HUMAN ANTIBODY RESPONSES TO TRYPANOSOMA CRUZI 70-kD HEAT-SHOCK PROTEINS

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Abstract. Heat-shock proteins of the 70-kD (hsp70) family are targets of humoral and cellular immune responses following bacterial or parasitic infections, including Chagas’ disease. In the present study, we measured antibodies in human sera reactive with hsp70s from the cytoplasm (cy-hsp70), mitochondrion (mt-hsp70), and endoplasmic reticulum (grp78) of Trypanosoma cruzi. Of the three hsp70s tested, only grp78 detected T. cruzi infection in more than 90% of nontreated (NT) patients, with cy-hsp70 and mt-hsp70 detecting only 78% and 25% of NT patients, respectively. Reactivity of leishmanial sera was 77% with cy-hsp70, 13% with grp78, and 5% with mt-hsp70. Therefore, considering sensitivity and specificity, the best candidate for T. cruzi serodiagnosis is grp78. Combination of grp78 with a T. cruzi 24-kD flagellar calcium binding protein (FCaBP) increased the diagnostic sensitivity from 90% to 97% but increased leishmanial reactivity from 3% to 8%. To determine whether hsp70s are useful for discriminating between cured and nontreated patients treated with trypanocidal drugs, we tested sera from treated nontreated (TNC) patients and cured patients who have positive conventional serology, termed treated dissociated (TD). The cy-hsp70 and grp78 reacted with 74% and 68% of TNC patient sera, respectively, but these antigens did not discriminate TNC from TD patients (52% and 45% positive, respectively). The mt-hsp70 was detected by sera from few TNC patients (18%) and no TD patients. Although individual hsp70s were not useful for determining the effect of trypanocidal drugs on T. cruzi infection in individual patients, the majority of TNC patient sera (70–80%) reacted with two or three of the hsp70s. In contrast, no TD sera reacted with all three hsp70s, and 40% did not react with any of the hsp70s, indicating that the number of hsp70s detected decreases following successful treatment. Considered together, these results show that grp78 has potential as a diagnostic antigen and that absence of reactivity to all three hsp70s may be indicative of effective treatment.

Chagas’ disease is a parasitic disease, highly prevalent in Central and South America, that is caused by infection with the protozoan Trypanosoma cruzi. The infection is characterized by an acute parasitemic phase of 1–2 months, followed by a life-long chronic phase in which bloodstream parasites are detectable only by indirect methods such as xenodiagnosis or hemoculture. Most patients are diagnosed during the chronic phase by conventional serology (CS) tests, such as indirect immunofluorescence, ELISA, complement fixation reaction, or indirect hemagglutination. Although CS tests using crude parasite extracts are very sensitive in detecting T. cruzi infections, a problem with these assays is that they yield a small but important number of false-positive results, especially when sera from patients with leishmanial infections are tested. This can make a correct diagnosis difficult, especially in areas in which both diseases are endemic. Therefore, recent efforts have attempted to identify antigens that can distinguish T. cruzi and leishmanial infections. Another important effort involves identifying antigens that may identify patients who have been successfully cured by treatment with trypanocidal drugs.

The treatment of T. cruzi infection with nitroimidazole derivatives in both acute and chronic phases has been shown to be effective at preventing fatal outcomes and pathologic effects in the later stages of the disease. Long-term studies of treated patients have shown that approximately 90% have positive CS results as long as seven years after treatment. However, up to 30% of patients appear to be cured and are termed treated dissociated (TD), defined by their having persistently negative test results for complement-mediated lysis (CoML) and hemocultures, but positive CS results. Therefore, negative CoML predicts elimination of T. cruzi in treated patients who remain positive by CS. One of the major goals of our group is to identify recombinant antigens that are recognized by sera from treated patients considered to have failed therapy because they have persistently positive hemocultures and CoML test results (treated nontreated [TNC]), but not by sera from TD patients. Such antigens could form the basis for a simple assessment of the effectiveness of trypanocidal drug therapy.

Recently, we characterized a 24-kD flagellar calcium binding protein (FCaBP), also termed Tc24, as a useful T. cruzi recombinant protein for diagnosis, and for follow-up of treated patients. In the present study, we determined the utility of recombinant T. cruzi heat-shock proteins of 70-kD (hsp70s) for serodiagnosis and ascertained whether hsp70 seroreactivity correlates with persistent infection in treated patients. Although hsp70s display high sequence conservation across evolutionarily diverse species, they are often major targets of humoral and cellular immune responses following bacterial or parasitic infections. The hsp70s are an important family of molecular chaperones involved in essential cellular functions such as protein folding and translocation. In addition, they are involved in cellular tolerance to thermal and chemical stresses. Members of this family in trypanosomatids are located in the cytoplasm (cy-hsp70), mitochondrion (mt-hsp70), and endoplasmic reticulum (grp78), as is true in other eukaryotic organisms. Immunologic screening of cDNA expression libraries to identify proteins for serodiagnosis of human parasitic infections such as Chagas’ disease, leishmaniasis, malaria, schistosomiasis, and filariasis, has identified antigens belonging to the hsp70 family. Although humoral immune responses to T. cruzi hsp70 have been described, this is
the first attempt to systematically evaluate the utility of each of the hsp70 family members (cy-hsp70, mt-hsp70, and grp78) for the serodiagnosis of a human infectious disease.

MATERIALS AND METHODS

Patient sera. Chagasic sera were obtained from 101 patients with chronic *T. cruzi* infections. These patients were diagnosed by CS, CoML, and hemoculture tests as described previously. Thirty patients were nontreated (NT) and 71 patients were treated with trypanocidal drugs (nifurtimox and/or benznidazole) and followed clinically and serologically for 10 years. Treated patients showed two diagnostic patterns, as defined by CS, CoML, and hemocultures: 1) 48 patients were persistently positive by all tests (TNC), being considered a therapeutic failure, and 2) 23 patients were considered cured (TD), showing persistently negative hemocultures and CoML, but positive CS. Leishmanial sera were obtained from 30 patients diagnosed with cutaneous disease (CL) and nine diagnosed with visceral disease (VL). Control sera were obtained from 36 nonchagasic individuals (NCh), including 26 individuals from an endemic area who had at least three negative serologic results for *T. cruzi*. The study was approved by the Human Experimentation Committee of the Universidad Federal de Minas Gerais and written informed consent was obtained from all patients.

Expression and purification of recombinant hsp70s and FCaBP. *Trypanosoma cruzi* hsp70s were produced as His-tagged proteins in the pET system (Novagen, Madison, WI). Primers specific for the 5′- and 3′- ends of coding regions, containing appropriate flanking restriction sites, were used for polymerase chain reaction (PCR) amplification of cy-hsp70, mt-hsp70, grp78, and FCaBP from previously cloned full-length cDNAs. An additional grp78 coding sequence was generated that lacks the 5′ 81 basepairs encoding the endoplasmic reticular targeting sequence. The PCR products were cloned into pET14b (cy-hsp70 and mt-hsp70) or pET23b (grp78 and FCaBP) to allow expression of the proteins as N-terminal (pET14b) or C-terminal (pET23b) His fusions. Expression of the His-tagged proteins and purification on HisBind resin were performed according to the manufacturer’s (Novagen) instructions. All hsp70s were purified under denaturing conditions using 6 M urea. The concentrations of recombinant hsp70s and FCaBP were determined using a Bradford assay (Bio-Rad, Hercules, CA). Purified proteins were analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and staining with Coomassie blue.

*Trypanosoma cruzi* epimastigote lysate. Epimastigotes of the Silvio X-10/4 clone of *T. cruzi* were grown in supplemented liver digest–neutralized medium and a lysate was produced as described previously. Briefly, epimastigotes were washed twice with cold phosphate-buffered saline (PBS) and the cell pellet was frozen on dry ice, lyophilized, and suspended in ice-cold 0.9% NaCl containing protease inhibitors. After sonication, the homogenate was centrifuged and the supernatant containing soluble antigens was stored at −20°C until use.

Enzyme-linked immunosorbent assay. The optimal concentrations of antigen, serum, and conjugate were determined by checkerboard titration. The hsp70s, FCaBP, recombinant antigen pools, and epimastigote lysate were used at a concentration of 1.25 μg/ml. Plates were coated with 50 μl/well of each antigen diluted in 0.05 M sodium carbonate buffer, pH 9.6. The plates were incubated at 4°C overnight, washed with PBS containing 0.05% Tween-20 (PBS-Tween), and then blocked with 200 μl/well of 1% bovine serum albumin (BSA) in PBS for 1 hr at 37°C. After three washes with PBS-Tween, 100 μl of sera diluted in PBS-Tween containing 1% BSA were added to the wells, and plates were incubated for 2 hr at 37°C. The plates were washed, and biotin-conjugated anti-human IgG was added to the wells. After incubation for 30 min at 37°C, the plates were washed and incubated for 30 min at 37°C with streptavidin-conjugated horseradish peroxidase. After an additional three washes, 100 μl of a 1:1 mixture of 3,3′,5,5′-tetramethylbenzidine peroxidase substrate and hydrogen peroxide (Kirkgaard and Perry Laboratories, Gaithersburg, MD) was added to each well and the plates were incubated for 5–10 min at room temperature. The reactions were stopped by the addition of 100 μl of 0.18 M HCl and the plates were read at an absorbance of 450 nm.

Analysis of ELISA data. A standard curve was included in every assay to compensate for within-run and run-to-run variation and to allow comparison of the reactivity of a given serum with different antigens. The serum used to establish the standard curve was from an NT patient positive by hemoculture, CS, and CoML tests. Concentrations of all serum antibodies were determined by semilogarithmic linear regression analysis of their optical density values and were expressed as relative titers based upon the standard curve as described by Moser and others. Cut-off values for positivity were defined as the median plus two standard deviations of the relative serum antibody titers of NCh individuals. Student’s t- and chi-square tests were used to determine the statistical significance of mean antibody titers and the percentages of serum positivity.

RESULTS

Engineering and expression of hsp70s and FCaBP as His-tagged proteins. During the screening of a *T. cruzi* amastigote cDNA expression library using a pool of sera from 10 chagasic patients, 43 primary clones were isolated. Of these, two highly positive clones were plaque purified by several rounds of immunoscreening. Sequence analysis of the positive clones showed that they encoded partial-length versions of grp78, first identified by Tibbetts and others and cy-hsp70, also described previously. Because of the high immunogenicity of these hsp70 proteins and our long-standing interest in diagnosis and monitoring of treatment efficacy, we set out to systematically test these two *T. cruzi* hsp70s and a third, mt-hsp70, for their potential as diagnostic antigens.

Although we had previously expressed cy-hsp70, mt-hsp70, and grp78 as glutathione S-transferase (GST) fusion proteins, we decided to subclone the coding regions into vectors that permit expression of proteins as hexahistidine fusions. The primary reasons for this are that immunity to GST can occur in some parasitic infections, and it would be more convenient not to have to include free GST as a negative control in all assays. We subcloned cy-hsp70,
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FIGURE 1. Coomassie blue-stained gel of purified recombinant heat-shock proteins of the 70-kD (hsp70s) family and flagellar calcium binding protein (FCaBP). Cy-hsp70, mt-hsp70, grp78, and FCaBP were produced as His6-tagged proteins in Escherichia coli, purified by nickel affinity chromatography, and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Trypanosoma cruzi epimastigote (epi) lysate was used as a positive control for all assays and is shown on the right. Positions of molecular size standards (in kilodaltons) are marked on the left. cy = cytoplasm; mt = mitochondrion; grp78 = endoplasmic reticulum.

Reactivity of sera from chagasic patients with recombinant hsp70s. Enzyme-linked immunosorbent assays were developed to compare the reactivities of chagasic sera with T. cruzi cy-hsp70, mt-hsp70 and grp78 to determine whether they might be used successfully for serodiagnosis of T. cruzi infection. We tested sera from 30 NT patients and from 36 NCh individuals (Figure 2). Two parameters were used to compare seroreactivity with the antigens: average titer and percentage of positivity. Mean ± SD antibody titers of NT patient sera (cy-hsp70: 1,560 ± 1,213; mt-hsp70: 950 ± 906; grp78: 2,376 ± 2,335) were significantly higher (P < 0.05) than average antibody titers of NCh patient sera (cy-hsp70: 379 ± 69; mt-hsp70: 484 ± 311; grp78: 312 ± 87). However, only grp78 was able to detect infection in more than 90% of the NT patients; positive cy-hsp70 responses were detected in 78% of NT patient sera and mt-hsp70 reactivity was seen in only 25% of the patients. The controls FCaBP and epimastigote lysate were recognized by 90% and 100%, respectively, of NT patient sera. Comparison of antibody responses of sera from individual NT pa-

FIGURE 2. Antibody responses to hsp70s (cy-hsp70, mt-hsp70, grp78) and epi lysate. ELISAs were used to determine hsp70-specific antibody responses in 30 sera from chronic, nontreated, chagasic (NT) patients, 30 sera from patients with cutaneous leishmaniasis (CL), nine sera from patients with visceral leishmaniasis (VL), and 36 negative control sera from nonchagasic (NCh) individuals. The antibody response for each serum sample is represented as relative titer in comparison with a positive serum standard of known titer. The percentage of positive sera is indicated above the data for each group. The cut-off value established for each antigen is represented as a horizontal line. See Figure 1 for definitions of other abbreviations.
and in a previous study\textsuperscript{13} strong potential for use in serodiagnosis. We expected that pooling grp78 with FCaBP (1:1 ratio, 1.25 \( \mu \)g/\( \mu \)l) might detect all sera from NT patients and decrease the problems of low-titer leishmanial seroreactivity with grp78. Indeed, the combination of grp78 and FCaBP allowed the detection of 29 of 30 NT patient sera, in comparison with 28 detected with grp78 alone and 27 detected with FCaBP alone. The only serum negative with the combination also failed to react with each protein tested individually. In terms of specificity, three of 39 leishmanial sera remained positive with the pooled antigens, in comparison with five positive with grp78 alone and one positive with FCaBP alone. Thus, combining grp78 with FCaBP increased sensitivity from 90\% to 97\% but increased leishmanial reactivity from 3\% to 8\% in comparison with FCaBP alone.

**Utility of hsp70 antigens for discriminating between cured and uncured patients treated with trypanocidal drugs.** In previous studies, several antigens have been used to determine the successful treatment of chagastic patients with trypanocidal drugs;\textsuperscript{14, 33, 37, 38} however, these antigens are either difficult to purify in sufficient amounts or are not 100\% effective in discriminating between treated TNC and TD patients. To determine the effectiveness of recombinant \textit{T. cruzi} antigens in patient follow-up, we measured seroreactivity to hsp70s in the hope of defining an antigen that would yield a high percentage of positivity with TNC patient sera but give a negative result with TD patient sera. Forty-eight sera from TNC patients and 23 sera from TD patients were tested with cy-hsp70, mt-hsp70, and grp78 (Figure 3). The TNC patient sera showed a high percentage of positivity to cy-hsp70 (74\%) and grp78 (68\%); however, these antigens did not discriminate TNC from TD patients, who also showed significant seropositivity (52\% and 45\%, respectively, with cy-hsp70 and grp78). The mt-hsp70 detected positivity in sera from few TNC patients (18\%) and no TD patients. Epimastigote lysate, as previously shown,\textsuperscript{33} did not discriminate TNC from TD patients since seropositivity in both groups was 100\% and 95\%, respectively. Thus, although grp78 appears to be useful for diagnosis of infection (as shown in Figure 2), no correlation could be established between reactivity to grp78 and treatment outcome.

**Diversity and magnitude of hsp70-specific antibody responses in TNC and TD patients.** Although hsp70s were not useful for determining the effect of trypanocidal drugs on \textit{T. cruzi} infection in individual patients, striking differences were seen between the TNC and TD groups when their relative titers and the diversity of responses to these antigens were compared. Sera from TNC patients had average titers to cy-hsp70 (1.157 \( \pm \) 1.271) and grp78 (1.201 \( \pm \) 1.141), which were significantly higher (\( P < 0.01 \)) than the average titers of TD patients (cy-hsp70: 643 \( \pm \) 377; grp78: 521 \( \pm \) 241). In fact, it appeared that effective treatment lowered average titers in TD patients to the levels of normal NCh patient background reactivity (cy-hsp70: 379 \( \pm \) 69; grp78: 312 \( \pm \) 73). Epimastigote lysate responses also appeared to be decreased in TD patient sera as compared with TNC patient sera; however, the TD patient responses remained at levels significantly higher than those measured in NCh patient sera. In contrast to antibody titers to cy-hsp70 and grp78, antibody titers to mt-hsp70 were not significantly different between TNC (827 \( \pm \) 885) and TD (490 \( \pm \) 198) pa-

![Table 1](image-url)

**Table 1**

Comparison of reactivities of sera from individual nontreated chagastic patients with Trypanosoma cruzi hsp70s and FCaBP*.

<table>
<thead>
<tr>
<th>Patient no.</th>
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<th>grp78</th>
<th>FCaBP</th>
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* hsp70s = heat-shock proteins of the 70-kD family; FCaBP = flagellar calcium binding protein; cy = cytoplasm; mt = mitochondrion; grp78 = endoplasmic reticulum.

\* Antibody titers of chagasic sera were compared with normal human sera. − = titer below cut-off value for positivity (mean titer of normal sera + 2 standard deviations); + = titer 500–1,500; ++ = titer 1,600–3,000; +++ = titer >5,000.

patients with the three hsp70s and the positive control antigen FCaBP is shown in Table 1. The highest titers were obtained with grp78, similar to those obtained with FCaBP. Only one serum was negative with all hsp70s and FCaBP, but positive with epimastigote lysate (titer: 22,230). Interestingly, all sera positive with mt-hsp70 were positive with all the other antigens.

We evaluated potential cross-reactivity of leishmanial sera with \textit{T. cruzi} hsp70s by testing sera from 39 patients with leishmaniasis (Figure 2). Average antibody titers of leishmanial sera to \textit{T. cruzi} hsp70s were significantly lower than titers from NT patient sera (\( P < 0.05 \)), from mt-hsp70 (505 \( \pm \) 215), and from grp78 (436 \( \pm \) 350), but not from cy-hsp70 (1,349 \( \pm \) 1,311). In fact, 77\% of leishmanial sera showed antibody titers above the cut-off value with cy-hsp70 (21 of 30 sera from CL patients and all nine sera from VL patients recognized cy-hsp70). On the other hand, only 13\% and 5\% of leishmanial sera reacted with grp78 and mt-hsp70, respectively; all were from patients with CL. The controls FCaBP and epimastigote lysate were recognized by 3\% and 30\% of leishmanial sera, respectively; all positive sera were from patients with CL. These data suggest that grp78 is the most immunogenic of the hsp70s during chronic \textit{T. cruzi} infection.

In these studies we found that grp78 was the best hsp70 for detecting \textit{T. cruzi} infection, having the lowest cross-reactivity with leishmanial sera. The FCaBP has also shown here
The differences in cy-hsp70 and grp78 responses between TNC and TD patients, as well as differences in epimastigote lysate responses, suggest that antibody responses to T. cruzi antigens decrease in TD patients. This is supported by our finding that fewer hsp70s are detected by TD patient sera than by sera from either TNC or NT patients (Table 2). Whereas the majority of NT and TNC patients (70–80%) possess antibodies reactive with two or three of the hsp70s, more than 40% of the TD patients showed no reactivity to hsp70s, and none of the TD patients showed reactivity to all three of the hsp70s. It is perhaps significant that the hsp70 responses in TD patients are comparable with those seen in patients with leishmaniasis and in nonchagasic individuals, whereas the responses in TNC patients are indistinguishable from responses in NT patients.

**Table 2**

Percentage of sera in different groups of patients and normal individuals reactive with none of the heat-shock proteins of the 70-kD (hsp70s) family or with one or more hsp70s

<table>
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<tr>
<th>Group (n)*</th>
<th>No hsp70s</th>
<th>1 or more hsp70s</th>
<th>2 or more hsp70s</th>
<th>All 3 hsp70s</th>
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<tr>
<td>NT (30)</td>
<td>3</td>
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<td>77</td>
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<td>TD (23)</td>
<td>43†</td>
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<td>NCh (36)</td>
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†P < 0.01 compared with the NT group.

**Discussion**

The hsp70s are well known for their immunogenicity in many parasitic infections such as leishmaniasis,27, 28, 39 malaria,29 schistosomiasis,30 and onchocerciasis.31 They are highly abundant proteins, which, although similar to their human homologs, possess many foreign determinants that induce strong humoral and cellular immune responses during infection. The identification of T. cruzi hsp70s upon screening of an amastigote cDNA expression library with sera from T. cruzi-infected individuals suggested that immune responses to hsp70s may also be important in Chagas’ disease; chagasic sera showed high reactivity with two clones expressing hsp70 family members (cy-hsp70 and grp78).

The diagnostic potential of each hsp70 can be assessed both by the percentage of chagasic patients showing reactivity and by the relative concentration of specific antibody. Our results in this study suggest that grp78 may be the most immunogenic of the hsp70s tested. Comparison of the antibody responses to cy-hsp70, grp78, and mt-hsp70 in sera from chronically infected patients showed that grp78 was detected by most of the sera (94%) with titers higher than those obtained with cy-hsp70 and mt-hsp70. This is consistent with our previous findings in chagasic mice, in which high levels of grp78 antibodies are produced and little or no reactivity with cy-hsp70 or mt-hsp70 is seen.23 Human antibody responses to Plasmodium falciparum grp78 (Pfgrp) and cy-hsp70 (Phsph) have been evaluated by ELISA using peptides with unique sequences for each protein. The Pfhspeptide was more sensitive (62% of positive sera) than Pfgrp (38% of positive sera) in detecting malaria infection. However, the investigators did not exclude the possibility that other peptides present in these proteins were immunogenic as well.40
In parasitic infections other than malaria and Chagas’ disease, only cy-hsp70 has been tested for diagnosis. Antibody responses to Schistosoma mansoni hsp70, for instance, were detected in 50% of patients with chronic T. cruzi infections, and cross-reactivity was observed with sera from filariasis and malaria patients. In leishmaniasis, studies of hsp70 seroreactivity had different outcomes. According to Wallace and others, L. donovani hsp70 is recognized by 92% of sera from patients with VL and no cross-reactivity is observed in sera from healthy individuals or patients with CL, leprosy, malaria, schistosomiasis, and Chagas’ disease. In contrast, Arora and others found that all sera from VL and 60% of sera from healthy individuals react with L. donovani hsp70. If chagasic patient sera seem not to react with L. donovani hsp70, the opposite is not necessarily true. In our ELISA system, T. cruzi cy-hsp70 was detected by 77% of leishmanial sera (70% of CL patients and 100% of VL patients), consistent with the earlier report that sera from patients infected with L. donovani can react with T. cruzi hsp70. This cross-reactivity can be expected since cy-hsp70s from Leishmania sp. and T. cruzi share more than 80% sequence identity, including the C-terminal repeats of the tetrapeptide GGMP. In contrast, only 13% of leishmanial sera reacted with grp78, and the antibody titers were significantly lower than those obtained with cy-hsp70. Although the sequence of the grp78 homolog in Leishmania has not been identified, we speculate that T. cruzi and Leishmania grp78 may be less similar in sequence than are T. cruzi and Leishmania sp cy-hsp70. We also found significant reactivity of normal human sera with mt-hsp70. Conserved hsp70 epitopes among a variety of human pathogens may contribute to cross-reactivity of normal human sera with T. cruzi hsp70. Although hsp70 immunodominant epitopes may be concentrated in the variable C-terminal region of the molecule as demonstrated by epitope mapping, some are also located in structurally and functionally conserved regions.

The sensitivity of a recombinant protein-based assay for detecting T. cruzi infection can be improved by using pooled antigens as reported previously. Since we found that ELISAs using each hsp70 alone were not 100% sensitive and specific for detection of T. cruzi infection, we tried to improve the results by combining the best hsp70 (grp78) with FCaBP, another useful diagnostic antigen. Although the pool improved sensitivity in comparison with grp78 and FCaBP alone, it did not improve specificity in comparison to FCaBP alone, as indicated by the reactivity of three cutaneous leishmanial sera with grp78.

In summary, grp78 is the only hsp70 antigen with potential utility for diagnosis of T. cruzi infection, and its sensitivity and specificity are improved when it is combined with FCaBP. None of the three hsp70 antigens tested in this study was effective in distinguishing cured and uncured treated patients; qualitative differences between TNC and TD patients were only apparent when responses to all three hsp70s were compared. The titers of hsp70-specific antibody responses were significantly lower in cured patients. This is in agreement with the finding that decreased antibody responses to purified native T. cruzi antigens is indicative of successful treatment. We also found that the numbers of different hsp70s detected by sera of TD patients were lower than in TNC and NT patients, further supporting the notion that antibody responses to T. cruzi antigens diminish upon effective treatment.

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