HSP70 from *Trypanosoma cruzi* is endowed with specific cell proliferation potential leading to apoptosis

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**Abstract**

The *Trypanosoma cruzi* HSP70 recombinant protein has the capacity to stimulate splenocytes or lymph node cells from naive mice in a non-haplotype-restricted way. The proliferative response is abolished by proteinase K digestion and by specific anti-HSP70 antibodies. The induced stimulation index was maximal after 24 h of incubation with the protein. This stimulation leads to cell death in a Fas–Fas ligand-independent way. The phenotype of the expanded cells was CD3⁺ TCRαβ⁺ CD4⁺. HSP70-responsive cells express a broad range of cytokines including IFN-γ, IL-2 and tumor necrosis factor-α. After 48 h of incubation with HSP70 there was a significant increase in relative intracellular levels of CD3 TCRαβ receptors. The expanded CD4⁺ cell population expressed CD25; however, in contrast to concanavalin A-treated culture, delayed CD44 expression was observed.

**Introduction**

In spite of their high degree of conservation, heat shock proteins (HSP) have been shown to behave as immunodominant antigens in a large number of pathologies including infectious diseases. The HSP70 proteins from some pathogenic organisms are involved in the development of autoimmune responses (1); however, they have also been implicated in immunoprotection (2). These HSP proteins show chaperone activity, and bind proteins and peptides in a non-covalent way in order to prevent their denaturation and promote translocation to their target organelles under conditions of stress (3). This peptide-binding capacity is reversible and occurs through an ATP-dependent active process (4). HSP70 has also been involved in regulatory processes associated with degradation or translation of mRNAs by binding to AU-rich regions of cytoplasmic RNA, probably through its ATP binding domain (5).

The binding properties of HSP70 protein confer to the proteins outstanding immunological properties, e.g. they have been implicated in class II (6) and in transporters associated with antigen processing (TAP)-independent way class I antigen presentation pathways (7). Thus, this property would allow peptides bound to HSP70 to be presented more efficiently and would thus promote stronger stimulation of antigen-specific T cells (8). Furthermore, chimeric proteins formed by antigens coupled to the C-terminus of the HSP70s in mammals (9), *Mycobacterium tuberculosis* (10) and *Leishmania infantum* (11) have been constructed to search in vivo for the immunostimulatory capacity of the HSP70 protein. The protein enhances the humoral and cellular response to the coupled antigen. The induced immune response is usually classified as type T₅,1, in terms of IgG isotype, induction of cytotoxic T lymphocyte activity and cytokine secretion.

The immunostimulatory effect of HSP70 may be a consequence of stimulation of previously generated memory T cells. However, it has been reported that the strong humoral response to *M. tuberculosis* HSP70 protein itself appears to be due to a strong αβ T cell response with no evidence of natural priming (12). An immunostimulatory activity of stripped HSP70 was also recently described: the protein was able to stimulate the antigen-specific cytotoxic activity of a CD8⁺ T cell clone in the presence of peritoneal macrophages as antigen-presenting cells through the active secretion of IFN-γ.
addition, we tested the capacity of the protein to stimulate Bertani medium. The protein was solubilized in mild SDS (0.025% SDS, pH 8. Soluble extracts were adjusted to reach data show, moreover, that a long period of activation of HSP70 expression and purification internalized by receptor-mediated endocytosis (14). In these T. cruzi HSP70 recombinant protein. E. coli M15 strain transformed with the pQE-TcHSP70 recombinant CD19 (1D3), PE–Fig. 1. Expression and purification of the T. cruzi recombinant HSP70 protein. (A) SDS–PAGE analysis of the pQE-TcHSP70 expression products stained with Coomassie brilliant blue. Lane 1, lysate of the E. coli M15 strain transformed with the pQE-TcHSP70 recombinant vector; lane 2, T. cruzi HSP70 protein after purification through the Ni²⁺ affinity column. M, mol wt marker. (B) Western blot analysis of T. cruzi HSP70 recombinant protein. αHis, mAb against the C-terminus region of the T. cruzi HSP70 protein. αGMPG, antibody against the histine tract.

and tumor necrosis factor (TNF)-α. This activity was not MHC dependent and was also functional in vivo, suggesting the existence of a new mode of T cell stimulation for these types of protein (13). Nevertheless, the mechanism of HSP70 action, as well as the nature of the primary target cells, remains uncertain. However, mouse HSP70 has the capacity to bind to the surface of dendritic cells and macrophages, and to be internalized by receptor-mediated endocytosis (14). In these cells, HSP70 are co-localized with MHC class I molecules in late endosomes, suggesting a direct interaction between HSP70 and MHC molecules. These studies indicated that antigen-presenting cells are capable of interacting with HSP70 and can be stimulated to adapt the immune response. In addition, endothelial cells also respond to HSP70, inducing the expression of adhesion molecules and cytokines (15) that attract lymphocytes to the HSP70-expressing focus.

The HSP70 protein from Trypanosoma cruzi, the protozoan parasite responsible for Chagas’ disease, has been found to be a dominant antigen in this human infection. We cloned and purified the T. cruzi HSP70 recombinant protein. In addition, we tested the capacity of the protein to stimulate splenocytes or lymph node cells from non-immunized mice and characterized the phenotype of the stimulated cells. The data show, moreover, that a long period of activation of proliferating cells with HSP70 leads to significant down-regulation of the surface expression of TCRαβ and CD3, leading to cell death. Apparently, cell death was not directly mediated by Fas–Fas ligand (FasL) interactions.

Methods

Mice and cell suspension preparation
C57BL/6 and BALB/c (CRIFFA, Lyon, France) and C57BL/6-derived gld⁻/⁻ (16) mice strains were used. All mouse strains were maintained in the animal facility. Mice aged 6–8 or 18 weeks old were sacrificed by CO₂ anoxia, and the spleen or inguinal lymph nodes were extracted in sterile conditions and homogenized. Splenocytes or lymph node cells were incubated 15 min at room temperature in ACK buffer (150 mM NH₄Cl, 1 mM KHCO₃ and 0.1 mM EDTA, pH 7.4). Then cells were washed with RPMI and resuspended in complete medium. All cells were cultured in RPMI supplemented with 10% FCS, 50 µM β-mercaptoethanol, 2 mM L-glutamine and 1 mM sodium pyruvate. To assay cell viability, the cell suspension was mixed with 0.4% Trypan blue (Sigma, St Louis, MO) and counted using a Neubauer chamber.

Antibodies

The following antibodies were purchased from PharMingen (San Diego, CA): FITC– and phycoerythrin (PE)–anti-CD3e (145-2C11), FITC–anti-CD4 (GK1.5), FITC–anti-CD8 (53-6.7), FITC–anti-β (H57-597), FITC–anti-NK1.1 (PK136), FITC–anti-CD19 (1D3), PE–anti-IL-2 (S4B6), PE–anti-IL-4 (BVD4-1D11), PE–anti-IL-5 (TRFK5), PE–anti-IL-6 (MP5-20F3), PE–anti-IL-10 (JESS-16E3), PE–anti-IFN-γ (XMG1.2), PE–anti-TNF-α (MP6-XT22), biotin–anti-CD45R/B220 (RA3-6B2), biotin–anti-CD44 (IM7) and biotin–anti-CD25 (PC61). Biotinylated antibodies were revealed using Red670-coupled streptavidin (PharMingen, San Diego, CA).

For the Western blot analyses the mouse mAb recognizing the 6×His tract (Clontech, Palo Alto, CA) was used. The rabbit hyperimmune sera recognizing the C-terminus of trypanosomal HSP70s was obtained and purified as described Martin et al. (17). As secondary antibodies, alkaline phosphatase-conjugated anti-mouse and anti-rabbit (Sigma) were used.

HSP70 expression and purification

The T. cruzi HSP70 genomic unit, coding for the cytoplasmic protein for which overexpression is inducible by a heat shock, was PCR-amplified from phage Tc70.6 DNA (18), using primers 70-U (5’-TCTTCTTCTCCCTATTC-3’) and 70-L (5’-CAGACACTACTGCTCTA-3’), and cloned in the pGEM-T vector (Promega, Madison, WI). The DraⅠ–SspI coding region was subcloned in the Smal site of pQE32 expression vector (Quiagen, Hilden, Germany), that allows the expression of the HSP70 gene in phase with a poly-histidine tract in the N-terminus of the recombinant protein. E. coli M15 strain was chosen as host bacteria and the recombinant HSP70 protein was induced during 3 h at 37°C with 0.1 mM IPTG in Luria–Bertani medium. The protein was solubilized in mild sonication conditions in 50 mM phosphate buffer, 60 mM NaCl and 0.025% SDS, pH 8. Soluble extracts were adjusted to reach final concentrations of 300 mM NaCl, 10 mM β-mercaptoethanol, 1 mM PMSF, 5 mM MgCl₂, 10% glycerol and bound to the Ni²⁺-NTA resin. The resin was washed with the same buffer at pH 8 followed by buffer at pH 7.5. The recombinant HSP70 protein was finally eluted with 50 mM phosphate buffer, 300 mM NaCl, 1 mM PMSF, 5 mM MgCl₂, 10% glycerol, 0.025% SDS, pH 6 and then extensively dialyzed against the same buffer without SDS. The purity of HSP70 preparation was evaluated by SDS–PAGE and Coomassie blue staining, and HSP70 concentration was measured using the Bradford method.

For HSP70 stripping, purified protein was incubated with
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Fig. 2. Proliferation assays of splenocytes from naive mice incubated with T. cruzi HSP70 protein. (A) BALB/c (open symbols) and C57BL/6 (filled symbols) splenocytes were stimulated with 1 µg/ml of T. cruzi HSP70 (circles) or 1 µg/ml of Con A (triangles). (B) C57BL/6 splenocytes were utilized to carry out a 24-h lymphoproliferation assay using T. cruzi (circles), human (rhombus) HSP70 and Con A (triangles) or an equivalent fraction of a parallel purification using the pQE32-transformed bacteria (cross). Antigens were added either untreated (filled symbols) or T. cruzi HSP70 and Con A proteinase K treated (open circles and open triangles respectively). Response to anti-GMPP antibody-preincubated T. cruzi HSP70 is represented as open squares. The plotted stimulation index values are the means of triplicates. (C) Intracellular synthesis of cytokines by C57BL/6 splenocytes non-stimulated (dotted line), stimulated with 5 µg/ml of T. cruzi recombinant HSP70 (solid line) or with 1 µg/ml of Con A (broken line). Staining was performed 48 h after the stimulation and after 24 h for TNF-α staining. The figures show a representative result out of three carried out.

10 mM ATP or ADP (as a control) in presence of 5 mM MgCl₂ for 30 min at 37°C and then dialyzed in a Centricom-50 spin column 3 times as described by Udono and Srivastava (19). The HSP70 protein concentration was measured by the Bradford method. For some experiments, the HSP70 or concanavalin A (Con A) preparations were digested with 50 µg/ml of proteinase K for 1 h at 50°C in a buffer containing 0.05% SDS and 5 mM EDTA.

Western blot analysis
HSP70 protein was loaded in 8% SDS–PAGE and transferred to PVDF membranes (Immobilon-P) following standard procedures. Membranes were blocked in TBS/0.1% Tween 20/5% non-fat milk. The antibody incubations were carried out in the same solution. The immunocomplexes were visualized using NBT/BCIP as substrate.

Lymphoproliferation assays
For cell proliferation assays, spleen or lymph node cells were split in flat-bottom 96-well plates (4×10⁵ cells/well) in the presence of the indicated quantities of antigen or Con A in triplicate wells. The final volume was 200 µl/well in the above-mentioned medium. Plates were incubated at 37°C in a CO₂ atmosphere for 24, 48, 72 or 96 h. After addition of [methyl-³H]thymidine (0.5 µCi/well) cells were incubated for another 6 h at 37°C. Genomic DNA was immobilized in glass fiber
filtermats using an Inothech harvester. The $^3$H incorporation was measured in a Wallac 1450 Microbeta counter device. Stimulation index was calculated using the formula: stimulation index = [c.p.m. (stimulated culture) – c.p.m. (control culture)]/c.p.m (control culture).

**Staining of cells and FACS analysis**

For extracellular staining the cells ($5 \times 10^5$–$10^6$ cells/sample) were treated with 2.4G2 hybridoma supernatant to block Fc receptors and then stained with the labeled antibodies diluted in PBS for 15 min at 4°C. Intracellular staining was performed on 2% paraformaldehyde-fixed cells permeabilized with 0.2% saponin before antibody staining. The cell cycle analysis was assessed on 70% EtOH-fixed cells incubating samples with 40 µg/ml propidium iodide and 100 µg/ml RNase A in PBS for 30 min at 37°C. Cells were gated according to FSC and SSC parameters in a Becton Dickinson FACScan, and calibration points were set by eye. Cytometric data were analyzed using the CellQuest software.

**Results**

**Purification of T. cruzi recombinant HSP70 protein**

The DNA fragment coding for the *T. cruzi* HSP70 protein was cloned in the pQE32 plasmid (see Methods). The profile of the proteins expressed is shown in Fig. 1(A, lane 1) in which an intensely stained band of ~75 kDa corresponding to the size of the HSP70 recombinant protein may be observed. Figure 1(A, lane 2) shows the PAGE band resulting from purification of the recombinant protein after passing the soluble fraction of the total expressed proteins through a Ni$^{2+}$-affinity chromatography column. The purity was >95% as assessed by Coomassie blue staining. In order to determine the integrity of the HSP70 recombinant protein, a Western blot assay was performed using antibodies against the histid-
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Fig. 4. Cell surface phenotype of HSP70 responsive cells analyzed by FACS. (A) Representative surface staining of splenocytes of C57BL/6 naive mice at different times upon HSP70 addition. At the bottom of each graph the corresponding markers analyzed are indicated (B) Double staining (anti-CD4/antiCD3 and anti-CD4/antiTCRαβ) of HSP70-stimulated splenocytes after 24 and 96 h of incubation. Contour plots represent the linear percentage of probability values.

Characterization of T. cruzi HSP70-responsive cells

To determine the phenotype of the cells induced to proliferate, C57BL/6 naive mouse splenocytes were incubated with the T. cruzi HSP70 recombinant protein, and analyzed after 24 and 96 h by flow cytometry using antibodies against a variety of lymphocyte surface markers. The results showed in Fig. 4(A) indicate a clear expansion of the CD3+ TCRαβ+ CD4+ cells after 24 h of incubation. Since it has been described that the HSP70 family of proteins activates different lymphocyte populations, we also searched for CD8+, TCRγδ+, NK1.1+ and CD19+ cell populations. We did not observed any significant expansion of these cells (Fig. 4A). The phenotype of the responding cells as well as the data obtained after inhibition of the proliferative response after addition of cyclosporin A

level of TNF-α which was not observed in the Con A-activated culture (Fig. 2C). The mitogenic-like character of the HSP70 protein is independent of species since both the human and

T. cruzi HSP70 induce the expansion of a non-adherent population of cells with a similar stimulation index (Fig. 2B). In order to analyze whether the proliferative response was due to putative protein-bound peptides, the T. cruzi HSP70 protein was treated with ATP/MgCl₂ or ADP/MgCl₂ (see Methods). The results shown in Fig. 3 clearly indicate that the treatment does not modify the mitogenic-like character of the protein.

Fig. 5. Effect of HSP70 incubation on the viability of the activated splenocytes. The percentage of cell death was measured by the Trypan blue viability test in non-stimulated splenocytes cultures (triangles) and in stimulated splenocytes treated with HSP70 protein (circles) or Con A (squares).

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Fig. 6. Apoptosis of the HSP70-responsive cells. The DNA content assessed by propidium iodide staining was analyzed in HSP70-stimulated splenocytes, Con A-stimulated splenocytes and the control sample after different times of treatment. The different subpopulations are indicated in the histogram corresponding to 24 h of incubation. For clarity the sub-G1 populations are indicated in all histograms.

(data not shown) suggested that the expanded cells are particularly of the T lineage. Interestingly, after 96 h of HSP70 incubation we observed a drastic reversion of CD3 and TCRαβ expression. Double-staining analysis of splenocytes, using anti-CD3 and anti-CD4 antibodies, showed a significant drop of the CD3 surface expression after 96 h of HSP70 incubation relative to the level present at the 24 h of incubation maintaining unmodified the CD4+ population (Fig. 4B). Similar results were also observed when antibodies against the TCRβ were used (Fig. 4B). Thus, after 96 h of HSP70 stimulation 60% of the expanded cells were CD3- TCRαβ+ CD4+. In order to study the down-regulation of the TCRαβ and CD3 surface expression incubation time/response kinetic staining was carried out in non-permeabilized and in saponin-permeabilized splenocytes. Table 1 shows a significant increase in the percentage of intracellular CD3 and TCRβ. The intracellular levels of TCRα and CD3 after 96 h represented ~65 and 52% respectively of the total detected amount of these molecules. This effect was not observed in Con A-treated or untreated splenocytes cultures.

HSP70 induces cell death

The rapid drop of the stimulation index of splenocytes after 48 h of incubation with the T. cruzi HSP70 recombinant protein suggested that either death or an anergic state of the expanded population is occurring. As observed in Fig. 5, the viability of the HSP70-stimulated culture significantly decreases after 72 h of incubation. In order to analyze whether apoptosis or necrosis caused the elevated percentage of non-viable cells we performed propidium iodide staining assays. Figure 6 shows that the peak corresponding to the S/G2 phase significantly increased after 24 h of stimulation maintaining unmodified the CD4+ population (Fig. 4B). Similar results were also observed when antibodies against the TCRβ were used (Fig. 4B). Thus, after 96 h of HSP70 stimulation 60% of the expanded cells were CD3- TCRαβ+ CD4+. In order to study the down-regulation of the TCRαβ and CD3 surface expression incubation time/response kinetic staining was carried out in non-permeabilized and in saponin-permeabilized splenocytes. Table 1 shows a significant increase in the percentage of intracellular CD3 and TCRβ. The intracellular levels of TCRα and CD3 after 96 h represented ~65 and 52% respectively of the total detected amount of these molecules. This effect was not observed in Con A-treated or untreated splenocytes cultures.

To analyze whether the induced cell death after HSP70 cellular activation is mediated by Fas–Fas ligand (FasL) interactions, we tested the ability of the HSP70 protein to induce the expansion of splenocytes from the FasL-deficient gld mice strain (16). The Fas–FasL system is known to
be overexpressed in activated T lymphocytes (16,20) and implicated in a wide number of activation-induced cell death (AICD) models (21). As seen in Fig. 7, T. cruzi HSP70 induces on naive gld splenocyte cultures high cellular proliferation followed by a drop in the stimulation index in a similar way to that observed in the wild-type mice. The same stimulation index pattern was observed in both young (6–8 weeks) and older (18 weeks) gld<sup>−/−</sup> mice. Also, after 72 h of incubation with the protein the sub-diploid cellular population was also significantly higher than that observed in Con A-treated cultures or in the untreated culture (data not shown). Thus, most likely the HSP70 activation-induced cell death pathway detected in non-gld mice is not directly mediated by Fas–FasL interactions.

**Activation markers of HSP70-responsive cells population**

FSC, and CD25, CD44 and B220 (CD45R) activation markers were used to determine the phenotype of the CD4<sup>+</sup> population of HSP70-stimulated cells. Figure 8 shows that the FSC pattern and the expression level of CD25 (IL-2R) were similar to that observed after Con A induction. However, in contrast to the Con A-treated culture, the B220 marker was not detectable in HSP70-stimulated CD4<sup>+</sup> T cells and CD44 expression is delayed. Thus, after 48 h of HSP70 incubation only a low level of CD44 was observed.

**Discussion**

High-molecular-weight HSP participate in a large number of biochemical and immunological pathways. They behave as chaperones (3), as immunodominant antigens (22) and are also implicated in the antigen-processing pathway (6,7). In addition they can be used as adjuvant-free carriers to induce T<sub>H1</sub> responses against fused antigens (9–11). Moreover, HSP70 can bind to professional antigen-presenting cells (13–15) and be recognized by a great variety of lymphocyte populations (21). We describe a novel immunological property of HSP70 proteins as inducers of cell proliferation and death in mature splenocytes from naive mice. Treatment with ATP of the purified T. cruzi recombinant HSP70 proteins showed that the protein itself, and not any bacterial antigen contaminants, was the actual triggering agent of the proliferative response and subsequent cell death. The proliferative response was very rapid: the stimulation index peaked after 24 h of incubation.

As in the study of Bonorino et al. (12) who used the M. tuberculosis HSP70 protein as the triggering agent, CD3<sup>+</sup> TCRαβ<sup>+</sup> CD4<sup>+</sup> T cells seem to be the target of T. cruzi HSP70 action. Analyses of the activation markers of CD4<sup>+</sup> cells after incubation with HSP70 showed no expression of B220 and delayed expression of CD44. The profile of these activation markers is a further indirect evidence of the singular mitogenic character of the T. cruzi HSP70 protein. The B220 marker is expressed in some peripheral activated T cells (23) and...
staphylococcal enterotoxin B-activated T cells before they undergo apoptosis (24). After an intense activation of splenocytes by HSP70, certain mechanisms may be switched on to prevent the putative negative consequences induced by the strong immune response generated against the HSP70 conserved antigen. This in turn may lead, for example, to severe autoimmunity. Such a mechanism may also explain why incubation with HSP70 is followed by down-modulation of the surface expression of TCR/CD3, most likely as a consequence of the increase in the percentage of the internal receptor and induction of apoptosis. These effects would lead to tolerance and anergy to the autoantigen. In fact, internalization of TCR/CD3 receptors has been reported to occur as a mechanism of anergy induced by the staphylococcal enterotoxin B (SEB) superantigen (25) and autoantigens (26). Apoptotic death has also been described in immature T lymphocytes during thymic selection (27) and in mature peripheral T cells in AICD via TCR aggregation (28) or superantigen activation (29). The rapid, strong T cell activation followed by death is compatible with an AICD process.

Although many reports have shown that the HSP70 protein induces CD8+ activation (10,13), there are also reports describing the interaction between HSP and MHC class II molecules (6), which would lead to the activation of CD4+ cells. Our data indicate that in naive mice, HSP70 protein-dependent activation seems to be directed to a subset of CD4+ T cells. The activation and apoptosis induced probably involve a broad range of cell types in a paracrine pathway. The pattern of cytokines observed in HSP70-responsive cells also suggests co-activation of other spleen populations, which might favor an extensive apoptosis. Because similar results were obtained in gld mice, we suggest that the process of cell death induced by activation of HSP70 may not be directly mediated by Fas–FasL interactions. It is known that other receptors belonging to the TNF-α family may also be involved in death signal transduction (30). The fact that HSP70-responsive cells expressed significant TNF-α led us to suggest that the AICD-like effect caused by HSP70 protein could be mediated by this molecule.

Our data indicate, moreover, that the proliferative response induced by T. cruzi HSP70 protein is not haplotype restricted, since splenocytes of both C57BL/6 H2b and BALB/c H2d mice respond in a similar way to the antigen. This phenomenon is typical of superantigens (31) and may be explained by assuming that HSP70 includes several T immunodominant epitopes that can be presented by a diverse range of antigen-presenting molecules. The hypothetical existence of multiple T epitopes in HSP70 would require these epitopes to map to the most highly conserved regions, given that both human and T. cruzi HSP70 are recognized by splenocytes of naive mice (see Fig. 2B). However, recent results demonstrate that the cellular response induced by immunization with mycobacterial HSP70 is directed mainly against the divergent region of the protein (32).

We believe that the non-species-specific, oligoclonal and autolimited capacity of HSP70 to activate splenocytes or lymph node cells from naive mice makes this protein family a good candidate for the development of new immunomodulatory therapies, as has been proposed for different polyclonal and autolimiting activator molecules (33). Moreover, since this type of cellular polyclonal activation and anergy have also been described in the early stages of experimental T. cruzi infection (34,35), the possibility that T. cruzi HSP70 is involved in acute-phase events of Chagas’ disease merits careful investigation. Recently, it has been described that a single in vivo injection of apoptotic, but not necrotic splenocytes in T. cruzi-infected mice resulted in a sudden rise in parasitemia (36). That this phenomenon may occur in vivo when the HSP70 is overexpressed in the infective form of the parasite (37) can be further supported by the fact that the HSP70 protein mimics this immunological disorder in vitro. In this context, HSP70 may interact with MHC class II molecules and subsequently be exposed as a surface complex that interacts with CD4 and TCR molecules in a superantigen-like way, or it may interact directly with the TCR, as has been described for the SEB superantigen (25). The presence of skewed TCR Vβ repertoires in infected animals suggests the existence of a superantigen-like response during T. cruzi infection (38).

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Abbreviations

AICD activation-induced cell death
Con A concanavalin A
FastL Fas ligand
HSP70 70 kDa heat shock protein
LPS lipopolysaccharide
PI propidium iodide
PE phycoerythrin
SEB staphylococcal enterotoxin B
TAP transporters associated with antigen processing
TBS Tris-buffered saline
TNF tumor necrosis factor

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