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The relative contribution of antibody production and CD8$^+$ T cell function to immune control of *Trypanosoma cruzi*

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SUMMARY

The life cycle of the protozoan parasite Trypanosoma cruzi *in mammalian hosts includes both non-dividing trypomastigote forms which circulate in the blood and replicating intracellular amastigotes which reside within the cytoplasm of a variety of host cells. In this study we have used mice with induced mutations in genes responsible for either antibody production or cytolytic T lymphocyte (CTL) function to examine the relative contributions of these effector mechanisms to control of* T. cruzi. *Mice deficient in the production of antibodies exhibited a delay in the rise in acute phase parasitaemia and an extended time to death relative to mice lacking CD8$^+$ T cells. Nevertheless, B cell deficient mice eventually succumbed to the infection. Prior infection with an avirulent strain of* T. cruzi *failed to protect either CD8$^+$ T cell-deficient mice or B cell deficient mice from challenge infection with virulent parasites. In contrast, mice with disruptions in the genes controlling perforin- or granzyme B-mediated cytolytic pathways had parasitaemia and mortality rates similar to wild-type mice and were protected from secondary infection by prior exposure to avirulent parasites. These results 1) confirm that antibody production, although secondary in importance to cellular responses, is nevertheless absolutely required and 2) perforin- or granzyme B-mediated lytic pathways are not required for control of* T. cruzi *infection.*

Keywords Trypanosoma cruzi, *Chagas' disease, immunoglobulins, cytotoxic T lymphocytes, CTL, perforin, granzyme B, vaccine*

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INTRODUCTION

Trypanosoma cruzi infects a wide variety of mammalian hosts and in humans can cause life threatening Chagas' disease. In mammals, *T. cruzi* cycles between intracellular amastigotes and extracellular trypomastigotes and evokes both humoral and cell mediated immune responses. Until recently, anti-*T. cruzi* antibodies and activated macrophages have been considered the major mechanisms of immune control of this parasite. The lytic (Lages-Silva *et al*. 1987), opsonic (Scott & Moyes, 1982), antibody dependent cellular cytotoxicity (ADCC) (Lima-Martins *et al*. 1985), complement activating (Mota and Umekita, 1989, Brodskyn *et al*. 1989, Spinella *et al*. 1992) and invasion blocking (Almeida *et al*. 1991) activities of antibodies have all been proposed to assist in immune control of *T. cruzi*. Likewise, activation of macrophage-mediated killing mechanisms by the production of Th1-type cytokines has also been shown to be critical to control of *T. cruzi* (Gazzinelli *et al*. 1992, Munoz-Fernandez *et al*. 1992, Metz *et al*. 1993, Vespa *et al*. 1994). Nevertheless, vaccination studies designed to activate primarily class II MHC-dependent T cell responses and antibody production have consistently failed to provide complete protection in experimental models (Rottenberg *et al*. 1988, Ruiz *et al*. 1986, Harth *et al*. 1994).

In recent years, class I MHC-restricted CD8$^+$ T cells have been added to the list of critical effectors in immunity to *T. cruzi* (Nickell *et al*. 1993). Support for the importance of CD8$^+$ T cells in immune control of *T. cruzi* comes from evidence that depletion of CD8$^+$ T cells or targeted disruption of genes necessary for the generation of mature CD8$^+$ T cells results in high susceptibility to *T. cruzi* in murine models (Tarleton, 1990, Tarleton *et al*. 1992, Rottenberg *et al*. 1993, 1995a, Tarleton *et al*. 1996). Further, transfer of CD8$^+$ T cells specific for TSA-1, a target of both murine and human anti-*T. cruzi* CTL responses, provides significant protection from *T. cruzi* infection (Wizel *et al*. 1997).

In this study we have utilized mice with targeted disruptions

in genes controlling B cell development and $CD8^+$ T cell function to address the question of the relative contribution of these effector mechanisms in control of *T. cruzi* infection. As predicted from earlier studies, the failure to produce antibodies significantly hinders the generation of a protective response to *T. cruzi* in either a primary or challenge infection. Surprisingly, however, B cell-deficient mice are able to survive for significantly longer than do mice with defects in either $CD4^+$ or $CD8^+$ T cell function. With respect to the effector function of $CD8^+$ T cells in *T. cruzi* infection, the high susceptibility of mice with a deficiency in $CD8^+$ T cells is confirmed in studies using mice lacking TAP-1 gene function. However, infections in mice with induced defects in the cytolytic function of $CD8^+$ T cells suggest that the control of *T. cruzi* infection by this population of T cells is not dependent on the granzyme/perforin cytolytic pathway.

MATERIALS AND METHODS

Mice

Wild-type C57Bl/6J mice (common name B6), B cell deficient mice (C57BL/6-Igh-6^{tm1Cgn}; common name muMT; (Kitamura *et al*. 1991)), beta-2-microglobulin deficient mice (C57BL/6J-$B2m^{tm1Unc}$; common name $b2m^{-/-}$; (Koller *et al*. 1990)), perforin deficient (C57BL/6-Pfp^{tm1Sdz}; common name $perforin^{-/-}$ (Kagi *et al*. 1994a) and granzyme B deficient (C57BL/6J-$Gzmb^{tm1Ley}$; common name granzyme $B^{-/-}$ (Heusel *et al*. 1994)) mice were either obtained from The Jackson Laboratory (Bar Harbor, ME, USA) or were bred in our facility from stocks obtained from The Jackson Laboratory. TAP-1 deficient mice (common name TAP-$1^{-/-}$; (Van Kaer *et al*. 1992)) on the B6 background were bred from mating pairs obtained from Dr Luc Van Kaer, Vanderbilt University.

Parasites

Blood-form trypomastigotes (BFT) of the *T. cruzi* Brazil strain were maintained in C3HHe/SnJ mice by biweekly passage and were injected at a dose of 10^3/mouse by the intraperitoneal (i.p.) route. Trypomastigotes of the *T. cruzi* M/80 Miranda clone were obtained from culture in bovine embryonic smooth muscle cells and injected i.p. at a dose of 10^6/mouse. Mice infected with this avirulent M/80 clone were subsequently challenge infected with 5×10^4 BFT Brazil strain *T. cruzi* 294 days later. The parasitaemias in infected mice were determined by weekly examination of tail blood in a haemacytometer and mortality was monitored daily.

Measurements of anti-*T. cruzi* immune responses

T. cruzi-specific IgM and IgG antibody levels were measured 25 and 35 days post-infection using a previously described procedure (Tarleton *et al*. 1996). Measurement of anti-*T. cruzi* TSA-1 peptide 77.2-specific cytolytic T cell activity was performed as previously described (Wizel *et al*. 1997). The percentage specific ^{51}Cr-release from triplicate wells was calculated as {(average experimental cpm – average spontaneous cpm)/(average maximum cpm – average spontaneous cpm)}$\times$100. Maximum and spontaneous ^{51}Cr-release in counts per minute (cpm) were determined from triplicate wells containing labeled target cells only with no effectors in the presence or absence of 5% SDS, respectively.

Histology

Mice were killed by CO_2 inhalation at various times post-infection and tissues from heart, spleen, and skeletal muscle were collected in 10 per cent buffered formalin. The five micron sections of paraffin embedded tissues were fixed, dehydrated and stained with haematoxylin and eosin and examined by light microscopy.

RESULTS

Previous studies have utilized mice with targeted deletions in genes encoding proteins of immunological importance to determine the role of different immune effector mechanisms in immunity to *T. cruzi* (Tarleton *et al*. 1992, Rottenberg *et al*. 1993, 1995a, Abrahamsohn and Coffman, 1996, Santos Lima and Minoprio, 1996, Tarleton *et al*. 1996, Hunter *et al*. 1997). To analyse further the mechanism of action of B cells and $CD8^+$ T cells in *T. cruzi* infection, we took advantage of mice with an induced mutation in the *mu* heavy chain immunoglobulin gene and mice with mutations in genes effecting the development and function of $CD8^+$ T cells. Our initial experiments compared the course of infection with the Brazil strain of *T. cruzi* in immunocompetent B6 mice, mice lacking *b2m* gene function, a defect previously shown to result in high susceptibility due to the absence of $CD8^+$ T cells (Tarleton *et al*. 1992), *TAP-1* deficient mice which also lack mature $CD8^+$ T cell function and muMT mice which are defective in immunoglobulin production. As expected, the course of *T. cruzi* infection in TAP-$1^{-/-}$ mice closely resembles that of $b2m^{-/-}$ mice with high parasitaemias and death between days 28-33 post-infection (Figure 1). In comparison muMT mice infected with same dose of *T. cruzi* displayed wild-type levels of parasitaemia early in the infection (up to day 28 post infection). However, by 42 days post infection, parasitaemias in the muMT mice had risen significantly above that of wild-type mice and all muMT mice were dead by day 55 post-infection. The absence of antibody production in the

muMT mice was confirmed by analysis of serum from infected mice for the presence of anti-*T. cruzi* IgM and IgG (Figure 2). Thus in the absence of an antibody response, mice infected with *T. cruzi* exhibit evidence of an initial control of the infection but eventually fail to contain parasite replication and succumb to the infection.

We next asked if the survival of B cell deficient mice could be extended further by initiation of the infection with lower doses of parasites. muMT mice infected with 10 or 100 BFT of the Brazil strain exhibited control of the infection and survival for as long as 89 days post-infection (Figure 3). However, all of these animals did eventually develop high parasitaemia levels and die. Therefore immunoglobulin production is absolutely required for control of an even low initial level infection with a virulent strain of *T. cruzi*, although defects in other effector populations, in particular $CD8^+$ (Figure 1 and Tarleton *et al*. 1992) and/or $CD4^+$ T cells (Rottenberg *et al*. 1995b, Tarleton *et al*. 1996), leads to an even earlier time to death.

The high susceptibility of the TAP-$1^{-/-}$ mice provides further confirmation of the requirement for the induction of parasite-specific $CD8^+$ T cells in immunity to *T. cruzi* . Both cytolytic T lymphocyte (CTL) activity and cytokine production has been documented in anti-*T. cruzi* $CD8^+$ T cells (Wizel *et al*. 1997) but the requirement for either or both of these activities in immune control of *T. cruzi* has not been studied. To begin to address this question, mice with targeted defects in the pathways considered to be the most important in cytolytic killing by CTL were infected with *T. cruzi*. Perforin- and granzyme B-deficient mice infected with 1000 BFT of the Brazil strain of *T. cruzi* exhibited parasitaemia levels (Figure 4) and survival rates (100% in all groups in this experiment) comparable to that of wild-type mice. Perforin$^{-/-}$ mice exhibited slightly higher peak blood parasite levels than either B6 or granzyme $B^{-/-}$ mice but parasitaemia levels in all groups were undetectable by day 56 PI and remained so until greater than 100 days post-infection when the experiment was terminated.

Despite the absence of either granzyme B or perforin, spleen cells obtained from these deficient mice at day 90 post-infection displayed significant cytolytic T cell activity versus cells pulsed with peptide 77.2 (Figure 5), a *T. cruzi* TSA-1-derived peptide which had been previously shown to be a target of anti-*T. cruzi* CTL (Wizel *et al*. 1997). No similar activity was detected against target cells pulsed with an irrelevant peptide (OVA) or incubated without peptide. Both the ability of mice with defects in perforin or granzyme to control *T. cruzi* infection and the retention of cytolytic activity in splenocytes from these same mice suggest that at least part of the control function of $CD8^+$ T cells in *T. cruzi* infection is independent of a granzyme/perforin cytolytic pathway.

The results of histopathological analysis of heart tissues from immunodeficient mice confirm the evidence from parasitaemia and longevity studies indicative of a heightened susceptibility of the TAP-$1^{-/-}$ and muMT mice and of a relatively unaltered infection in the granzyme $B^{-/-}$ and perforin$^{-/-}$ mice (Figure 6). b2m$^{-/-}$ (Tarleton *et al*. 1992, 1996) and TAP-$1^{-/-}$ (Figure 6a) mice showed high tissue parasitism both in skeletal muscles (not shown) and heart with negligible inflammation in the acute phase of the infection (day 23). However, muMT mice displayed low levels of tissue parasites and moderate inflammation in heart and skeletal muscles at a comparable time point in the infection (day 25; Figure 6c), a response very similar to that of the wild-type B6 mice (Figure 6d). However the tissue parasite burden and inflammation increased in heart muscle of muMT mice and was significantly more intense than that in B6 mice at 45 days post infection (Figures 6e and 6f, respectively). Tissue parasitism and inflammation in perforin$^{-/-}$ (Figure 6b) and granzyme $B^{-/-}$ (not shown) mice at day 45 post-infection was comparable to wild type B6 mice.

The ability of B cell deficient mice to survive for a significant period of time following infection with a virulent strain of *T. cruzi* suggests that the antibody response is relatively less important, in comparison to the responses mediated by $CD4^+$ or $CD8^+$ T cells, in control of the acute infection. This result prompted the question of whether or not protection from infection could be induced by vaccination of animals which lack the ability to generate an antibody response. To address this question immunodeficient mice were infected with a low virulence strain of *T. cruzi* which had previously been shown to allow for survival in immunodeficient mouse strains (Tarleton *et al*. 1996). At approximately 300 days after this primary immunizing infection, the mice were challenged with virulent Brazil strain parasites and the parasitaemia and longevity monitored (Figure 7). The challenge infection was lethal in all b2m$^{-/-}$ and TAP-$1^{-/-}$ mice by 32 and 45 days post infection, respectively. B cell deficient mice again exhibited a delay in the rise of parasitaemia and a significant increase in longevity relative to the $CD8^+$ T cell deficient mice. However these mice eventually died between 60 and 100 days post infection. Granzyme $B^{-/-}$ and perforin$^{-/-}$ mice were protected by the prior infection with avirulent parasites and had no detectable parasites in the blood at any point post-infection with the virulent Brazil strain.

DISCUSSION

Immune control of *T. cruzi* infection involves multiple immune effector mechanisms (reviewed in Tarleton, 1997)). Previous studies using cell depletion protocols and/or mice with induced mutations in genes controlling

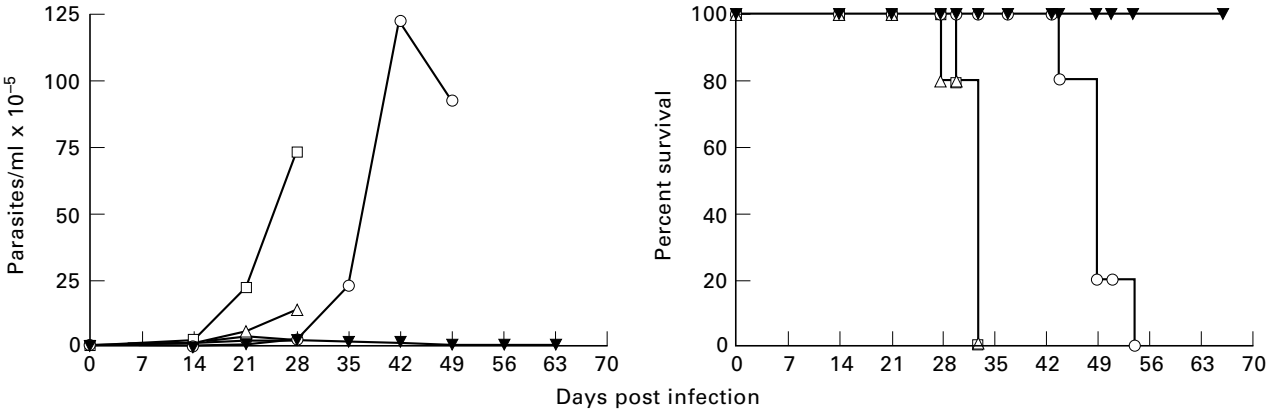

Figure 1 Parasitaemia and mortality curves in immunoglobulin- and $CD8^+$ T cell-deficient mice infected with *T. cruzi*. △ $bzm^{-/-}$; □ TAP-$1^{-/-}$; ○ muMT; ▼ B6.

either $CD4^+$ (Araujo, 1989, Rottenberg *et al*. 1993, 1995a, Tarleton *et al*. 1996) or $CD8^+$ (Tarleton, 1990, Tarleton *et al*. 1992, Rottenberg *et al*. 1993, 1995a, Tarleton *et al*. 1996) T cell function have documented the absolute requirement for the activation of both of these T cell subpopulations for survival of mice during the acute phase of *T. cruzi* infection. Mice with deficiencies in both T cell subsets achieve higher parasite loads and die earlier in the infection than mice with single defects (Tarleton *et al*. 1996), indicating a non-overlapping mechanism of action for $CD4^+$ and $CD8^+$ T cells in control of *T. cruzi* infection. In addition, previous studies also provided strong evidence of an important role for antibodies in regulating *T. cruzi* in the infected host: anti-μ suppressed rats (Rodriguez *et al*. 1981) and mice (Trischmann, 1983, 1984) are more susceptible to infection than are immunologically intact hosts.

The present study extends these findings by directly comparing the course of *T. cruzi* infection in mice lacking either $CD8^+$ T cells or antibody producing B cells. Mice unable to make antibodies to *T. cruzi* live substantially longer than mice lacking either $CD4^+$ (Tarleton *et al*. 1996) or $CD8^+$ T cell function, suggesting a more critical role for these T cell populations than for antibody production in the initial control of the infection. Nevertheless, even mice infected with very low numbers of virulent parasites or with an avirulent strain prior to challenge with virulent parasites, are unable to control parasite growth and succumb during the acute parasitaemic phase of the infection. Thus, in agreement with previous studies (Trischmann, 1983, 1984) T cells appear to be required for the initial control of the infection but for long-term survival, an effective antibody response is also critical. These results are also consistent with the failure to achieve vaccine-induced immunity with protocols based solely or primarily on induction of antibody responses (Brener 1986). Elicitation of antibody production alone is insufficient to control the infection and effective vaccines are likely to additionally require activation of both the $CD4^+$ and $CD8^+$ T cell compartments.

The critical effector functions necessary for parasite control by anti-*T. cruzi* $CD4^+$ and $CD8^+$ T cells are not yet known. $CD4^+$ T cells likely have multiple roles,

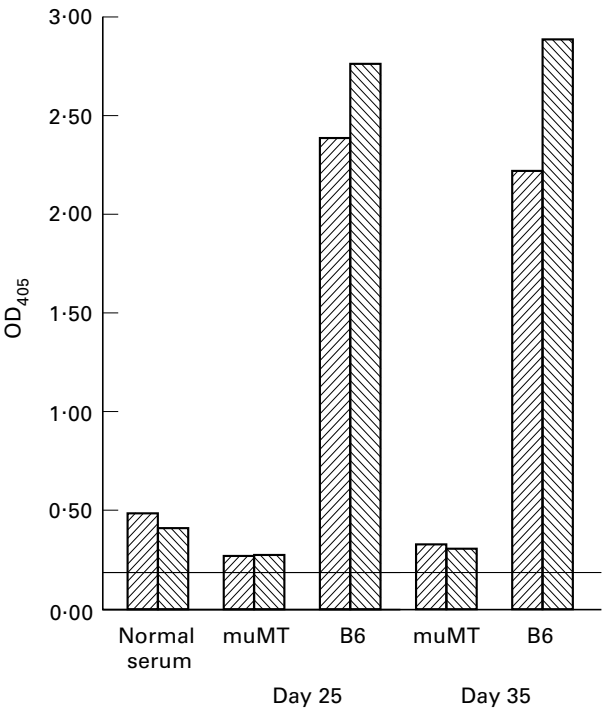

Figure 2 *Trypanosoma cruzi*-specific IgM and IgG levels in muMT and wild type B6 mice at day 25 and 35 post-infection. ▨ IgM; ▧ IgG. Horizontal line indicates background OD of normal mouse serum.

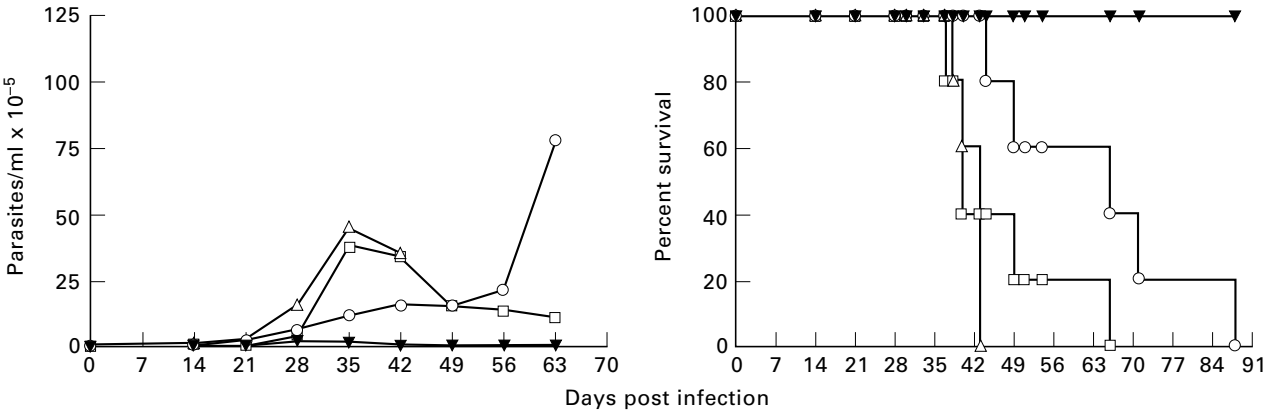

Figure 3 Parasitaemia and mortality in muMT mice infected with varying doses of *T. cruzi* Brazil strain blood-form trypomastigotes (BFT). △ muMT + 1000 BFT; □ muMT + 100 BFT; ○ muMT + BFT; ▼ B6 + 1000 BFT.

including providing help to B cells and $CD8^+$ T cells and as activators of effector cells such as macrophages. $CD8^+$ T cells have been shown to lyse *T. cruzi*-infected target cells (Nickell *et al*. 1993, Wizel *et al*. 1997) and this cytolytic function has been presumed to be the major mechanism of action of these cells. Recently the understanding of the role of $CD8^+$ T cells in immunity to *T. cruzi* has been extended with the presentation of information on the protective capacity of parasite-specific CTLs and the identification of antigen targets for some of these anti-*T. cruzi* $CD8^+$ T cells. Three members of the trans-sialidase family of surface proteins have now been identified as targets of anti-*T. cruzi* CTL responses in both mice (Low *et al*. 1997, Wizel *et al*. 1997) and humans (Wizel and Tarleton, in preparation) and short-term $CD8^+$ T cell lines specific for one of these molecules, TSA-1, have been shown to transfer protection to naive mice (Wizel *et al*. 1997). These T cell lines were highly cytolytic for peptide-pulsed or *T. cruzi*-infected target cells and were potent producers of both IFN-gamma and TNF (Wizel *et al*. 1997). The relative contribution of the cytolytic activity or cytokine production of these protective $CD8^+$ T cells is not known.

$CD8^+$ T cells kill and/or mediate immune control via at least three distinct mechanisms. The perforin/granzyme cytolytic pathway was the first described mechanism of cytolysis by $CD8^+$ CTL and is still considered the primary cytolytic mechanism in most systems (Atkinson & Bleackley, 1995). Perforin/granzyme mediated cytolysis involves the release of granules containing perforin, which polymerizes to form pores in the target cell membrane, and granzymes which may enter through these pores and induce apoptosis of the target cell (Berke, 1995, Kagi *et al*. 1996b). The generation of knockout mice lacking perforin or granzyme B has allowed investigators to explore the role of this pathway in $CD8^+$ T cell response in a number of systems (Heusel *et al*. 1994, Kagi *et al*. 1994a, b, Guidotti and Chisari, 1996, Denkers *et al*. 1997, Laochumroonvorapong *et al*. 1997, Renggli *et al*. 1997, Tang *et al*. 1997). In the present work we show that in *T. cruzi* infection, perforin/granzyme-mediated cytolysis plays a rather minor role in the protective capacity of $CD8^+$ T cells. While mice deficient in $CD8^+$ T cells were highly susceptible to virulent strains of *T. cruzi* and uniformly die in the acute stage of the infection, mice deficient in either perforin or granzyme B function have little to no increase in susceptibility to primary infection, can be protected by prior infection with an avirulent strain of *T. cruzi* and continue to exhibit cytolytic activity for host cells displaying *T. cruzi* peptides. *T. cruzi* infection is not unique with respect to the relative

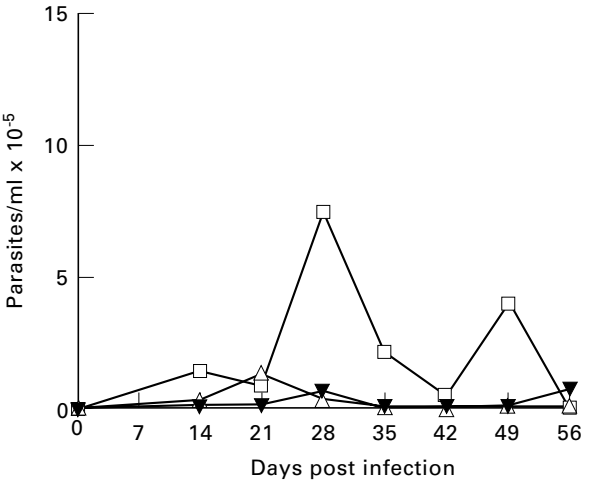

Figure 4 Parasitaemia in mice with targeted deletions in the granzyme and perforin genes and infected with 10^3 BFT of the Brazil strain of *T. cruzi*. Wild-type B6 mice serve as controls. △ granzyme $B^{-/-}$; □ $perforin^{-/-}$; ▼ B6.

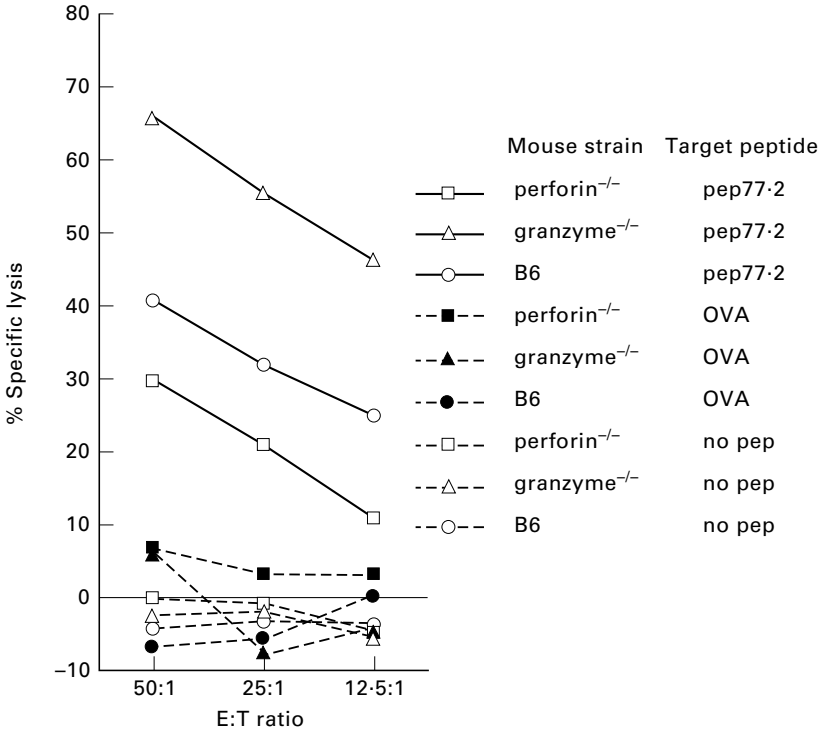

Figure 5 Percent specific lysis as an indicator of CTL activity in spleen cells obtained from wild-type (B6), perforin$^{-/-}$or granzyme B$^{-/-}$ mice on day 90 post infection. Spleen cells were stimulated in vitro with *T. cruzi* TSA-1 derived pep77.2 for six days and tested for lytic activity (^{51}Cr release) on peptide-pulsed 5aKb target cells in a six h assay as previously described (Wizel *et al*. 1997). 5aKb target cells were either pulsed with 1 μM pep77.2 (open symbols, solid lines) 1 μM ovalbumin peptide SIINFELK (Rotzschke *et al*. 1991) (closed symbols, dashed lines) or were incubated in the absence of peptide (open symbols, dashed lines).

lack of importance of the perforin/granzyme pathway in CD8$^+$ CTL-mediated killing. In a number of viral systems, particularly cytopathic viruses, killing via the perforin/granzyme pathway is also a minor factor in protection mediated by CTL (Guidotti *et al*. 1996, Kagi *et al*. 1996b, Kagi & Hensgartner, 1996a). Also early control of infection with either *Toxoplasma gondii* (Denkers *et al*. 1997) or *Mycobacteria tuberculosis* (Laochumroonvorapong *et al*. 1997) and irradiated sporozoite-induced protection in *Plasmodium berghei* infection (Renggli *et al*. 1997) are all perforin-independent.

CD8$^+$ CTL may also kill by Fas/Fas-L interactions or via the surface display or secretion of cytokines such as TNF and IFN-gamma (Berke, 1995, Kagi *et al*. 1996b). The Fas/Fas-L pathway of cytolysis has generally been considered to be of primary importance as an immune regulatory pathway (Kagi *et al*. 1994b, Atkinson *et al*. 1995, Griffith *et al*. 1995, Kagi *et al*. 1996b). However, a number of recent studies have documented a prominent role for Fas/Fas-L interactions in CTL-mediated killing of infected or tumor targets (Kagi *et al*. 1994b, Lowin *et al*. 1994, Clark *et al*. 1995, Frost *et al*. 1997, Garcia *et al*. 1997b, Stenger *et al*. 1997). Additionally, CTL activity and/or immune control mediated by CD8$^+$ T cells has been shown in some cases to be independent of both the perforin/granzyme and Fas/Fas-L pathways and has in most of these cases been ascribed to cytokine production, usually TNF and/or IFN-gamma (Guidotti *et al*. 1996, Kagi *et al*. 1996a, Elkon *et al*. 1997, Laochumroonvorapong, 1997, Sutton *et al*. 1997). The contribution of these other cytolytic pathways to control of *T. cruzi* infection are the focus of on-going studies in our laboratory.

The retention in perforin and granzyme B deficient mice of cytolytic activity specific for class I MHC-presented *T. cruzi* peptides suggests that the Fas/Fas-L or cytokine dependent cytolytic mechanisms are involved in the killer activity of *T. cruzi*-specific CTL. It is also possible that other activities of cytokines, in addition to or rather than cytolysis of target cells, are critical to the protective activity of CD8$^+$ T cells in *T. cruzi* infection. The virus inhibitory activity of IFN-gamma and possibly other cytokines are thought to be of greater importance than cytolysis in immunity to cytopathic viruses (Guidotti *et al*. 1996, Kagi *et al*. 1996a, Ando *et al*. 1997, Elkon *et al*. 1997). Studies in hepatitis B virus transgenic mice (Nakamoto *et al*. 1997) suggest that *in vivo*, both lytic (including perforin/granzyme, Fas/Fas-L and TNF dependent) and non-lytic (involving IFN-gamma and TNF production) mechanisms contribute to CD8$^+$ CTL-mediated target cell destruction. In this latter model it is the nature of the target cells which is proposed to be the primary determinant of the most efficient killing mechanism (Nakamoto *et al*. 1997).

Preliminary studies in our laboratory confirm that cytokines produced by *T. cruzi*-specific CTL can induce nitric

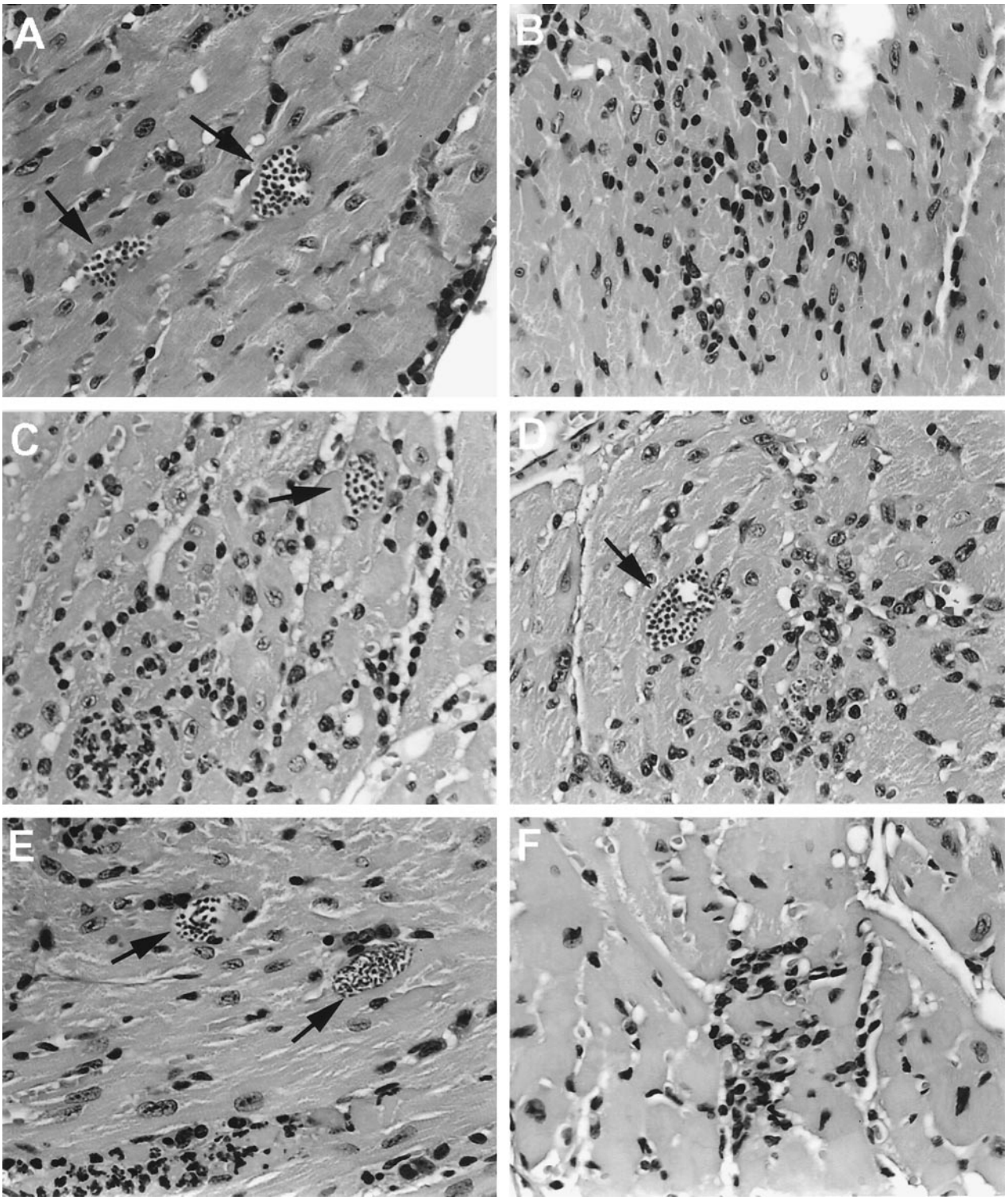

Figure 6 Haematoxylin and eosin-stained tissue sections from hearts of mice infected with 10^3 BFT of the Brazil strain of *T. cruzi*. A) TAP-$1^{-/-}$ day 23 post infection, B) perforin$^{-/-}$ day 45 post infection, C) muMT day 25 post infection, D) B6 day 25 post infection, E) muMT day 45 post infection, and F) B6 day 45 post infection. Note the absence of inflammation in the TAP-$1^{-/-}$ (A) mice in contrast to perforin$^{-/-}$ (B) muMT (C and E), and B6 (D and F) mice. Parasite-infected cells (arrows) are readily detected at 25 days post infection in all strains. However parasites are not obvious at 45 days post infection in perforin$^{-/-}$ and B6 mice but continue to increase in number in muMT mice until the eventual death of these animals. Tissues from granzyme $B^{-/-}$ mice (not shown) are similar in appearance to perforin$^{-/-}$ mice. Original magnification 400×.

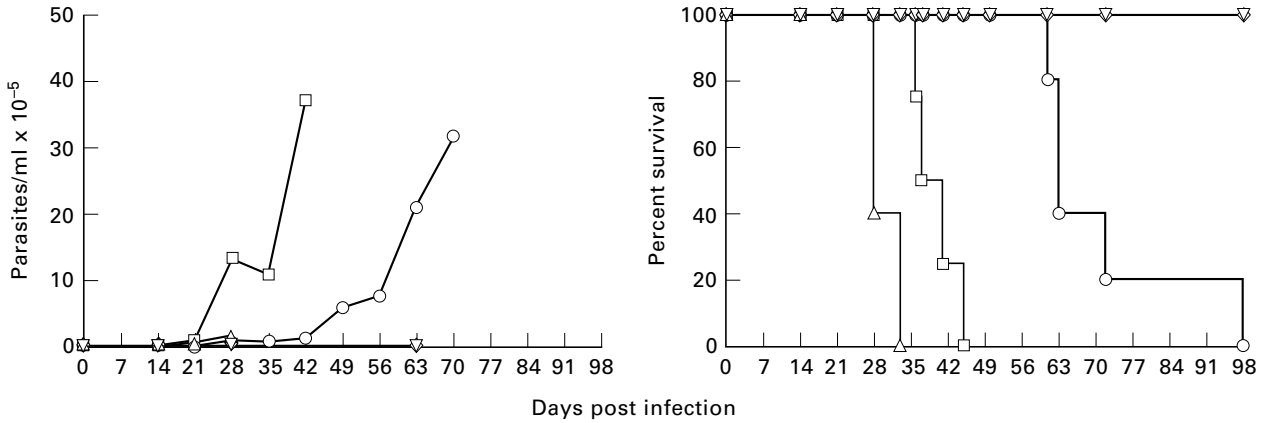

Figure 7 Parasitemia and mortality in immunodeficient and wild type B6 mice immunized with avirulent M80 Miranda clone parasites and challenged with virulent Brazil strain of *T. cruzi.* Mice were infected with 10^6 cultured derived trypomastigotes of *T. cruzi* M80 Miranda clone. After 294 days the mice were challenged with 5×10^4 BFT of the Brazil strain of *T. cruzi*. △ b2m$^{-/-}$; □ TAP-1$^{-/-}$; ○ muMT; ▽ perforin$^{-/-}$; ◇ granzyme B$^{-/-}$.

oxide-dependent regulation of the growth of parasites in non-macrophage cell lines (Rosario & Tarleton, unpublished). In conjunction with the studies reported in the present study, these finding suggest the following model for immune control in *T. cruzi* infection. CD4$^+$ and CD8$^+$ cells have a primary role in regulation of the infection. CD4$^+$ T cells likely act through a number of mechanisms, primarily via providing the appropriate helper function for both antibody-producing B cells and CD8$^+$ CTL. CD4$^+$ T cells may also serve an important role in the activation of macrophages for killing of intracellular parasites. However in this latter function, the importance of CD4$^+$ T cells is probably secondary to that of CD8$^+$ T cells since the majority of the cells infected by *T. cruzi in vivo* are likely to be non-macrophage, non-MHC class II bearing cells. As reviewed above, the CD8$^+$ T cells have a variety of mechanisms by which to regulate parasite growth. The cytokines produced by CD8$^+$ T cells can significantly limit intracellular replication of *T. cruzi* but appear to be insufficient to completely control the infection. Likewise cytolysis of infected cells by CTL would be expected to result in the premature release, but not necessarily the destruction, of intracellular amastigotes. The role of antibody production as a secondary mechanism of immune control can be explained by the requirement for these antibodies to mediate or potentiate the clearance of released amastigotes. Antibodies may also be important in the killing of trypomastigotes although a considerable body of literature documents the multiple mechanisms by which trypomastigotes of *T. cruzi* may evade such antibody-mediated destruction (De Miranda-Santos and Compos-Neto, 1981, Krettli and Brener, 1982, Schenkman *et al*. 1986, Sher *et al*. 1986, Joiner *et al*. 1988, Murfin and Kuhn, 1989, Norris *et al*. 1989, Rimoldi *et al*. 1989, Hall and Joiner, 1993, Tomlinson *et al*. 1994, Garcia *et al*. 1997a). If our model is correct, then efforts toward the production of anti-*T. cruzi* vaccines should focus on protocols which elicit strong type 1 biased T helper and CTL responses and antibody responses to amastigote-derived molecules.

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