel electrophoresis and protein blotting. The discontinuous buffer system described by Laemmli (14) was used with 13% acrylamide slab gels measuring 140 by 120 by 1.5 mm. The stacking gel was 4.5% acrylamide and had one small lane 10-mm wide and one wide (120 mm) lane. The lanes were obtained by using a tooth in a blank Teflon (E. I. du Pont de Nemours & Co., Inc., Wilmington, Del.) comb. The small lane was used for the M_r markers, and the wide lane was used for the sample, which was 500 μ l of freshly solubilized epimastigote preparation per gel. Two to four gels were processed at one time. A 50- μ l amount of 1% bromophenol blue was added to the sample, which was layered into the wide lane. Running buffer (14) was added, and the sample was electrophoresed at 20 mA per gel until the stain front reached the bottom of the gel. Thereafter, the gels were mounted in a protein transfer apparatus (Bio-Rad Laboratories, Richmond, Calif.), and the separated polypeptides in the gels were transferred to nitrocellulose paper sheets by the method of Towbin et al. (21). The sheets were treated with a solution of 3% defatted milk in PBS (pH 7.2) for 1 h at room temperature with constant shaking, rinsed with PBS, and used for antigen detection as previously described (4). Briefly, vertical strips (10 by 120 mm) were cut from the sheets and treated overnight at room temperature with serum samples diluted 1:50 in PBS containing 1% bovine serum albumin (BSA) and 0.05% Tween 20 (PBS-BSA-T). After being washed with PBS, the strips were

treated for 1 h at room temperature with a peroxidase-labeled, affinity-purified rabbit anti-human immunoglobulin G (IgG) conjugate (Tago Laboratories, Burlingame, Calif.) diluted 1:1,000 in PBS-BSA-T. After another washing, the substrate, H_2O_2 , and the chromogen, diaminobenzidine, were added. Color was allowed to develop for 30 min. Thereafter, the strips were rinsed with tap water, dried, and photographed.

Elution of separated polypeptides from sodium dodecyl sulfate-polyacrylamide gel electrophoresis gels. To retrieve the electrophoresed antigens from gels, the following procedure was used. After completion of electrophoresis, the gels were layered on a template, and a 25-mm-wide, 120-mm-long strip of the gel area containing the M_r markers and part of the separated epimastigote polypeptides was cut vertically and stained with Coomassie blue. The remainder of the gel was cut horizontally into 12 strips. Each strip was minced into small fragments which were put into a glass tube containing 5 ml of sterile distilled water. Elution of the polypeptides from the minced gels was conducted at 4°C for 3 to 5 days with occasional vortexing.

Demonstration of antigens in gel eluates. The demonstration of antigens in gel eluates was done by an ELISA as previously described (2). Briefly, 100 μ l of each gel eluate, concentrated five times with CX-10 ultrafiltration and concentration units (Millipore Corp., Bedford, Mass.), was mixed volume to volume with 0.1 M carbonate buffer (pH 9.6) and added to triplicate wells of ELISA plates (Immulon; Dinattech Laboratories, Alexandria, Va.) which were incubated overnight at 4°C. Thereafter, the plates were washed with PBS containing 0.05% Tween 20 and post-coated with 3% BSA in PBS for 1 h at 37°C. Another washing with PBS containing 0.05% Tween 20 was followed by the addition to each well of 100 μ l of human serum with antibodies to *T. cruzi* diluted 1:40 in PBS-BSA-T. Incubation was for 1 h at 37°C, followed by a washing as described above and the addition of the peroxidase-labeled, affinity-purified rabbit anti-human IgG conjugate (Tago Laboratories) diluted 1:1,000 in PBS-BSA-T. Incubation was for 1 h at 37°C, followed by a washing and the addition of the substrate, H_2O_2 , and the chromogen, diaminobenzidine. Results were recorded as the average of the absorbancies of the triplicate wells as compared with the average of the absorbancies of the triplicate wells containing negative serum and tested against the eluate from a blank gel.

Biochemical assays. To avoid interference of sodium dodecyl sulfate in some of the biochemical assays, this detergent was removed from the gel eluates as previously described (13). Protein determinations were done by the protein dye binding assay (8) with rabbit gamma globulin as standard, and the anthrone method (7) was used to determine the concentration of carbohydrates. Treatment of the isolated antigens with pronase and sodium periodate (Sigma Chemical Co., St. Louis, Mo.) was performed as previously described (19). Staining of gels for carbohydrates was performed as previously described (24).

Preparation of antiserum against polypeptides eluted from gels. Antiserum against polypeptides eluted from gels was prepared by injection of mice with 50 μ g of the eluted polypeptides mixed with 50 μ g of saponin. Each mouse received three weekly injections and was bled 1 week after the last injection. Sera from three mice were pooled and examined for the presence of antibodies by the protein blotting technique described above. These sera were also used to perform an indirect immunofluorescent antibody (IFA) test with killed or live epimastigotes and tissue cul-

ture-derived amastigotes. Organisms were treated with 1% Formalin in PBS for 1 h in the refrigerator, washed three times with PBS, and layered onto a microscope slide. The indirect IFA test was performed as previously described (5), with serum diluted 1:40. The indirect IFA test with live organisms was done as follows. A suspension of washed organisms in microfuge tubes was treated with serum diluted 1:40 in PBS containing 0.001 M sodium azide for 60 min at 4°C (3). Thereafter, the organisms were washed three times with PBS and treated with an optimal dilution of fluorescein-conjugated rabbit anti-mouse IgG for 60 min at 4°C. After three more washings, the organisms were resuspended in PBS, and a drop was examined by fluorescence microscopy. The test was performed at 4°C and in the presence of sodium azide to prevent antibody-induced cell surface movement (capping).

Sera. Fifty sera from 50 individuals infected with *T. cruzi* and seven sera from 7 noninfected individuals from the same geographical area were used. These sera were kindly provided by M. E. Camargo, Instituto Medicina Tropical of Sao Paulo, Brazil, and were part of a panel of standard human antisera against *T. cruzi* distributed by the World Health Organization. Ten sera from 10 individuals infected with *L. braziliensis*, as demonstrated by isolation of the organism from cutaneous lesions, and two sera from 2 individuals infected with *L. donovani* were kindly provided by E. Nascimento and M. Norma Melo, Institute for Biological Sciences, Belo Horizonte, Brazil. All sera were mixed volume to volume with glycerol and were kept at 4°C.

Demonstration of antibodies to *T. cruzi* and *L. braziliensis*. Antibodies against *T. cruzi* and *L. braziliensis* in the serum from individuals infected with either or both organisms were demonstrated by ELISA as previously described (2).

Microtiter plates were coated with a previously determined optimal protein concentration (30 μ g/ml) of either a lysate of epimastigotes of *T. cruzi* (Y strain) or a lysate of promastigotes of *L. braziliensis* (kindly provided by M. Norma Melo and C. A. Costa, Institute for Biological Sciences). Coating of the microtiter wells with antigen diluted in 0.1 M carbonate buffer (pH 9.6) was performed overnight at 4°C. All sera were tested diluted 1:45 in PBS-BSA-T. The conjugate was an affinity-purified rabbit anti-human IgG used as described above.

In addition, each serum sample was tested in an ELISA in which the microtiter plates were coated with antigens that reacted with antibodies against (*T. cruzi* only or with antigens that reacted with antibodies against *T. cruzi*, *L. braziliensis*, and *L. donovani*, as demonstrated by protein blotting. These antigens were eluted from gels and concentrated, and sodium dodecyl sulfate was removed from them (13). They were used at a protein concentration of 30 μ g/ml in 0.1 M carbonate buffer (pH 9.6). In these experiments, most sera were used diluted 1:30, but some were tested serially diluted beginning at 1:10.

RESULTS

Cross-reactive antibodies. Representative results of the ELISA with antigens of *L. braziliensis* or *T. cruzi* and sera from individuals infected with one or both of the organisms are shown in Table 1. In general, the sera from infected individuals had higher titers when tested with antigens of the organism causing the respective infection. However, in a number of cases, the sera from infected individuals when tested with antigens of the infecting organism had titers equal to or slightly higher than the titers of sera from infected

individuals tested with antigens of the noninfecting organism. This was not a peculiarity of the ELISA technique since very similar results were noted when the sera were tested by an indirect IFA test.

The antigens of epimastigotes of *T. cruzi* recognized by antibodies formed after infection with *L. braziliensis* or *L. donovani* were visualized by the protein blotting technique. Results representative of experiments with 30 different sera are shown in Fig. 1. A large number of bands representing antigen-antibody reactions were noted where antibodies in sera from individuals infected with *T. cruzi* reacted with antigens of the epimastigote stage of *T. cruzi* (Fig. 1, lanes A and D). Lanes B to I of Fig. 1 show the results of the reaction between *T. cruzi* epimastigote antigens and antibodies in sera from individuals infected with *L. braziliensis* (lanes B to G and lane I) or with *L. donovani* (lane H). In these protein blots, a smaller number of bands than in blots A or D were noted. The results shown in lane D are quite interesting because the serum was from an individual with proven (by isolation of the causative organisms) infection with both *T. cruzi* and *L. braziliensis*. The pattern of the bands in blot D more closely resembles the pattern in blot A (*T. cruzi* infected) than it resembles the pattern in the other blots (*L. braziliensis* or *L. donovani* infected). Most of the *T. cruzi* epimastigote antigens recognized by antibodies against *L. braziliensis* or *L. donovani* were located just above the 68,000- M_r marker. In this area, a major band that was darkly stained and wide in four blots but that was present in all seven blots of sera from *L. braziliensis*- or *L. donovani*-infected individuals was conspicuously absent from the blots of sera from *T. cruzi*-infected individuals (Fig. 1, lane A and, particularly, D). A wide and diffuse band below the 68,000- M_r marker was present in all blots. None of the blots of each one of the sera from *L. braziliensis*- or *L. donovani*-infected individuals revealed bands between the 31,000- and

21,000- M_r markers. However, each one of the blots of sera from *T. cruzi*-infected individuals revealed numerous (lane A) or two or three (lane D) bands in this area, suggesting that the antigens recognized were specific for *T. cruzi* and therefore strong candidates for isolation attempts. A cluster of at least three bands was clearly detected in all blots, except lane D, in the area between the 21,000- and 14,000- M_r markers.

Determination of the epimastigote antigens specific for antibodies against *T. cruzi*. The results described above prompted us to examine the cross-reactive and specific antigenic polypeptides in an ELISA for detection of antibodies. The epimastigote preparation was electrophoresed in 13% polyacrylamide slab gels which were then cut into horizontal strips as shown in Fig. 1 (strips 1 to 12, to right of figure). Antigens eluted from the gel strips were standardized on the basis of protein content and used to coat wells of microtiter plates for the ELISA. Representative results with serum from individuals infected with *T. cruzi* only, *L. braziliensis* only, or both organisms are shown in Fig. 2. Sera from individuals infected with *T. cruzi* only or with *T. cruzi* and *L. braziliensis* reacted with the antigens eluted from each one of the gel strips. In contrast, all 10 sera from *L. braziliensis*-infected individuals and the 2 sera from *L. donovani*-infected individuals reacted with antigens eluted from gel strips 1 to 7, 11, and 12 (Fig. 1). No reaction was noted with antigens eluted from gel strips 8, 9, and 10. These results were in accord with those obtained with the protein blots, which indicated that epimastigote antigens in the area of the gel just below the 68,000- M_r markers strongly cross-reacted with antibodies against *L. braziliensis* and *L. donovani*, and those in the area between the 31,000- and 21,000- M_r markers were specific for antibodies against *T. cruzi*.

ELISA with antigens isolated from polyacrylamide gels. To confirm the observation described above, the cross-reactive antigen eluted from gel strip 5 and the *T. cruzi*-specific antigens eluted from gel strips 9 and 10 (Fig. 1) were used in an ELISA for detection of antibodies against *T. cruzi*. The results are shown in Fig. 3. All sera from individuals infected with *T. cruzi*, *L. braziliensis*, or *L. donovani* gave a positive result with the cross-reactive antigen eluted from gel strip 5, whereas only sera from *T. cruzi*-infected individuals gave a positive result with the specific antigens eluted from gel strips 9 and 10. All sera were used diluted 1:30. Similar results were obtained with lower dilutions (1:10 or 1:20) of the test serum, but the absorbancy readings with the negative controls were not acceptable at these serum dilutions.

Characteristics of the specific antigen. Mice injected with antigens eluted from gel strips 5 or 9 and 10 formed antibodies that reacted in the protein blots mainly with the respective antigens (Fig. 4). The antibodies formed against antigens in gel strip 5 recognized an antigen on the surface of the membrane of epimastigotes, as evidenced by an indirect IFA test performed with live organisms (data not shown). In contrast, the antibodies formed against antigens in gel strips 9 and 10 apparently reacted with cytoplasmic antigens only, since the indirect IFA test with live organisms did not reveal any surface fluorescence. The IFA test with Formalin-fixed parasites was positive (Fig. 5). Of interest was the observation that the antigens were also present in tissue culture-derived, formalinized amastigotes of *T. cruzi* (Fig. 5).

Treatment with pronase of both antigen preparations from gel strips 5 or 9 and 10 reduced considerably their ability to react with antibodies in the ELISA (Table 2). Because staining of the gels for carbohydrates had indicated their

TABLE 1. ELISA titers of sera from individuals infected with *T. cruzi*, *L. braziliensis*, or both

Serum source/serum no.	Titer of serum with antigens of ^a :	
	<i>L. braziliensis</i>	<i>T. cruzi</i>
<i>L. braziliensis</i> -infected individual		
1	80	80
2	160	80
3	320	80
4	80	80
5	160	80
6	640	160
7	160	40
8	160	80
<i>L. braziliensis</i> - and <i>T. cruzi</i> -infected individual		
9	640	5,120
<i>T. cruzi</i> -infected individual		
10	640	640
11	40	160
12	160	2,560
13	160	640
14	80	640
15	160	5,120

^a Titers are expressed as the reciprocal of the highest serum dilution in which the average absorbance of two replicate wells of the test serum was higher than the average absorbance plus two standard deviations of eight wells of negative serum control diluted 1:20.

FIG. 1. Protein blots of antigens of *T. cruzi* epimastigotes treated with serum from *T. cruzi*-infected (lanes A and D), *L. braziliensis*-infected (lanes B to G and lane I), or *L. donovani*-infected (lane H) humans. All strips were cut from a single protein blot. Numbers to the left of the figure are M_r markers. Numbers to the right of the figure indicate where gels were cut horizontally for elution of the separated polypeptides as described in the text.

presence in the area of the gels corresponding to strips 5, 9, and 10, eluates were treated with sodium periodate to determine the effects of this treatment on the ability of the antigens to bind antibodies. The results revealed a decrease of 47% in the absorbance of the ELISA performed with eluates of gel strip 5, whereas the decrease with pooled eluates from gel strips 9 and 10 was only 12% (Table 2).

DISCUSSION

In this report, we demonstrated that a large number of antigenic polypeptides of *T. cruzi* epimastigotes are bound by antibodies against *L. braziliensis* and *L. donovani*. Most polypeptides were of an M_r between 92,000 and 35,000 and below 21,000, and there was a considerable variation in the number of polypeptides recognized by antibodies from each of the individuals infected with either *L. braziliensis* or *L.*

donovani. This variation may reflect differences in the strains of the infecting organisms or differences in the antigen recognition process of the immune system of infected individuals. Some antigenic polypeptides were recognized by antibodies in serum from each one of the *L. braziliensis*- or *L. donovani*-infected individuals. This was the case for an antigen or a group of antigens of an approximate M_r of 72,000 that was recognized as one wide dark band and various thin bands with some sera (Fig. 1, lanes B, C, E, and G) and as a thin line with other sera (Fig. 1, lanes F, H, and I). This antigen was not detected at all (Fig. 1, lane D) or was, apparently, faintly detected (Fig. 1, lane A) by antibodies against *T. cruzi*. It apparently has an M_r similar to that of a glycoprotein that has been reported to be present in the epimastigote stage but absent from the trypomastigote and amastigote stages of *T. cruzi* (16, 20). Our results may substantiate this observation. Thus, because epimastigotes

FIG. 2. ELISA with eluates of each gel strip and sera from individuals infected with *T. cruzi* (x), *L. braziliensis* (○), or both (Δ). Each point represents the mean A_{495} of two duplicate wells coated with the respective gel eluate. Dilution of the sera was 1:30. The shaded area represents the average absorbancies of eight negative controls plus two standard deviations. Eluates were obtained from gel strips cut as shown in Fig. 1. Numbers under arrows indicate M_r markers.

FIG. 3. Scattergram of the ELISA results of the reaction between sera from individuals infected with *L. braziliensis* or *L. donovani* (x) or with *T. cruzi* (●) and epimastigote antigens from gel eluates. Each point represents the average absorbance of replicate wells. The broken line indicates the average A_{495} plus two standard deviations of eight wells of negative control serum diluted 1:30. The in-run and between-run variations in the ELISA were minimal.

FIG. 4. (A) Coomassie blue-stained part of the gel used for elution of the antigens showing (to the left) the M_r markers (lane 1) and the separated polypeptides of epimastigotes (lane 2). (B) Protein blots of epimastigote antigens treated with serum from mice immunized with eluates 5 (lane 1) and 9 and 10 (lane 2) from gel strips. M_r markers are shown to the left of the gel.

FIG. 5. Indirect IFA test with formalinized epimastigotes and amastigotes and serum from mice immunized with eluates of gel strips 9 and 10.

TABLE 2. Effect of pronase and sodium periodate on the capacity of antigens eluted from gels to bind antibodies against *T. cruzi*^a

Reagent	A ₄₉₅ with eluate(s) of gel strip no.:	
	5	9 and 10
PBS	0.256	0.212
Pronase	0.072	0.064
Sodium periodate	0.135	0.186
Control ^b	0.042	0.038

^a Gel eluates were treated with reagents and used to coat microtiter plates for an ELISA with human serum containing antibodies against *T. cruzi*.

^b ELISA performed as described above, with a negative human serum.

are not present in the vertebrate host, antibodies against the 72,000-*M_r* glycoprotein were not formed. However, antigens with determinants similar to those of the 72,000-*M_r* glycoprotein of *T. cruzi* epimastigotes are present in the stages of *L. braziliensis* or *L. donovani* present in infected humans.

Antibodies in the serum from the individual infected with both *T. cruzi* and *L. braziliensis* (Fig. 1, lane D) apparently did not recognize the 72,000-*M_r* antigen; the reasons for this are not clear at this time. Of the antigenic polypeptides recognized by antibodies against all three trypanosomatids, those located just below the 68,000-*M_r* marker gave the strongest reaction with antibodies against each one of the three protozoa. In the protein blots, these antigens appeared as a wide and undefined band when reacted with antibodies from infected individuals. Immunization of mice with these antigens, however, resulted in the formation of antibodies that defined the wide band into at least six bands.

Antibodies against each one of the trypanosomatids reacted in the protein blots and ELISA with antigens in eluates of gel cut from the 90,000-*M_r* area. This was of interest because one antigen of *T. cruzi* epimastigotes, the 90,000-*M_r* glycoprotein isolated by affinity chromatography with *Lens culinaris* lectin, has been shown to be specific for *T. cruzi* antibodies (12, 18). Our method for isolating the antigens from the gels provided not a purified 90,000-*M_r* glycoprotein but a mixture of polypeptides in the area of the 90,000-*M_r* glycoprotein. Thus, the cross-reactivity we noted may have been due to a contaminant. It is of interest, however, that the ELISA we used to determine cross-reactivity between antibodies against *L. braziliensis* or *L. donovani* and antigens of *T. cruzi* was performed with serum diluted 1:30. When serum dilutions equal to those used by Schechter et al. (18) and Dragon et al. (12) were used, little or no binding of *Leishmania* antibodies to antigens eluted from the 90,000-*M_r* area of the gel was noted.

Several antigenic polypeptides located between the 31,000- and 21,000-*M_r* markers were recognized by antibodies against *T. cruzi* but not by antibodies against *L. braziliensis* or *L. donovani* in either the protein blots or the ELISA. The number of polypeptides recognized varied from serum to serum, but at least one was noted in each one of more than 30 protein blots of serum from individuals infected with *T. cruzi*. One of the recognized polypeptides may represent the 25,000-*M_r*, *T. cruzi*-specific glycoprotein that was described by Scharfstein et al. (17) as being located in the cell membrane surface of all developmental stages of *T. cruzi*, as well as in several different strains of the organism. Our results indicated that there are a number of antigenic polypeptides between the 21,000- and 31,000-*M_r* markers and that all of them are *T. cruzi*-specific since antibodies against any of the *Leishmania* species did not recognize

them. Immunization of mice with polypeptides from this area of the gel resulted in the formation of antibodies that recognized two major and two or three minor antigens. One of these antigens may be a breakdown product or may share a determinant with a heavier-*M_r* antigen. This is because one antigen in the area of the 45,000-*M_r* marker was recognized by antibodies in the sera from mice immunized with gel eluates from the area between 31,000 and 21,000 *M_r*. Antibodies against polypeptides in this area of the gel reacted in the IFA test with formalinized but not with live epimastigotes and amastigotes, suggesting that they were directed against cytoplasmic antigens or against cryptic membrane antigens that are exposed only when the normal structure of the membrane of the organism is disrupted by some chemical treatment, such as formalinization. This result was unexpected, since the 25,000-*M_r* glycoprotein described by Scharfstein et al. (17) is a cell surface antigen and must have been eluted from the gels. It is possible that this glycoprotein is present in small quantity in relation to the other antigens eluted from the gels. Therefore, the formation of antibodies against it may have been hampered because of antigenic competition. Two lines of evidence suggest that glycosylated polypeptides may represent a minor component of the array of antigens eluted from the 31,000- to 21,000-*M_r* area of the gels. One is the result of the anthrone test, which indicated that the concentration of carbohydrates comprised less than 10% of the concentration of proteins; another is that treatment of the eluted antigens with sodium periodate had a minor deleterious effect upon their ability to bind to antibodies, whereas treatment with pronase abolished this ability almost completely.

It has been shown that each of the three stages of the *T. cruzi* life cycle presents stage-specific antigens (6, 16, 23, 25). The epimastigote stage, however, is the one that appears to possess the most varied array of antigenic components. Thus, they offer the largest number of antigenic determinants for binding by antibodies against *T. cruzi* (1, 2). Our results, in conjunction with the results reported by others (12, 17, 18), indicate that antigens specific for antibodies against *T. cruzi* can be obtained from the organism at the epimastigote stage. This and the fact that epimastigotes are easily grown and obtained in large quantities indicate that this stage is the one best suited for the isolation of specific antigens for the serodiagnosis of Chagas' disease.

ACKNOWLEDGMENTS

I thank Douglas R. Guptill and Robert Sadler for expert technical help and Jessica Erickson for help in the preparation of the manuscript.

This work was supported by Public Health Service grant 18794 from the National Institutes of Health and by the United Nations Development Program/World Bank/World Health Organization Special Programme for Research and Training in Tropical Diseases.

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