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Serodiagnosis of Chronic and Acute Chagas' Disease with *Trypanosoma cruzi* Recombinant Proteins: Results of a Collaborative Study in Six Latin American Countries

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An enzyme-linked immunosorbent assay to diagnose Chagas' disease by a serological test was performed with *Trypanosoma cruzi* recombinant antigens (JL8, MAP, and TcPo). High sensitivity (99.4%) and specificity (99.3%) were obtained when JL8 was combined with MAP (JM) and tested with 150 serum samples from chagasic and 142 nonchagasic individuals. Moreover, JM also diagnosed 84.2% of patients in the acute phase of *T. cruzi* infection.

A serological test is the most reliable and practical method for the diagnosis of Chagas' disease, an illness that is caused by the protozoan *Trypanosoma cruzi* and that affects millions of people in Latin America (22). The risk of *T. cruzi* transmission by transfusion in areas where Chagas' disease is endemic is assessed by performing at least two different tests to detect specific antibodies (23, 25, 26). In countries where Chagas' disease is not endemic, it is advisable to use serological tests on persons born in or given blood transfusions in countries where Chagas' disease is endemic (8, 9, 12, 14, 27). The acute phase of Chagas' disease is rarely diagnosed, because it is often without symptoms (22). Moreover, natural transmission by triatomine bugs is under control in some Latin American countries. Furthermore, there is still a need for continuing epidemiological surveillance in countries where transmission has not yet been controlled (5, 22). Conventional serological tests for Chagas (CSC tests) (e.g., indirect immunofluorescence [IIF], indirect hemagglutination [IHA], and enzyme-linked immunosorbent assay [ELISA]) usually employ semipurified antigens from the epimastigote form of *T. cruzi*. Consequently, CSC tests yield relatively large numbers of inconclusive and false-positive results (4, 19, 23, 25), mainly when a concomitant infection, such as leishmaniasis, is present (4, 31), and the sensitivities of CSC tests are far from ideal in the diagnosis of the early acute phase of disease (3, 28, 30) or in patients with low titers of anti-*T. cruzi* antibodies (31). This nonideal performance may have social, legal, and economic implications. To overcome these problems, several laboratories developed new serodiagnostic tests using antigens from infective trypomastigote forms (1, 28, 30) or a combination of *T. cruzi* recombinant proteins and/or synthetic peptides (4, 6, 7, 13, 20, 21, 24, 31).

The International Atomic Energy Agency organized a collaborative study to develop an ELISA with a mixture of *T. cruzi* recombinant antigens for immunodiagnosis of the acute and chronic phases of Chagas' disease. In this study, we evaluated the performance of three *T. cruzi* recombinant antigens (JL8, MAP, and TcPo) with serum samples from patients living in six Latin American countries (Table 1). Previous studies showed that JL8 and TcPo react with immunoglobulin G (IgG) antibodies of patients with chronic Chagas' disease (15–18), and assays with JL8 showed high sensitivity and specificity (4, 7, 13, 15–18). MAP is recognized by IgG antibodies from chronic and acute chagasic patients (11; unpublished data).

Mixtures of recombinant antigens perform better than single proteins. Several studies have shown that the use of a single antigen in an assay does not confer the required sensitivity (4, 7, 16, 18, 21, 29, 31). In this study, the reactivities of recombinant antigens, used singly or in different combinations, were compared with the reactivities of the following tests: (i) CSC tests (IIF, IHA, epi-ELISA [ELISA that uses semipurified antigens from the epimastigote form of *T. cruzi*], and EAE-ELISA [in-house ELISA that uses the epimastigote form of *T. cruzi*]) (31); (ii) a test using a mixture of recombinant proteins (BHF) developed for diagnosis of chronic cases (31); and (iii) a Western blot assay that uses antigens from *T. cruzi* trypo-

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TABLE 1. Geographical origin and distribution of serum samples of *T. cruzi*-infected individuals and nonchagasic individuals

Country	No. of serum samples	No. of individuals			
		T. cruzi infected		Nonchagasic	
		Acute phase	Chronic phase	Other diseases	Healthy
Bolivia	51	0	15	0	36
Brazil	172	10	81	53	28
Honduras	40	0	35	0	5
Mexico	5	0	3	0	2
Panama	38	9	20	9	0
Venezuela	16	0	7	0	9
Total	322	19	161	62	80

mastigotes (TESA blot assay) (28, 31). The diagnostic performance of ELISA with JL8, MAP, and TcPo antigens used singly or in various combinations of two or three antigens was evaluated first, using a panel of serum samples from 11 Brazilian patients with the chronic phase of Chagas' disease that were positive by CSC tests. The optimal concentration of each component was determined by cross-titration: the optimal serum and conjugate dilutions were determined to be 1:50 and 1:6,000, respectively. Microtiter plates (high binding; Costar) were coated with 50 μl of antigen/well. The antigens used follow: antigens JL8 (1,000 ng ml^{-1}), MAP (200 ng ml^{-1}), and TcPo (200 ng ml^{-1}) alone; mixtures of two antigens, such as JL8 and MAP (JM) (250 ng ml^{-1}), MAP and TcPo (MT), and JL8 and TcPo (JT) (300 ng ml^{-1}); or all three antigens together, namely, MAP, JL8, and TcPo (MJT) (350 ng ml^{-1}). Titration of antigen binding to microtiter plates was performed by recombinant proteins labeled with iodine (^{125}I), as previously described (29). Higher average absorbance (A_{492}) (mean ± standard deviation [SD]) and sensitivity values were obtained with JL8 (0.89 ± 0.41 and 100%, respectively), followed by MAP (0.85 ± 0.56 and 82%, respectively) and TcPo (0.56 ± 0.42 and 73%, respectively) (Fig. 1A). Using different combinations of antigens in ELISAs resulted in a sensitivity of 100%, with higher reactivities than those of the single recombinant antigens. The reactivities of the antigen combinations were 1.34 ± 0.50 for JM, 1.19 ± 0.59 for MT, and 1.17 ± 0.48 for MJT. BHF and EAE-ELISA had a sensitivity of 100%, with averages of 1.26 ± 0.42 and 1.13 ± 0.22, respectively (Fig. 1A). The JT combination had a low sensitivity (not shown), so its use was discontinued.

Some mixtures of recombinant proteins also detect anti-*T. cruzi* IgG antibodies from acute-phase patients. The capacities of mixtures of recombinant antigens (JM, MT, and MJT) to detect acute-phase antibodies were tested. JM and MJT were able to detect 84.2% and MT was able to detect 78.9% of acute cases (9 samples from Panama and 10 from Brazil) (Table 1 and Fig. 1B). JL8 and MAP antigens are made up of 14- and 38-amino-acid repeats, respectively, that are strongly conserved in strains and isolates of *T. cruzi* (11, 15), which improved the sensitivity of diagnosis of acutely infected individuals. These results were quite similar to those described for recombinant SAPA (shed acute-phase antigen) that has been employed in the diagnosis of the acute phase of Chagas' disease (2, 7, 16, 20); its sensitivity varied from 80.8 to 90% (2, 16). The specificities for acute-phase sera were 15.8% by BHF, 94.7% by EAE-ELISA (Table 2 and Fig. 1B), and 100% by TESA blotting (IgG and IgM [not shown]).

Mixtures of recombinant proteins are more specific than whole-epimastigote antigens. The specificities of recombinant mixtures were determined with sera from 142 nonchagasic individuals (62 of these individuals had other diseases) (Table 2). The specificities of JM, MT, and MJT recombinant proteins were 99.3, 96.5, and 98.6%, respectively (Table 2). BHF and TESA blotting presented specificities of 99.3 (Table 2) and 100% (not shown), respectively. The sensitivity of EAE was 95.8%, since 6 of 10 sera from patients with leishmaniasis showed a cross-reaction (Table 2). The cross-reactivity of sera from individuals infected with *Trypanosoma rangeli* in CSC tests has been the subject of controversy (32), but our samples from *T. rangeli*-infected individuals were not reactive in assays that use *T. cruzi* recombinant proteins or in CSC tests.

Evaluation of the JM mixture to diagnose *T. cruzi*-infected individuals from different geographical areas. The JM mixture gave a sensitivity of 99.3% (Table 2 and Fig. 2) with sera from

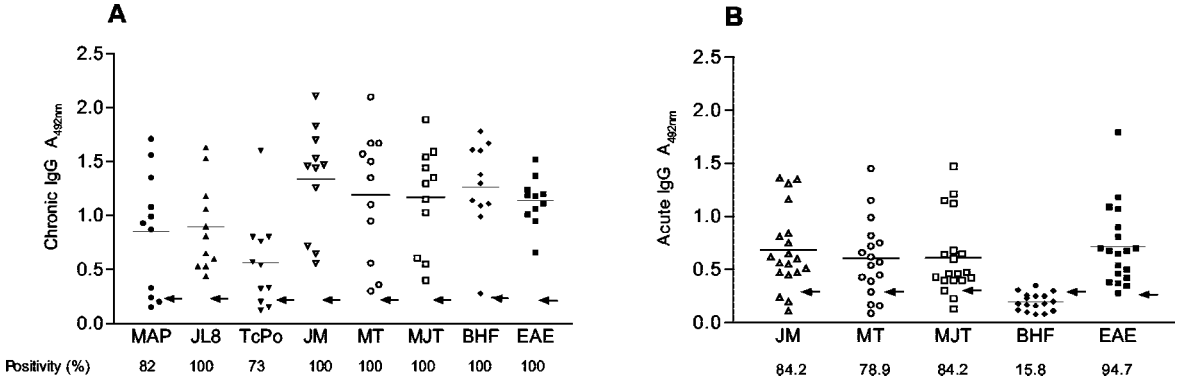

FIG. 1. (A) Reactivity data of *T. cruzi* recombinant antigens MAP, JL8, and TcPo individually or in various combinations of two or three proteins (JM, MT, and MJT) with sera from 11 Brazilian patients with well-defined chronic-phase Chagas' disease. (B) Reactivity data of recombinant mixtures JM, MT, MJT, and BHF with 19 acute-phase sera. The sensitivities of the different antigens or tests are shown at the bottom of the figure. EAE-ELISA data are shown in panels A and B. For each antigen, the average is indicated by the short horizontal line, and the arrow indicates the cutoff value.

TABLE 2. Sensitivity of ELISA with mixtures of recombinant proteins JM, MT, and MJT compared with those of BHF and EAE-ELISA

Individual	No. of individuals	Sensitivity (%)				
		ELISA with:			BHF[a]	EAE[b]
		JM	MT	MJT		
Chagasic	180					
Acute	19	84.2	78.9	84.2	15.8	94.7
Chronic	11	100.0	100.0	100.0	100.0	100.0
Chronic	150[c]	99.4	ND[d]	ND	ND	100.0
Nonchagasic	142					
Healthy	80	0	0	0	0	0
Other diseases[e]	43	2.3	7.0	2.3	2.3	0
T. rangeli	9	0	0	0	0	0
Leishmaniasis	10	0	20.0	10.0	0	60.0
Specificity[f]	142	99.3	96.5	98.6	99.3	95.8

[a] Mixture of recombinant antigens (BHF) developed for diagnosis of chronic patients (31).
[b] EAE-ELISA, an in-house test with the epimastigote form of *T. cruzi* (31).
[c] Sera positive in three CSC tests (IIF, IHA, and epi-ELISA).
[d] ND, not determined.
[e] Five individuals had toxoplasmosis, four had malaria, five had paracoccidioidomycosis, five had schistosomiasis, 19 had connective tissue disorders positive for antinuclear antibodies, and 5 had rheumatic fever.
[f] Specificity data obtained with 142 samples from nonchagasic individuals.

chagasic individuals (n = 150) (Table 1) that showed a sensitivity of 100% in three CSC tests (not shown) and with EAE-ELISA (Table 2). The average absorbance (± SD) or reactivity obtained with JM was high for chagasic patients from Bolivia (1.54 ± 0.33), Brazil (1.50 ± 0.63), Honduras (1.67 ± 0.59), and Mexico/Venezuela (1.58 ± 0.62) but low for Panama (0.75 ± 0.64) (Fig. 2). The sensitivity presented by JM (99.4%) was similar to those described with mixtures of recombinant antigens and/or synthetic peptides (4, 10, 20, 21, 31). Notwithstanding the fact that several reports have reported greater diagnostic ability of recombinant protein mixtures, there are always a few chagasic patients in whom anti-*T. cruzi* antibodies were not detected or the reactivity was near the cutoff value (10, 20, 21, 24, 31). This could be due to the heterogeneity of different parasite strains and/or variability in individual host

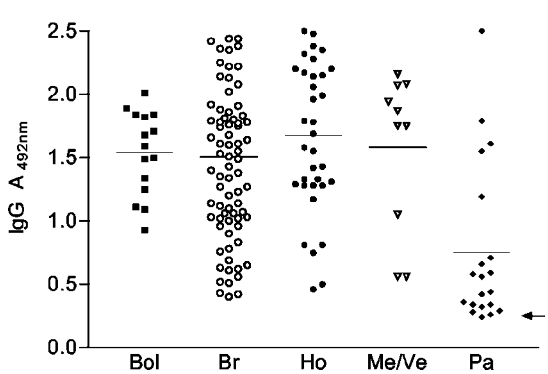

FIG. 2. Reactivity data of recombinant antigens JM with 150 serum samples from patients infected with *T. cruzi* from regions of Bolivia (Bol), Brazil (Br), Honduras (Ho), Mexico and Venezuela (Me/Ve), and Panama (Pa) where Chagas' disease is endemic. For each country, the average is indicated by the short horizontal line, and the arrow indicates the cutoff value.

immune responses. In conclusion, our results show that recombinant proteins in mixtures, such as JM, are very useful as antigens for immunodiagnosis of acute and chronic Chagas' disease and that the specificity was superior to that of CSC tests that employ whole or semipurified fractions from epimastigote forms of *T. cruzi*.

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REFERENCES

1. **Almeida, I. C., D. T. Covas, L. M. T. Soussumi, and L. R. Travassos.** 1997. A highly sensitive chemiluminescence enzyme-linked immunosorbent assay for diagnosis of active *Trypanosoma cruzi* infection. Transfusion **37:**850–857.
2. **Breniere, S. F., N. Yaksic, N. Telleria, M. F. Bosseno, F. Noireau, P. Wincker, and D. Sanchez.** 1997. Immune response to *Trypanosoma cruzi* shed acute phase antigen in children from an endemic area for Chagas' disease in Bolívia. Mem. Inst. Oswaldo Cruz **92:**503–507.
3. **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.
4. **Carvalho, M. R., M. A. Krieger, W. Oeleman, M. A. Shikanai-Yasuda, A. W. Ferreira, J. B. Pereira, A. Saez-Alquezar, D. F. Dorlhiac-Llacer, D. F. Chamone, and S. Goldenberg.** 1993. Chagas' disease diagnosis: evaluation of several tests in blood bank screening. Transfusion **33:**830–834.
5. **Feliciangeli, M. D., D. Campbell-Lendrum, C. Martinez, D. Gonzalez, P. Coleman, and C. Davies.** 2003. Chagas' disease control in Venezuela: lessons for the Andean region and beyond. Trends Parasitol. **19:**44–49.
6. **Ferreira, A. W., Z. R. Belem, E. A. Lemos, S. G. Reed, and A. Campos-Neto.** 2001. Enzyme-linked immunosorbent assay for serological diagnosis of Chagas' disease employing a *Trypanosoma cruzi* recombinant antigen that consists of four different peptides. J. Clin. Microbiol. **39:**4390–4395.
7. **Franco da Silveira, J., E. S. Umezawa, and A. O. Luquetti.** 2001. Chagas' disease: recombinant *Trypanosoma cruzi* antigens for serological diagnosis. Trends Parasitol. **17:**286–291.
8. **Frank, M., B. Hegenscheid, K. Janitschke, and T. Weinke.** 1997. Prevalence and epidemiological significance of *Trypanosoma cruzi* infection among Latin American immigrants in Berlin, Germany. Infection **25:**355–358.
9. **Galel, S. A., and L. V. Kirchoff.** 1996. Risk factors for *Trypanosoma cruzi* infection in California blood donors. Transfusion **36:**227–231.
10. **Houghton, R. L., D. R. Benson, L. D. Reynolds, P. D. McNeill, P. R. Sleath, M. J. Lodes, D. A. Leiby, R. Badaro, and S. G. Reed.** 1999. A multi-epitope synthetic peptide and recombinant protein for the detection of antibodies to *Trypanosoma cruzi* in radioimmunoprecipitation-confirmed and consensus-positive sera. J. Infect. Dis. **179:**1226–1234.
11. **Kerner, N., P. Ligeard, M. J. Levin, and M. Hontebeyrie-Joskowicz.** 1991. *Trypanosoma cruzi*: antibodies to a MAP-like protein in chronic Chagas' disease cross-react with mammalian cytoskeleton. Exp. Parasitol. **73:**451–459.
12. **Kirchoff, L. V.** 1993. American trypanosomiasis (Chagas' disease)—a tropical disease now in the United States. N. Engl. J. Med. **329:**639–644.
13. **Krieger, M. A., E. Almeida, W. Oelemann, J. J. Lafaille, J. B. Pereira, H. Krieger, M. R. Carvalho, and S. Goldenberg.** 1992. Use of recombinant antigens for the accurate immunodiagnosis of Chagas' disease. Am. J. Trop. Med. Hyg. **46:**427–434.
14. **Leiby, D. A., R. M. Herron, E. J. Read, B. A. Lenes, and R. J. Stumpf.** 2002. *Trypanosoma cruzi* in Los Angeles and Miami blood donors: impact of evolving donor demographics on seroprevalence and implications for transfusion transmission. Transfusion **42:**549–555.
15. **Levin, M. J., E. A. Mesti, R. Benarous, G. Levitus, A. Schijman, P. Levy-Yeyati, A. Ruiz, A. Kahan, M. B. Rosenbaum, H. N. Torres, and E. L. Segura.** 1989. Identification of major *Trypanosoma cruzi* antigenic determinants in chronic Chagas' heart disease. Am. J. Trop. Med. Hyg. **41:**530–539.
16. **Levin, M. J., J. Franco da Silveira, A. C. C. Frasch, M. E. Camargo, S. Lafon, W. M. Degrave, and R. Rangel-Aldao.** 1991. Recombinant antigens and Chagas' disease diagnosis: analysis of a workshop. FEMS Microbiol. Immunol. **89:**11–20.
17. **Lopez Bergami, P., J. Scaglione, and M. J. Levin.** 2001. Antibodies against the C-terminal end of *Trypanosoma cruzi* ribosomal P proteins are pathogenic. FASEB J. **15:**2602–2612.

18. **Moncayo, A., and A. O. Luquetti.** 1990. Multicentre double blind study for evaluation of *Trypanosoma cruzi* defined antigens as diagnostic reagents. Mem. Inst. Oswaldo Cruz **85:**489–495.
19. **Oelemann, W. M. R., M. G. M. Teixeira, G. C. Verissimo-da-Costa, J. Borges-Pereira, J. A. De Castro, J. R. Coura, and J. M. Peralta.** 1998. Evaluation of three commercial enzyme-linked immunosorbent assays for diagnosis of Chagas' disease. J. Clin. Microbiol. **36:**2423–2427.
20. **Pastini, A. C., S. R. Iglesias, V. C. Carricarte, M. E. Guerin, D. O. Sanchez, and A. C. Frasch.** 1994. Immunoassay with recombinant *Trypanosoma cruzi* antigens potentially useful for screening donated blood and diagnosing Chagas' disease. Clin. Chem. **40:**1893–1897.
21. **Peralta, J. M., M. G. M. Teixeira, W. G. Shreffler, J. B. Pereira, J. M. Burns, P. R. Sleath, and S. G. Reed.** 1994. Serodiagnosis of Chagas' disease by enzyme-linked immunosorbent assay using two synthetic peptides as antigens. J. Clin. Microbiol. **32:**971–974.
22. **Prata, A.** 2001. Clinical and epidemiological aspects of Chagas' disease. Lancet Infect. Dis. **1:**92–100.
23. **Saez-Alquézar, A., M. M. Otani, E. C. Sabino, G. Ribeiro-dos-Santos, N. Salles, and D. F. Chamone.** 1998. Evaluation of the performance of Brazilian blood banks in testing for Chagas' disease. Vox Sang. **74:**228–231.
24. **Saez-Alquézar, A., E. C. Sabino, N. Salles, D. Chamone, F. Hulstaert, H. Pottel, E. Stoops, and M. Zrein.** 2000. Serological confirmation of Chagas' disease by a recombinant and peptide antigen line immunoassay: INNO-LIA Chagas. J. Clin. Microbiol. **38:**851–854.
25. **Salles, N. A., E. C. Sabino, M. G. Cliquet, J. Eluf-Neto, A. Mayer, C. Almeida-Neto, M. C. Mendonça, P. Dorliach-Llacer, D. F. Chamone, and A. Saez-Alquézar.** 1996. Risk of exposure to Chagas' disease among seroreactive Brazilian blood donors. Transfusion **36:**969–973.
26. **Schmunis, G. A.** 1999. Prevention of transfusional T. cruzi infection in Latin America. Mem. Inst. Oswaldo Cruz **94:**93–101.
27. **Shulman, I. A.** 1999. Intervention strategies to reduce the risk of transfusion-transmitted *Trypanosoma cruzi* infection in the United States. Transfus. Med. Rev. **13**:227–234.
28. **Umezawa, E. S., M. S. Nascimento, N. Kesper, Jr., J. R. Coura, J. Borges-Pereira, A. C. V. Junqueira, and M. E. Camargo.** 1996. Immunoblot assay using excreted-secreted antigens of *Trypanosoma cruzi* in serodiagnosis of congenital, acute, and chronic Chagas' disease. J. Clin. Microbiol. **34:**2143–2147.
29. **Umezawa, E. S., S. F. Bastos, M. E. Camargo, L. M. Yamauchi, M. R. Santos, A. Gonzalez, B. Zingales, M. J. Levin, O. Souza, R. Rangel-Aldao, and J. Franco da Silveira.** 1999. Evaluation of recombinant antigens for Chagas' disease serodiagnosis in South and Central America. J. Clin. Microbiol. **37**:1554–1556.
30. **Umezawa, E. S., M. S. Nascimento, and A. M. Stolf.** 2001. Enzyme-linked immmunosorbent assay with *T. cruzi* excreted-secreted antigens (TESA-ELISA) for serodiagnosis of acute and chronic Chagas' disease. Diagn. Microbiol. Infect. Dis. **39:**169–176.
31. **Umezawa, E. S., S. F. Bastos, J. R. Coura, M. J. Levin, A. Gonzalez, R. Rangel-Aldao, B. Zingales, A. O. Luquetti, and J. Franco da Silveira.** 2003. An improved serodiagnostic test for Chagas' disease employing a mixture of *Trypanosoma cruzi* recombinant antigens. Transfusion **43**:91–97.
32. **Vasquez, J. E., J. Krusnell, A. Orn, O. E. Souza, and R. A. Harris.** 1997. Serological diagnosis of *Trypanosoma rangeli* infected patients. A comparison of different methods and its implications for the diagnosis of Chagas' disease. Scand. J. Immunol. **45:**322–330.