Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 1239–1243, February 1992
Medical Sciences

Identification and synthesis of a major conserved antigenic epitope of *Trypanosoma cruzi*

(Chagas disease/repetitive epitope/peptide/parasite/diagnosis)

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Communicated by Paul B. Beeson, October 24, 1991

ABSTRACT **A gene sequence encoding an immunodominant protein with a repetitive epitope from the protozoan *Trypanosoma cruzi*, the causative agent of Chagas disease, was cloned and expressed. The identified 10-amino acid repeat is present within a high-molecular-weight trypomastigote antigen that appears specific to and conserved among *T. cruzi* isolates. More importantly, >95% of *T. cruzi* infection sera, including both chronic and acute Chagas disease, contained elevated levels of antibody to a 15-amino acid synthetic peptide bearing the repetitive B-cell epitope. Considering the wide diversity of *T. cruzi* parasites, as well as the broad spectrum of clinical manifestations of Chagas disease, such a prevalent immune response among patients is significant and applicable to the control of Chagas disease through the diagnosis of *T. cruzi* infection.**

Immune responses to protozoan infection are complex, involving both humoral and cell-mediated responses to an array of parasite antigens. Infection often involves multiple life-cycle stages of these parasites, which adds to the diversity of antigens potentially important for the development of protective immunity. To examine the molecular basis of the immune responses elicited during these infections, recent efforts have focused on evaluating responses to defined parasite B- and T-cell epitopes. Such evaluation is fundamental for controlling infection by developing sensitive and specific diagnostic tests and constructing subunit vaccines.

Infection with the protozoan parasite *Trypanosoma cruzi*, the causative agent of Chagas disease, occurs in an estimated 18 million persons throughout South America and is a major cause of chronic heart disease (1). Immune responses after *T. cruzi* infection are particularly complex due to the biochemical diversity of multiple parasite strains (2–6) and the influence of host genetic factors (7, 8). The result is a wide diversity in the clinical manifestations of Chagas disease and, in some cases, the disruption of immune regulation leading to immunosuppression and/or the development of autoimmunity (for review, see ref. 9). Thus, a greater understanding of the responses to specific parasite antigens is needed. Although several antigens of *T. cruzi* have been identified and characterized biochemically, limited data are available on the evaluation of human immune responses to these molecules.

In this paper, we report the cloning and expression of a *T. cruzi* antigen gene sequence encoding an immunodominant protein with a repetitive epitope.§ This gene sequence is conserved among diverse *T. cruzi* isolates, and the antigen is predominantly expressed by trypomastigotes, the infective form of the parasite. Evaluation of the human immune responses to this molecule revealed readily detectable levels of antibody in >95% of *T. cruzi* infection sera to a 15-amino acid synthetic peptide.

MATERIALS AND METHODS

Parasites. Trypanosome isolates used in this study were as follows: *T. cruzi* strains Tulahuen, Y, CL, Esmeraldo, PB295, VL1467, VL2067; *Trypanosoma rangeli* (obtained from Antonio D'Alessandro, Tulane University of Louisiana, New Orleans); and *Trypanosoma brucei* (IHRI 1). Isolates of leishmania included *Leishmania chagasi* (MHOM/BR/82/BA-2,C1), *Leishmania amazonensis* (MHOM/BR/84/BA32c5), and *Leishmania donovani* (MHOM/Et/67HU3). Promastigotes and epimastigotes were cultured in axenic media.

Patient Sera. Sera were from well-characterized (clinical and/or xenodiagnosis confirmed, as well as seropositive) patients with acute or chronic Chagas disease or indeterminate *T. cruzi* infection from South American countries, including Northern and Southern Brazil, Bolivia, and Argentina. Other patient sera were from individuals living in nonendemic *T. cruzi* areas. Sera from Sudanese patients with parasitologically confirmed visceral (n = 24) or cutaneous (n = 15) leishmaniasis were used. Mycobacterial sera were from Seattle (tuberculosis) or Haiti (leprosy), and malaria sera were from Thailand. Normal sera were from Seattle (n = 24) or from uninfected individuals living in Brazil (n = 8). These sera have been described (10).

Production of Recombinant and Synthetic Antigens from Clone TcD. A genomic library was constructed in λZAPII (Stratagene) with mechanically sheared DNA of *T. cruzi* (Tulahuen). Construction and immunoscreening of the library and excision of the pBSK(−) phagemid sequences were done according to the manufacturer's protocols. Recombinants were screened with a pool of Chagas patient sera, preadsorbed to remove anti-*Escherichia coli* reactivity (11). The 59-kDa recombinant antigen of clone TcD was purified from a soluble lysate of induced bacterial cultures by ammonium sulfate fractionation, preparative isoelectric focusing with the Bio-Rad Rotofor IEF cell and 1% 3/10 ampholytes in the presence of 8 M urea, followed by SDS/PAGE (12) and electroelution as described (13). Protein concentrations were determined by using the Pierce BCA protein assay.

Peptides were synthesized on an Applied Biosystems instrument model 430A by using *N-tert*-butoxycarbonyl (t-Boc) chemistry and were purified by reverse-phase HPLC. Peptides were characterized by amino acid analysis with a

Abbreviation: TcD, antigen from clone TcD specific in detecting antibody to *Trypanosoma cruzi*.

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§The sequence reported in this paper has been deposited in the GenBank data base (accession no. M82834).

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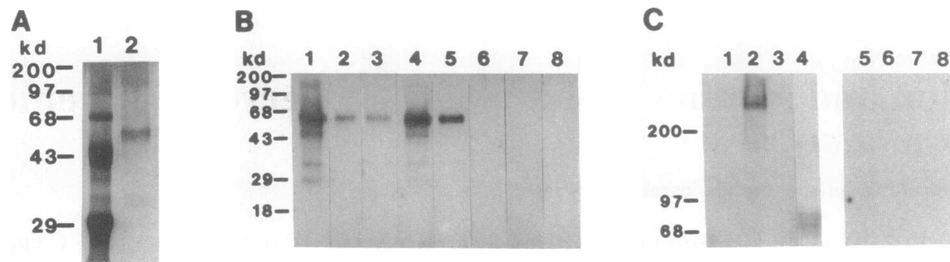

FIG. 1. Identification of recombinant and native TcD antigens. (A) Silver-stained SDS/12% PAGE gel of purified recombinant TcD (lane 2, 2 μg) with markers (lane 1). (B) Reactivity of recombinant TcD with patient sera. Blots containing recombinant TcD (20 ng per lane) were probed with individual Chagas patient sera (lanes 1–5) or pooled acute visceral leishmaniasis patient sera (lane 6, $n = 3$). Pooled normal human sera ($n = 3$) and no primary antibody controls are shown (lanes 7 and 8, respectively). (C) Immunoblot of *T. cruzi* epimastigote (lanes 1 and 5, 20 μg), trypomastigote (lanes 2 and 6, 20 μg) and amastigote (lanes 3 and 7, 20 μg) lysates or purified recombinant TcD (lanes 4 and 8, 20 ng) transferred from SDS/5% PAGE gels and probed with a rabbit antiserum against recombinant TcD antigen (lanes 1–4) or with preimmune rabbit sera (lanes 5–8). kd, kDa. Trypomastigotes and amastigotes were cultured in L_6E_9 myoblast cells.

Beckman 6300 system and by plasma desorption mass spectrometry on a Bio-Ion 20 spectrometer.

Immunoblot Analysis. Immunoblots with patient and rabbit sera diluted 1:400 were done as described (13) with ^{125}I-labeled protein A. The rabbit anti-TcD serum was raised by immunization of an adult New Zealand White rabbit with purified recombinant TcD antigen.

TcD Gene Sequence Analysis. DNAs from several protozoa were isolated, digested with *Sst* I or *Pst* I, separated by agarose gel electrophoresis, and analyzed by Southern blotting with standard protocols (11). No *Sst* I or *Pst* I restriction sites are present within the TcD insert. The blot was probed with the 636-base-pair (bp) insert of TcD, radiolabeled by using random oligonucleotides as primers (Boehringer Mannheim) in the presence of [α-^{32}P]dCTP to a specific activity of 5×10^8 cpm/μg. The final wash was done in 0.1× standard saline citrate/0.5% SDS at 65°C.

The TcD insert of the phagemid pBSK(−) was subcloned into pBSK(+) to obtain the complete sequence of both the coding and noncoding strands. A series of clones containing overlapping deletions of both constructs was generated by controlled exonuclease III digestion (14). After infection with the helper phage M13K07, single-stranded template was prepared from *E. coli* (strain XL-1 Blue) transformed with the deleted constructs and sequenced by the Sanger dideoxynucleotide chain-termination method (15) and ^{35}S-labeled dATP.

TcD Antigen ELISAs. Microassay plates (Probind, Falcon) were coated with recombinant TcD antigen (20 ng per well) or with *T. cruzi* epimastigote lysate (100 ng per well) by overnight incubation at 4°C in coating buffer (15 mM Na_2HCO_3/28 mM $NaHCO_3$, pH 9.6). Wells were blocked by a 1-hr incubation in phosphate-buffered saline (PBS)/1% Tween 20 (Sigma). Sera (diluted 1:100 in PBS/0.1% Tween 20) were added to each well and incubated for 30 min at room temperature. Bound antibody was detected by using protein A–horse radish peroxidase (Zymed Laboratories) as described (10).

The ELISA using the TcD peptide differed as follows: peptide (2 μg per well) was added to Corning Easy Wash microassay plates and blocked with PBS/1% Tween 20/5% bovine serum albumin; primary antibody was diluted (1:50) in high-ionic-strength PBS (185 mM NaCl)/0.4% bovine serum albumin for 15 min. All absorbance values are expressed relative to the mean of five normal sera run on each assay.

RESULTS

Identification of Clones Expressing *T. cruzi* Antigen Genes. To begin to evaluate Chagas patient immune responses to defined antigens, a genomic expression library was constructed, and recombinants expressing *T. cruzi* antigen genes were selected based on their reactivity with patient sera. Of 12 clones identified, one clone, TcD, was exceptionally reactive with patient sera. The purified recombinant antigen of clone TcD migrated at ≈59 kDa on SDS/PAGE (Fig. 1*A*, lane 2). As shown by immunoblot analyses, the TcD antigen was strongly recognized by Chagas patient sera (Fig. 1*B*, lanes 1–5) but was not recognized by normal sera (Fig. 1*B*, lane 7) or by high-titer sera from patients with acute visceral leishmaniasis (Fig. 1*B*, lane 6), an infection known to induce antibodies cross-reactive with *T. cruzi*.

Southern Blot Analysis of TcD Gene Sequences. The 636-bp insert of clone TcD was used to probe Southern blots of *T. cruzi* DNA and DNA from several other protozoan parasites of humans. The probe hybridized to multiple restriction fragments of *T. cruzi* DNA (Fig. 2*A*, lane 1) but not to *T. brucei*, *L. chagasi*, *L. amazonensis*, or *L. donovani* DNA (Fig. 2*A*, lanes 2–5). Noteworthy was the lack of homologous sequences in the genome of *T. rangeli* (Fig. 2*A*, lane 6), a related but nonpathogenic American trypanosome present in many *T. cruzi* endemic areas. Analysis of DNA from seven geographically diverse *T. cruzi* isolates indicated that the TcD gene sequence was a conserved, multicopy element (Fig. 2*B*, lanes 1–7), with isolates showing restriction fragment length polymorphism and variability in gene-copy number.

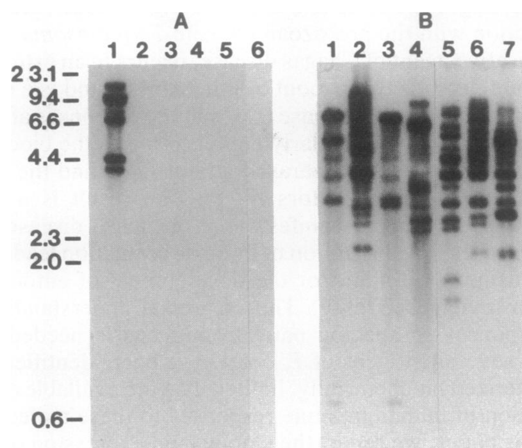

FIG. 2. Southern blot analysis of TcD gene sequences. (A) DNA from several protozoa were digested with *Sst* I and analyzed by Southern blotting with the radiolabeled 636-bp insert of TcD as a probe. Digested DNAs (2.5 μg per lane) were loaded in the following lanes: 1, *T. cruzi* (Tulahuen); 2, *T. brucei*; 3, *L. chagasi*; 4, *L. amazonensis*; 5, *L. donovani*; and 6, *T. rangeli*. (B) Genomic DNA was extracted from various isolates of *T. cruzi*, digested with *Pst* I, and analyzed by Southern blotting as in *A*. *T. cruzi* isolate DNAs were loaded in the following lanes: 1, Tulahuen; 2, Y; 3, CL; 4, Esmeraldo; 5, PB295; 6, VL1467; and 7, VL2067.

Clone TcD Encodes a 10-Amino Acid Repetitive Sequence. DNA sequence analysis of clone TcD predicted an amino acid sequence comprised entirely of a 10-amino acid repeat arrayed in tandem, present in 20.5 copies, with minor degeneracies in five positions (Fig. 3*A*). The predicted molecular mass of the recombinant TcD antigen was 36.3 kDa; 60% was encoded by the TcD insert, and 40% was encoded by plasmid fusion sequences. The aberrant migration of the TcD recombinant antigen at 59 kDa observed during SDS/PAGE most likely reflects the high proline content (28%).

Reactivity of Patient Sera with Recombinant TcD. The reactivity of a panel of individual *T. cruzi* infection sera with the recombinant TcD antigen was tested. Sera were obtained from patients from Northern and Southern Brazil, Bolivia, and Argentina. Patient sera with ELISA values at least five SDs greater than the mean absorbance value of the normal controls were considered positive. Of confirmed *T. cruzi* infection sera, 95.3% (121 of 127) were positive for anti-TcD antibody; absorbance values ranged from 0.12 to >3.0 ($\bar{x}$ = 1.244) (Fig. 4*A*). This seroreactivity was highly specific, as the percentage of reactive non-Chagas patient sera was reduced from 31.9% to 6.6% when *T. cruzi* lysate was replaced with the recombinant TcD in the ELISA.

Reactivity of Patient Sera with Synthetic TcD. An evaluation of 5-, 10-, 15-, and 20-amino acid synthetic peptides containing sequences of the TcD repetitive element was done to map the immunodominant B-cell epitope of TcD. From these results, a peptide was synthesized containing 15 residues of the repeat (Fig. 3*B*). The reactivity of patient sera with the synthetic TcD was comparable to that obtained with the recombinant molecule—96.7% (116 of 120) of Chagas sera gave positive absorbance values from 0.03 to >2.0 ($\bar{x}$ = 0.712) (Fig. 4*B*). In addition, positive TcD-specific antibody responses were detected in eight of nine acute Chagas disease patients, indicative of an early immune response upon *T. cruzi* infection to this epitope. All positive absorbance values were at least two SDs greater than the mean absorbance value of the normal controls. The specificity of this synthetic peptide-based assay improved on that seen with the recombinant TcD antigen; only 3.3% of non-Chagas patient sera were positive with synthetic peptide.

Identification of Native TcD Antigen. To identify the native TcD molecule, a polyclonal rabbit antiserum was raised against the recombinant antigen and used to probe blots of *T. cruzi* epimastigote (insect form), trypomastigote (infective and blood form), and amastigote (tissue form) lysates. This antiserum bound specifically to the recombinant TcD antigen (Fig. 1*C*, lane 4) and to a trypomastigote antigen migrating at 260 kDa (Fig. 1*C*, lane 2), with gel composition influencing its mobility. A similar-sized antigen was detected in epimastig-

A

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1                                                   GAATTCA

8    GCA GAG CCC AAA CCA GCG GAG CCG AAG TCA   GCA GAG CCT AAA CCA GCG GAG CCG AAA TCG
     ALA GLU PRO LYS PRO ALA GLU PRO LYS SER   ALA GLU PRO LYS PRO ALA GLU PRO LYS SER

68   GCA GAG CCC AAA CCA GCG GAG CCG AAA TCG   GCA GAG CCC AAA CCA GCG GAG CCG AAA TCA
     ALA GLU PRO LYS PRO ALA GLU PRO LYS SER   ALA GLU PRO LYS PRO ALA GLU PRO LYS SER

128  GCG[GGG]CCT AAA CCA GCG GAG CCG AAG TCA   GCG GAG CCT AAA CCA GCG GAG CCG AAA TCA
     ALA[GLY]PRO LYS PRO ALA GLU PRO LYS SER   ALA GLU PRO LYS PRO ALA GLU PRO LYS SER

188  GCA GAG CCC AAA CCA GCG GAG CCG AAA TCG   GCA GAG CCC AAA CCA GCG GAG CCG AAG TCA
     ALA GLU PRO LYS PRO ALA GLU PRO LYS SER   ALA GLU PRO LYS PRO ALA GLU PRO LYS SER

248  GCA GAG CCC AAA CCA GCG GAG[TCG]AAG TCA   GCA GAG CCT AAA CCA GCG GAG CCG AAA TCA
     ALA GLU PRO LYS PRO ALA GLU[SER]LYS SER   ALA GLU PRO LYS PRO ALA GLU PRO LYS SER

308  GCA GAG CCC AAA CCA GCG GAG[TCG]AAG TCA   GCA GAG CCC AAA CCA GCG GAG CCG AAG TCA
     ALA GLU PRO LYS PRO ALA GLU[SER]LYS SER   ALA GLU PRO LYS PRO ALA GLU PRO LYS SER

368  GCA GAG CCC AAA CCA GCG GAG CCG AAG TCA   GCA GAG CCC AAA CCA GCG GAG CCG AAA TCA
     ALA GLU PRO LYS PRO ALA GLU PRO LYS SER   ALA GLU PRO LYS PRO ALA GLU PRO LYS SER

428  GCG GAG CCC AAA CCA GCG GAG CCG AAA TCA   GCA GAG CCC AAA CCA GCG GAG[TCG]AAA TCA
     ALA GLU PRO LYS PRO ALA GLU PRO LYS SER   ALA GLU PRO LYS PRO ALA GLU[SER]LYS SER

488  GCG[GGG]CCT AAA CCA GCG GAG CCG AAG TCA   GCG GAG CCA AAA CCA GCG GAG CCG AAA TCA
     ALA[GLY]PRO LYS PRO ALA GLU PRO LYS SER   ALA GLU PRO LYS PRO ALA GLU PRO LYS SER

548  GCG GAG CCA AAA CCA GCG GAG CCG AAA TCG   GCA GAG CCC AAA CCA GCG GAG CCG AAG TCA
     ALA GLU PRO LYS PRO ALA GLU PRO LYS SER   ALA GLU PRO LYS PRO ALA GLU PRO LYS SER

608  GCA GAG CCA AAA CCA GCG GAG CCGAATTC
     ALA GLU PRO LYS PRO ALA GLU
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B

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Ac-ALA GLU PRO LYS SER ALA GLU PRO LYS PRO ALA GLU PRO LYS SER GLY CYS GLY-NH2
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FIG. 3. Amino acid sequence of the recombinant and synthetic TcD antigens. (*A*) DNA sequence and deduced amino acid sequence of the 636-bp TcD insert are shown with residues blocked to indicate the 10-amino acid repetitive unit and the number of repeats. Boxed amino acids mark degeneracies in the repeat unit. (*B*) Sequence of the synthetic TcD peptide containing the 10-residue repeat unit plus 5 residues of the next repeat; Gly-Cys-Gly facilitates conjugation to carrier molecules.

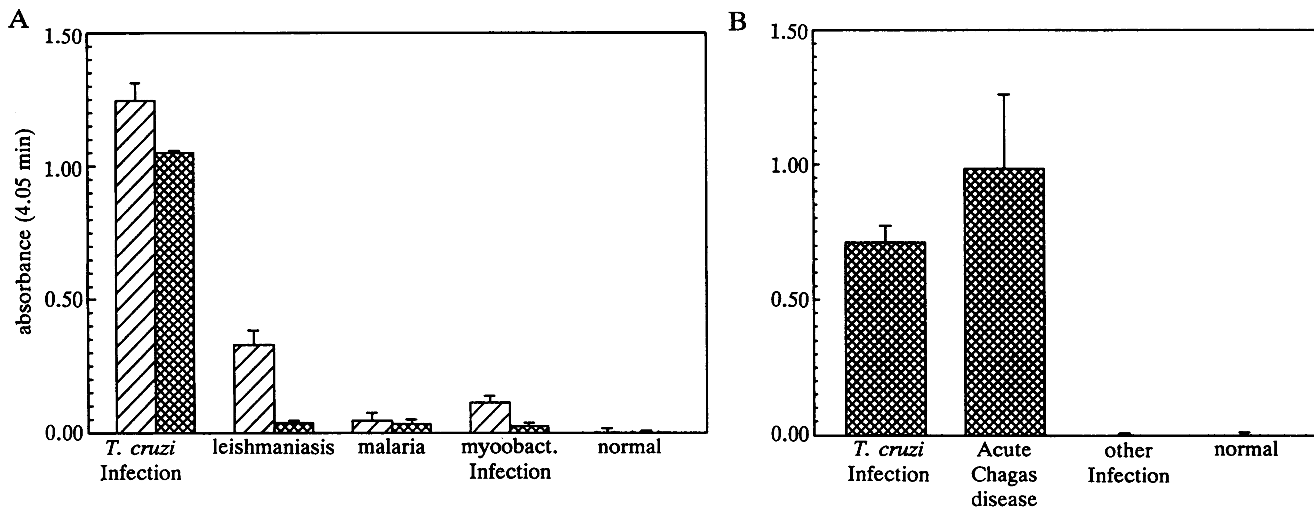

FIG. 4. ELISA evaluation of recombinant TcD and synthetic TcD peptide. (*A*) Absorbance values ($\bar{x}$ + SEM) of *T. cruzi* infection (n = 127), leishmaniasis (n = 34), malaria (n = 10), mycobacterial (myobact.) infection (n = 17), and normal (n = 32) sera against *T. cruzi* lysate (hatched bars) and recombinant TcD (double-hatched bars). (*B*) Absorbance values ($\bar{x}$ + SEM) of *T. cruzi* infection sera (n = 120); acute Chagas disease sera (n = 9); other infection sera including leishmaniasis (n = 15), malaria (n = 10), mycobacterial infection (n = 16); and normal (n = 32) sera evaluated on synthetic TcD peptide.

ote and amastigote antigen preparations at very low levels (Fig. 1*C*, lanes 1 and 3). Similar results were obtained by using sera obtained from mice immunized with the TcD peptide coupled to keyhole limpet hemocyanin and with TcD-specific antibodies affinity-purified from Chagas patient sera (data not shown).

DISCUSSION

We have identified and synthesized a major antigenic epitope of a 260-kDa *T. cruzi* antigen expressed predominantly by trypomastigotes. The antigen is conserved among geographically diverse *T. cruzi* isolates. Conservation of the antigen was further indicated by the presence of TcD-specific antibodies in sera from Chagas patients having great clinical and geographical diversity, produced as a result of natural infections with *T. cruzi* parasites expressing the TcD repetitive epitope. This finding is of interest because repeat domains identified in other protozoan parasite antigens have, for the most part, shown intraspecies variability (16, 17). The importance of the strong immune response to this determinant during *T. cruzi* infection is unclear. However, we have preliminary evidence indicating that mice immunized with the synthetic TcD peptide can be partially protected from lethal *T. cruzi* infection.

The extremely high prevalence of seroreactivity to TcD indicates its usefulness as a serodiagnostic. A number of *T. cruzi* antigens, both native (18–21) and recombinant (22–27), have been evaluated for potential use in the diagnosis of Chagas disease. To date, there have been no reports of defined epitopes with wide serodiagnostic potential, making TcD unique in this regard. Some of the previously reported cloned *T. cruzi* antigens contain repetitive domains, with one domain reportedly present in an 85-kDa antigen, showing similarity to the TcD sequence (22, 23). Although the 5-amino acid repeat present in this recombinant antigen is identical to the second half of the TcD repeat, reactivity was demonstrated with only 40% of Chagas patient sera (23, 28). This result is consistent with our mapping studies, which indicated that peptides containing only the 5-residue repeat lacked an essential portion of the dominant B-cell epitope of TcD. Nevertheless, given their sequence similarity, these clones may represent members of a large gene family.

The identification of a recombinant or synthetic antigen such as TcD, which is sensitive and specific in detecting antibody to *T. cruzi*, is a priority of the World Health Organization (29). *T. cruzi* infections are often subtle and long-lasting, making diagnosis crucial and problematic. Detecting antibodies against parasite antigens is the most common and reliable method of determining clinical and subclinical infections. Presently, serological tests use whole or lysed *T. cruzi* and require positive results on two of three tests—including complement fixation, indirect immunofluorescence, passive agglutination, or ELISA to accurately detect *T. cruzi* infection. The expense as well as the difficulty in performing such tests reliably prevents the screening of blood or sera in many endemic areas.

The ELISA with synthetic TcD is easy to perform, allows for the standardization of reagents, permits the screening of large numbers of samples, and can be used with either blood or serum. Blood bank screening is particularly important in Latin America, where 0.1–62% of samples may be infected and where the parasite is frequently transmitted by blood transfusion (for review, see ref. 30). It is also an increased concern that the blood supply in certain U.S. cities is contaminated with *T. cruzi* parasites (31, 32). Considering these findings, the TcD peptide appears a promising candidate for application in the serodiagnosis of *T. cruzi* infection. Efficiency may be further increased with the use of additional *T. cruzi* antigens in combination with the TcD repeat. These assays will be useful for the control of disease transmission by blood transfusion, for the diagnosis of infection, and for monitoring the effectiveness of Chagas disease control programs.

We thank Drs. Roberto Badaró, Aldina Barral, Hashim Ghalib, Richard Miller, Miercio Pereira, Moyses Sadigursky, H. Kyle Webster, and especially M. Lourdes da Silva for providing sera for these studies. We also thank Drs. Jerry Manning and Wesley Van Voorhis for providing *T. cruzi* DNA, Dr. Marilyn Parsons for providing *T. brucei* DNA, and Dr. Antonio D'Alessandro for providing *T. rangeli*. Lastly, we thank Drs. Marilyn Parsons and Kenneth Stuart for a critical review of the manuscript. This work was supported by the National Institutes of Health, grants AI22726 and AI16282.

1. Moncayo-Medina, A. (1987) in *Tropical Disease Research: A Global Partnership*, eds. Maurice, J. & Pearce, A. M. (WHO, Geneva), pp. 87–98.

2. Miles, M., Souza, A., Povoa, M., Shaw, J., Lainson, R. & Toye, P. (1978) *Nature (London)* **272,** 819–821.
3. Tibayrenc, M., Ward, P., Moya, A. & Ayala, F. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 115–119.
4. Dvorak, J. A., Engel, J. C., Leapman, R. D., Swyt, C. R. & Pella, P. A. (1988) *Mol. Biochem. Parasitol.* **31,** 19–26.
5. Tibayrenc, M. & Ayala, F. (1988) *Evolution* **42,** 277–292.
6. Avila, H., Goncalves, A. M., Nehme, S. N., Morel, C. M. & Simpson, L. (1990) *Mol. Biochem. Parasitol.* **42,** 175–188.
7. Wrightsman, R., Krassner, S. & Watson, J. (1982) *Infect. Immunol.* **36,** 637–644.
8. Trischmann, T. M. & Bloom, B. R. (1982) *Infect. Immunol.* **35,** 546–551.
9. Kuhn, R. E. (1981) in *Parasitic Diseases: The Immunology*, ed. Mansfield, J. M. (Dekker, New York), Vol. 1, pp. 137–166.
10. Reed, S. G., Shreffler, W. G., Burns, J. M., Jr., Scott, J. M., da Gloria Orge, M., Ghalib, H. W., Siddig, M. & Badaró, R. (1990) *Am. J. Trop. Med. Hyg.* **43,** 632–639.
11. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) in *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
12. Laemmli, U. H. (1979) *Nature (London)* **227,** 680–685.
13. Reed, S. G., Carvalho, E. M., Sherbert, C. H., Sampaio, D. P., Russo, D. M., Bacelar, O., Pihl, D. L., Scott, J. M., Barral, A., Grabstein, K. H. & Johnson, W. D., Jr. (1990) *J. Clin. Invest.* **85,** 690–696.
14. Henikoff, S. (1984) *Gene* **28,** 351–359.
15. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5463–5467.
16. Kemp, D. J., Coppel, R. L. & Anders, R. F. (1987) *Annu. Rev. Microbiol.* **41,** 181–208.
17. McCuthan, T. F., de la Cruz, V. F., Good, M. F. & Wellems, T. E. (1988) *Prog. Allergy* **41,** 173–192.
18. Schechter, M., Flint, J. E., Voller, A., Guhl, F., Marinkelle, C. J. & Miles, M. A. (1983) *Lancet* **ii,** 939–941.
19. Dragon, E. A., Brothers, V. M., Wrightsman, R. A. & Manning, J. (1985) *Mol. Biochem. Parasitol.* **16,** 213–229.
20. Scharfstein, J., Luquetti, A., Murata, A. C. M., Senna, M., Rezende, J. M., Rassi, A. & Mendonca-Previato, L. (1985) *Am. J. Trop. Med. Hyg.* **34,** 1153–1160.
21. Kirchhoff, L. V., Gam, A. A., d'A Gusmao, R., Goldsmith, R. S., Rezende, J. M. & Rassi, A. (1987) *J. Infect. Dis.* **155,** 561–564.
22. Ibañez, C. F., Affranchino, J. L. & Frasch, A. C. C. (1987) *Mol. Biochem. Parasitol.* **25,** 175–184.
23. Ibañez, C. F., Affranchino, J. L., Macina, R. A., Reyes, M. B., Leguizamon, S., Camargo, M. E., Aslund, L., Pettersson, U. & Frasch, A. C. C. (1988) *Mol. Biochem. Parasitol.* **30,** 27–34.
24. Affranchino, J. L., Ibañez, C. F., Luquetti, A. O., Rassi, A., Reyes, M. B., Macina, R. A., Aslund, L., Pettersson, U. & Frasch, A. C. C. (1989) *Mol. Biochem. Parasitol.* **34,** 221–228.
25. Lafaille, J. J., Linss, J., Krieger, M. A., Souto-Padron, T., de Souza, W. & Goldenberg, S. (1989) *Mol. Biochem. Parasitol.* **35,** 127–136.
26. Hoft, D. F., Kim, K. S., Otsu, K., Moser, D. R., Yost, W. J., Blumin, J. H., Donelson, J. E. & Kirchhoff, L. V. (1989) *Infect. Immunol.* **57,** 1959–1967.
27. Paranhos, G. S., Cotrim, P. C., Mortara, R. A., Rassi, A., Corral, R., Freilij, H. L., Grinstein, S., Wanderley, J., Camargo, M. E. & da Silveira, J. F. (1990) *Exp. Parasitol.* **71,** 284–293.
28. Frasch, A. C. C. & Reyes, M. B. (1990) *Parasitol. Today* **6,** 137–139.
29. World Health Organization (1991) in *Tropical Diseases: Progress in Research, 1989–1990* (WHO, Geneva), pp. 69–77.
30. Schmuñis, G. A. (1991) *Transfusion* **31,** 547–557.
31. Kerndt, P., Waskin, H., Shulman, I., Nelson, J., Steurer, F., Kirchoff, L., Gellert, G. & Waterman, S. (1988) *Transfusion* **28,** 31S (abstr.).
32. Grant, I. H., Gold, J. W. M., Wittner, M., Tanowitz, H. B., Nathan, C., Mayer, K., Reich, L., Wollner, N., Steinherz, L., Ghavimi, F., O'Reilly, R. J. & Armstrong, D. (1989) *Ann. Intern. Med.* **111,** 849–851.