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Antigen-Specific T Cells Maintain an Effector Memory Phenotype during Persistent Trypanosoma cruzi Infection

Diana L. Martin* and Rick L. Tarleton2*†

Infection with the parasitic protozoan parasite Trypanosoma cruzi is a major cause of morbidity and mortality in Central and South America. Adaptive immune responses allow for control of parasite levels in the acute phase of the infection but are insufficient to completely clear the infection. Thus, most individuals are infected for life, with parasites persisting primarily in muscle cells, and ~30% of individuals develop chronic Chagas’ disease (1, 2).

Both CD4+ and CD8+ T cells are involved in control of T. cruzi infection, as demonstrated by the high susceptibility of mice lacking a fully competent T cell compartment, such as β2-microglobulin and MHC class I or II knockout mice (3, 4). Parasite-specific CD4+ T cells inhibit T. cruzi replication in vitro (5) and confer protection against lethal challenge in vivo (6). Induction of T. cruzi-specific CD4+ and CD8+ T cells is a critical component of vaccine-induced immunity to T. cruzi (7–9). Additionally, depletion of CD8+ T cells during the late acute or chronic phase of infection results in an increased inflammatory response accompanied by an increase in the parasite load in muscle tissue (10). These results, coupled with the observation that the predominant lymphocyte population in the heart is CD8+ T cells (11), suggest a crucial role for these cells in the control of T. cruzi in both the acute and chronic stages of infection.

Several lines of investigation have led to speculation that the nature of immune responses following T. cruzi infection is largely nonspecific and polyclonal rather than parasite Ag specific. Among these data are the lack of altered TCR Vβ distribution in T. cruzi-infected mice, the high proportion of lymphocyte blasts during the acute phase of infection, the low percentage of hybridomas isolated from infected mice that secrete Abs that bind to T. cruzi, and the expression of B cell mitogens by T. cruzi (12–15). Until relatively recently, similar conclusions had been drawn with regard to T cell activation during viral infection, because limiting dilution analysis estimated that ~95% of responding cells were non-Ag specific (16, 17). However, the use of MHC class I (MHC I)3 tetramers to monitor peptide-specific CD8+ T cells has shown that up to 50% of CD8+ T cells responding during the peak of viral infection can be specific for a single epitope (18). Parasite epitopes recognized by CD4+ or CD8+ T cells from T. cruzi-infected mice and humans have been identified (19–23), and Ag-responsive CD8+ T cells have been isolated from T. cruzi-infected mice (24).

In this study, we have used recently developed, highly sensitive assays to more fully examine the Ag specificity of T cell responses during T. cruzi infection.

Ag-experienced T cells expressing high or low levels of the lymph node-homing receptors CCR7 and CD62L have been identified in infected mice and humans (25). The origins of these central memory T cells (Tcm, CD62Lhigh) and effector memory T cells (TeM, CD62Llow) are the focus of much study and debate. One model proposes that these phenotypes are generated independently after Ag exposure at different anatomical sites in the host (26–28). An alternate view holds that these subsets arise in the process of an alternate differentiation pathway of CD8+ T cells (29, 30). Studies using Ag-specific and polyclonally activated human T cells suggest that Tcm cells are not fully differentiated and recirculate to lymph nodes where they can be rapidly activated and expand following re-exposure to Ag by dendritic cells (DC) after secondary

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infections (25, 29, 30). However, in mice infected with lympho-
cytic choriomeningitis virus (LCMV), T_{EM} convert to T_{CM} fol-
lowing homeostatic proliferation in vivo, lending evidence to a
linear differentiation model in which memory CD8^+ T cells are
maintained as T_{CM} following Ag clearance (31).

Although the development of memory CD8^+ T cells is well
studied in nonpersistent infections, less is known about the de-
velopment of memory in the presence of persistent antigenic stimu-
lation. Parasite persistence in _T. cruzi_-infected mice and humans
has been well documented and provides an opportunity to examine
the nature of the T cell response in chronically infected animals.
The current studies sought to examine several issues related to
_T. cruzi_ infection and persistent infections in general: the Ag speci-
ficity of the T cell response following _T. cruzi_ infection, whether
memory responses develop in persistently infected mice, and if
memory cells develop, what is the phenotype of these cells.

**Materials and Methods**

**Mice and parasites**

C57BL/6 (B6) mice were bred in our animal facility in microisolator cages
under specific pathogen-free conditions. Tissue culture trypomastigotes
(TCT) of the Brazil or Y strains of _T. cruzi_ were obtained from passage
through Vero cells. Mice were infected i.p. with 1000 Brazil or Y strain
TCT and sacrificed by CO_2 inhalation at >150 days postinfection.

**Peptides**

Peptides from _T. cruzi_ trans-sialidase family genes with homology to pre-
viously identified CD8^+ T cell target epitopes (20, 21) were synthesized at
the Molecular Genetics Instrumentation Facility. Approximately 70 such
peptides were pooled and used to stimulate memory CD8^+ T cells.

**Generation of _T. cruzi_ lysate (TCL)**

TCT of the Brazil strain of _T. cruzi_ were transformed to amastigotes in
acidic DMEM/10% FBS for 24 h at 37°C (32). Parasites were pelleted,
washed in PBS, and subjected to greater than five rounds of freeze-thawing
followed by sonication. Cellular debris was removed by centrifugation at
12,000 rpm, and the soluble fraction was boiled for 5 min to denature the
proteins. Protein concentrations were determined using a Bio-Rad protein
assay.

**In vitro stimulation of splenocytes**

Single-cell suspensions of splenocytes from uninfected or _T. cruzi_-infected
mice were generated by gently crushing the spleens with the blunt end of
a syringe, lysing RBC using a hypotonic solution (0.83% NH_4Cl), and
removing clumps by passing cells through a mesh screen. Splenocytes were
cultured 16–20 h at 37°C. Cells were washed twice in PAB, fixed in 2% formaldehyde,
and analyzed using a FACS-Channnel (BD Biosciences).

**Surface staining for memory markers**

Single cell suspensions of spleen cells (SC) were washed in staining buffer
(2% BSA, 0.02% azide in PBS (PAB)) incubated for 15 min in Fc Block
(anti-CD16/CD32; BD Pharmingen), then incubated with the following
appropriately labeled Abs: anti-CD25 FITC, anti-CD11a FITC, anti-
CD62L FITC, anti-CD43 FITC (S7), anti-CD11b PE, anti-CD69 PE, anti-
CD43 PE (1B11), anti-CD8 PerCP, anti-CD4 PerCP, anti-CD45.2 Per-
CPCy5.5, anti-CD45.1 PE, and anti-CD44 allophycocyanin (all BD
Pharmingen). Cells were stained for 30 min at 4°C, the washed
twice in PAB, fixed in 2% formaldehyde, and analyzed using a FACS-
Calibur (BD Biosciences) and CellQuest 3.0 software or FlowJo (Tree
Star).

**FACS and adoptive transfer**

Single-cell suspensions from chronically _T. cruzi_-infected mice were gen-
erated, RBC lysed, and SC stained with anti-CD8 allophycocyanin and anti-
CD62L FITC (both BD Pharmingen) for 30 min at 4°C. Cells were washed
and sorted on a MoFlo cell sorter (DakoCytomation). CD8^-CD62L^- and
CD8^-CD62L^high cells were collected and re-run after sorting to determine
the purity of the sort. Sorted cells were stimulated overnight with bone
marrow-derived DC pulsed with the _T. cruzi_ peptide mix and assayed
the next day for intracellular accumulation of IFN-γ. In separate experi-
ments, SC from naive or chronically infected mice were stained with anti-
CD4, anti-CD8, and CD62L, sorted into CD4^+ CD8^-CD62L^low and
CD4^-CD8^+ CD62L^high populations, and then labeled with 5 μM CFSE.
Briefly, cells were washed twice in PBS, and then incubated for 3 min
in 5 μM CFSE in PBS at room temperature. After extensive washing in
serum-containing medium, cells were washed one last time in PBS, and
50 × 10^5 SC were transferred i.v. to uninfected congenic Ly5.1^+ B6.SIL
mice. For determination of Ag-driven proliferation, mice were infected
the same day with 10^5 Brazil strain _T. cruzi_ trypomastigotes. After 21 days,
mice were sacrificed, and SC were stained for expression of CD4, CD8,
and Ly5.2. Proliferation of cells was determined by examining CFSE di-
lution in the CD8^+ Ly5.2^+ and the CD8^-Ly5.2^+ population following
acquisition and analysis with a FACS caliber flow cytometer.

**Generation of bone marrow-derived DC**

Bone marrow was removed from the femurs of B6 mice and cultured in
RPMI 1640/10% FBS containing glutamine, sodium pyruvate, and genta-
micin, supplemented with 20 ng/ml GM-CSF (R&D Systems). Fresh cul-
ture medium containing GM-CSF was added every other day, and the
development of DC was monitored by gross microscopic evaluation for the
stellate appearance of DC.

**Intracellular cytokine staining**

In vitro-stimulated splenocytes were assayed for intracellular IFN-γ levels
using the Cytofix/Cytoperm intracellular staining kit (BD Pharmingen).
Cells were washed in PAB, incubated for 15 min on ice in Fc Block (anti-
CD16/32; BD Pharmingen), and stained for surface expression of CD4 and
CD8 using anti-CD4 PE and anti-CD8 PerCP (BD Pharmingen). The cells
were fixed and permeabilized using Cytofix/Cytoperm (BD Pharmingen)
on ice for 15 min, and then washed in PermWash (BD Pharmingen). The
cells were then stained with anti-IFN-γ allophycocyanin (BD Pharmingen)
for 20 min on ice. Cells were washed and fixed in 2% formaldehyde for
>20 min at 4°C, and then washed and resuspended in PAB for flow cy-
tometric analysis.

**MHC I tetramer staining**

Single-cell suspensions of splenocytes were washed in PAB. Nonspecific
staining of cells was blocked using Fc Block. Cells were stained 45 min
with anti-CD8 PerCP and allophycocyanin-labeled _T. cruzi_ TSA-1 pep-
tide 77.2-bearing MHC I tetramers consisting of biotin-conjugated H-2K^d/
β_2-microglobulin molecules bearing VDYNFTIV, which were synthesized at
the Tetramer Core Facility (Emory University, Atlanta, GA). Relative fluores-
cence levels were analyzed on 500,000 cells acquired on a FACS-
Calibur (BD Biosciences).

**In vivo cytotoxicity assay**

SC from naive mice were either incubated with a mixture of _T. cruzi_ pep-
tides or with no peptide for 1 h at 37°C. Cells were washed twice with PBS,
and then incubated with 2.5 μM CFSE (CFSE^high) for peptide-loaded cells
or 0.25 μM CFSE (CFSE^low) for unpulsed cells for 3 min at room temper-
ature. The CFSE was quenched with FBS, and the CFSE^low and CFSE-
^high cells were combined and transferred i.v. to naive and chronically
infected mice. After 16 h, spleens were harvested, and CFSE^high and CFSE-
^low cells were detected using a cyan flow cytometer (DakoCytomation).
Percentage of specific killing was determined using the formula: ([1
− (%CFSE^low naive/%CFSE^high naive)/(%CFSE^low chronic/%CFSE^high
chronic)] × 100%).

**Results**

Phenotypic analysis of _T_ cells from chronically _T. cruzi_-infected
mice

Questions remain as to whether _T_ cells fully differentiate to memory
cells if Ag is not cleared. To determine whether _T_ cells from mice
with chronic _T. cruzi_ infection develop into memory _T_ cells or main-
tain an activated phenotype, we examined _T_ cells for evidence of prior
Ag activation. Mice with chronic _T. cruzi_ infections exhibit a 3- to
5-fold increase in the percentage of CD4^+CD11a^high cells in both
CD4^+ and CD8^+ _T_ cell subsets (Fig. 1A). The expression of CD44
and CD11a indicates Ag-experienced cells (33), but these cells do not
show signs of recent activation, because only a minor population ex-
presses the activation markers CD25 or CD69 (Fig. 1B) (33, 34),

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Additionally, CD44<sup>high</sup>CD8<sup>+</sup> T cells do not express the activation-associated isoform of CD43 (35–37) (Fig. 1C). However, a substantial percentage of CD4<sup>+</sup> and CD8<sup>+</sup> T cells from chronically T. cruzi-infected mice, but not uninfected mice, express the low m.w. isoform of CD43 associated with resting memory T cells (37) (Fig. 1D). These data suggest that the vast majority of T cells present in chronically T. cruzi-infected mice are resting, not activated, memory cells.

Memory T cells can be further divided based upon CD62L isoform of CD43 associated with resting memory T cells (37) (Fig. 1E). CD4<sup>+</sup>CD44<sup>high</sup>CD11a<sup>high</sup> memory T cells are CD62L<sup>low</sup> (Fig. 1). Approximately 80–90% of CD4<sup>+</sup>CD44<sup>high</sup>CD11a<sup>high</sup> cells from naive mice. CD11a<sup>high</sup>(left) and CD8<sup>+</sup>CD44<sup>high</sup>CD11a<sup>high</sup>(right) lymphocytes were examined for expression of CD62L. Only data from chronic mice are shown in this group due to the low frequency of CD4<sup>+</sup>CD11a<sup>high</sup> cells in naive mice. Data are representative of > 10 (A and E) or of 3 (B, C, and D) mice.

The use of MHC I tetramers has allowed for the phenotypic and functional analysis of peptide-specific T cells in a wide array of infections (29, 38, 39). We sought to determine the phenotype of functional analysis of peptide-specific T cells in a wide array of infections (29, 38, 39). We sought to determine the phenotype of recent activation of these cells in chronically infected mice. Furthermore, the low frequency of CD69- or CD25-expressing cells among the CD4<sup>+</sup>CD69<sup>low</sup>CD25<sup>low</sup> population of T cells (Fig. 1B) strongly suggests that the CD62L<sup>low</sup> phenotype is not the result of recent activation of these cells in chronically infected mice.

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Although a number of studies have identified T. cruzi Ags that are the targets of T cell responses, other studies suggest that the parasite-specific T cell response in T. cruzi infection is weak (13, 14, 22, 24). This is surprising considering the absolute requirement for the T cell compartment in the control of infection. We tested the ability of a T. cruzi Brazil strain Ag preparation (TcL) to induce IFN-γ production by T cells from T. cruzi-infected mice. Approximately 10% of CD4<sup>+</sup> T cells from mice acutely (data not shown) or chronically infected with T. cruzi (Fig. 3A) produce IFN-γ in response to TcL, compared with 0.57% of CD4<sup>+</sup> T cells from chronically infected mice produce IFN-γ in response to T. cruzi Ags.

**FIGURE 1.** T cells from chronically infected mice display a surface memory phenotype. Spleens were removed from naive B6 or chronically T. cruzi-infected mice and stained immediately ex vivo for memory markers as described in Materials and Methods. A. Cells were gated on CD4<sup>+</sup> (top) and CD8<sup>+</sup> (bottom) lymphocytes and examined for coexpression of CD44 and CD11a. B. Cells were gated on CD4<sup>+</sup>CD44<sup>high</sup>CD11a<sup>high</sup> (top) and CD8<sup>+</sup>CD44<sup>high</sup>CD11a<sup>high</sup> (bottom) lymphocytes and examined for expression of CD25 (top) and CD69 (bottom). Solid lines represent cells from naive mice; dashed lines represent cells from chronically T. cruzi-infected mice. C, CD8<sup>+</sub>CD44<sup>high</sub> cells from naive (left) and chronically T. cruzi-infected (right) mice were stained for the activation-associated isoform of CD43. D, Cells were gated on CD4<sup>+</sup>CD44<sup>high</sup>CD11a<sup>high</sup> (top) and CD8<sup>+</sup>CD44<sup>high</sup>CD11a<sup>high</sup> (bottom) lymphocytes and examined for expression of CD43. E, CD4<sup>+</sup>CD44<sup>high</sup>CD11a<sup>high</sup> (left) and CD8<sup>+</sup>CD44<sup>high</sup>CD11a<sup>high</sup> (right) lymphocytes were examined for expression of CD62L. Only data from chronic mice are shown in this group due to the low frequency of CD4<sup>+</sup>CD11a<sup>high</sup> cells in naive mice. Data are representative of > 10 (A and E) or of 3 (B, C, and D) mice.

**FIGURE 2.** Low occurrence of CD8<sup>+</sup> T cells specific for T. cruzi trypomastigote surface Ag-1 peptide 77.2 during chronic T. cruzi infection. SC from naive or chronically T. cruzi-infected mice were stained with anti-CD8 FITC and peptide 77.2-Kb allophycocyanin, then analyzed using a FACSCalibur (BD Biosciences) cytometer. Data represent the only two tetramer-positive mice from > 35 mice tested. Chronic/reinfected, chronically Brazil-infected mice (1000 TCT) reinfected at > 150 days postinfection with 10<sup>6</sup> Brazil TCT. Numbers represent percentage of CD8<sup>+</sup> T cells that are tetramer-positive.
uninfected B6 controls. Greater than 80% of IFN-γ-producing CD4+ T cells are CD62Llow (Fig. 3C), suggesting that Ag-responsive CD4+ T cells in mice carrying T. cruzi infection are T EM. IFN-γ was produced only in response to T. cruzi Ags and not other complex parasite Ags, such as Schistosoma mansoni-soluble worm Ag preparation (data not shown).

Approximately 4% of CD8+ T cells from chronically T. cruzi-infected mice produced IFN-γ after overnight stimulation with TcL, compared with <0.5% with medium alone (Fig. 3B). IFN-γ-producing CD8+ T cells also exhibit a T EM phenotype, because the majority are CD44highCD62Llow (Fig. 3D). These data demonstrate that a robust Ag-specific CD4+ and CD8+ T cell-derived type 1 cytokine response is generated and maintained during chronic experimental T. cruzi infection.

We exploited our prior knowledge of and ability to identify additional MHC I-restricted epitopes from T. cruzi (20, 21) to confirm that T. cruzi-responsive T cells reside in the CD62LlowCD8+ population. CD8+ T cells from chronically infected mice were sorted into CD62Llow and CD62Lhigh populations, and then stimulated with a mixture of predicted MHC I binding peptides with sequence homology to peptide 77.2, using bone marrow-derived DCs as APCs. Only CD62LlowCD8+ T cells produced IFN-γ in response to T. cruzi peptides (Fig. 4), confirming that cytokine-producing CD8+ T cells in T. cruzi-infected mice are maintained as effector memory cells.

**T cells from chronically T. cruzi-infected mice undergo Ag-driven and homeostatic proliferation**

Proliferative capacity is a defining characteristic of memory T cell responses (31, 40). Self-renewal through Ag-independent basal homeostatic proliferation is thought to maintain memory T cell numbers, whereas Ag-driven proliferation assures rapid responses to secondary infection. We used adoptive transfer to examine the ability of CD62Lhigh and CD62Llow T cells from chronically infected mice to undergo homeostatic proliferation (Fig. 5, no infection) and to proliferate in response to secondary infection (Fig. 5, 10^5 Brazil). As shown in Fig. 5A, CD4+ T cells recovered from uninfected recipient mice were primarily CFSEhigh in both the CD62Llow and CD62Lhigh compartments, indicating no difference in the level of homeostatic proliferation between these cell populations. Both the CD62Lhigh- and CD62Llow-transferred CD4+ cell populations exhibited similar degrees of expansion (4- to 7-fold) 21 days postinfection compared with uninfected recipients (Fig. 5A). Examination of CFSE dilution of transferred CD8+ T cell subsets from uninfected recipient mice reveals that T EM but not T CM cells have the capacity for self-renewal (Fig. 5B), confirming data obtained from viral infection (31). However, both CD62Lhigh and CD62Llow CD8+ T cells proliferate in response to secondary Ag challenge (Fig. 5B). Virtually all of the transferred T cells were CFSEneg following secondary Ag exposure, indicating that cells capable of proliferating in response to Ag are present in both the CD62Llow and CD62Lhigh T cell subsets.

**T. cruzi-specific CTL activity in chronically infected mice**

To assess the functional activity and specificity of CD8+ T cells in chronically infected mice, we determined the in vivo cytolytic activity of T cells using an in vivo CTL assay. A pool of T. cruzi-derived peptides from the trans-sialidase family of proteins was used to pulse SC from naive mice. This peptide pool included
epitopes previously identified using conventional chromium release assays (20, 21), as well as peptides from other trans-sialidase family members that were predicted to bind to H-2Kb. After a 1-h incubation with 10 μM peptide mix, pulsed SC were labeled with 2.5 μM CFSE, whereas unpulsed SC were labeled with 0.25 μM CFSE. Peptide-pulsed and unpulsed cells were then combined and transferred to naive or chronically infected mice. After 16 h, SC from recipient mice were examined for the presence of transferred cells by flow cytometric detection of CFSE-stained cells. Equivalent numbers of peptide-pulsed and unpulsed cells were recovered from naive mice, whereas >90% of T. cruzi peptide-pulsed SC were eliminated in chronically infected mice (Fig. 6). Cells loaded with Sendai virus nucleoprotein epitope 324–332, or with several individual T. cruzi peptides (including peptide 77.2), were not killed (data not shown). These data confirm the immediate effector function of CD8+ T cells in persistently T. cruzi-infected mice and attest to the parasite Ag specificity of the response.

Discussion

Infection of C57BL/6 mice with the Brazil strain of T. cruzi results in the activation of cellular and humoral immune responses that control but do not cure the infection, resulting in parasite persistence in muscle. This model closely mimics the course of infection in humans, in which control but not clearance is also the norm (2).

Infection with T. cruzi results in persistent infection in mice, providing a useful system for examining these models of memory cell development in the absence of Ag clearance. We followed TCM and TEM based on CD62L expression on CD8+ T cells with a memory phenotype (CD44<sup>hi</sup><sup>/</sup>CD11a<sup>hi</sup> or CD44<sup>hi</sup><sup>/</sup>CD43<sup>int</sup>). These cell populations encompass both TCM and TEM cells in LCMV-infected mice (31) and are present at very low levels in naive mice. Both CD4+ and CD8+ T cells producing IFN-γ in response to T. cruzi Ags in a recall response were found to be almost exclusively CD62L<sup>low</sup>, suggesting that Ag-reactive cells maintain a TEM phenotype in chronic T. cruzi infection. Additionally, expansion of both CD62L<sup>hi</sup> and CD62L<sup>low</sup> T cell subsets in response to secondary antigenic challenge was observed, indicating that this function is not limited to the TCM compartment in CD4+ or CD8+ T cells. However, in accord with current models from viral infection (31), homeostatic proliferation was restricted to the TCM population of CD8+ T cells. CD62L<sup>hi</sup> and CD62L<sup>low</sup> CD8+ T cells from T. cruzi-infected mice underwent homeostatic proliferation to similar degrees. Our phenotypic and functional data demonstrate the presence of a large pool of effector memory cells that produce IFN-γ in response to T. cruzi Ag and expand upon secondary Ag exposure, and a smaller pool of CD8+ TCM with the high proliferative capacity.

The models of TCM and TEM functionality continue to undergo revisions. For example, early reports indicated that TCM lacked immediate effector function, but recent reports in mice and humans demonstrate that TCM are capable of both cytokine production and cytolytic capacity ex vivo (44, 45). During experimental T. cruzi infection, it appears that two populations of memory CD8+ T cells emerge and persist: cytokine-producing TEM cells with an extant but restricted capacity to expand and rapidly proliferating self-renewing TCM. Therefore, it may not be unexpected that the proliferative capacity of memory CD8+ T cells with different surface phenotypes would vary depending on the infection. And it is unlikely that T. cruzi infection represents a unique situation. Indeed, much of the world’s population carries some parasitic burden or chronic infection, and these complex infections may not follow the

FIGURE 4. T. cruzi peptide-responsive CD8<sup>+</sup> T cells are CD62L<sup>low</sup>. SC from chronically T. cruzi-infected mice were stained with Abs against CD8 and CD62L<sup>low</sup>, and then sorted into CD8<sup>+</sup>CD62L<sup>hi</sup> and CD8<sup>+</sup>CD62L<sup>low</sup> populations. Sorted cells were incubated overnight on bone marrow-derived DC that had been pulsed with a T. cruzi peptide mix. As a control, CD8<sup>+</sup> T cells were also incubated on unpulsed DC. Cells were stained for expression of IFN-γ as described in Materials and Methods. Cells shown are gated on CD8<sup>+</sup> lymphocytes. Numbers represent the percentage of CD8<sup>+</sup> T cells producing IFN-γ.
rules established in model viral infections where Ag clearance is the norm. Therefore, it may be informative to attempt to understand the effectiveness of different memory subsets within a given infection rather than striving to develop a universal rule for memory T cell development.

The third goal of this study was to begin to address the questions of the Ag specificity, functionality, and the potency of the *T. cruzi*-specific T cell response. The T cell responses to *T. cruzi* are often described as polyclonal, nonspecific, and anergic (13–15, 46). These attributes are a result of findings from a number of studies, but primarily the combination of extensive (“polyclonal”) expansion of the T cell compartment in the infection and the relatively poor proliferative response of these T cells to *T. cruzi* Ags (14, 15).

The advent of MHC I tetramers to analyze peptide-specific CD8\(^+\) T cells at the single-cell level has redefined our thinking about the nature of the immune response to infection. Massive expansion of CD8\(^+\) T cells following infection with intracellular pathogens has traditionally been presumed to be due to the proliferation of cells not specific for the infectious agent. However, analysis of the CD8\(^+\) T cell response to immunodominant viral epitopes using peptide-bearing MHC I tetramers revealed that a greater percentage of activated CD8\(^+\) T cells during acute viral infection was in fact virus specific than had previously been thought (18). Unfortunately, we have to date been unable to make use of MHC tetramers to consistently identify *T. cruzi*-specific CD8\(^+\) T cells in either mice (this study) or humans (47) with *T. cruzi* infection. Given the high antigenic complexity of *T. cruzi* and the multitude of potential MHC I-binding peptides it expresses, it would not be surprising that the frequency of T cells specific for a single epitope would be relatively low and thus difficult to detect with MHC tetramers. Therefore, we turned to the use of a crude parasite preparation and pools of potential peptide epitopes to demonstrate that a significant proportion of T cells in chronically infected mice produced IFN-γ in response to parasite Ags. These responses were specific for *T. cruzi* Ags and not a generalized activation of cells during infection. Furthermore, we showed that MHC I-restricted T cells have potent in vivo cytolytic activity for target cells pulsed

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**FIGURE 5.** Ag-driven and homeostatic proliferation of T cells from chronically *T. cruzi*-infected mice. SC from naive and chronically *T. cruzi*-infected B6 mice were isolated and stained with CFSE as described in Materials and Methods. Labeled SC were transferred i.v. to naive congenic B6.SJL mice, which were then allowed to rest or were infected with 10\(^5\) Brazil strain TCT on the same day as transfer. A, On day 21 posttransfer, SC were isolated and stained with Abs against CD45.2 and CD4 (left panel). Numbers represent the -fold increase of CD45.2\(^+\)CD4\(^+\) cells (gated) in infected vs noninfected recipient mice. Histograms are gated on CD4\(^+\)CD45.2\(^+\) cells and display CFSE vs cell counts (right panel). B. On day 21 posttransfer, SC were isolated and stained with Abs against CD45.2 and CD8. Numbers represent the -fold increase of CD45.2\(^+\)CD8\(^+\) cells (gated) in infected vs noninfected recipient mice. Histograms are gated on CD8\(^+\)CD45.2\(^+\) cells and display CFSE vs cell counts (right panel). Data are representative of three experiments.

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**FIGURE 6.** Target cells loaded with a mix of *T. cruzi*-derived peptides are killed in chronically *T. cruzi*-infected mice. SC from naive B6 mice were loaded with *T. cruzi* peptides and labeled with CFSE as described in Materials and Methods. Cells were recovered from naive and chronic B6 mice 16 h after transfer and examined using a DakoCytoMax cyan flow cytometer. The number in the right panel represents percentage of specific killing and was calculated as described in Materials and Methods. Data are representative of eight experiments.
with *T. cruzi* peptides. Models for CD8+ T cell development derived from examining Ag-specific T cells from several chronic human infections show that perforin expression and cytotoxicity are part of the final phase of differentiation of memory cells (29). Thus, the presence of cytolytic T cells in chronically *T. cruzi*-infected mice suggests that these cells are fully functional, fully differentiated memory cells in vivo. To our knowledge, this is the first report of an in vivo cytotoxic response maintained in a protozoa infection.

Collectively, these results demonstrate that parasite-specific T cells are not depleted or anergic but rather mediate potent and functionally relevant parasite-destructive immune responses in chronic *T. cruzi* infection. We are continuing our attempts to identify targets of the anti-*T. cruzi* response to gain a better understanding of the magnitude of the anti-*T. cruzi* response during infection. We hypothesize that, as in viral infections, the bulk of the immune response generated in *T. cruzi* infection is directed against the parasite. However, the number and diversity of Ags that are the targets of these responses is likely to be much greater in protozoal infections than in viral or bacterial infections. Any bystander activation that occurs in this response may not be an inherent property of *T. cruzi* infection but may be a generalized phenomenon of infection due to cross-reactivity of TCR to different peptides and responses of pre-existing memory T cells that respond to cytokines or chemokines produced during the response to pathogens in the absence of TCR ligation (48, 49). Finally, the antiparasite CD8+ T cell pool in persistently infected mice maintains properties of both TC1 and TC2 cells, bolstering the argument that models of T cell memory derived from sterile viral infections may not be applicable to persistent infections.

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References


