JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 1996, p. 2143–2147 Vol. 34, No. 9
0095-1137/96/$04.00+0

Immunoblot Assay Using Excreted-Secreted Antigens of *Trypanosoma cruzi* in Serodiagnosis of Congenital, Acute, and Chronic Chagas' Disease

EUFROSINA S. UMEZAWA,[1]* MARILDA S. NASCIMENTO,[1] NORIVAL KESPER, JR.,[1] JOSÉ R. COURA,[2] JOSÉ BORGES-PEREIRA,[2] ANGELA C. V. JUNQUEIRA,[2] AND MARIO E. CAMARGO[1]

Instituto de Medicina Tropical de São Paulo, Faculdade de Medicina da Universidade de São Paulo, CEP 05403-000, São Paulo,[1] and Departamento de Medicina Tropical, Instituto Oswaldo Cruz, Rio de Janeiro,[2] Brazil

Received 22 January 1996/Returned for modification 8 April 1996/Accepted 21 June 1996

Immunoblotting with trypomastigote excreted-secreted antigens (TESA blot) of *Trypanosoma cruzi* was evaluated as a method for diagnosis of chronic and acute phases as well as congenital (in newborn children) Chagas' disease. Serum samples from acute-phase and congenital infections were considered to be positive when they reacted with ladder-like bands of 130- to 200-kDa antigens, recognized by immunoglobulin M (IgM) and IgG antibodies, while IgG from chronic-phase sera recognized a broad band antigen of 150 to 160 kDa. Nonchagasic sera were not reactive to these antigens. The study was carried out on 512 patients, 111 of whom were nonchagasic but included cases of leishmaniasis or other pathologies, and 401 chagasic patients. The latter group comprised 361 chronic cases, 36 acute cases, and 4 congenital cases in newborn children. Among the chronic cases, 256 were from areas in which *T. cruzi* is endemic but which differed widely in the pathogenic expression of *T. cruzi* infection and in parasitemia levels. These patients at the same time showed a broad range of low, medium, and high reactivity to conventional enzyme-linked immunosorbent assays and indirect immunofluorescence serotests for Chagas' disease. For these reasons they may better represent the universe of chagasic patients than would a sample of highly reactive sera obtained from chagasic patients in a single area endemic for *T. cruzi*. All acute and congenital cases showed positivity in the IgM and IgG TESA blots, while chronic cases were 100% positive for IgG antibodies. In nonchagasic sera, including 30 cases of visceral and muco-cutaneous leishmaniasis, the specificity index was 1.000, and no cross-reactions were observed. The TESA blot thus seems to be useful as a sensitive and specific diagnostic assay in cases of suspected acute or congenital *T. cruzi* infection and as a general confirmatory test for conventional Chagas' disease serology.

Chagas' disease affects several million people in Latin America. It is characterized by a short highly parasitic acute phase, which is not diagnosed in most cases, and a subsequent chronic phase that persists for life (3, 28), though only a few parasites are found in the blood. The agent, *Trypanosoma cruzi*, is normally transmitted by infected triatomid bugs; however, blood transfusions (28) and, infrequently, congenital origins (7, 17) are of increasing importance as ways of acquiring the disease.

Serological diagnosis of Chagas' disease by using extracts of *T. cruzi* epimastigotes as antigens, although of limited specificity (3, 5, 19, 27), displays high sensitivity in the chronic phase of the disease but low sensitivity in the acute phase (14, 19, 24, 25) and in congenital (7, 17) infection. For these cases, greater sensitivity is obtained by use of shed acute-phase antigen/*trans*-sialidase (SAPA/TS), an enzyme released from the surface of *T. cruzi* trypomastigotes (1, 10, 20) and expressed as a recombinant peptide (1, 12, 13, 17). The fact that trypomastigotes spontaneously release into the medium polypeptides of different molecular weights (1, 8, 15, 16, 30) which are recognized by chagasic sera (1, 10, 16) has led us to evaluate the usefulness of these antigens, in their native form, for immunodiagnosis of acute, congenital, and chronic Chagas' disease. Trypomastigote excretion-secretion antigens (TESA) of *T. cruzi* Y, collected in supernatants of infected LLC-MK_2 cells, were used as antigens for an immunoblot assay.

To evaluate the diagnostic efficiency of the test during these different phases of Chagas' disease, two groups of patients were studied. The first comprised 145 chagasic patients (acute, congenital, and chronic stages) and 111 nonchagasic individuals. The second group was composed of 256 chronic chagasic patients from three areas in Brazil; 244 of these patients were also examined by xenodiagnosis and PCR parasitological tests (2, 11, 29).

MATERIALS AND METHODS

Antigens. TESA from *T. cruzi* Y were obtained from the supernatant of infected LLC-MK_2 cells as described elsewhere (10), but with some modifications. Four days after infection with 5×10^6 trypomastigotes, the cells were washed twice and reincubated for 18 to 20 h, at 37°C in 5% CO_2, on RPMI 1640 medium (Sigma), with or without 2% fetal calf serum (FCS). Different batches of supernatants (containing 10×10^6 to 20×10^6 trypomastigotes per ml) were recovered, centrifuged at $2,800 \times g$ for 10 min at 4°C, filtered through a cellulose acetate membrane (pore size, 0.20 μm), and used immediately or stored at −70°C. Protein contents of 30 to 40 μg/ml (the range of four different batches) were quantified in supernatants obtained without FCS (micro-bicinchoninic acid protein assay reagent kit; Pierce).

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting. Supernatants (150 μl) from infected cultures were diluted (vol/vol) in SDS-sample buffer (60 mM Tris-HCl [pH 6.8], 5% 2-mercaptoethanol, 10% glycerol, and 0.01% bromophenol blue), and boiled for 5 min at 100°C. Samples were loaded in a 7% polyacrylamide minigel (Mini-Protean II; Bio-Rad) using 2-D/preparative comb (1.0 by 1 mm). Lysates of 30×10^6 *T. cruzi* tissue culture trypomastigotes (TCT) were processed in the same way (26). Separated antigens were electrophoretically transferred on 0.45-μm-pore-size polyvinylidene difluoride (PVDF; Millipore) or nitrocellulose membranes (Bio-Rad), in a semidry system (Hoefer Scientific Instruments), for 1 h. The blotted

* Corresponding author. Mailing address: Instituto de Medicina Tropical de São Paulo, Faculdade de Medicina da Universidade de São Paulo, Av. Dr. Enéas de Carvalho Aguiar 470, CEP 05403-000, São Paulo, Brazil. Fax: 55-11-852-3622.

antigens were blocked with Tris-buffered saline (TBS) (10 mM Tris-HCl [pH 7.5], 150 mM NaCl) containing 5% defatted milk (blocking buffer) for 1 h. Membrane strips (5 mm) were later incubated with sera diluted 1:200 in TBS–1% milk, for 2 h, with mechanical agitation. After four (5-min) washes in TBS, the bound antibodies were detected with peroxidase-conjugated goat anti-human immunoglobulin M (IgM) (Biosys, Compiègne, France), anti-human IgG, or anti-rabbit IgG (Sigma), diluted in TBS–1% milk, for 2 h. After new cycles of washes the immune complexes were revealed by addition of H_2O_2 and 4-chloro-1-naphthol. The reaction was stopped with deionized water. The molecular mass standards (Sigma) used were 205 kDa, rabbit muscle myosin; 116 kDa, *Escherichia coli* β-galactosidase; 97 kDa, rabbit muscle phosphorylase *b*; 66 kDa, bovine albumin; and 45 kDa, ovalbumin.

Parasitological tests. Acute patients in Group 1 (see below) were examined by xenodiagnosis; chronic patients in Group 2 were examined by PCR and/or xenodiagnosis tests. Xenodiagnosis was carried out with 40 *Panstrongylus megistus* and was performed as described elsewhere (11); to detect parasite DNA, PCR amplifications with *T. cruzi* minicircle-specific primers were performed as described (11, 29). Parasitological diagnosis of the four congenital cases was carried out by direct examination of the buffy coat after centrifugation of heparinized blood.

Serological tests. Sera diluted at 1:200 were assayed by epimastigote alkaline extract–enzyme-linked immunosorbent assay (EAE-ELISA) to detect anti-*T. cruzi* IgM and IgG antibodies; a polyantigenic extract of epimastigote forms of *T. cruzi* Y strain (obtained after treatment with 0.3 M NaOH) (24) was used. The indirect immunofluorescence (IIF) test was performed with formaldehyde-fixed epimastigote forms of the Y strain, with sera diluted at 1:10 to 1:1,280 by using fluorescein-labeled anti-human IgG or IgM immunoglobulins (Hoechst-Behring, Marburg, Germany) (14).

Study populations. (i) Group 1. Serum samples were collected from 145 chagasic seropositive patients (as determined by ELISA and IIF) and 111 nonchagasic seronegative patients (with confirmed clinical, epidemiological, and serological diagnoses of their respective pathologies). The chagasic sera from 40 patients in the acute phase of Chagas' disease included sera from 4 parasitologically positive congenitally infected newborn children (5 to 27 days after birth) and sera from 36 patients who had been infected orally, by transfusion, by laboratory accident, or by vectorial transmission. The diagnoses of infection were based on clinical symptoms, parasitological assays, and the detection of anti-*T. cruzi* IgM antibodies (24). The 105 patients in the chronic phase of Chagas' disease, diagnosed according to data from clinical evolution, electrocardiography, and radiology, plus a positive serology for Chagas' disease, were classified as follows: (i) indeterminate form (n = 57), (ii) cardiac form (n = 40), or (iii) digestive form (n = 8) (14, 21). The 111 nonchagasic seronegative patients included (i) 35 blood donors from an area of endemic *T. cruzi*; (ii) 30 cases of leishmaniasis from a region where Chagas' disease is nonendemic (6 mucocutaneous cases and 24 active visceral cases); (iii) 2 patients infected with *Trypanosoma rangeli*; and (iv) 44 patients with unrelated diseases, as defined by their respective clinical and laboratory diagnoses (5 with syphilis, 5 with connective tissue diseases and positive for anti-nuclear antibodies, 6 with systemic lupus erythematosus, 8 with malaria, 4 with paracoccidioidomycosis, 4 with nonchagasic idiopathic cardiomyopathy, 4 with nonchagasic megaesophagus, 4 with a positive anti-streptolysin O test, and 4 with rheumatic fever).

(ii) Group 2. Serum samples were collected in three areas of Brazil where Chagas' disease is endemic: in the State of Minas Gerais (Virgem da Lapa), and in the North-Eastern States of Paraíba (Sertão da Paraíba) and Piauí (Sertão do Piauí). Serum samples were taken from 256 patients in the chronic phase of the disease, with confirmed clinical, epidemiological, and serological diagnoses (ELISA and IIF); 244 patients were also examined by PCR and/or xenodiagnosis (PCR+Xe) parasitological tests. These different areas present clear-cut differences in parasitemia levels and above all in the pathogenic expression of Chagas' disease. In Virgem da Lapa, cardiac and digestive forms of the disease are frequent, but the indeterminate form is more prevalent in the areas investigated in Sertão do Piauí and in Sertão da Paraíba (2, 6). Positivity results for parasitological tests (PCR+Xe) were as follows: 95% in Virgem da Lapa (n = 86), 67% in Sertão do Piauí (n = 81), and 32% in Sertão da Paraíba (n = 77). Patients in Group 2 were also reactive by the results of conventional serotests (EAE-ELISA) for Chagas' disease and were classified according to the range of optical density (OD) results as low (Ch1, OD = 0.20 to 0.99), medium (Ch2, OD = 1.00 to 1.99), or high (Ch3, OD > 2.00) titer.

All sera were stored after dilution (vol/vol) with glycerin at −20°C.

Rabbit serum raised against SAPA/TS was kindly donated by A. C. C. Frasch (Instituto de Investigaciones Bioquímicas, Fundação Campomar, Buenos Aires, Argentina). Serum samples from a rabbit infected with *T. cruzi* Y were collected in the acute phase (16th day after infection) and the chronic phase (1 year after infection), as described elsewhere (26), and rabbit anti-epimastigote serum was collected 5 months after immunizations as described elsewhere (26).

Data analysis. TESA blot results were defined as positive by naked-eye observation of bands. For EAE-ELISA, samples were recorded as positive or negative in relation to the cutoff value, calculated as the mean OD of the blood donor serum plus 3 standard deviations (SD). The cutoff value for IIF results was recorded as 1:20 (14).

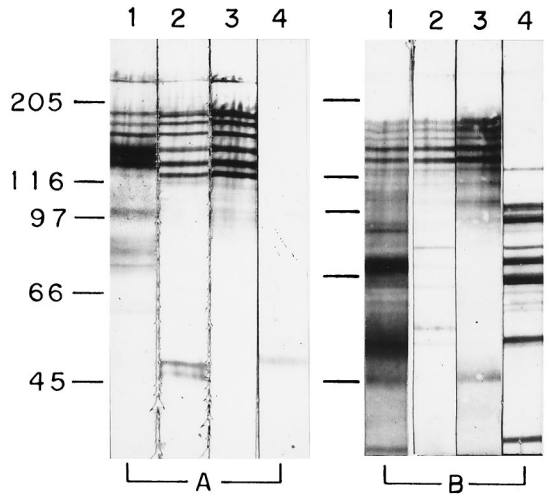

FIG. 1. Patterns recognized in TESA blot (A) and immunoblot (B) of lysed TCT by IgG antibodies from chronic-phase (lane 1) and acute-phase (lane 2) rabbit sera. The ladder-like 130- to 200-kDa antigens were recognized by rabbit serum raised against SAPA/TS (lane 3) and by rabbit serum immunized with total extract of the epimastigote form of *T. cruzi* (lane 4). Molecular mass markers are on the left.

RESULTS

The secreted-excreted antigens are spontaneously and continuously shed from the surface membrane of TCT into the medium, and they were used for immunoblotting (TESA blot) to diagnose chronic and acute phases of Chagas' disease.

In this assay, polypeptides of between 130 and 200 kDa were recognized by acute-phase rabbit serum as a ladder-like pattern (Fig. 1A, lane 2) and also by a rabbit serum raised against SAPA/TS (Fig. 1A, lane 3), which clearly indicates the presence of these molecules in the TESA blot. On the other hand, the chronic-phase rabbit serum reacted with a broad band of 150 to 160 kDa (Fig. 1A, lane 1), although this reactivity was lower than that in chronic-phase human sera (see Fig. 3, lanes 1 to 6). The presence of the same pattern of excreted-secreted antigens was visible in different supernatant batches (Fig. 2, lanes 1 to 3); these batches contained antigens with a ladder-like pattern, recognized by acute-phase patient serum (Fig. 2B), and a 150- to 160-kDa band, recognized by chronic-phase patient serum (Fig. 2A).

On the other hand, a Western blot (immunoblot) of lysed TCT showed complex reactivity patterns. IgG from acute-phase rabbit sera recognized mainly high-molecular-weight components (Fig. 1B, lane 2), while chronic-phase sera reacted mainly with low-molecular-weight components (Fig. 1B, lane 1). This complexity impairs a clear distinction between acute and chronic cases, which is not the case with the TESA blot.

The lysed TCT also gave rise to a ladder-like pattern of SAPA/TS, as shown when the anti-homologous serum was used (Fig. 1B, lane 3). Anti-epimastigote hyperimmune rabbit serum recognized several antigens in the TCT immunoblot (Fig. 1B, lane 4) but did not react with the 130- to 200-kDa or with the 150- to 160-kDa antigens in the TESA blot (Fig. 1A, lane 4).

TESA blot assays were considered to be positive when samples reacted with the ladder-like antigens of 130- to 200-kDa and/or with the 150- to 160-kDa antigen; sera from some chagasic patients also produced weak recognition of bands of between 80 and 120 kDa (Fig. 3, lanes 1 to 3).

When the supernatants containing TESA were collected

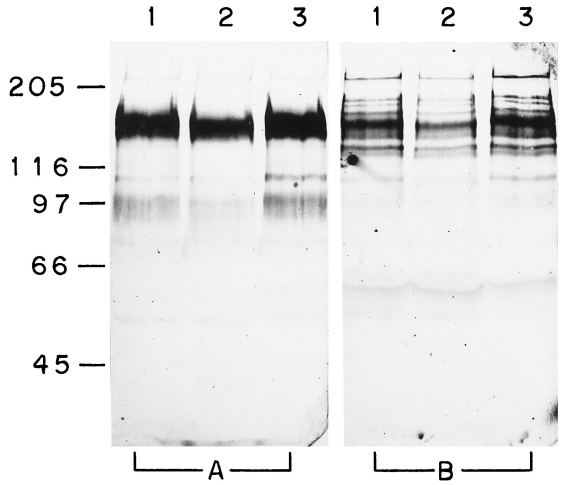

FIG. 2. Reproducibility of TESA blot, shown in three different batches of supernatants (lanes 1 to 3). The 150- to 160-kDa antigen is recognized by serum from a chronic chagasic patient (A), and the ladder-like antigens, with molecular masses of 130 to 200 kDa, are recognized by serum from a chagasic acute patient (B). Each lane of the TESA blot was prepared by loading 10 μl of supernatants of *T. cruzi* Y. Molecular mass markers are on the left.

from cultures supplemented with 2% FCS, IgG or IgM antibodies in some chronic-phase or acute-phase sera reacted with bands between 45 and 55 kDa (Fig. 3, lanes 7 and 8′); this was not observed, however, in supernatants without FCS (not shown).

Acute patients (n = 36) in Group 1 showed 100% positivity in the TESA blot, both for IgG (Fig. 3, lanes 7 and 8) and IgM (Fig. 3, lanes 7′ and 8′), which recognized ladder-like antigens with molecular masses of 130 to 200 kDa as well as certain other minor and inconstant bands which are not considered in this evaluation. Conventional serology with EAE-ELISA displayed a positivity of 89% for IgG assays (OD = 0.68 ± 0.39) (Fig. 4A, column ChA) and 97% for IgM (OD = 0.57 ± 0.26); these assays not infrequently displayed low reactivity near the

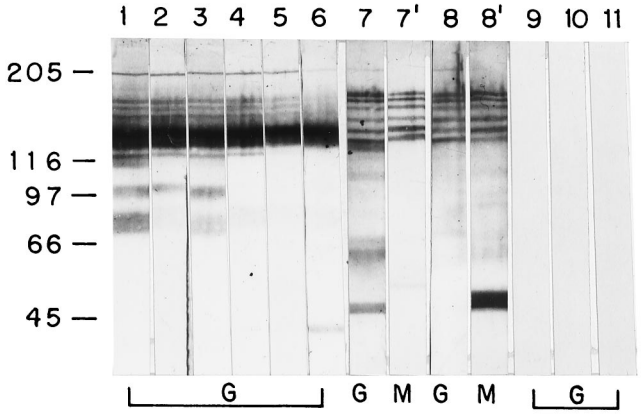

FIG. 3. TESA blot polypeptides recognized by IgG (G) from chronic (lanes 1 to 6) or acute chagasic patients (lanes 7 and 8) or by IgM (M) from acute chagasic patients (7′, 8′). Patients in lanes 1, 5, and 6 were classified as having indeterminate forms and patients in lanes 2 to 4 were classified as having cardiac forms of chronic Chagas' disease. Patients in lanes 1, 3, and 6 were positive for PCR+Xe. Reactivities of IgG from nonchagasic groups are shown as follows: normal (lane 9), *T. rangeli* (lane 10), and leishmaniasis (lane 11). The lanes in this figure represent the patterns found in the 512 serum samples analyzed. Molecular mass markers are on the left.

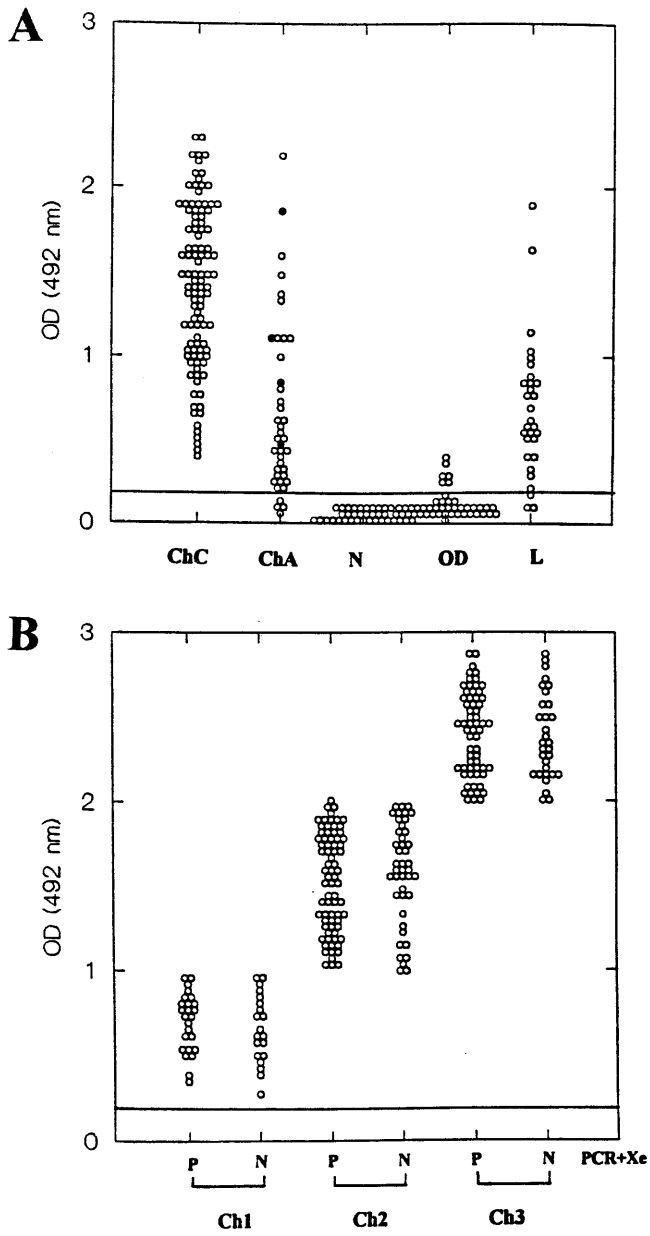

FIG. 4. Evaluation of IgG reactivity by EAE-ELISA. (A) Group 1 patients (n = 256) were classified according to the chronic (ChC) or acute (○) and congenital (●) (ChA) phases of Chagas' disease; nonchagasic individuals were classified as N, normal (blood donors); OD, other diseases; and L, leishmaniasis. (B) Group 2 patients (n = 256) in the chronic phase of Chagas' disease were classified as Ch1, Ch2, and Ch3, respectively, according to the following titers: low (OD = 0.20 to 0.99), medium (OD = 1.00 to 1.99), and high (OD = >2.00). Classifications according to parasitological tests (PCR+Xe) were N, negative, and P, positive. The horizontal line in each panel represents the cutoff value.

cutoff value and are often considered as giving doubtful results. Similar results were obtained with the IIF test (data not shown).

The four congenital patients studied had parasites circulating in their bloodstreams and were reactive in the EAE-ELISA with IgG (OD = 1.09 ± 0.53) but nonreactive in the EAE-ELISA with IgM (OD = 0.14 ± 0.08). A doubtful result for IgM was seen in an IIF test in one case, but the other three cases were negative (not shown). However, all four cases were

undoubtedly positive in the TESA blot; antigens of 130 to 200 kDa were recognized by both IgM and IgG antibodies, while there was no detectable reactivity to the 150- to 160-kDa antigen.

In the IgG TESA blot survey, sera from all of the 105 chronic patients in Group 1 and from 256 patients in Group 2, a total of 361 chronic chagasics, reacted with the 150- to 160-kDa antigen (Fig. 3, lanes 1 to 6); a few serum samples also recognized the 130- to 200-kDa antigens. In the EAE-ELISA (Fig. 4A, column ChC, and B) and IIF (not shown), 100% of patients were also positive, but no reactivity to IgM antibodies was found in these patients. No correlation was observed among different clinical forms of the chronic phase or between the presence of circulating parasites and the TESA blot patterns (Fig. 3, lanes 1 to 6).

Chronic patients in Group 2 were classified according to the mean OD reactivity in EAE-ELISA as being of low (Ch1), medium (Ch2), or high titers (Ch3). Positivities of the PCR+Xe parasitological tests for these subgroups were as follows: Ch1 = 56%, Ch2 = 67.5%, and Ch3 = 68%. No differences were seen among the TESA blot patterns for these groups.

Specificity of the TESA blot was evaluated in the 111 nonchagasic serum samples from Group 1. No reactivity was observed either for the 130- to 200-kDa antigen or the 150- to 160-kDa antigens (Fig. 3, lanes 9 to 11). A specificity index of 1.000 was thus found. Meanwhile, conventional serology using *T. cruzi* epimastigote antigens in ELISA and IIF tests showed lower indices of specificity, 0.700 and 0.750, respectively, mainly due to cross-reactions with sera from leishmaniasis patients, which showed a positivity of 90% in EAE-ELISA (OD = 0.68 ± 0.40) (Fig. 4A, column L).

DISCUSSION

The presence of IgM antibodies in the course of Chagas' disease confirms the infection to be in the acute phase (4, 17, 24, 25). Yet in this same phase low sensitivity to IgG and IgM antibodies is displayed by the conventional serological techniques for the diagnosis of the disease (14, 19, 24, 25), and it is common for detection of IgM not to occur in congenital cases (7, 17). It has been argued that this failure might be due to insufficient sensitivity in relation to the antigens usually employed (extracts of *T. cruzi* epimastigotes or whole forms) in the conventional tests (ELISA and IIF) (24). Although efficient for serological diagnosis in the chronic phase, these antigens unfortunately show a limited specificity and frequent cross-reactions (3, 9, 27), mainly with leishmaniasis sera (5, 14, 19).

Purified antigens from *T. cruzi* epimastigotes have been described as presenting good specificity and high sensitivity in the diagnosis of chronic cases of Chagas' disease (18, 21, 22). However, there is no reference to satisfactory sensitivity in acute or congenital cases (18, 21, 22); satisfactory sensitivity has been observed for a lipopeptidophosphoglycan of *T. cruzi* epimastigotes (24), but unfortunately it is of low specificity. All antigens described as serologically able to discriminate acute- from chronic-phase infections, and which apparently react preferentially with antibodies from acute-phase sera, are located at the surface of the trypomastigotes (1, 16, 17, 25). The TESA blot, containing shed trypomastigote specific antigens, shows high sensitivity not only to chronic cases of Chagas' disease but also to acute and congenital cases; recognition of these is a further outstanding characteristic of the TESA blot and has already been described for a few cases (25). The 150- to 160-kDa antigen seems to be well conserved in different strains, and our preliminary results show that it can be detected by human chronic-phase serum in a TESA blot prepared from four *T. cruzi* strains (Y, CL-Brener, José-IMT, and BS-IMT) (23).

T. cruzi trypomastigotes are known to spontaneously release polypeptides of different molecular weights into the culture medium, and these antigens have been characterized by different researchers (1, 8, 15, 16, 30). Soluble antigens are shed from the parasite surface independently of the presence or absence of FCS or bovine serum albumin in the culture medium (8). As shown previously by our group, the loss of polypeptides from *T. cruzi* of different strains correlates well with the appearance of these antigens in the cultures (8).

One of the shed polypetides already characterized and cloned (20) is SAPA/TS, the antigenic epitopes of *T. cruzi trans*-sialidase enzyme, that upon denaturation migrate as multiple bands ranging from 100 to 220 kDa in SDS-PAGE (1, 20). The bands vary in number and relative molecular weight, depending on the strain of the parasite (20). The presence of SAPA/TS in the TESA blot has been confirmed with homologous rabbit immune serum (Fig. 1), as well as with IgG and IgM from acute-phase human sera (Fig. 3).

Antibodies from acute and congenital patients always react in the TESA blot with a ladder-like pattern, which for *T. cruzi* Y comprises six bands from 130 to 200 kDa. As observed in the 36 acute and 4 congenital cases studied, the TESA blot for IgG and IgM antibodies depicts a higher sensitivity than do the conventional tests such as ELISA or IIF, which use the epimastigote forms of *T. cruzi.*

Positivity for IgG, recognizing the 150- to 160-kDa antigen, has been described for chronic patients (10, 25) and is here confirmed in the 361 chronic cases studied; several of these also show a less intense IgG reactivity to the ladder-like 130- to 200-kDa antigens. This is not surprising, since this reactivity has been found in 37% of chronic indeterminate chagasic patients when the recombinant SAPA/TS was employed as antigen (13). In our analysis we included a sample made up of 256 patients from areas of endemic disease in Brazil; these areas significantly differ in the pathological expression of Chagas' disease (2, 6) and in the percentages of positivity for parasitological tests (11, 29) (Fig. 4B). These patients were divided into groups of low, medium, and high reactivity, according to titers in the ELISA with the epimastigote antigen (Fig. 4B). In this way they are certainly more representative of the universe of chronic chagasics than are panels of highly reactive serum samples from a particular area of endemic disease, which are not infrequently employed for evaluating tests and antigens.

Our data agree with previous data (10, 25) in that the presence of a ladder-like pattern of SAPA/TS antigens in the TESA blot is responsible for the observed sensitivity in the diagnosis of congenital and acute infections. On the other hand, the 150- to 160-kDa antigen is recognized by all patients in the chronic phase of Chagas' disease; this confirms previous data obtained from a small number of serum samples (10, 25). The high sensitivity of the TESA blot in chronic cases, even when these cases are of low reactivity in conventional assays, in combination with the absence of cross-reactions with leishmaniasis or *T. rangeli* infections (9), suggests that the TESA blot is a reliable serological alternative to conventional tests in cases which show low sensitivity and that it may be used as a specific confirmatory assay to exclude cross-reactions after serum screening by EAE-ELISA.

ACKNOWLEDGMENTS

This investigation was supported by grants from FAPESP and FMUSP-LIM-49.

We thank M. A. Shikanai-Yasuda, K. Takei, and V. L. da Matta for supplying some of the sera used in this study, and we thank A. R. Ferreira for the photography.

REFERENCES

1. **Affranchino, J. L., C. F. Ibañez, A. O. Luquetti, A. Rassi, M. B. Reyes, R. A. Macina, L. Aslund, U. Pettersson, and A. C. C. Frasch.** 1989. Identification of a *Trypanosoma cruzi* antigen that is shed during the acute phase of Chagas' disease. Mol. Biochem. Parasitol. **34:**221–228.
2. **Borges-Pereira, J., R. V. Cunha, H. P. Willcox, and J. R. Coura.** 1990. Evolução da cardiopatia chagásica crônica humana no sertão do estado da Paraíba, Brasil, no período de 4, 5 anos. Rev. Soc. Bras. Med. Trop. **23:**141–147.
3. **Camargo, M. E.** 1992. An appraisal of Chagas' disease serodiagnosis, p. 165–178. *In* S. Wendel, Z. Brener, M. E. Camargo, and A. Rassi (ed.), Chagas' disease (American trypanosomiasis): its impact on transfusion and clinical medicine, ISBT Brazil 92, Sociedade Brasileira de Hematologia e Hemoterapia, São Paulo, Brazil.
4. **Camargo, M. E., and V. Amato-Neto.** 1974. Anti-*Trypanosoma cruzi* IgM antibodies as serological evidence of recent infection. Rev. Inst. Med. Trop. São Paulo **16:**200–202.
5. **Chiller, T. M., M. A. Samudio, and G. Zoulek.** 1990. IgG antibody reactivity with *Trypanosoma cruzi* and *Leishmania* antigens in sera of patients with Chagas' disease and leishmaniasis. Am. J. Trop. Med. Hyg. **43:**650–656.
6. **Coura, J. R., and J. Borges-Pereira.** 1984. A follow-up evaluation of Chagas' disease in two endemic areas in Brazil. Mem. Inst. Oswaldo Cruz Rio J. **79:**107–112.
7. **Freilij, H., and J. Altech.** 1995. Congenital Chagas' disease: diagnostic and clinical aspects. Clin. Infect. Dis. **21:**551–555.
8. **Gonçalves, M. F., E. S. Umezawa, A. M. Katzin, W. De Souza, M. J. M. Alves, B. Zingales, and W. Colli.** 1991. *Trypanosoma cruzi*: shedding of surface antigens as membrane vesicles. Exp. Parasitol. **72:**43–53.
9. **Guhl, F., L. Hudson, C. J. Marinkelle, C. A. Jaramillo, and D. Bridge.** 1987. Clinical *Trypanosoma rangeli* infection as a complication of Chagas' disease. Parasitology **94:**475–484.
10. **Jazín, E. E., A. O. Luquetti, A. Rassi, and A. C. C. Frasch.** 1991. Shift of excretory-secretory immunogens of *Trypanosoma cruzi* during human Chagas' disease. Infect. Immun. **59:**2189–2191.
11. **Junqueira, C. V., E. Chiari, and P. Wwincker.** 1996. Comparison of the polymerase chain reaction with two classical parasitological methods for the diagnosis of Chagas' disease patients in a north-eastern endemic region of Brazil. Trans. Roy. Soc. Trop. Med. Hyg. **90:**129–132.
12. **Levin, M. J., J. F. da Silveira, A. C. C. Frash, M. E. Camargo, S. Lafon, W. M. Degrave, and R. Rangel-Aldao.** 1991. Recombinant *Trypanosoma cruzi* antigens and Chagas' disease diagnosis: analysis of a workshop. FEMS Microbiol. Immunol. **89:**11–20.
13. **Lorca, M., A. Gonzalez, C. Veloso, V. Reyes, and U. Vergara.** 1992. Immunodetection of antibodies in sera from symptomatic and asymptomatic Chilean Chagas' disease patients with *Trypanosoma cruzi* recombinant antigens. Am. J. Trop. Med. Hyg. **46:**44–49.
14. **Matsumoto, T. K., S. Hoshino-Shimizu, P. M. Nakamura, H. F. Andrade, Jr., and E. S. Umezawa.** 1993. High resolution of *Trypanosoma cruzi* amastigote antigen in serodiagnosis of different clinical forms of Chagas' disease. J. Clin. Microbiol. **31:**1486–1492.
15. **Norris, K. N., and J. E. Schrimpf.** 1994. Biochemical analysis of the membrane and soluble forms of the complement regulatory protein of *Trypanosoma cruzi*. Infect. Immun. **62:**236–243.
16. **Ouaissi, M. A., A. Taibi, M. Loyens, U. Martin, D. Afchain, C. Maidana, C. Caudioti, J. Cornette, A. Martelleur, P. Velge, B. Marty, and A. Capron.** 1991. *Trypanosoma cruzi*: a carbohydrate epitope defined by a monoclonal antibody as a possible marker of the acute phase of human Chagas' disease. Am. J. Trop. Med. Hyg. **45:**214–225.
17. **Reyes, M. B., M. Lorca, P. Muñoz, and A. C. C. Frasch.** 1990. Fetal IgG specificities against *Trypanosoma cruzi* antigens in infected newborns. Proc. Natl. Acad. Sci. USA **87:**2846–2850.
18. **Scharfstein, J., A. Luquetti, A. C. M. Murta, M. Senna, J. M. Rezende, A. Rassi, and L. Mendonça-Previato.** 1985. Chagas' disease serodiagnosis with purified GP 25 antigen. Am. J. Trop. Med. Hyg. **34:**1153–1160.
19. **Schattschneider, W., E. R. Lopes, J. E. Alencar, U. Bienzle, and H. Feldmeier.** 1992. A comparative study of four serological methods for diagnosis of acute and chronic Chagas' disease in Brazilian patients. Trop. Geogr. Med. **44:**210–218.
20. **Schenkman, S., D. Eichinger, M. E. A. Pereira, and V. Nussenzweig.** 1994. Structural and functional properties of *Trypanosoma cruzi* trans-sialidase. Annu. Rev. Microbiol. **48:**499–523.
21. **Solana, M. E., A. M. Katzin, E. S. Umezawa, and C. S. Miatello.** 1995. High specificity of *Trypanosoma cruzi* epimastigote ribonucleoprotein as antigen in serodiagnosis of Chagas' disease. J. Clin. Microbiol. **33:**1456–1460.
22. **Teixeira, M. G. M., J. Borges-Pereira, E. Netizert, M. L. N. X. Souza, and J. M. Peralta.** 1994. Development and evaluation of an enzyme linked immunotransfer blot technique for serodiagnosis of Chagas' disease. Trop. Med. Parasitol. **45:**308–312.
23. **Umezawa, E. S., K. A. Almeida, and N. Kesper, Jr.** Unpublished data.
24. **Umezawa, E. S., M. A. Shikanai-Yasuda, A. Gruber, V. L. Pereira-Chiocolla, and B. Zingales.** 1996. *Trypanosoma cruzi* defined antigens in the serological evaluation of an outbreak of acute Chagas' disease in Brazil (Catolé do Rocha, Paraíba). Mem. Inst. Oswaldo Cruz Rio J. **91:**87–93.
25. **Umezawa, E. S., M. A. Shikanai-Yasuda, and A. M. S. Stolf.** Changes in isotype composition and antigens of anti-*Trypanosoma cruzi* antibodies from acute to chronic Chagas' disease. J. Clin. Lab. Anal., in press.
26. **Umezawa, E. S., A. M. S. Stolf, and B. Zingales.** 1993. *Trypanosoma cruzi*: different surface antigens of trypomastigotes are targets of lytic antibodies. Acta Trop. **54:**41–53.
27. **Velásquez, E., L. Reyes, C. Thors, A. Miettimen, M. Chinchilla, and E. Linder.** 1993. Autoantibodies give false reaction in the serodiagnosis of *Trypanosoma cruzi* infection. Trans. Roy. Soc. Trop. Med. Hyg. **87:**35.
28. **Wendel, S., and A. L. Gonzaga.** 1993. Chagas' disease and blood transfusion: a new universe problem? Vox Sang. **64:**1–12.
29. **Wincker, P., C. Britto, J. Borges-Pereira, M. A. Cardoso, W. Oblemann, and C. Morel.** 1994. Use of a simplified polymerase chain reaction procedure to detect *Trypanosoma cruzi* in blood samples from chronic patients in a rural endemic area. Am. J. Trop. Med. Hyg. **51:**771–777.
30. **Yokayama-Yasunaka, J. K. U., E. M. F. Pral, O. C. Oliveira, Jr., S. C. Alfieri, and A. M. S. Stolf.** 1994. *Trypanosoma cruzi*: identification of proteinases in shed components of trypomastigote forms. Acta Trop. **57:**307–315.