Trypanosoma cruzi infection in MHC-deficient mice: further evidence for the role of both class I- and class II-restricted T cells in immune resistance and disease

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Abstract

The role of T cell populations in immune control of Trypanosoma cruzi infection and subsequent development of disease has been examined using gene knockout mice deficient in the expression of either or both class I and class II MHC. Mice deficient in either class I- or class II-restricted T cell populations show a striking similarity in their mortality rate, parasite load and tissue inflammatory response following infection with the Brazil strain of T. cruzi. In both cases, all animals died during the acute phase of the infection with high parasitemias and high parasite loads in the heart and skeletal muscle, but with reduced tissue inflammatory responses. Mice deficient in both class I and class II MHC expression demonstrated even higher numbers of circulating and tissue parasites, essentially non-existent tissue inflammatory responses, and succumbed to infection earlier than single-deficient mice. MHC class I-deficient mice which survive into the chronic phase following infection with the M/78 or M/80 clones of T. cruzi have both relatively higher tissue parasite loads and more extensive and severe inflammatory responses than control immunocompetent mice. Immunologically, the acute infection in the double-deficient mice was accompanied by a marked increase in CD4+CD8− αβ TCR+ cells in the spleen. Surprisingly, both class I- and class II-deficient mice produced detectable but sub-normal levels of anti-parasite antibodies while double-deficient mice produced little to no detectable anti-parasite antibody. These results establish the importance of both class I- and class II-restricted T cells in immune control of circulating blood stages and intracellular states of T. cruzi. In addition, this work reinforces the relationship between tissue parasite load and the severity of the inflammatory lesions in chronically infected animals.

Introduction

Trypanosoma cruzi is an obligate cytoplasmic parasite of mammals and the causative agent of Chagas' disease, the major source of human heart disease in Latin America. The infection has an acute phase, characterized by relatively high parasite burden, and a chronic phase, during which parasite proliferation is largely contained but disease symptoms may occur. Although an effective immune response correlates with the progression from the high parasitemic acute phase to virtually undetectable parasitemia in chronic phase, sterile immunity and complete parasite clearance by immune mechanisms are not known to occur in either human infections or in other permissive mammalian hosts.

The immune response elicited by T. cruzi infection is thought to play a role not only in the control of parasite growth in the acute and chronic phase of the infection but also in disease development. Immune control of T. cruzi probably involves a number of effector mechanisms, of which lytic antibodies, CD4+ T cells (possibly through their production of cytokines such as IFN-γ and subsequent macrophage activation) and, more recently, CD8+ T cells, have been best

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studied (1-3). The participation of the immune response in pathogenesis is less well documented, although a dominant hypothesis in the literature is that Chagas' disease has an autoimmune etiology (reviewed in 4). Most of the experimental evidence presented to date argues for a participation of either antibody or CD4+ T cells in the disease process (5-10).

Recently developed gene knockout mice lacking MHC antigens represent excellent systems in which to study the contribution of MHC class I- and class II-restricted T cells to immunity in infectious diseases (11-16). In this study, we have extended our previous investigations in β2-microglobulin (β2m) knockout mice (11), by using MHC class II-deficient and MHC double-deficient mice to more fully characterize the contribution of T cell subsets to immunity and pathogenesis in a murine model of T. cruzi infection. Using lower virulence strains of T. cruzi, we have been able to extend the period of survival of MHC-deficient mice and hence examine disease development in the absence of the CD8+ T cells which normally populate the inflammatory site (17). These studies confirm the participation of CD4+ and CD8+ T cells in immunity during the acute phase of T. cruzi infection and lend further support to the hypothesis that tissue parasites, and not self antigens, drive the chronic inflammatory response in T. cruzi-infected hosts (18,19).

Methods

Parasites and mice

The Brazil strain of T. cruzi was maintained by biweekly serial subinoculation of C3H/HeSnJ mice. Miranda M/78, Miranda M/80, and Sylvio X10/4 clones of T. cruzi were maintained by serial passage in bovine embryo skeletal muscle cell cultures (20). Infected mice received 10^3 blood-form trypomastigotes of the Brazil strain or 10^6 cell-culture-derived trypomastigotes for the Miranda and Silvio X10/4 clones by the i.p. route as an infective dose. Parasitemia levels were determined by hemacytometer counting of diluted tail vein blood and mice were monitored daily for deaths. Statistical significance of differences in death dates was determined by a paired t-test.

β2m-deficient (class I-deficient) (21), class II-deficient (22) and double-MHC-deficient (23) mice were generated as described. The animals used in this study all had the 129 genetic background and control mice consisted of +/+ or +/− littermates of the MHC-deficient (−/−) mice. All animals were maintained in autoclaved microisolator cages (Lab Products, Maywood, NJ) and provided with autoclaved food and water.

Histology

Mice were killed either in the acute phase (~25 days post-infection) or in the chronic phase (9-14 months), and heart, spleen and skeletal muscle tissue was removed and fixed in Carnoy's fixative for 3 h, dehydrated in absolute ethanol, cleared in xylene and embedded in paraffin. Five micron sections were stained with hematoxylin and eosin. For phenotyping of lymphocytes populations, tissues were frozen, fixed and stained as previously described (24).

Measurement of antibody responses

The level of anti-T. cruzi antibodies in the serum of infected mice was determined using a modification of a standard ELISA technique (25). A clarified sonicate of T. cruzi amastigotes harvested from infected Vero cells was used to coat the wells of microtiter plates (Falcon; Becton Dickinson, Oxnard, CA) at a concentration of 1 μg protein/well. Serum samples diluted 1/100 were applied and bound antibody detected with biotinylated rabbit anti-mouse IgG, peroxidase-labeled streptavidin and ATBS substrate (all from Kierkegaard and Perry, Gaithersburg, MD) For production of anti-TNP antibody, mice were immunized i.p. with 200 μg of TNP-keyhole limpet hemocyanin (KLH) (26) emulsified in complete Freund's adjuvant (Sigma, St Louis, MO) 10 days prior to being killed and harvesting of sera. Sera were diluted 1/100 and assayed for anti-TNP IgG by ELISA using TNP-BSA-coated (26) microtiter plates (1 μg/well) and the same detecting antibodies and substrates as described above for anti-T. cruzi antibodies.

Lymphocyte populations

The total numbers of spleen cells were obtained by hemacytometer counts, and the percentages of T cells and T cell subpopulations were determined by antibody staining and flow cytometric analysis as previously described (18). Spleen cells were incubated consecutively with fluorescein-labeled anti-CD8 (H35 17.2), phycoerythrin-labeled anti-CD4 (Phar-

![Fig. 1. Parasitemia and mortality in MHC-deficient mice.](image-url)

Parasitemia (A) and mortality (B) curves for β2m (class I−/−), class II MHC (class II−/−) and β2m and class II MHC (double −/−) gene-deletion mice in comparison with heterozygous (+/−) or homozygous (+/+) littermate controls (control) following infection with 10^3 blood-form trypomastigotes of the Brazil strain of T. cruzi. Each group consisted of six mice.
Mingen, San Diego, CA), biotinylated anti-TCR α (PharMingen), and TriColor-avidin (Caltag, San Francisco, CA), prior to analysis on an EPICS 752 flow cytometer (Coulter Electronics, Hialeah, FL). Cursor settings for the location of negative populations was determined by staining with fluorescein-labeled rat Ig, phycoerythrin-labeled rat Ig and biotinylated hamster Ig (all from PharMingen).

Immunization with attenuated parasites
Mice were immunized three times at 2 week intervals with 3 x 10⁷ 8-methoxypsoralen-inactivated (27) trypomastigotes of the Brazil strain of T. cruzi. Two weeks following the third immunization, mice were challenged with 10⁴ blood-form trypomastigotes of the Brazil strain, and parasitemias and death dates monitored.

Results
Parasitemia and mortality
We have previously reported that class I MHC-deficient mice display high parasitemia and 100% mortality when infected with the Brazil strain of T. cruzi (11). Class II MHC-deficient mice exhibited an almost identical susceptibility to T. cruzi infection as the class I-deficient mice (Fig. 1). The parasitemia and mortality curves for mice deficient in either class I or class II MHC are indistinguishable, and the mean death dates for class I- (29.7 ± 2.9) and class II- (29.8 ± 4.4) deficient mice are equivalent. Mice which lack both class I and class II MHC expression display an even greater susceptibility to T. cruzi infection than either class I or class II MHC single-deficient mice, as signified by a nearly 3-fold higher parasitemia at 3 weeks of infection and an earlier mean day of death (25.7 ± 1.5) (Fig. 1).

The heightened susceptibility of the class I and/or class II MHC-deficient mice is also observed when the infections are initiated with the lower virulence Miranda clones of T. cruzi (Table 1) Infection with parasites of either the M/78 and M/80 clones resulted in the earliest death in the double-deficient mice while the single-deficient mice had survival times which were intermediate between wild-type and double-deficient mice. Infections with the M/80 clone, the double-deficient mice but not the single-deficient mice, died significantly earlier than the wild-type mice. Class I-deficient mice were also highly susceptible to infection with the Sylvio X10/4 clone of T. cruzi, dying prior to day 30 of infection (data not shown). These results re-emphasize the importance of both class I- and class II-restricted T cells in the initial control of T. cruzi infection.

Histopathology
The class I-, class II- and double-deficient mice all display heavy tissue parasite burdens in the heart and skeletal muscle in the virtual absence of inflammatory responses in the acute phase of the infection with the Brazil strain (Fig. 2). This result is in distinction to the low numbers of parasite-infected cells and extensive inflammatory response observed in MHC-competent mice (Fig. 2a–c). Inflammatory cells in the heart and skeletal muscle are more apparent in the single-deficient mice than in double-deficient mice, which exhibit essentially no inflammatory cells in the heart or skeletal muscle. In addition to the extensive parasitization of the muscle seen in all of the MHC-deficient strains, the double-deficient mice also display a high number of parasitized cells in the spleen (Fig. 2).

Because CD8+ T cells normally compose the majority of the lymphocytes at the site of disease in chronic T. cruzi infections (17,18,28,29), it was of interest to also study the inflammatory response in chronically infected mice which had a deficiency in the ability to generate CD8+ T cells. The ability of some class I MHC-deficient mice infected with the M/78 and M/80 clones to survive beyond the acute phase of the infection allowed us this opportunity. As is common for infection with the Miranda clones (30), inflammation was more intense in the skeletal muscles than in the hearts of both wild-type and MHC-deficient mice (Fig. 3). Wild-type mice largely resolve the infection and exhibit few signs of a chronic inflammatory response at 14 months post-infection (Fig. 3a). However, class I-deficient mice showed signs of severe inflammation which were accompanied by an increase in the tissue parasite load (Fig. 3b–d). Thus, despite the deficiency in CD8+ T cells, chronically infected class I MHC-deficient mice nevertheless exhibited significant and severe inflammatory responses. Immunohistochemical staining of the skeletal muscle of the class I-deficient mice with chronic Miranda clone infections found the majority of the inflammatory cells to share the T cell markers Thy-1.2 and TCRαβ but lack either CD4 or CD8 expression (data not shown).

Lymphocyte populations
The spleen cell populations in the MHC-deficient mice were examined to confirm the absence of the respective T cell subpopulations and to monitor the changes in this compartment during the acute phase of the infection with the Brazil strain of T. cruzi. The spleens of non-infected class I- and double-deficient mice were found to contain higher numbers of cells than non-infected immunocompetent mice (Fig. 4). Spleen cell recoveries from the class I-, class II- and double-deficient mice increased during the course of the acute infection, as occurs in the non-deficient mice. This increase in spleen cell numbers of the MHC-deficient mice keeps pace with that of non-deficient mice through at least the first 18 days of infection, suggesting that class I- and class II-restricted T cells may not be required for the initial stages of cellular expansion in the spleens of T. cruzi-infected mice (31).

Examination of the T cell composition of the spleens from

| Table 1. Survival times [mean (SD)] of MHC-normal and MHC knockout mice infected with clones of the Miranda strain of T. cruzi |
|----------------------------------|-----------------|-----------------|-----------------|
| Infecting clone                | Survival time (days) |
| MHC normal | Class I knockout | Class II knockout | Double knockout |
| M/78     | 214.8 (75) | 146.3 (35) | 41.6 (3)⁴ |
| M/80     | 161.8 (17) | 150.8 (6) | 128 (31) | 114.8 (18)⁵ |

⁴Significantly different from MHC-normal mice at P < 0.01.
Fig. 2. Histopathology of heart, skeletal muscle and spleen of *T. cruzi*-infected, MHC-deficient mice. Hematoxylin & eosin stained sections of heart (a, d, g and j), skeletal muscle (b, e, h and k) and spleen (c, f, i and l) from heterozygous (+/-) or homozygous (+/+ ) littermate controls (a-c), β2m −/− (d-f), class II MHC −/− (g-i) and double-deficient (j-l) mice infected for 25 days with 10³ blood-form trypomastigotes of the Brazil strain of *T. cruzi*. Arrows in (d), (e) and (l) identify some of the many parasite-infected host cells.
acutely infected MHC-normal and -deficient mice revealed the expected absence of CD4+ T cells in the class II- and double-deficient mice and of CD8+ T cells in class I- and double-deficient mice (Fig. 5). These profiles were not altered by *T. cruzi* infection. In addition, uninfected mice lacking either or both class I and class II MHC contained a small population (5–10%) of TCRαβ bearing cells which expressed neither CD4+ or CD8+. These CD4−CD8− αβTCR+ were also a minor population of the T cells in infected class I- or class II-deficient mice, representing 7–8% of the total spleen cells, but were dramatically increased in number (to 30% of the total) in double-deficient infected mice. Similar results were obtained when cells were stained with anti-CD3 antibodies in place of anti-TCR (data not shown).

**Antibody responses in *T. cruzi*-infected, MHC-deficient mice**

Class-I, class II- and double-deficient mice were killed at various times post-infection and assayed for *in vivo* and *in vitro* responses. Serum anti-parasite antibody responses were measured by ELISA using an amastigote sonicate as the antigen. As expected, the non-deficient mice exhibited the highest anti-*T. cruzi* IgG levels and these antibody levels increased as the acute phase of the infection progressed (Fig. 6). Mice deficient in either class I or class II MHC generated more moderate antibody responses, while mice deficient in both class I- and class II-restricted T cells failed to generate any anti-*T. cruzi* antibody above background levels (Fig. 6B). The antigen-specificity of response in class I-deficient mice was not different from that of wild-type mice, as determined by Western blot analysis (data not shown).

The lower antibody response of class I-deficient mice to infection with *T. cruzi* was surprising. To confirm that these mice could respond normally to simple, non-parasite antigens, they were immunized with TNP-KLH (with or without *T. cruzi* infection) and the level of anti-TNP antibodies were measured. As in previous experiments, class I-deficient mice
responded poorly to *T. cruzi* but made antibody responses to TNP-KLH which were indistinguishable from wild-type mice (Fig 7). Infection with *T. cruzi* did not alter the level of the TNP-specific antibody response in either the wild-type or class I-deficient mice.

**Immunization studies**

The ability of class I-deficient mice to be protectively immunized against *T. cruzi* infection was determined by immunizing mice with three doses of chemically attenuated trypomastigotes of *T. cruzi* and challenging with virulent Brazil strain blood-stage parasites (Fig 8). All wild-type mice had moderate (non-immunized controls) or barely detectable (immunized group) parasitemias and no mortality. Class I-deficient non-immunized mice had the expected very high parasitemia and a mean day of death of 25.7. Although immunization moderated the mean parasitemia level of class I-deficient mice, all but one of these mice died between days 27 and 38 of the infection, and a single mouse survived until day 62.

**Discussion**

Gene knockout mice which lack particular immunological functions are valuable tools for the study of the contribution of these particular immune functions to control of infectious diseases. We have made further use of MHC-deficient mice to study various aspects of the murine infection with *T. cruzi* and the course of experimental Chagas' disease.

The results of this study demonstrate the critical dependence on both CD8+ and CD4+ T cell-mediated responses for control of the acute phase of *T. cruzi* infection in mice. Infections with the relatively virulent Brazil strain of *T. cruzi* in MHC class I- or class II-deficient mice are uniformly fatal with parasitemia levels and mortality rates which are nearly identical between these two groups. This result is in stark contrast to the low parasite burden and 100% survival in wild-type mice. Mice deficient in both class I and class II MHC succumb to infection earlier than single-deficient mice, again reinforcing the importance of the contribution of both class I- and class II-restricted T cells to anti-*T. cruzi* immunity, which had been suggested from previous studies using anti-MHC and anti-CD4 or -CD8 antibodies (3, 18, 32, 33) and mice with CD4 or CD8 gene deletions (34).

This requirement for competence in both the class I- and class II-restricted T cell compartment extends to infection with parasite strains which exhibit a low virulence phenotype in immunocompetent mice (30). In the case of infections with either the M/78 or M/80 clones of *T. cruzi*, the double-deficient mice die earliest, followed by the class II-deficient and then the class I-deficient mice. The broadest range of survival times is seen in infections with the M/78 clone in which double-deficient mice die relatively early in the infection while class I- and class II-deficient mice do not succumb until what is normally considered the chronic stage of the infection (>100 days). In the M/80 infections, even the double-deficient mice survive on average >100 days.

Previous studies of mechanisms of immunity to *T. cruzi* infection have focused primarily on the activity of CD4+ T cells, and their role in facilitating production of lytic antibodies and activation of macrophages for parasite killing (10, 35). The possible participation of class I-restricted T cells in control of *T. cruzi* has been only recently appreciated with the demonstration of the high susceptibility of CD8-depleted animals (3, 11, 18) and the presence of parasite-specific cytotoxic T cells in infected mice (2). The current study provides additional, unequivocal evidence that mice must have the ability to recognize and respond to both class I-associated and class II-associated parasite epitopes in order to control *T. cruzi* infection. In addition, the generation of protective immunity through vaccination may depend on the stimulation of the CD8+ T cell compartment: β2m-deficient mice, unlike their control littermates, cannot be protectively immunized with attenuated parasites.

The requirement for both a CD4+ and CD8+ T cell-mediated response in *T. cruzi* infection is consistent with the fact that *T. cruzi* has both an extracellular stage which is accessible to antibody-dependent responses as well as an intracellular stage. The cytoplasmic location of the amastigote stage of *T. cruzi* and the fact that the parasite often resides in class II MHC-negative cells *in vivo* makes it likely that the CD8+ T cell contribution to immunity is via the recognition and lysis of infected cells. However, it is also interesting to note that class I-deficient mice infected with *T. cruzi* exhibit depressed antibody responses to *T. cruzi* when compared with wild-type animals. Whether this lower level of antibody production is
T. cruzi infection in MHC-deficient mice

Fig. 5. Flow cytometric analysis of spleen cells from mice infected from 24 days with $10^3$ blood-form trypomastigotes of the Brazil strain of T. cruzi. The percentage of cells in each single- and double-positive quadrant are indicated. The x-axis shows log_{10} fluorescence. Data were collected on 10,000 cells per sample.

Due to factors related to the alteration in antigen presentation to T<sub>H</sub> cells in class I-deficient mice (i.e., how much parasite antigen is presented and how that antigen is presented) or to a direct role for class I-restricted T cells in providing help to the anti-T. cruzi antibody response is not known.

Class I-deficient mice are capable of mounting anti-TNP-KLH antibody responses which are comparable to that of wild-type mice, indicating that class I-restricted T cells are not essential for generation of antibody responses to classical hapten-carrier complexes. However, the moderate anti-T. cruzi antibody response of class II-deficient mice might argue for a participation of class I-restricted T cells as helper cells in the antibody responses to the more complex antigenic challenge presented by T. cruzi. Spriggs et al. have reported significantly lower antibody responses to vaccinia virus in β<sub>2m</sub>-deficient mice but a marginal if any effect of the β<sub>2m</sub> mutation on the response to TNP-KLH (36). In contrast, class II MHC-deficient mice have been reported to have normal

Fig. 6. Antibody production in MHC-deficient mice following T. cruzi infection. Total anti-T. cruzi IgG levels in the serum of uninfected (day 0) or T. cruzi-infected mice at various times post-infection with $10^3$ blood-form trypomastigotes of the Brazil strain of T. cruzi. Control = +/- littermates; class I +/- = β<sub>2m</sub>-negative mutants; class II +/- = class II MHC-negative mutants; double +/- = mice homozygous for both the β<sub>2m</sub> and class II MHC mutations. Each bar represents the mean (± SD) of sera from three mice assayed separately in triplicate.
B cell development (37) and to produce antibody in response to T-independent but not to T-dependent hapten-carriers (22,37). The decreased level of anti- \( T. cruzi \) antibody production in class I-deficient mice was consistent in four separate experiments involving a total of 18 mice. However, the ability of class II-deficient mice to produce anti- \( T. cruzi \) antibody was not as consistent in all experiments. Class I-restricted T cells have previously been shown to be capable of acquiring a type 2 cytokine production pattern (38,39), expressing the ligand for CD40 which is important in B cell activation (39) and providing help for antibody production by resting B cells (38,39). Determination of whether or not class I-restricted T cells serve as true helper T cells in the antibody response to \( T. cruzi \) will require further study.

The heightened susceptibility and decreased level of antibody production and vaccine-induced protection in \( \beta_2 \)m knockout mice could also be explained by the absence of other lymphocyte populations which are dependent on \( \beta_2 \)m expression for their maturation. CD1-restricted CD4\(^{-}\)CD8\(^{-}\)NK1.1\(^{+}\), \( \alpha \beta \)TCR\(^{+}\) lymphocytes also fail to develop in \( \beta_2 \)m knockout mice (40). This subpopulation of lymphocytes is known to produce significant amounts of IL-4 (40,41) and thus could also contribute to antibody production, and hence immune protection, in \( T. cruzi \)-infected mice. MHC normal mice infected with \( T. cruzi \) show an increase in the number of CD4\(^{-}\)CD8\(^{-}\) \( \alpha \beta \)TCR\(^{+}\) T cells in the spleen (Fig. 5). However, these cells are unlikely to be CD1-restricted since they are present in even greater numbers in class I- and double-deficient mice (which lack expression of the CD1 restricting element) as well as in class II MHC-deficient mice.

The different course of infection in MHC-deficient mice infected with strains of \( T. cruzi \) which vary in virulence allowed us to also study the participation of class I and class II MHC-restricted T cells in the acute and chronic phase inflammatory response. Infection in immunocompetent hosts normally results in a substantial inflammatory response in tissues infected by \( T. cruzi \). As the parasite load is controlled by the T cell-dependent immune response, the stimulus for the parasite-specific component of the inflammatory response is thought to diminish. However, the inflammatory response itself does not subside, instead persisting and eventually resulting in tissue destruction and the disease state known as Chagas’ disease. Whether the disease-causing component of this chronic inflammatory response is directed against the parasite or is an autoimmune response is the source of considerable controversy. In both human Chagas’ disease and in rodent models CD8\(^{+}\) T cells have been shown to dominate the inflammatory site (17,18,28,29). In our studies, this CD8 dominant pattern is consistent throughout the acute and chronic \( T. cruzi \) infection and in the heart and skeletal muscle in a variety of parasite-host strain combinations (17).

The participation of the infiltrating CD8\(^{+}\) cells in the control of parasite load in the acute phase of \( T. cruzi \) is supported by the near absence of inflammation and high tissue parasite burden in class I-deficient mice (11 and this study). The tissue inflammatory response in the class II- and double-deficient mice is similar to that of class I-deficient mice; all three MHC-deficient groups exhibit very high tissue parasite burdens but
with inflammatory responses significantly lower than that of MHC-normal mice. The acute phase inflammatory response in class II-deficient mice is slightly more intense than in the class I-deficient mice and the inflammatory response in the double-deficient mice is less than that of the class I-deficient mice. The reduced inflammatory response in the class II-deficient mice during the acute phase of infection suggests that although CD4+ T cells are a relatively minor component of the lesion itself, they either enhance the influx of CD8+ T cells into the lesion site or the expansion of CD8+ T cells once they reach the lesion. In the absence of either or both of the class I- or class II-restricted T cells, the tissue inflammatory response is greatly reduced and tissue parasites appear to replicate largely unchecked.

In contrast to the decrease inflammatory response observed in MHC-deficient mice in the acute phase, the tissue inflammatory response of MHC class I-deficient mice to chronic T. cruzi infection is greatly enhanced relative to that observed in immunocompetent littermates. This increased inflammatory response is also associated with a higher tissue parasite burden. These results are consistent with our previous report on the exacerbating effects of CD8+ T cell depletion in chronically infected mice using antibody treatment (18) and gives further support to the theory that T. cruzi antigens are the main focus of the chronic inflammatory response. In the absence of the class I MHC-restricted T cells which normally populate the chronic inflammatory site, a compensatory inflammatory response ensues. The resulting infiltrate is composed largely of CD4+CD8+ (double negative) T cells which are clearly less effective in controlling the parasite at the level of the infected host cell. Thus, the CD8+ T cells present at the site of disease in chronic T. cruzi infection serve a protective function there which cannot be provided by other lymphocyte or non-lymphocyte populations. Consequently, limiting the severity of chronic disease in T. cruzi infection might be accomplished by enhancing this protective CD8+ T cell response.

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**Abbreviations**

- β2m: β2-microglobulin
- KLH: keyhole limpet hemocyanin

**References**

22. T. cruzi infection in MHC-deficient mice


